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Hot Topic Themes

CHEMOGENETIC INHIBITION OF ACCUMBENS CHOLINERGIC INTERNEURONS INHIBITS CUE-INDUCED NICOTINE SEEKING

J. M. Leyrer, M. Holter, M. Brickner, J. Newbern, P. F. Overby, M. F. Olive, C. D. Gipson. Psychology, Sch. of Life Sci., Arizona State Univ., Tempe, AZ; Psychology, Univ. of North Carolina, Chapel Hill, Chapel Hill, NC; Psychology, Arizona State university, Tempe, AZ. (859)489-2100. cdgipson@gmail.com

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Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: DP11/X10
Presentation Time: 1:00 PM - 5:00 PM
Session Title: Nicotine, Reward, and Dependence

Our research indicates that cholinergic interneurons, which make acetylcholine and represent less than 1% of neurons within the nucleus accumbens - a brain region highly associated with drug addiction – have outsized control over motivation for nicotine.

Nicotine is the primary addictive substance in tobacco and is widely abused. Cigarette smoking remains the leading cause of preventable death within the United States, accounting for over 400,000 premature deaths each year. Currently, 45 million Americans smoke tobacco, and 70% report that they would like to quit smoking. However, over 80% of individuals who attempt to quit smoking relapse within the first month. Further, only 3% of smokers quit smoking successfully, indicating that relapse to nicotine-containing products is an impactful health issue. Importantly, use of nicotine-containing e-cigarettes, especially among adolescents, has drastically risen in recent years. From 2017-2018, there was a rapid increase in vaping prevalence among adolescents, with nicotine vaping rates translating to roughly an additional 1.3 million adolescent users in 2018 compared to 2017. These statistics indicate that we are in the midst of a nicotine use epidemic.

The NAc has long been associated with drug seeking and relapse. Drug use causes changes the connectivity among neurons and increases the cellular excitability within this brain region. There are multiple neuronal subtypes in the nucleus accumbens, and cholinergic interneurons represent a small subset. The function of these neurons in nicotine relapse is unknown. We determined the role of cholinergic interneurons in nicotine relapse and whether bi-directional control of their excitability could alter nicotine relapse in a preclinical rodent model. This study used a 'chemogenetic' technique in which Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are expressed within specific groups of neurons. DREADDs are genetically modified receptors that can only be activated by human-designed drugs, and they can turn on or off specific cell populations. We expressed two types of DREADDs within nucleus accumbens cholinergic interneurons that allowed us to either activate (excitatory/G_s DREADD) or inhibit (inhibitory/G_i DREADD) cell activity using the designer drug Clozapine-N-Oxide (CNO). We also used a control DREADD, which neither activates nor inhibits these neurons when CNO is given. Before nicotine use via a model termed "self-administration", a cannula was placed within the nucleus accumbens, and DREADDs were directly administered into this brain region to express these designer receptors. Rats then underwent nicotine self-administration procedures, where they learned to press a lever to receive intravenous infusions of nicotine, which were paired with nicotine-associated cues including a tone and a light. Because of the pairings, the tone and light cues took on value that was associated with nicotine. Following 10 days of nicotine self-administration, lever pressing was extinguished, where a press on the lever no longer resulted in nicotine infusions, the light, or the tone. After 14 days of extinction training, animals were given intra-nucleus accumbens CNO injections to manipulate cholinergic cellular activity. Rats were then placed into a 15-minute relapse session, in which lever presses were again paired with the tone and light.

We found that inhibition of cholinergic interneuronal activity prevented nicotine relapse, while activation of these cells did not prevent relapse. By inhibiting cholinergic interneurons, we were also able to inhibit synaptic plasticity, which is a drug-induced change in the brain that increases cross-talk between cells and drives motivated drug seeking behavior. These findings lay the groundwork for identifying new treatment strategies that target cholinergic interneuronal activity to treat nicotine addiction and contribute to understanding nicotine relapse.

Addiction

CIRCUIT-SPECIFIC CRISPR/CAS9 GENE EDITING REVEALS AN EXTENDED AMYGDALA NEUROPEPTIDE RECEPTOR SIGNALING MECHANISM DRIVING ALCOHOL DRINKING, ANXIETY, AND AVOIDANCE

W. J. Giardino, H. Yamaguchi, L. De Lecea. Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA. (509) 869-7415. willgiar@stanford.edu

Program Number: 237.11
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: U13
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Our research implemented revolutionary gene-editing neurotechnology to study molecular targets within brain stress pathways that control alcohol addiction. Together with cutting-edge methods for *monitoring*, *manipulating*, and *mapping* neural circuits in freely-behaving mice, we identified precise receptor signaling mechanisms within unique cellular populations, and characterized their differential contributions to behavioral processes present in substance use disorders.

Alcohol use disorder is prevalent in the United States, where alcohol drinking imposes a massive burden on public health, including an annual death toll of ~90,000 people. Alcohol dependent individuals often achieve extreme intoxication through repeated cycles of binge drinking, and maintain high blood alcohol levels to avoid withdrawal symptoms appearing upon detoxification. In particular, individuals attempting to abstain from alcohol following chronic excessive drinking typically endure withdrawal symptoms including compulsive reward-seeking, irritability, anxiety, insomnia, sleep fragmentation, and in severe cases, fatal seizures. Thus, major efforts aim to elucidate the physiological and genetic mechanisms within neural stress and sleep/wake arousal systems that will inform innovative new strategies for remedying the negative emotional states and disrupted sleep patterns associated with alcohol misuse.

The neural networks responsible for stress, addiction, and sleep disturbances are immensely challenging to study because they encompass an enormous diversity of cell groups that are *physically intermingled* and yet *functionally distinct*. To address these complexities, we performed intricate classification of neuronal subtypes based on 1) long-range axonal projections targeting explicit downstream brain structures, and 2) distinctive genetic markers indicating a cell's molecular contents. Yet, even upon accurately categorizing myriad cell types within given circuits, we considered that neurons rely on innumerable electrical and chemical signals to communicate. Therefore, our studies used advanced strategies to directly manipulate exclusive signaling components in specific families of brain-active "peptide modulators." These so-called "neuropeptide" transmitter molecules bind to specialized receptors that trigger cascading events of protein interactions, resulting in profound modulation of neural activity patterns that can dramatically influence emotional behaviors.

To study addiction, we utilized a well-established paradigm of long-term recurrent alcohol exposure that captures key aspects of human alcohol use disorder. In this procedure, mice that are given unrestricted access to normal food and water repeatedly choose to voluntarily drink intoxicating quantities of alcohol. During the development of this free-choice excessive alcohol consumption, we used a transformative gene-editing technique called *CRISPR/Cas9* to disrupt the expression of specific neuropeptide receptor genes within specific neural circuits, uncovering a detailed molecular mechanism that profoundly controls binge alcohol intake. Additional studies of sucrose drinking, anxiety-like behavior, and approach/avoidance behaviors were used to further define the phenotypic characteristics associated with circuit-specific receptor signaling events. Collectively, our findings established a framework in which chronic alcohol drinking enhances negative emotional states through specific receptor signaling in precisely-defined neurocircuit connections. These outcomes have considerable implications for the multifaceted challenges faced in the search for effective therapeutic strategies to treat addiction.

[Negative emotional states linked to addiction are thought to arise from dysregulated activity and detrimental neuroplasticity in limbic brain systems. In particular, the effects of stress on addiction are proposed to occur via long-lasting adaptations in reciprocally connected circuits of the hypothalamus and amygdala. Our studies focused on lateral hypothalamus (LH) neurons containing the neuropeptide hypocretin (Hcrt; also known as orexin), which are critical for stabilizing wakefulness and driving motivated behaviors. Our previous research identified dense connectivity between Hcrt-LH neurons and "extended amygdala" neurons of the bed nuclei of stria terminalis (BNST) containing the prototypical stress neuropeptide corticotropin-releasing factor (Crf). Our prior studies characterized Hcrt-LH neurons and Crf-BNST neurons as tightly coupled nodes in a stress-promoting neurocircuit, suggesting their involvement in the dysregulated emotional states observed in addiction. The current studies advanced our previous discoveries by successfully identifying the specific cell types and receptor signaling mechanisms through which LH-BNST circuits mobilize the behavioral processes and physiological adaptations underlying voluntary excessive alcohol consumption.

NOCICEPTIN ATTENUATES ALCOHOL DRINKING IN A SEX DEPENDENT MANNER

P. Ghazal, K. Pleil. Dept. of Pharmacol., Weil Cornell Med. Col., Newyork, NY. (929)5295535. pag4003@med.cornell.edu

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Room Number: Hall A
Board Number: U28
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Addiction Treatment

Our research suggests that sex hormones in females may attenuate the protective anti-drinking effect of the endogenous neuropeptide Nociceptin.

Alcohol use disorder (AUD) is one of the most prevalent neuropsychiatric diseases globally, with enormous socioeconomic and health consequences. While alcohol addiction is more prevalent in men, this gender gap is closing and, indeed, has already vanished in the teenage population due to increased alcohol abuse in young women. This is extremely problematic, as women experience a more rapid development of addiction from first use and suffer greater biological and psychological consequences of alcohol use.

Accumulating evidence from both human and rodent studies show that the gonadal sex hormone estrogen, the primary reproductive hormone that fluctuates across the estrous/menstrual cycle in females, contributes to enhanced drug seeking behavior, elevates mood and decreases anxiety, and increases sensitivity to the rewarding effects of alcohol. Interestingly, clinical studies have shown that treatments available for alcohol addiction like Naltrexone are less efficacious for female alcoholics than males, potentially due to the drug reward-promoting actions of estrogen. Consequently, there is urgent need for identification of novel and potentially sex-specific targets for the prevention and treatment of alcohol addiction.

Nociceptin (NOP) is a naturally occurring endogenous signaling molecule in the central nervous system that is recruited during physiological stress. NOP has been shown to reduce conditioned and stress-responsive alcohol consumption, reduce alcohol withdrawal symptoms including withdrawal-induced anxiety, and mitigate relapse behavior in models of addiction. Consequently, NOP agonists have emerged as potential therapeutic candidates for alcohol addiction treatment, however there are off-target effects and most studies have been conducted in males, with few examining NOP effects on risky alcohol drinking prior to dependence.

In this study, we evaluated the effect of NOP signaling on binge alcohol drinking, the riskiest type of alcohol consumption behavior characterized by rapid drinking to intoxication followed by acute withdrawal, in gonadally-intact male and female mice. Using the Drinking in the Dark (DID) model that elicits binge drinking behavior similar to that observed in humans, mice were given home cage access to 20% alcohol for two hours per day for four days, with three days forced abstinence between cycles of access. We found that NOP administered systemically before alcohol access acutely suppressed binge drinking in both sexes, but the effect was greater in males than cycling females, implicating a role of endogenous estrogen in modulating NOP effect on alcohol consumption. Further, direct administration of NOP to the bed nucleus of the stria terminalis (BNST), a sexually-dimorphic brain region known to be involved in the regulation of binge alcohol drinking, stress responsivity, and anxiety, recapitulated the blunting effects of systemic NOP. Ongoing experiments are evaluating the interactions between estrogen and NOP within the BNST to evaluate the hypothesis that estrogen promotes binge drinking behavior by functionally competing with NOP signaling in the BNST using behavioral pharmacology, *in situ* hybridization, and other converging approaches. Our study may provide a new combinatorial pharmacological target (estrogen + NOP signaling) for females at risk for or expressing AUD.

Addiction

CHRONIC PATERNAL THC IN RATS PRIOR TO MATING CAUSES LONG-LASTING BEHAVIORAL DISRUPTION IN THEIR OFFSPRING

E. D. Levin, Z. Holloway, A. B. Hawkey, E. Pippen, H. White, B. Kenou, J. Kim, E. Greengrove, A. H. Rezvani, S. Murphy. Duke Univ. Med. Ctr., Durham, NC. (919) 681-6273. edlevin@duke.edu

Program Number: 112.07
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 9:45 AM
Room Number: Room S401
Board Number: N/A
Presentation Time: 9:30 AM - 9:45 AM
Session Title: Modeling of Schizophrenia Relevant Risk Factors

As cannabis becomes legalized, risks of expanded cannabis and THC use on neurodevelopment need to be better understood. Abundant research has characterized the risks of maternal cannabis and THC exposure during pregnancy for producing adverse neurobehavioral effects in the offspring. However, comparatively little is known about the risks engendered by paternal exposure to cannabis or THC prior to conception. Even though the father does not share physiological processes during early development like the mother does, he can convey to his offspring effects of his chemical exposure by way of epigenetic imprints on the DNA of his sperm.

We have previously shown that preconception delta-9-tetrohydrocannabinol (THC) exposure in male rats significantly alters sperm epigenetic marks, i.e. altered DNA methylation. We also found altered sperm DNA methylation in human males who smoke cannabis. The current study investigated the intergenerational effects of chronic THC exposure of young adult male Sprague-Dawley rats (0, 2 or 4 mg/kg/day SC for 28 days) prior to mating with drug naïve female rats. This paternal THC exposure was not seen to significantly impact the overt health of the offspring. However, the offspring of THC exposed fathers did show significant long-term alterations of behavioral function. Paternal THC exposure caused significant locomotor hyperactivity in adolescent offspring as well as significant cognitive dysfunction when these offspring became adults. Specifically, during adolescence there was significant locomotor hyperactivity in the offspring of fathers exposed to 2 mg/kg/day of THC prior to mating. This hyperactivity diminished as the animals matured, similar to what is seen with attention deficit hyperactivity disorder (ADHD). There were also significant long-term cognitive effects of paternal THC exposure. During the novel object recognition task, the controls maintained their relative preference for the novel object across the duration of the ten-minute session, while the rats whose fathers received THC (2 mg/kg/day) showed a significantly greater drop-off in interest in the novel object during the second half of the session. In the 16-arm radial maze, the 4 mg/kg/day paternal THC dose caused a significant delay in learning. This study found that that chronic paternal THC exposure before conception can cause detrimental neurobehavioral effects in the offspring, including locomotor hyperactivity, and cognitive impairment. Future studies are needed to investigate the underlying mechanisms driving these aberrant developmental outcomes and seek to identify possible behavioral or pharmacological treatments.

This research was supported by grant 60564 from the John Templeton Foundation.

NUCLEUS ACCUMBENS NEURONAL ENSEMBLES IN CUE-INDUCED REWARD SEEKING

A.-C. Bobadilla, E. Dereschewitz, M. D. Scofield, J. A. Heinsbroek, P. W. Kalivas. Neurosci., Anesthesia and Perioperative Med., Med. Univ. of South Carolina, Charleston, SC; Anesthesiol., Univ. of Colorado Denver - Anschutz Med. Campus, Aurora, CO. 1(843)534-7410. bobadilla@musc.edu

Program Number: 453.02
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 11:00 AM
Room Number: Room S401
Board Number: N/A
Presentation Time: 8:15 AM - 8:30 AM
Session Title: Effects of Cocaine Use

Our research indicates that different groups of neurons respond to drugs of abuse (cocaine) compared with natural rewards (sugar).

The ability of positive feelings or outcomes to reinforce a behavior is a common evolutionary strategy across species for establishing reward seeking behaviors. However, in certain mental disorders such as drug addiction, reward seeking becomes unmanageable and causes perseveration on maladaptive behaviors, such as a high vulnerability to relapse to drug use even after long periods of recovery. Over 1.5 million Americans aged 12 and older report current cocaine use, and chronic, life-long substance abuse has been estimated to cost the US over $600 billion annually. Understanding the differences between how the human brain processes natural rewards and drugs of abuse is necessary to develop therapies that specifically target maladaptive drug seeking, but not adaptive seeking of natural rewards.

In brain circuits, different ensembles of neurons are thought to mediate different behaviors, and we compared cocaine-seeking and sucrose-seeking ensembles. To measure both ensembles within the same brain, we developed a new model of dual cocaine and sucrose self-administration in mice. Drug self-administration and reinstatement model is one of the most accepted models for relapse in preclinical research. In our adaptation of this model, mice learned to nose-poke for cocaine one day and sucrose the next, each reward being paired with a different cue. This alternate training procedure was repeated until mice learned to self-administer and discriminate between rewards. The mice then underwent extinction training, during which animals didn't receive rewards or cues. This part of the training models a drug-free, abstinence period. After this training, presenting cues specific for cocaine induced cocaine seeking, while sucrose cues induced sucrose seeking.

Armed with the ability to generate reward-specific seeking, we used genetically modified mice that allow us to identify neurons activated by cocaine or sucrose cues. We first permanently tagged with a red dye neurons activated by cocaine cues, then presented sucrose cues to the same animals and tagged the sucrose seeking ensemble of neurons with a green dye. Using this approach, when looking in the nucleus accumbens, a key region of the reward circuitry, we were able to determine the cocaine-seeking ensemble (red cells) and the sucrose-seeking ensemble (green cells). We found that only 30% of cells expressed both red and green dyes, meaning only a minority of neurons are activated during both cocaine and sucrose seeking, while the majority of neurons selectively responded to either cocaine or sucrose.

These results suggest a finely tuned specificity of neuronal ensembles that code for drug seeking versus seeking a natural reward. The comparison of the two types of reward was critical to determine whether or not drugs "highjack" the biological reward circuitry, or generate their own ensemble of neurons, and we can conclude that drugs are coded by their own ensemble. Importantly, since we find that mice prefer to seek the cocaine cue over the sucrose cue, we are now examining the two ensembles for cellular differences that might cause cocaine seeking to dominate over seeking biological rewards in addiction.

Adolescent Development

DOSAGE SENSITIVITY INTOLERANCE OF VIPR2 DUPLICATION IS CAUSATIVE IN MANIFESTATION OF SCHIZOPHRENIA-LIKE STRIATAL DOPAMINE ABNORMALITY, COGNITIVE, SOCIAL, AND DEVELOPMENTAL DEFICITS IN A NOVEL BACTERIAL ARTIFICIAL CHROMOSOME TRANSGENIC MOUSE MODEL

X. Tian, M. Ren, A. D. Richard, M. Elsaadi, D. S. Dwyer, R. L. Klein, N. E. Goeders, X. W. Yang, X.-H. Lu. Dept. of Pharmacology, Toxicology & Neurosci., Psychiatry, Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA; Dept Psych, UCLA, Los Angeles, CA. (804)503-3104. xtian1@lsuhsc.edu

Program Number: 550.05
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: A50
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Animal Models II

A novel genetic mouse model of schizophrenia identifies a therapeutic target and provides a preclinical tool for drug discovery:

Based on the solid human genetic findings in schizophrenia patients, we developed a genetically modified mouse model of schizophrenia to help understand the cause of the disease. Our findings have important implications for the identification of a novel therapeutic target, and the etiology-relevant mouse model provides a useful preclinical tool for drug discovery.

Schizophrenia is a chronic and often disabling neuropsychiatric disorder that affects 1% of the population, with a lifetime prevalence of 4.0 per 1000 individuals. The economic burden of schizophrenia to the United States was estimated at $155.7 billion, with substantial non-health care and indirect costs. Current pharmacologic agents primarily manage the positive symptoms (responsive only in a small percentage of patients), while cognitive and negative symptoms are largely refractory. Novel disease-modifying therapeutics that rationally target cellular and molecular substrates of cognitive and social deficits, beyond D2 and 5-HT2A receptors, should be developed. However, the lack of understanding the pathogenesis and genetics of schizophrenia and the absence of valid in vivo models are the biggest hurdle for the development of next-generation of antipsychotic therapeutics targeting disease process.

Recent large-scale genome-wide association studies (GWAS) have identified a single-gene duplication of Vasoactive intestinal peptide receptor 2 (VIPR2) on chromosome 7 significantly contribute to the risk of schizophrenia. To confirm the human genetic finding and to understand the pathogenic mechanism of the disease, we have developed a series of transgenic mouse models to mimic the human gene copy number variation. Furthermore, to pinpoint the brain circuits involved in the pathogenic mechanism of schizophrenia, we have designed a genetic switch to remove the genetic defect in different time points of development and different brain regions. The transgenic mouse model recapitulates gene expression and neuronal signaling abnormalities as seen in human patients with the mutation. The genetic insult also manifests schizophrenia-like brain developmental, dopamine dysfunction, cognitive, sensorimotor gating, and social-behavioral deficits in mice. Genetic removal of the mutation rescued the schizophrenia-like dopamine and multiple behavioral deficits. Our results provide further evidence to support the human genetic studies that the increased gene copies of VIPR2 is disease causative to manifest schizophrenia-like symptoms in mice. We have developed a novel genetic strategy to facilitate the genetic dissection of when/where/how the genetic vulnerability affects the development, structure, and function of the brain. We are using this unique animal model to test small-molecule drugs targeting VIPR2 receptor to ameliorate schizophrenia symptom. Furthermore, we have used CRISPR/Cas9 to remove the whole chromosomal duplication as a novel gene therapy strategy. Our animal model will be critical to define the efficacy, safety of the approach, and to establish optimal mechanisms for delivery and efficiency in order to support eventual clinical trials in patients. The translational significance of the study does not just limit to schizophrenia but affords new strategies for the treatment or prevention of other gain-of-gene copies related neurodevelopmental disorders, such as autism, bipolar disorders, OCD, speech and language delay, birth defect, epilepsy, and intellectual disability.

FUNCTIONAL INTEGRITY OF SYNAPSES IS ASSOCIATED WITH ABSENCE OF SYNAPTIC TAU OLIGOMERS IN THE CNS OF COGNITIVELY-INTACT INDIVIDUALS WITH HIGH ALZHEIMER'S NEUROPATHOLOGY

B. Tumurbaatar, A. Singh, B. Krishnan, C. Natarajan, A. Limon, P. Scaduto, R. Woltjer, R. Kayed, G. Taglialatela. Univ. of Texas Med. Br., Galveston, TX; Oregon Hlth. & Sci. Univ., Portland, OR. (409) 772-1679. gtaglial@utmb.edu

Program Number: 015.01
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 3:45 PM
Room Number: Room S401
Board Number: N/A
Presentation Time: 1:00 PM - 1:15 PM
Session Title: Neurodegeneration and Injury I

In this study we discovered that humans that do not succumb to Alzheimer's Disease (AD) despite presenting with the disease's hallmark neuropathology, have neurons that resist the disrupting attack of the pathological Tau protein, the most toxic species in the AD brain.

AD is the most common and devastating age-associated dementia for which there is currently no resolving cure. While the accumulation of neurofibrillary tangles and amyloid plaques (primarily formed by the toxic Tau and Aβ proteins, respectively) is a pathological hallmark of AD, early disruption of neuronal synapses by toxic small aggregates (oligomers) of these proteins ultimately leads to the overt neuron death characterizing the terminal stages of AD.

However, not everyone affected succumbs to the havoc of AD. Indeed, evidence has revealed that some individuals, here referred to as Non-demented with Alzheimer's disease Neuropathology (NDAN), remain cognitive intact despite the presence of AD neuropathology normally associated with terminal stages of AD. This remarkable discovery suggests that there is a natural way for the human brain to resist the devastating impact of AD. It follows that understanding the involved mechanisms could lead the way to the development of a novel therapeutic concepts centered on inducing resistance in anyone affected by AD. With this goal in mind, here we used autopsy brain specimens from AD patients and NDAN subjects to determine the synaptic presence of toxic Tau oligomers and evidence of synapse functional integrity.

We found that overall levels of Tau oligomers were decreased in the brains of NDAN versus AD patients and determined that increasing levels of Tau oligomers correlate with dysfunctional synapses and cognitive deterioration. Most importantly, while abundant tau oligomers were observed associated with synapses in AD patients, there were no Tau oligomers onto synapses in non-demented NDAN subjects. Even more remarkably, we discover a novel, non-amyloid hybrid protein aggregate comprising Tau and Aβ within the synapses of NDAN subjects that appears to sequester Tau from forming toxic oligomers.

These results reveal one possible mechanism for the preserved cognition found in NDAN individuals, linking the absence of toxic Tau oligomers at synapses to maintenance of synaptic function and retention of cognitive integrity despite the conspicuous presence of AD neuropathology, thus giving further credence to tau oligomers as important therapeutic targets in AD. Current studies in our group are exploring the possibility of inducing the formation of the newly discovered Tau/Aβ hybrid aggregated species as a therapeutic strategy to prevent the formation of toxic Tau oligomers so as to save synapses from disruption and ultimately protect the brain from the devastation brought about by AD neuropathology.

Alzheimer's Disease and Other Dementias

SINGLE-CELL TRANSCRIPTOME IDENTIFIES CONSERVED TRANSCRIPTOMIC ALTERATIONS IN ALZHEIMER'S DISEASE

S. J. Morabito, E. Miyoshi, N. Michael, V. Swarup. Grad. Program in Mathematical, Computat. and Systems Biology., Interdepartmental Neurosci. Program, Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA. 1(949)824-3182. vivek.swarup@live.com

Program Number: 014.03
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 4:00 PM
Room Number: Room S104
Board Number: N/A
Presentation Time: 1:30 PM - 1:45 PM
Session Title: Proteome Dysfunction in Aging, Neurodegenerative Disorders, and Alzheimer's Disease

Our findings identify fundamental biological processes that are altered in Alzheimer's disease (AD) but that are unaffected in normal human aging process, the most common risk factor for the disease.
AD is a neurodegenerative disease affecting more than 5 million people in the United States. AD is marked by memory impairment caused due to massive loss of neurons. Currently there is no known cure for this devastating disease, and it is critical that we identify new and improved drug targets for the disease. While aging is been known to be the most common (non-genetic) risk factor of the disease, the changes that occur during normal aging seems to be distinct from that occurring in AD. Using big data analysis, we now identify biological changes which are unique to AD that do not alter during normal aging. While several other research have also pointed to the fact that AD is distinct from accelerated aging, our analysis uses over a thousand individuals from different AD brain banks to arrive at this conclusion. This finding is of immense importance as targeting genes and pathways which are specific to AD and distinct from healthy aging is a prudent approach to develop effective therapeutics.
Our work focusses on identifying biological processes that are at the core of the disease. We performed transcriptional profiling (RNA sequencing) at single-cell resolution on thousands of brain cells from postmortem human patients and used that information to quantify the changes in neuronal and non-neuronal population in human AD patients. We used datasets from more than one thousand human AD and control samples from different brain regions and integrated multiple layers of data comprising of transcriptomics, genetics and epigenetics to identify key biological processes associated with the disease. We also analyzed several brain regions using this approach and found that the changes occurring in AD vary widely according to brain region and we note that this regional diversity is an important aspect of the disease.
Studying network of genes, rather than single genes in complex diseases like AD has been at the forefront of disease research. Using cutting edge data analysis, we found networks of genes that are changed only in the progression of disease. Not only these gene networks co-express together in disease, but also, they are co-regulated, implying that there are special groups of genes, called transcription factors, which are likely modulating these gene-networks. These gene-networks can be thought to be similar to airline networks and also have "gene"-hubs resembling properties of airline hubs. These network hub genes are the most connected genes and it stands to reason that disrupting them using drugs can have profound impact on the progression of disease itself.
We have used these findings to determine which pathways and processes are evolutionarily conserved in mouse models of AD. As a next step, we plan to profile single-cells from human AD and control individuals and understand how the changes in heterogenous groups of brain cells differ between AD and control human subjects. Finally, we plan to take this approach to mouse and stem-cell models of AD and integrate across multiple model systems to a) help us better model the disease in the lab and b) find smarter drug targets which are most likely to be successful against the disease. Overall, we believe our study will usher way for new and powerful therapeutics for this devastating disease.

TREM2 FUNCTION IMPEDES TAU SEEDING IN NEURITIC PLAQUES

C. E. Leyns, M. Gratuze, S. Narasimhan, N. Jain, L. J. Koscal, M. Manis, H. Jiang, M. Colonna, V. M. Y. Lee, J. D. Ulrich, D. M. Holtzman. Neurol., Hope Ctr. for Neurolog. Disorders, Knight Alzheimer's Dis. Res. Ctr., Pathology, Washington Univ. Sch. of Med., St. Louis, MO; Pathology and Lab. Med., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA. 2625108812. cheryl.leyns@merck.com

Program Number: 106.05
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 11:15 AM
Room Number: Room S103
Board Number: N/A
Presentation Time: 9:00 AM - 9:15 AM
Session Title: Alzheimer's Disease: Neuroinflammation and Immune Actions

Our research, using a newly developed mouse model of Alzheimer's disease (AD), has revealed that the brain's immune cells, called microglia, are important in preventing disease progression.

Scientists distinguish AD from other dementias by the accumulation of two proteins in the brain: amyloid-beta (Aβ) and tau. Aβ starts to accumulate first, forming aggregates called plaques outside of brain cells. Tau aggregates appear later in the disease, forming tangles inside of neurons. Yet for many years, exactly how Aβ plaques influence tau aggregation remained an enigma. Then, in 2018, researchers isolated tau aggregates from human AD brain tissue and injected them into mice that had been genetically engineered to develop plaques. Shortly after injection, the human AD tau caused the normal mouse tau to form small ball-like aggregates in damaged neurons surrounding the plaques. This early "seeding" of tau pathology in the mice even led to the appearance of tangles at later time-points. This model demonstrated that plaques can create a permissive environment for tau aggregation.

However, there is more to AD than just plaques and tangles. Recent human genetic studies have found a number of inherited gene mutations that increase an individual's risk of developing AD. Interestingly, many of these newly identified genetic risk factors affect microglia, the resident immune cells of the brain, and the most influential of them is TREM2 (triggering receptor expressed on myeloid cells 2). We were interested in how TREM2 facilitates microglial interactions with Aβ and tau to better understand why mutations that cause a loss of TREM2 function, such as the 'R47H' mutation ($TREM2^{R47H}$), increase risk for AD.

In our study, published earlier this year in *Nature Neuroscience*, we used the model described above to "seed" tau in plaque-bearing mice that had been genetically altered to either not have TREM2 or to have the $TREM2^{R47H}$ AD-risk mutation. Startlingly, mice with either no TREM2 or $TREM2^{R47H}$ had significantly more seeded tau aggregates surrounding their plaques than mice with functional TREM2. This occurred regardless of plaque size or number. Further analyses confirmed that when TREM2 was absent or non-functional, microglia failed to cluster around Aβ plaques, leading to increased damage to surrounding neurons which strongly correlated with the amount of seeded tau aggregates. Furthermore, absent or dysfunctional TREM2 was associated with an increase in a particularly toxic form of Aβ around the outer edges of the plaques, which could possibly contribute to the nearby neuronal damage. Finally, we assessed human brain tissue from AD patients with the $TREM2^{R47H}$ mutation and found they also had more aggregated tau surrounding their plaques compared to AD patients without the mutation, similar to our mouse studies.

This research offers new evidence of how TREM2 function in microglia limits Aβ plaque-mediated tau pathogenesis in AD. It also suggests that loss of plaque-associated microglia in TREM2 mutation carriers increases AD-risk and disease progression via increasing neuronal susceptibility to tau seeding. Future studies will investigate if this vulnerability to these early forms of tau aggregation around plaques accelerates later-stage tau tangle formation and neurodegeneration.

Aβ plaques are considered an early contributor to AD, accumulating 15-20 years prior to cognitive symptoms, whereas tau aggregates are thought to initiate the downward slope towards degeneration and dementia. Our new research suggests TREM2 in microglia may be the missing link between Aβ and tau. TREM2 helps microglia respond to and contain plaques, preventing damage to nearby neurons that would create a tau pool vulnerable to seeding and aggregation. This data will aid current drug discovery efforts to target TREM2 and microglia which could hopefully yield a treatment that slows the progression of AD for patients.

THE EFFECTS OF INSULIN AND INSULIN-LIKE GROWTH FACTOR-I ON AMYLOID PRECURSOR PROTEIN PHOSPHORYLATION IN *IN VITRO* AND *IN VIVO* MODELS OF ALZHEIMER'S DISEASE

B. Kim, S. E. Elzinga, R. E. Henn, L. M. Mcginley, E. L. Feldman. Neurol., Dept. of Neurol., Univ. of Michigan, Ann Arbor, MI. (734) 615-7225. bhumsoo@umich.edu

Program Number: 127.21
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: D38
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Our research suggests that insulin resistance, a key component of diabetes and obesity, may hinder the effective treatment of Alzheimer's disease.

Alzheimer's disease is the most common form of dementia, accounting for over 70% of all cases, and it currently affects 5.4 million Americans. The incidence is expected to reach over 13.8 million by 2050, and it is estimated that the cost for caring for people with Alzheimer's disease will dramatically increase from $203 billion in 2013 to $1.2 trillion by 2050. Meanwhile, the prevalence of obesity and diabetes is rapidly growing; currently over 30 million Americans have diabetes and 2 in 3 adults are overweight or obese. Compounding these alarming statistics, multiple studies report that patients with diabetes and obesity are at increased risk of developing Alzheimer's disease. Despite these associations, however, the mechanisms connecting these conditions are unknown, and effective treatments for both conditions remain elusive.

Insulin and the related protein insulin-like growth factor-I are currently being investigated as treatments for Alzheimer's disease. Some preclinical studies show promising results in improving cognition; however, outcomes are inconsistent, especially for the patients with diabetes and obesity. We believe that this is due the presence of insulin resistance in these patients. Insulin resistance is the key component of diabetes and obesity and can interfere with the normal action of insulin and insulin-like growth factor-I. While clinical studies link insulin resistance and Alzheimer's disease, however, currently little is known about how insulin resistance may influence the effectiveness of either insulin or insulin-like growth factor-I for the treatment of Alzheimer's disease.

In this study, we examined how insulin resistance influences the effect of insulin and insulin-like growth factor-I on the regulation of amyloid precursor protein, one of the critical proteins involved in the development of Alzheimer's disease. Using neurons derived from rodent brains, we show that insulin and insulin-like growth factor-I reduce amyloid precursor protein. However, this effect was greatly blocked when the cells were conditioned to mimic insulin resistance. In support of the clinical relevance of these effects on amyloid precursor protein, we also discovered that obese rats fed with high fat diet display increased amyloid precursor protein in the brain.

Together, these results suggest that insulin resistance may prevent the effectiveness of insulin or insulin-like growth factor-I treatment in Alzheimer's disease, especially for the patients with diabetes and obesity. Additional studies using animal models with diabetes and obesity are needed to fully understand the use of these treatments alone, or in combination, as therapeutic agents in Alzheimer's disease patients with insulin resistance. We are currently studying whether drugs that improve insulin sensitivity may regulate amyloid precursor protein in additional neuronal cell types and animal models.

The findings from our study suggest that insulin resistance can greatly impact the effectiveness of certain treatments designed to prevent or slow down the development or progression of Alzheimer's disease in obese or diabetic individuals. Considering the almost epidemic increase in people with diabetes and obesity combined with the longer life expectancy due to improved healthcare, these results can provide a breakthrough to improve the quality of life for generations to come.

DISSECTING AMYLOID B PHYSIOLOGICAL FUNCTION AT THE SYNAPSE

W. Gulisano, M. Melone, C. Ripoli, M. Tropea, D. D. Li Puma, S. Giunta, S. Cocco, D. Marcotulli, N. Origlia, A. Palmeri, F. Conti, C. Grassi, D. Puzzo. Biomed. and Biotechnological Sci., Univ. of Catania, Catania, Italy; Exptl. and Clin. Med., Univ. Politecnica Delle Marche, Ancona, Italy; Ctr. for Neurobio. of Aging, IRCCS INRCA, Ancona, Italy; Inst. of Human Physiol., Univ. Cattolica Sch. of Med., Roma, Italy; Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy; CNR- Neurosci. Inst., PISA, Italy; Oasi Res. Institute-IRCCS, Troina, Italy. 39(095)4781335. walter.gulisano@gmail.com

Program Number: 354.02
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 4:30 PM
Room Number: Room S103
Board Number: N/A
Presentation Time: 1:15 PM - 1:30 PM
Session Title: Amyloid-Beta: Novel Insights Into Function, Toxicity, and Animal Models

Our research provides novel insight into the physiological role of Amyloid-beta peptide in the healthy brain laying the basis for a better understanding of Alzheimer's disease pathophysiology.

Alzheimer's disease is the most common neurodegenerative disorder of the elderly with 44 million people affected worldwide and representing the 6th leading cause of death in the United States. Although the massive economic and scientific effort towards a better understanding and management of the disease, the lack of efficacious therapies and the increasing number of patients represent a serious problem for the healthcare system.

Alzheimer's disease patients exhibit abnormal accumulation of Amyloid-beta and tau proteins in the brain. Aggregates of these proteins, named oligomers, induce a progressive and irreversible damage of neuronal functions leading to memory loss and, ultimately, to dementia and death. In the last decades, most of the studies have focused on the pathological role of increased Amyloid-beta levels and, as a consequence, several drugs have been developed aiming at clearing it from the brain. However, so far, none of these therapeutic approaches has been effective. In this context, we believe that understanding the physiological function of Amyloid-beta in the nervous system is necessary to interpret its role in Alzheimer's disease.

It has been previously demonstrated that Amyloid-beta is released during neural activity and it is needed for memory formation since blocking its function results in memory impairment. Furthermore, several reports have indicated that oligomers exert opposite effects depending upon their dose, with high concentrations being extremely toxic and low concentrations exerting a beneficial neuromodulatory role.

In our study, we investigated the molecular mechanisms underlying the physiological role of Amyloid-beta in synaptic plasticity, a phenomenon characterized by functional and structural changes occurring at the synapse, i.e. the connection between neurons, during learning and memory. In particular, we studied the effects of Amyloid-beta oligomer administration at low concentrations, resembling their physiological content in the brain, on different models (from cell cultures to animal models). Electrophysiological and electron microscopy experiments indicated that Amyloid-beta oligomers increased the neurotransmitter release at the synapse. This treatment also enhanced the expression of key proteins involved in synaptic plasticity and long-term memory formation. Consistently, low concentration of Amyloid-beta oligomers strengthened long-term potentiation, a form of synaptic plasticity thought to be the molecular correlate of memory, thus converting short-term memory into long-term memory and reinforcing the idea that the protein acts as a cognitive enhancer. These Amyloid-beta effects were mediated by a subtype of receptors belonging to the cholinergic system, a well-known circuitry that modulates cognitive functions in the brain. Interestingly, several studies have demonstrated that cholinergic transmission is impaired in Alzheimer's disease and different drugs have been developed to restore its function, e.g. *cholinesterase inhibitors* which are among the few approved drugs against the disease.

Taken together, our results indicate that Amyloid-beta is a neuromodulator whose function is needed in the healthy brain to make new memories. Understanding why and how a protein that is normally produced in the brain to ensure memory formation accumulates thus leading to memory disruption is still an unanswered question. An intriguing hypothesis is that the pathological increase of Amyloid-beta is a compensatory mechanism caused by a failure of its physiological function that might be due to a malfunction of cholinergic receptors. Thus, we are now focusing on the crosstalk between Amyloid-beta and cholinergic system trying to propose a novel model of the disease. Thus, our findings give new perspectives into Alzheimer's disease pathophysiology and could explain why clearing Amyloid-beta from the brain might not be a good therapeutic strategy.

ALTERED HEME METABOLISM IN ALZHEIMER'S DISEASE PATHOGENESIS

L. Zhang, C. Vidal, K. Daescu, K. E. Fitzgerald, A. Starokadomska, I. Bezprozvanny. Univ. of Texas at Dallas, Richardson, TX; Univ. of Texas Southwestern Med. Ctr., Dallas, TX; Dept Physiol, UT Southwestern Med. Ctr. Dallas, Dallas, TX. li.zhang@utdallas.edu

Program Number: 377.17
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: D32
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Our research indicates that altered heme homeostasis is likely the early, initiating event underlying the pathogenesis of Alzheimer's disease.

Alzheimer's disease is the most common cause of dementia. An estimated 5.7 million Americans of all ages are living with Alzheimer's dementia. It is progressive and degenerative. The disease causes problems with memory, thinking, and behavior. A major hallmark of Alzheimer's disease is amyloid plaques, which are extracellular deposits of a peptide called amyloid beta. Aggregated amyloid beta is an invariable signature of the molecular pathology of the disease. Despite decades of intense research on the basic biology and clinical pathophysiology of Alzheimer's disease, there is still a lack of effective strategies to prevent and treat Alzheimer's disease. All clinical trials designed to lower levels of amyloid beta generation and aggregation or promoting its clearance by immunotherapy have failed.

However, amyloid beta can influence many molecular and cellular functions in the brain. The failures of previous clinical trials may suggest that drugs were being tested too late in the disease process or that the drug-targeted event is not the initiating or causal event for disease pathogenesis. It is worth noting that mitochondrial bioenergetic deficits coupled with increased oxidative stress are consistent antecedents leading to Alzheimer's disease pathology. Importantly, heme, iron protoporphyrin IX, is crucial for mitochondrial bioenergetic function and can supply potent antioxidants, biliverdin and bilirubin, which are heme degradation products. Intriguingly, limited experimental evidence has suggested that altered heme homeostasis accompanies Alzheimer's disease pathogenesis.

In this study, we evaluated the link between altered heme metabolism and Alzheimer's disease using data from human patients, mouse models, and neuronal cell lines. Firstly, computational analysis of gene expression data from human patient brains showed that the levels of several heme synthesis and degradation enzymes, as well as enzymes involved in the mitochondrial bioenergetic process, oxidative p hosphorylation, are inversely correlated with the severity of Alzheimer's disease. Secondly, immunohistochemistry analysis of brain tissues from mice with Alzheimer's disease pathology showed that the levels of heme synthesis and degradation enzymes are decreased in early stages of pathogenesis. Thirdly, using human neuronal cell lines, we found that the levels of heme synthesis, uptake, and degradation, as well as the relevant enzymes and transporters, strongly increase as neuronal differentiation proceeds. Likewise, the levels of subunits of mitochondrial oxidative phosphorylation complexes are elevated as neuronal cells differentiate. These results support the idea that heme flux is highly elevated in neuronal cells, thereby increasing the synthesis of mitochondrial oxidative phosphorylation complexes for bioenergetic needs and enhancing the generation of heme degradation products for antioxidant power. Further, our studies showed that amyloid beta strongly reduces the level of the heme degradation enzyme, heme oxygenase 2, as well as the rate of heme degradation.

Together, these results indicate that proper neuronal functioning requires high levels of heme synthesis, uptake, and degradation, in order to promote the production of oxidative phosphorylation complexes and to supply potent antioxidants in neuronal cells. By lowering the levels of heme degradation and the heme degradation enzyme, amyloid beta alters cellular heme homeostasis and perturbs the production of both cellular energy and reducing power, two crucial factors in the maintenance of neuronal functions. Such perturbation has the potential to initiate a cascade of neurotoxic events leading to Alzheimer's disease pathogenesis. The findings from this study provide promise for further investigation of the link between altered heme homeostasis and Alzheimer's disease pathology. Such investigations are likely to fundamentally advance the understanding of Alzheimer's disease pathogenesis and to discover novel strategies for the treatment and prevention of Alzheimer's disease.

PHOSPHORYLATION AND CYTOPLASMIC TRANSLOCATION OF TDP-43 IN RESPONSE TO A NEUROTOXIC FRAGMENT OF APOLIPOPROTEIN E4 (APOE4): A MECHANISM THAT MAY UNDERLIE LOSS OF COGNITION IN ALZHEIMER'S DISEASE AND ALZHEIMER'S DISEASE RELATED DEMENTIAS (ADRD)

P. Mcdonough, K. L. Gordon, R. C. B. Basa, S. Ramesh, C. Handley, T. T. Rohn, A. Ward, J. Wang, J. H. Price. Vala Sci. Inc, SAN DIEGO, CA; Boise State Univ., Boise, ID; Allele Biotech., San Diego, CA; Scintillon Inst., San Diego, CA. (858)461-6863. pmcdonough@valasciences.com

Program Number: 375.11
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: DP06/C67
Presentation Time: 1:00 PM - 5:00 PM
Session Title: Alzheimer's Disease: APOE and Associated Pathways

We have discovered that when a fragment of the protein ApoE4 (apolipoprotein E-epison-4) is applied to cultured human neurons, a protein named TDP-43 (transactive response DNA binding protein 43 kDa) is modified (a phosphate group is added to it) and moves from the nucleus to the cytoplasm (the main region of the neuron, that surrounds the nucleus). This effect is associated with a toxic effect of the ApoE4 fragment on the neurons. This is important because ApoE4 and TDP-43 have previously been implicated in dementia resulting from Alzheimer's Disease, Parkinson's Disease, stroke, AIDs, and traumatic brain injury, but the details of how these proteins participate in the death of brain neurons is not understood. We are performing preclinical research in neuroscience and we are presenting our research on this subject for the first time. Our findings suggest that ApoE4 and TDP-43 are involved in a common pathway that leads to loss of the neurons in the brain leading to defects in memory and cognition (thinking properly). Our work is novel and has the potential to link recent observations by other researchers into a framework for better understanding neurodegenerative diseases and brain injury. ApoE is a protein previously implicated in transporting cholesterol and other substances to the tissues and organs. Every person has two copies of the ApoE gene (one copy is inherited from each parent) but the copies may not be identical as there are 3 different types of ApoE (named ApoE2, ApoE3, and ApoE4). ApoE3 is most common and most people have two copies of the ApoE3 gene. Unfortunately, people that inherit the ApoE4 gene are more likely to develop dementia from Alzheimer's Disease or from other causes, than people with ApoE3. Previous research has shown that ApoE4 is cut into fragments within the brain, and one fragment of ApoE4 is toxic to neurons, but the mechanism of toxicity has not been established. Modification and movement of TDP-43 to the cytoplasm was first implicated in loss of muscle control in Amyotrophic Lateral Sclerosis (ALS). However, recent results show that TDP-43 is also modified and cytoplasmic in brain sections, obtained post-mortem, from people with dementia, and the extent of TDP-43 modification correlates with the severity of the dementia. Our experiments use human neurons produced from stem cells that are derived from skin cells (human embryos are not used to produce the stem cells). We culture the neurons in clear plastic dishes (up to 384 wells per dish) and we quantify the results by photographing the neurons with digital cameras attached to robotic microscopes. About 5 million people in the USA have dementia and these numbers will rise in the future as the population grows and people live longer. There is no known cure and the burden to society is enormous (about $277 billion per year is spent in the USA to take care of people with dementia as reported by CBS news). Our methods can be used to test thousands of drug candidates per week, and will accelerate the discovery of new medicines for dementia.

ENDOSOMAL PH DROP AMELIORATES APOE4-MEDIATED SYNAPTIC IMPAIRMENTS AND REDUCES AMYLOID PLAQUE LOAD *IN VIVO*

T. Pohlkamp, X. Xian, M. Durakoglugil, C. H. Wong, F. Plattner, J. Herz. Univ. of Texas Southwestern Med. Ctr., Dallas, TX. (469)321-7531. theresa.pohlkamp@utsouthwestern.edu

Program Number: 375.22
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C78
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Alzheimer's Disease: APOE and Associated Pathways

Combating Alzheimer's Disease by Endosomal Acidification

Endosomal acidification restores endosomal trafficking of Apolipoprotein E (ApoE) isoform E4, the most important genetic risk factor for Alzheimer's disease. We now show that endosomal acidification attenuates E4-associated deficits in memory formation and reduces Alzheimer's patholgy.

Alzheimer's disease is a condition of memory loss for which no cure is available. E4-genotype decreases the age of onset for this devastating disease by up to 10 years. Currently, it is not well understood how ApoE isoform E4 contributes to dementia. The main function of ApoE in the brain is to nurture neurons with lipids and cholesterol, the major components of cell membranes. To deliver its cargo, ApoE gets taken up into intracellular membrane compartments called endosomes, which also carry membrane receptors. Memory formation requires the communication of neurons at their junctions, called synapses, through receptors and neurotransmitters. The amount of membrane receptors ath the synaptic surface is tightly regulated depending on neuronal activity. Receptors can get internalized from the cell surface by budding off membrane to form endosomes. Inversely, endosomes can fuse back with the cell membrane to insert receptors. Importantly, ApoE traffics through the same endosomal system as synaptic receptors do. After cargo delivery ApoE recycles back to the cell surface, whereas its cargo undergoes further sorting for delivery. The luminal pH is a key feature in the endosomal sorting process: mild acidification in early endosomes allows ligands like ApoE to detach from their receptors; stronger acidification in late endosomes and lysosomes allows further sorting and degradation of biomolecules. In humans E4 is one of three common ApoE isoforms and in comparison to the others E4 is most positively charged. The charge of E4, but not the other ApoE isoforms, neutralizes at the pH present inside early endosomal compartments. Importantly, a charge-neutralized protein is prone to self-aggregation. Thus, we have proposed that E4 aggregates in the pH-environment of early endosomes and prevents these compartments from recycling back to the surface, holding back synaptic relevant receptors. In order to prevent E4-neutralization and aggregation we chose to acidify the endosomal lumen by disrupting the gene of a protein named NHE6. NHE6 is embedded in the endosomal membrane and acts to alkalize the lumen. We previously found that loss of NHE6 in neurons ameliorates E4-mediated delays in endosomal receptor trafficking. To validate our findings in an animal model we used a mouse mutant that is deficient for NHE6. With this mouse model we now show that loss of NHE6 improves E4-mediated deficits in long-term potentiation, a paradigm of memory formation. Additonal new findings show that endosomal acidification reduced Alzheimer's pathology in a mouse model of this disease.

This is the first time that endosomal acidification is investigated as a potential treatment strategy against Alzheimer's disease. Traditional treatment strategies that are based on antibodies raised against a toxic peptide that causes Alzheimer's pathology have all failed in clinical studies. Endosomal acidification opens a new avenue in the search for a drug in this devastating disease. Since there are no specific NHE6-drugs available, small molecules need to be found and/or developed that are capable to enter the brain efficiently and selectively block NHE6.

ASSOCIATION OF AB WITH ASTROCYTE-DERIVED AND CERAMIDE-ENRICHED EXOSOMES MEDIATES AB MITOTOXICITY IN NEURONS WHICH IS PREVENTED BY NOVEL CERAMIDE ANALOGS

A. Elsherbini, H. Qin, Z. Zhu, S. Karki, S. Crivelli, P. Tripathi, G. Wang, E. Bieberich. Univ. of Kentucky, Lexington, KY. ael239@uky.edu

Program Number: 560.07
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C92
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Alzheimer's Disease: Amyloid-Beta Toxicity

Alzheimer Disease (AD) is the most common form of dementia and it represents the sixth leading cause of death among Americans with annual cost of 220 billion dollars. One of the hallmarks of AD is the accumulation of neurotoxic protein called beta-amyloid (Abeta) which gets in between neurons, interferes with the relay of information and subsequently, leads to the deposition of amyloid plaques. The exact molecular mechanism underlying the disease progression is still unclear, posing a critical barrier in AD research. Despite significant research efforts in the field of AD, the success rate of clinical trials in the past two decades is below 0.4%. The U.S. Food and Drug Administration (FDA) approved only one drug in the last 15 years. Therefore, there is a need for a paradigm shift in approaching AD in research and therapeutic strategies.In the past decade, exosomes have risen as new player in AD progression. Exosomes are nano-sized lipid vesicles formed inside cells and they are known to serve as means of intercellular communication by moving cargos between different types of cell. In the context of AD, exosomes have been implicated in the progression of the disease by facilitating amyloid plaque nucleation, and increasing neurotoxicity, among other effects. Ceramide- a pro-apoptotic sphingolipid that is involved in a variety of cellular processes- is part of the lipid component of exosomes and involved in exosome biogenesis and secretion. In an AD mouse model, we previously showed that ceramide depletion leads to decreased level of brain exosomes, lowered total Abeta peptide, decreased plaque burden and reduced glial activation.Knowing that exosomes can readily cross the blood brain barrier (BBB), we sought to capture these exosomes in the blood and study them as a window to the complex environment of the brain.In this work, we used an AD mouse model (5xFAD) that expresses human Abeta in neurons. We show that exosomes from the 5xFAD mice sera, but not from wild type mice, contains a proportion of exosomes that are enriched in the lipid ceramide. These ceramide-enriched exosomes are also shown to originate from astrocytes- glial cells in the brain closely associated with neurons. We coined these exosomes as "astrosomes" and we show that the novel ceramide analog N-oleoyl serinol (S18) prevented Abeta association with astrosomes suggesting that ceramide mediated binding of Abeta to astrosomes. We also show that combining Abeta with astrosomes causes significantly more harm to neurons in tissue culture compared to Abeta alone, suggesting that association with astrosomes enhanced Abeta neurotoxicity. Upon treating our neurons with astrosomes from 5xFAD mice serum, we found that they are taken up by neurons and transported to mitochondria. At mitochondrial level, astrosomes induced clustering which is a known evidence of mitochondrial dysfunction.We also report that Aβ-associated astrosomes mediated binding of Aβ to voltage gated anion channel 1 (VDAC1) a mitochondrial gatekeeper localized in the outer mitochondrial membrane. We hypothesize that Abeta-associated astrosomes induce complex formation between Aβ and VDAC1 concurrent with caspase activation and apoptosis. Caspase activation is the first step in the induction of neuronal cell death, a process leading to neurodegeneration and cognitive decline in Alzheimer's disease. Complex formation and caspase activation were not observed with exosomes from wild type and human control serum. By preventing association of Abeta to exosomes, novel ceramide analogs such as S18 may interfere with neurodegeneration induced by Abeta-associated exosomes. In summary, our data suggests that association of Aβ with ceramide in astrosomes enhances Aβ interaction with VDAC1 and mediates Aβ neurotoxicity in AD, which can be prevented novel ceramide analogs.This work was supported by funding from the National Institutes of Health (R01AG034389 and R01NS095215)

PROTECTIVE EFFECT OF A_{11} GOLD NANOPARTICLES AGAINST AGGREGATION AND TOXICITY MEDIATED BY B-AMYLOID: POTENTIAL IMPLICATIONS FOR ALZHEIMER'S DISEASE

B. G. Anand, Q. Wu, K. Shejale, S. Amidian, H. Wille, S. Kar. Med., Biochem., Ctr. for Prions and Protein Folding Diseases, Univ. of Alberta, Univ. of Alberta, Edmonton, AB, Canada; Dept. of Metallurgical Engin. and Materials Sci., Indian Inst. of Technol., Bombay, Powai, India, India. 1(780)242-8363. banand@ualberta.ca

Program Number: 447.06
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 11:15 AM
Room Number: Room S103
Board Number: N/A
Presentation Time: 9:15 AM - 9:30 AM
Session Title: Alzheimer's Disease and Related Dementia: Therapeutic Strategies

Alzheimer's disease (AD) is an unremitting neurodegenerative disorder characterized by a gradual loss of memory followed by deterioration of higher cognitive functions such as language, praxis, and judgment. AD afflicts ~7% of the population over 65yrs of age and its prevalence doubles every 5yrs thereafter. These facts, together with a steady increase in life expectancy, make AD one of the most serious health problems of our time. While most AD cases are not associated with any known genetic abnormalities, some cases segregate with mutations of specific genes. The pathological features associated with AD include the presence of abnormal clumps of proteins within the neurons (i.e., tau protein as neurofibrillary tangles) and outside the neurons [i.e., β-amyloid (Aβ) peptide as neuritic plaques] along with loss of selected neurons in the brain. Pathological changes that characterize AD indicate that an increase in production and/or decreased clearance may enhance Aβ levels which then aggregate to become toxic to neurons. Accumulation of toxic Aβ leads to neuronal death and subsequent development of AD. Thus, preventing the aggregation/toxicity of Aβ peptide has long been considered as a promising treatment strategy for averting or delaying the onset of AD.

Despite extensive research, at present, there is no effective treatment to arrest the progression of AD. The cholinesterase inhibitors (which prevent the breakdown of the neurotransmitter acetylcholine) and memantine (which inhibits the function of the glutamate receptor NMDA) that have been approved for the treatment provide symptomatic relief for only a fraction of AD patients. Other strategies that are under development for AD treatment include novel inhibitors of cholinesterase, neuroprotective agents, drugs that can inhibit Aβ aggregation/toxicity and vaccination against Aβ peptide.Over the last decade, nanoparticles, which are engineered materials less than 100nm in length with unique physio-chemical properties, have been explored extensively as an area of novel therapeutic modalities for the treatment of AD. Strategies utilizing functionalized/conjugated nanoparticles that are in different stages of development for AD include targeted delivery of cholinesterase inhibitors (donepezil, galantamine), phytochemicals (resveratrol, curcumin), hormones (estradiol, melatonin), antibodies and various drugs/agents (memantine, selegiline) to sequester Aβ peptide or to interfere with Aβ aggregation and toxicity. However, very little is known on surface engineered stealth-based nanoparticles.

In this current work, we show that gold nanoparticles once surface functionalized with a phenolic compound A11 was able to reduce aggregation as well as the toxicity of Aβ, thus indicating the therapeutic potential of these nanoparticles in the treatment of AD pathology. First, we have successfully synthesized phenolic A11 compound-based 5nm sized gold nanoparticles (i.e., A_{11} AuNps) and then demonstrate that these nanoparticles can able to attenuate the aggregation of Aβ as measured using Thioflavin-T assay. Subsequently using various biophysical techniques such as Circular dichroism, Fourier Transform Infra-Red, Raman spectroscopy and Dynamic Light Scattering we showed that these nanoparticles prevented self-assembly of Aβ peptide and retained them in non-toxic monomeric forms.

Additionally, using Fluorescence quenching, Isothermal Titration Calorimetry, and molecular docking studies, we revealed a strong binding interaction between A_{11} AuNps and Aβ peptide hydrophobic domain, which plays a critical role in Aβ aggregation. Interestingly, these effects were not observed with unfunctionalized A_{11} molecule or control AuNps. We further depicted that A_{11} AuNps was able to protect mouse brain neurons grown in Petri-dishes against Aβ-induced toxicity. The protective effect of A_{11} AuNps is partly mediated by decreasing cellular mechanisms that underlie neuronal death following Aβ treatment. Thus, we have successfully functionalized a new phenolic compound-based gold nanoparticle, which can suppress the aggregation of Aβ and also can protect neurons against Aβ toxicity - thus highlighting its potential implication in the treatment of AD-related pathologies.

ASSAY BASED ON TAU-RD-GFP FRET PAIRS DOES NOT REPRESENT TEMPLATED ASSEMBLY OF PHFS

S. Kaniyappan, K. Tepper, J. Biernat, R. R. Chandupatla, S. Hübschmann, S. Irsen, S. Bicher, C. Klatt, E. M. Mandelkow, E. Mandelkow. German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany; CAESAR Res. Ctr., Bonn, Germany. 49(228)43302630. mand@mpasmb.desy.de

Program Number: 446.07
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 11:15 AM
Room Number: Room S106
Board Number: N/A
Presentation Time: 9:30 AM - 9:45 AM
Session Title: Tau Protein in Alzheimer's Disease and Other Dementia: Biochemistry and Cellular/ Animal Models

Summary:

This abstract questions the hypothesis that the spreading of tau pathology in Alzheimer Disease is caused by a prion-like propagation of misfolded tau protein.

Background:

Alzheimer Disease, the major brain disease at advanced age, is recognized by abnormal changes in two proteins, **"amyloid-beta"** and **"tau"**, which accumulate as sticky clumps (**"aggregates"** composed of fibers). A focus of research for diagnosis and therapy is to detect aggregates at early stages and prevent or dissolve them. One clue is the sequential appearance of tau clumps **("spreading")** across the brain (**Braak** stages **[1]**). Spreading proceeds between areas connected by neurons, as if pathology were passed from one cell to the next.

Brain regions can communicate in various ways, e.g. electrical signals, neurotransmitters, or exchange of proteins. A leading hypothesis holds that tau pathology spreads by spreading of tau protein, which can adopt a misfolded structure in one cell, then pass into another cell where it imposes (**templates)** its pathological structure onto native tau molecules **[2]**. The process bears similarity to prion disease where spreading from donor to acceptor cells is caused by prion protein; hence the spreading of tau pathology is thought to be "prion-like".

Experimental:

A key approach to test the hypothesis is to prepare misfolded tau protein, add it to cells containing native tau, and check if this becomes misfolded and aggregated in the acceptor cells. The problem is that tau fibers with amyloid structure are difficult to observe in living cells. To overcome this problem, a common procedure uses surrogate markers expressed in acceptor cells **[3]**. They consist of short tau fragments (the "repeat domain", molecular weight ~13kDal, less than 30% of full-length tau, ~46kDal), with mutations enhancing amyloid structure, and tagged with fluorescent reporter proteins (variants of Green Fluorescent Protein, GFP, ~28kDal). Local accumulations of reporter molecules are observed by light microscopy as peaks of fluorescence or "fluorescence resonance energy transfer" (FRET), which is taken as evidence of pathological aggregation of tau.

The problem of this interpretation is that FRET signals are not specific for the β-sheet packing of amyloids (separation of strands 0.47nm). Rather, FRET measures a general vicinity of two reporter molecules up to ~5nm, without requiring a strict order. Considering this, we studied the properties of tau reporter proteins by various biophysical and cell biological methods, including scanning transmission electron microscopy (STEM), atomic force microscopy (AFM).

Conclusions:

Results show that tau repeat domains linked to GFP cannot form Alzheimer-like pathological fibers. The likely reason is a steric clash between the reporter protein (size ~4.8nm) which is ~10 times larger than the packing distance in the amyloid fiber (0.47nm) and blocks the assembly of fibers typical of Alzheimer. This is consistent with similar observations on amyloid-β peptides tagged to reporter proteins which also inhibits aggregation **[4]**. This suggests that the apparent transmission of tau pathology into acceptor cells is not based on templating a native tau structure onto a misfolded structure in a prion-like fashion. In fact it may not even require misfolded tau at all, since similar fluorescence signals from TauRD-GFP reporters can be induced by other signalling molecules such as cytokines (TNF-α) which elicit a stress response **[5]**. Implications for therapy are that the spreading of tau pathology may depend on factors other than propagation of a misfolded tau structure, including even other cell components. **Therefore efforts aimed at neutralizing the spreading of misfolded tau may have to be re-evaluated.**

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TAU INDUCES NEURODEGENERATION IN ALZHEIMER'S DISEASE THROUGH THE SEQUESTRATION AND INHIBITION OF LSD1 FUNCTION

D. J. Katz, A. K. Engstrom, A. C. Walker, R. A. Moudgal, D. A. Myrick, S. M. Kyle. Cell Biol., Emory Univ., Atlanta, GA. (404)727-3403. djkatz@emory.edu

Program Number: 472.07
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: E8
Presentation Time: 10:00 AM - 11:00 AM
Session Title: Tau: Animal and Cellular Models I

GENE THERAPY-BASED CELL THERAPY FOR NEURAL REGENERATION AND REPAIR

G. Chen, Z. Wu, X. Hou, Z. Guo, Y. Chen, J. Yin, N. Ma, L. Zhang. Biol., Penn State Univ., University Park, PA. 1(814)865-2488. guc2@psu.edu

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Room Number: Hall A
Board Number: E31
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Alzheimer's Disease and Other Dementias: Therapeutic Strategies II

We have invented an *in vivo* neuroregeneration technology to treat neurodegenerative disorders through NeuroD1-based gene therapy. Specifically, by overexpressing a neural transcription factor NeuroD1 in the reactive astrocytes of the mouse brain modeling Alzheimer's disease (AD), we can convert reactive astrocytes directly into functional neurons and increase the learning and memory capability in an AD mouse model. Our *in vivo* neuroregeneration technology provides a powerful new approach for the treatment of AD and many other neurodegenerative disorders through replenishing new neurons and restore lost brain functions.

Alzheimer's disease is a devastating disease that not only has profound effect on the patients themselves but also impacts the entire family of the patients with enormous financial and emotional burdens. Unfortunately, recent clinical trials on AD have failed one after another, raising serious concerns whether we have been working on the right target in searching for a cure for AD. There is a growing cacophony that perhaps the amyloid and tau hypotheses are wrong, and that we shall start from scratch to find new drug targets. While the failed clinical trials on Aβ and tau certainly warrant a deep thinking of what might have gone wrong, denial of the importance of Aβ and tau is also short-sighted.

What might have gone wrong may not be the wrong targets, but rather imperfect strategy. When a patient goes to a clinic and is diagnosed to have Alzheimer's disease, the likelihood of the patient's brain having Aβ plaques and tau tangles is very high. Therefore, reducing Aβ plaques and tau tangles is not a far-fetched approach. The problem with the failed clinical trials is because they did not consider what consequences caused by the Aβ plaques or tau tangles. AD is a called neurodegenerative disorder because many neurons have degenerated after being attacked by the Aβ plaques and tau tangles. It is the downstream effect of Aβ plaques and tau tangles, the neurodegeneration, that results in cognitive functional decline. Without functional new neurons to replace those degenerated neurons, how can one expect AD patients regain their lost brain functions? Missing functional new neurons is the key to the failed clinical trials on AD.

One approach to generate new neurons is of course stem cell therapy. But when considering AD treatment, one has to ask how many new neurons are required to treat AD patients? Human brain has 86 billions of neurons. If only 5% of neurons degenerated in an AD patient brain, that would be equivalent to more than 4 billions of neurons died! If we assume 10% of transplanted stem cells can survive in the brain, one would have to transplant 40 billions of stem cells into human brain, which is obviously not practical.

What is an alternative approach to regenerate billions of new neurons in a patient brain? We have recently developed an innovative *in vivo* cell conversion technology to directly convert reactive glial cells into functional neurons inside adult mouse brains, including in a mouse model with Alzheimer's disease. Using AAV-based gene therapy to express a single neural transcription factor NeuroD1 in astrocytes, a type of glial cells that surround every single neuron, we demonstrate that these astrocytes can be efficiently converted into functional new neurons in mouse AD brains. Moreover, after NeuroD1-mediated *in vivo* astrocyte-to-neuron conversion, the remaining astrocytes can proliferate and replenish themselves, making them an ideal cell source for generating billions of new neurons inside patients' brains.

Together, our studies demonstrate that *in vivo* cell conversion technology shows a great potential as a neuroregenerative approach to treat neurodegenerative disorders.

INEFFICIENT THERMOGENIC MITOCHONDRIAL RESPIRATION DUE TO FUTILE PROTON LEAK IN A MOUSE MODEL OF FRAGILE X SYNDROME

R. J. Levy, K. Griffiths, A. Wang, L. Sun, G. Yang, P. Licznerski, E. A. Jonas. Anesthesiol., Columbia Univ., New York, NY; Dept. of Anesthesiol., Columbia Univ. Med. Ctr., New York, NY; Intrnl. Medicine/Section Endocrinol., Yale Univ. Sch. of Med., New Haven, CT; Yale Univ. Sch. Med., New Haven, CT. rl2740@cumc.columbia.edu

Program Number: 103.09
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 11:00 AM
Room Number: Room S403
Board Number: N/A
Presentation Time: 10:00 AM - 10:15 AM
Session Title: Behavioral Analysis of Developmental Disorders

Our findings demonstrate that mitochondria are in inefficient in the developing Fragile X Syndrome brain and suggest that dysfunctional mitochondria may cause the intellectual disability and autistic behaviors seen in the syndrome.

Fragile X Syndrome is the leading known inherited intellectual disability and the most common genetic cause of autism. Approximately 90% of males with Fragile X Syndrome demonstrate one or more features of autism. The syndrome is caused by a gene mutation that results in a complete loss of fragile X mental retardation protein (FMRP) expression. This specific protein is thought to regulate the synthesis of other proteins that are important for synapse development within the brain. However, despite enhanced knowledge of the biological complexities of FMRP, the exact mechanisms of how the gene mutation disrupts synapse maturation, impairs cognition, and results in behavioral abnormalities in Fragile X Syndrome remain unknown.

Mitochondria are the cellular structures mainly responsible for making energy within the cell. Neurons within the developing brain rely on the energy generated by mitochondria to meet the substantial demands and energetic costs of making new synapses during critical periods of normal brain development. Because the immature brain is uniquely vulnerable during critical windows of maturation, impairments in mitochondrial efficiency during such periods have the potential to disrupt neurodevelopment. In fact, mitochondrial defects are known to cause a variety of other neurodevelopmental disorders. Prior work described alterations in metabolism in fruit fly and mouse models of Fragile X Syndrome. However, mitochondrial integrity has not been thoroughly assessed in this disease and the role of mitochondria in the manifestation of the various Fragile X Syndrome features is unknown.

In this study, we aimed to systematically assess the integrity of mitochondria in the brain of newborn Fragile X Syndrome mice during a critical period of neurodevelopment. We found that Fragile X Syndrome mitochondria were inefficient and generated heat due to a leak in one of their membranes. The leak was caused by coenzyme Q deficiency and a pathologically open leak channel. Restoring coenzyme Q within Fragile X Syndrome brain mitochondria closed the channel, blocked the leak, restored rates of protein synthesis, and normalized key features of the syndrome later in life.

The findings demonstrate that loss of FMRP results in inefficient mitochondria in the brain during a critical period of neurodevelopment. Furthermore, our work suggests that dysfunctional mitochondria may cause some of the abnormal behaviors seen in Fragile X Syndrome. The next steps are to determine how the gene mutation causes coenzyme Q deficiency in the developing brain and how coenzyme Q regulates the leak channel.

The results of our study provide a foundation for translation of our approach to the human disease. For example, we will determine if fever can be developed as a biomarker for inefficient and leaky mitochondria in infants and children with Fragile X Syndrome. Furthermore, we plan to develop coenzyme Q as a novel therapy designed to restore mitochondrial function in the developing brain and normalize behaviors in affected patients.

SHANK3 SOCIAL DEFICITS AND REPETITIVE BEHAVIOR REFLECT PREVENTABLE CONTEXT-INDUCED ANXIETY

S. Krüttner, P. Caroni. Friedrich Miescher Inst. For Biomed. Res., Basel, Switzerland. sebastian.kruettner@fmi.ch

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Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: A42
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Behavioral Study and Animal Models for Autism Spectrum Disorders

In our currently unpublished research we show, for the first time, that experience and altered learning strongly contribute to onset of Autism Spectrum Disorder (ASD) symptoms. We provide a novel, unifying framework to understand social and behavioral deficits in ASD. Our findings suggest effective behavioral therapies involving familiarity and enrichment to prevent their emergence.

Autism Spectrum disorders (ASD) are a mental condition with a strong genetic component that affect 1% of the population. Despite the identification of many risk genes involved, the underlying mechanisms are still poorly understood. One of the best described risk gene is Shank3. Accordingly, we generated a mouse model lacking this gene and mimicking aspects of ASD symptoms. ASD is difficult to diagnose due to the complex spectrum of core symptoms and co-morbidities involved. These include social deficits, anxiety, stereotypic and repetitive behaviors and impaired cognitive capabilities. To date, it is unclear whether all symptoms and co-morbidities described involve shared underlying genetic mechanisms. Furthermore, patients harboring mutations in the Shank3 gene can display surprisingly high resilience to disease onset, despite their genetic predisposition. Thus, onset and development of symptoms can vary even among patients harboring the same genetic defect, suggesting additional mechanisms might be acutely involved in disease onset.

We aimed to device novel rational therapies to prevent and treat core symptoms of ASD. To this end, we investigated experience-related mechanisms which might acutely control the manifestation of ASD-like characteristic behavioral dysfunction in our Shank3 mouse model.

To test such experience-dependent related mechanisms we exposed mice to a novel arena containing two identical objects. On the next day, one of the objects has been replaced by either a novel object or a novel social intruder mouse trapped underneath a cage. The animal's approaching behavior towards the novel cue or within the arena itself is quantified and is indicative for exploration versus repetitive behavior. In contrast to healthy individuals, which always respond with an engagement towards novel cues, mutant animals fail to interact with the novel cues when re-exposed to the same arena. Instead, they respond with increased anxiety and stereotypic repetitive behaviors.

Importantly, these behavioral alterations are not initially observed in mutant animals during the first day in a new arena. Instead the symptoms are delayed and induced by enhanced learning and consequent consolidation of the novel environment. Manipulating the reward pathway during memory consolidation prevented aberrant context learning and subsequently ASD symptom onset.

The most promising approaches to alleviate ASD symptoms in patients involve social skill interventions and environmental enrichment. However, to date such interventions lead to only modest and short-term relief of symptoms. It is thought that such interventions are most effective in younger patients and that it might be difficult to long-lastingly relief symptoms in later in life. Therefore, a better understanding about the underlying mechanisms that lead to improved social skills in patients will be required to further improve such intervention strategies. To shed more light on this subject, we established analogous protocols to mimic human interventions in our mouse model of ASD. In our environmental enrichment protocols, animals are exposed to constantly changing cues within their familiar environment and social group. Notably, this environmental enrichment protocol long-lastingly and permanently improved major aspects of induced behavioral symptoms onset. Additionally, we could restore changes in novel identified molecular biomarkers for ASD and learning related plasticity. In the future, such protocols will shed further light on the molecular and neuronal circuit mechanisms underlying ASD onset and possible therapeutic interventions.

Autism and Other Developmental Disorders

ACUTE P-CRESOL INDUCES AUTISM-LIKE BEHAVIORS AND ACTIVATES DOPAMINE TURNOVER IN BTBR MICE: A GENE X ENVIRONMENT INTERACTION PARADIGM FOR AUTISM SPECTRUM DISORDER

T. Pascucci, M. Colamartino, E. Fiori, A. Coviello, R. Ventura, S. Puglisi-Allegra, R. Sacco, C. Lintas, M. Canali, C. Tabolacci, S. Mirabelli, L. Turriziani, F. Cucinotta, A. Ricciardello, G. Calabrese, M. Briguglio, M. Lamberti, P. Tomaiuolo, M. Boncoddo, F. Bellomo, A. M. Persico. Dept. of Psychology & Ctr. "Daniel Bovet, Sapienza Univ., Rome, Italy; IRCCS "Fondazione Santa Lucia", Rome, Italy; Cell Biol. & Neurobio. Inst., CNR, Rome, Italy; EBRI, Rome, Italy; Univ. Campus Bio-Medico, Rome, Italy; Univ. of Messina, Messina, Italy. 39(090)2213143. apersico@unime.it

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Room Number: Hall A
Board Number: B6
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Genetic and Environmental Factors for Autism Spectrum Disorders

This study shows that exposure to an environmental factor, *p*-cresol, acting upon a genetically predisposed background, can induce autism-like behaviors in the mouse. These behavioural abnormalities are accompanied by changes in levels of the neurotransmitter dopamine, occurring in brain regions involved in anxiety, pleasure, and movement.

P-cresol is an organic compound exclusively of environmental origin, because the human body does not possess the enzymes necessary for its production. It is present in many contexts, foods and man-made products, from solvents to perfumes. Environmental exposure occurs through inhalation, skin contact and ingestion of food and beverages. Another important source of *p*-cresol is represented by some gut bacterial strains, such as *Clostridium difficile*, responsible for the most severe forms of antibiotic-associated diarrhea. These intestinal bacteria, when present, promote the formation of *p*-cresol from the amino acid tyrosine, a constituent of all food proteins.

Autism Spectrum Disorder (ASD) is characterized by deficits in social interaction and communication, repetitive behaviors, restricted interests, and abnormal sensory processing. Its incidence has dramatically risen during the last few decades and many cases remain unexplained even after advanced genetic testing. Investigators have thus begun exploring possible contributions by environmental factors acting on genetically vulnerable individuals.

We initially found urinary *p*-cresol significantly elevated in young autistic children compared to sex- and age-matched controls (Altieri et al., Biomarkers 2011). This finding was later replicated in several targeted and unbiased urinary or foecal metabolomic studies. Autistic children with chronic constipation were then found to display the highest urinary *p*-cresol levels, indicating that slow intestinal transit fosters greater absorption of *p*-cresol. Since *p*-cresol and its metabolite *p*-cresylsulphate are two known neuroactive toxins, excessive *p*-cresol could contribute to the clinical severity of ASD in young autistic children with chronic constipation.

In this study, we have assessed the effects of a single injection of low- or high-dose (1 or 10 mg/kg intravenous, respectively) *p*-cresol in BTBR mice, a reliable animal model of human ASD. Low-dose *p*-cresol significantly increased anxiety in the elevated plus maze and hyperactivity in the open field. In addition, high-dose *p*-cresol also produced repetitive behaviors in the open field and complete loss of preference for social interaction in the three-chamber test. Importantly, anxiety and hyperactivity represent the two symptoms most commonly associated with ASD in clinical settings, while deficits in social interaction and stereotypic behaviors are the two pivotal core symptoms of ASD, according to DSM-5.

The brains of these same mice unveiled significantly elevated levels of the neurotransmitter dopamine and of its metabolites (HVA and DOPAC) in the amygdala after low-dose *p*-cresol, and in the dorsal and ventral striatum after high-dose *p*-cresol. The amygdala is critically involved in anxiety and restlessness, whereas the ventral and dorsal striatum are pivotal for pleasure and for triggering the onset of motor activity, respectively. Dopamine is also critical for anxiety, hyperactivity, motivational drive and movement. This parallel between behavioural abnormalities and brain neurochemical alterations is indeed striking.

These experiments describe a new gene x environment interaction model of ASD, demonstrating that a specific compound present in the environment or produced by some gut bacteria can promote autistic behaviors in genetically predisposed individuals. Secondly, they help explain some mechanisms underlying autistic behaviors, such as the loss of preferential interest toward other human beings as compared to inanimate objects. Thirdly, they raise concern over the origin of the dramatic increase in ASD incidence recorded during the last three decades. Finally, this study spurs interest in the correction of chronic constipation and in microbiota transfer therapy, as approaches potentially able to at least partly ameliorate anxiety, hyperactivity, and core autistic symptoms by abating *p*-cresol levels.

OXYTOCIN NORMALIZES ALTERED SOCIAL CIRCUIT CONNECTIVITY IN THE CNTNAP2 KNOCKOUT MOUSE

K. Y. Choe, M. Safrin, R. A. Bethlehem, N. G. Harris, D. H. Geschwind. Neurosci., UCLA, Los Angeles, CA; Dept. of Psychiatry, Univ. of Cambridge, Autism Res. Ctr., Cambridge, United Kingdom; Neurosurg., Dept. Neurosurgery, UCLA, Los Angeles, CA. 424-465-0137. choekatr@ucla.edu

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Room Number: Hall A
Board Number: B10
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Genetic and Environmental Factors for Autism Spectrum Disorders

Our research indicates that oxytocin may be capable of increasing social behavior in autism spectrum disorders (ASD) by restoring normal patterns of brain activity.

ASD is a highly prevalent neurodevelopmental disorder (1 in 58 children in the United States). Previous research has firmly established that ASD has a strong genetic component, and identified a large number of gene mutations associated with the disorder. However, we still do not know how these mutations lead to development of abnormal brain functions that give rise to the debilitating social symptoms of ASD, for which there currently is no cure. Oxytocin, a hormone critical for social bonding and trust,has been suggested as a promising treatment option due to reports of lowered levels in children with ASD. Encouraging this view, several clinical studies successfully demonstrated that oxytocin admistration in children with ASD significantly improved their social behavior. However, many other studies have failed to obtain similar results, raising doubts as to whether oxytocin could be developed as a useful therapeutic option. Unfortunately, our understanding of exactly how oxytocin influences social behavior is still limited, both in normal and ASD-related contexts. This information could be critical to further develop oxytocin as a potential therapeutic for ASD.

Our previous work has utilized mice that harbor a mutation in a gene named *Cntnap2* as a model organism to study the mechanisms of how ASD-related gene mutations can lead to abnormal brain development and behaviors. In humans, mutations in this gene have been strongly linked to ASD, including a syndromic form called Cortical Dysplasia Focal Epilepsy (CDFE). These mutant mice have social deficits as well as lowered brain levels of oxytocin. Strikingly, we could temporarily normalize the social behavior of these mutant mice with a single injection of oxytocin.

In this study, we sought to further understand how oxytocin improves the social behavior of *Cntnap2* mutant mice. To do this, we used functional magnetic resonance imaging (fMRI) to first identify abnormal patterns of brain activity in these mice and then measured the effects of injected oxytocin on these patterns. We found that these mutant mice have decreased communication between a set of brain regions with established roles in social behavior (for example, paraventricular nuclei, nucleus accumbens, and medial prefrontal cortex) when compared to normal mice. In contrast, the mutant mice have increased communication between these and other regions that are not directly involved in social functions (for example, sensory cortices and thalamus). Strikingly, both of these abnormal brain activity patterns disappeared after oxytocin injection. Further investigation revealed that oxytocin selectively strengthens brain connections that involve the nucleus accumbens, a brain region critical for social reward.

These results suggest that the social deficits of *Cntnap2* mutant mice could be due to abnormal patterns of brain network activity involving social brain regions, and that oxytocin may improve their social behavior by restoring normal patterns of activity. To further investigate the significance of oxytocin activation of the nucleus accumbens and its connected brain regions, we are currently testing the behavioral impact of selectively activating of this circuit with optogenetics, an experimental strategy that allows one to control neuronal spiking using light.

The findings of this study provide information on how abnormal network activity patterns in the brain could be responsible for social impairments in ASD. These results also demonstrate that the pro-social effects of oxytocin in *Cntnap2* mutant mice may involve normalizing communication between social brain regions, providing additional support for utilizing the oxytocin system as a potential therapeutic target. Additional work is necessary to extend these observations to future clinical studies involving oxytocin.

MAGNETIC RESONANCE ASSESSMENT OF LEARNING DEFICIENCY INDUCED BY NEONATAL EXPOSURE TO ISOFLURANE

D. P. Aksenov, P. Venkatasubramanian, C. Dixon, L. Li, M. Miller, A. Wyrwicz. NorthShore Univ. HealthSystem, Evanston, IL. (224) 364-1405. daksenov@northshore.org

Program Number: 549.24
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: A44
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Neural Mechanisms for Developmental Disorders II

Our findings show that exposure to general anesthesia during infancy resulted in a variety of changes to brain structure and function in adolescence, which were detected using magnetic resonance imaging (MRI). Impairment to learning and memory for a simple learning task was also observed following anesthesia exposure. Furthermore, our results indicate that some of these changes may be more severe in cases where anesthesia is delivered in combination with higher-than-air levels of oxygen, which is a common practice during surgery and other procedures.

Each yearmillions of children in the USA undergo general anesthesia during the course ofsurgeries, imaging and other diagnostic procedures, and there is an increasingconcern about the potential harmful effects of anesthesia on the developing brain. Such effects depend upon the length of anesthesia, frequency of exposure and other possible factors. A number of previous studies from human andanimal subjects have indicated that exposure to anesthesia, especially at an early age, can affect a variety of aspects of brain development, leading to learning and behavioral deficiencies. For example, it was found that children who underwent anesthesia were more than twice as likely to exhibit behavioral or other developmental deficits in young adulthood. Animal studies provide a better-controlled analysis of anesthesia-related effects and allow for more precise comparisons between anesthetized subjects and unanesthetized controls. These previous studies have revealed associations between early anesthesia exposure and specific developmental abnormalities in the brain including increased cell loss in brain regions which are important for learning and memory, defects in the formation of myelin, which is critical for the normal function of neurons, and disruption of learning in adult subjects.

We examined a common general anesthetic (isoflurane) delivered to newborn rabbits in both air and 80% oxygen. We evaluated learning and acquired MRI data 3 months after anesthesia exposure, which is approximately equivalent to human adolescence. Diffusion Tensor MR Imaging (DTI) was used to measure changes in the cellular environment in several key regions of the brain. Changes in the volume of these regions were also assessed using MRI. Magnetic resonance spectroscopy was used to assess regional changes in the biochemistry of the brain. Functional MRI was used to measure responses of the brain to sensory stimulation. Memory function was tested using one of the most reliable and quantifiable classical conditioning techniques, called trace eyeblink conditioning. Our results demonstrated that learning was significantly affected in anesthesia-exposed groups as compared to the control group which did not receive anesthesia. The volumes of learning-related brain structures decreased in anesthesia-exposed groups. DTI results indicated that the cellular environment (for example, orientation and shape of neuronal branches) in some regions was also affected by anesthesia. Surprisingly, the long-term effect of anesthesia on the biochemistry of the brain was found to be comparatively mild. The functional responses of the brain were the same in all groups before learning. However, after learning the functional responses were affected in anesthesia groups, and most severely when anesthesia was delivered with 80% oxygen.

In summary, these findings support previous studies that have pointed to a link between early anesthesia exposure and the development of learning and behavioral deficiencies, and demonstrate that MRI is a reliable approach to evaluate anesthesia-related changes in the developing brain. Furthermore, we found that the use of supplemental oxygen may induce additional changes which manifest at the functional level. Further work will be necessary to understand the mechanisms through which isoflurane and other anesthetics interact with the brain in order to produce these pathological effects. However, our research suggests that care should be taken to avoid excessive use of general anesthesia in neonates.

USING HUMAN HPSC-DERIVED CEREBRAL ORGANOID TO MODEL DEFICITS OF CORTICAL NEUROGENESIS IN DOWN SYNDROME

X. Tang, L. Xu, Y. Hong, M. Xu, Y. Hu, Y. Liu. Stem Cell and Neural Regeneration Inst., Nanjing, China; Inst. for Stem Cell and Neural Regeneration, Sch. of Pharmacy, Nanjing Med. Univ., Nanjing, China. txy13777@njmu.edu.cn

Program Number: 459.01
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: A47
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Neural Mechanisms for Developmental Disorders I

IMPAIRED NEOCORTICOGENESIS AND GLYMPHATIC-MEDIATED CSF FLOW IN THE NEW GENETIC RAT MODEL OF NEONATAL HYDROCEPHALUS

J. Goto, S. Emmert, E. Iwasawa, C. Shula, D. Lindquist, F. Mangano. Pediatric Neurosurg., Radiology, Cincinnati Children's Hosp., Cincinnati, OH. (617)355-9033. june.goto@cchmc.org

Program Number: 627.06
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 10:15 AM
Room Number: Room N228
Board Number: N/A
Presentation Time: 9:15 AM - 9:30 AM
Session Title: Genetics and Neural Mechanisms of Developmental Disorders

Our research indicates that motile cilia function is needed for cerebrospinal-fluid circulation through a glymphatic system using a novel rodent model of neonatal hydrocephalus.
Hydrocephalus is the most common brain anomaly at birth, in which surgically-treated patients suffer from global developmental delays and neuropsychiatric problems. Children with surgically-treated hydrocephalus suffer from several neuropsychological problems, the mechanisms of which remain elusive. Therefore, although the surgical interventions of accumulated cerebrospinal fluid significantly improve their outcomes, including mortality rate, a better understanding of the cellular mechanisms driving the negative effects on brain maturation and function in hydrocephalus during the perinatal period is needed.
The glymphatic system is a recently re-discovered perivascular network within the brain that accelerates interstitial solute clearance and cerebrospinal fluid transport. Previous reports indicated that glymphatic dysfunction is involved mainly in natural brain aging and brain injury, including Alzheimer's disease, traumatic brain injury, stroke, and normal-pressure hydrocephalus. Interestingly, although the glymphatic system develops during the early postnatal days in rodents, the function of this fluid network in perinatal brain development and function is not known.
In this study, we generated a novel rat genetic model of perinatal hydrocephalus using the CRISPR genome editing system. We introduced a mutation in an essential motile cilia gene in rats and found that the mutants develop very severe hydrocephalus in their early postnatal life. We tested them in the glymphatic system-mediated cerebrospinal fluid transport assay and found that they have a significantly delayed cerebrospinal fluid circulation rate as they develop hydrocephalus. Interestingly, we also found monocyte/macrophage recruitment in the inflamed subpial glymphatic space between the pia membrane and the neuropil.
These results suggest that the development or function of the glymphatic system is significantly impaired in rats that lack functional motile cilia. We are currently in the process of analyzing the motor skills and the glymphatic function of the mutant rats that received the cerebrospinal fluid diversion surgery. This future analysis will help to determine if the surgical treatment of hydrocephalus leads to long-term problems in the glymphatic system with respect to young brain development and functions.
The findings from this study provided a possible fundamental basis for molecular mechanisms that are responsible for neurodevelopmental problems in neonatal hydrocephalus. These results also opened a new avenue for testing anti-inflammatory drugs that supplement cerebrospinal fluid diversion surgery in a neonatal hydrocephalus model.

REGULATION OF INSULIN SECRETION BY HYPOTHALAMIC NEURONS

I. Papazoglou, J.-H. Lee, Z. Cui, C. Li, M. J. Krashes, S. Rane. NIH/NIDDK, Bethesda, MD. (202)509-3099. ioannis.papazoglou@nih.gov

Program Number: 071.05
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: R15
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Our study shows that a group of neurons in the hypothalamus communicate with insulin-producing cells in the pancreas to regulate insulin secretion.

The prevalence of diabetes has significantly increased in the last few decades. Insulin, the glucose-lowering hormone produced by pancreatic β-cells, is in the center of diabetic research as its production and action is pivotal in the onset of the disease. Insulin secretion has been long thought to be controlled by the nervous system but there hasn't been a clear demonstration of a functional circuit connecting the brain to the β-cells. Claude Bernard, a French physiologist, was the first one to propose the neural control of glucose production in the mid-19th century and half century later, in the early 1900's, Ivan Pavlov provided evidence of the "psychic secretions" of the pancreas. A few decades later, insulin would be discovered by Frederick Banting and change the lives of millions. Since then, β-cells have been extensively studied and today we have a very good understanding of their biological function. Nevertheless, the fact that blood glucose levels and pancreatic secretions have been long known to be regulated by the brain, suggests that this could be the case for insulin secretion too. If indeed we get substantial proof that the brain regulates insulin secretion to a significant extend, it could improve the understanding of diabetes pathogenesis and lead to better treatment approaches. It is essential thus to investigate if the brain- β-cell interaction exists and what is the importance of such neuronal circuit in the regulation of insulin secretion.

To study the brain-pancreas connection, we used a novel tracing approach that allowed us to visualize by the presence of a green fluorescent protein (GFP) the neuronal cells that are exclusively connected to β-cells, in a preclinical model. This way we have been able to trace brain to β-cell circuits and identify a number of neurons within the brain. The brain region with the most β-cell-projecting cells was the hypothalamus, which has been already known to play an important role in metabolism. Among these hypothalamic regions, the paraventricular nucleus and the lateral hypothalamic area were found to be more closely connected to the β-cells. A subgroup of neurons that we identified within the paraventricular nucleus of the hypothalamus are producing the neuropeptide oxytocin and thus are called "oxytocin neurons".

The first step in investigating a potential role of oxytocin neurons was to see what will happen to insulin secretion if we stimulate or silence these neurons. We found that by stimulating oxytocin neurons, insulin secretion was suppressed which resulted in increased glucose levels. On the other hand, when these neurons were silenced, mice failed to reduce their insulin levels during long fasting resulting in hypoglycemia. Finally, using two independent approaches, we found that these neurons were activated by hypoglycemia.

In addition to oxytocin neurons, we have identified other neuronal groups that communicate with β-cells with diverse roles in insulin secretion. Our goal is to characterize the role of all these neuronal circuits in insulin secretion and expand to circuits that regulate other pancreatic hormones too, including glucagon. Eventually, this will lead to a comprehensive network which integrates all brain to pancreas communications and functions.

This study reveals that the brain communicates with pancreatic β-cells to regulate insulin secretion contributing to the maintenance of normal glycemia. These findings open a new field for the research of diabetes and expand our view of this pathology to one that involves the brain.

THERMOREGULATION VIA TEMPERATURE DEPENDENT PROSTAGLANDIN D2 PRODUCTION IN MOUSE PREOPTIC AREA

T. A. Wang, C. Teo, M. Åkerblom, C. Chen, M. T. Fontaine, V. J. Greiner, A. Diaz, M. T. Mcmanus, Y. N. Jan, L. Y. Jan. Physiol., Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA; UCSF Diabetes Ctr., San Francisco, CA; UCSF Diabetes Ctr., San francisco, CA; HHMI/UCSF, San Francisco, CA; Dept Physiol, Univ. of California San Francisco Dept. of Physiol., San Francisco, CA. tongfei.wang@ucsf.edu

Program Number: 072.12
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: T14
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Our research revealed that the brain temperature sensor in the anterior hypothalamus uses prostaglandin D2 ($PGD_{2)}$ as a messenger to signal local brain temperature increase so as to protect the animal from hyperthermia.

It is essential for the survival and health of animals to maintain their core body temperature (T_c) at the optimal level. In mammals, thermoregulation is mediated by the preoptic area of the anterior hypothalamus (POA), one of the very few brain regions that are “thermosensitive”. Brain temperature sensation is important for endothermic (warm-blooded) animals to ensure the maintenance of brain temperature at a steady and optimal level during exercise or intake of hot or cold fluids, and it is crucial for survival upon prolonged exposure to extreme thermal conditions rendering thermoregulation of peripheral body parts difficult. Moreover, thermoregulation involving temperature sensation within the brain is important for adjusting the Tc over the day-night cycle or in the process of pathogen-induced fever. Interestingly, transgenic mice engineered to have their hypothalamic temperature slightly raised by 0.3 °C exhibit a corresponding reduction of T_c, leading to an increased life span. Brain temperature change alters neuronal firing of ~30% of the neurons in the POA, and elicits a range of thermoregulatory responses in live animals. Intermingled with the temperature-sensitive neurons in the POA are various temperature-insensitive neurons with diverse homeostatic functions such as feeding, drinking, sleep, and parental behaviors. The temperature-sensitive POA neurons are thought to play a pivotal role in thermoregulation; this hypothesis, however, cannot be tested in the past 80 years, because electrophysiological recording has been the only way to identify the temperature-sensitive neurons since their discovery. Without a marker for these neurons, the neural circuit for thermoregulation in response to brain temperature changes remained inaccessible to modern genetic-based approaches.

Our research surpassed this critical barrier by applying cutting-edge technology of single-cell RNA-seq combined with whole-cell patch-clamp recording to profile POA neurons based on their transcriptomes and temperature-sensitivity. This screen identified the gene *Ptgds* as a marker for temperature-sensitive neurons in the POA. Using the mouse line that expresses Cre recombinase in cells expressing *Ptgds*, we demonstrated for the first time that the temperature-sensitive POA neurons are involved in thermoregulation. This genetic tool also allows for cell-type specific labeling, recording, and manipulation of neuronal activity of temperature-sensitive neurons in POA, which will greatly facilitate the dissection of neural circuit of thermoregulation.

We further showed that the gene *Ptgds* is not only a marker for temperature-sensitive neurons in the POA, but also functionally involved in thermoregulation. This gene encodes the enzyme that synthesizes PGD_2, a hormone in reproduction as well as a neuromodulator in the brain. In response to brain temperature increase, the temperature-sensitive POA neurons become more active in producing PGD_2, which in turn activates its receptors (DP1) expressed in neurons of the ventral median POA. These downstream neurons integrate inputs from both ambient and central temperature sensors, and mediate Tc decrease by inhibiting thermogenesis and facilitating heat loss.

In future research, it is important to identify brain regions that act downstream of temperature-sensitive POA neurons and respond to signals regarding ambient and/or brain temperature decrease by mediating T_c increase, to elucidate the neural circuits that act in parallel to mediate bi-directional homeostatic thermoregulation. Our research paves the way towards this goal.

The translational impact of our research involves the opening of new avenues in the development of novel therapeutic approaches for the treatment of medical conditions associated with abnormal T_c, such as infection, anesthesia, epilepsy, and diseases arising from metabolism or endocrine disruptions.

KETOGENIC DIET AMELIORATES COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF TBI

M. Har Even, V. Rubovitch, S. Schreiber, C. G. Pick. Tel Aviv Univ., Tel Aviv, Israel; Psychiatry, Tel Aviv Med. Ctr., Tel Aviv, Israel. 972 972-3-6. pickc@tauex.tau.ac.il

Program Number: 480.07
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: I6
Presentation Time: 10:00 AM - 11:00 AM
Session Title: Traumatic Brain Injury: Mechanisms and Therapeutic Strategies

Our present study demonstrates the potential capabilities of the ketogenic diet in providing neuroprotection and improving cognitive deficit impairments following a traumatic brain injury.
Due to the varying causes and effects associated with traumatic brain injury, making each case different, a standard treatment for traumatic brain injury is yet to be found. Another problem encountered when treating this condition is the necessity of a pharmacological treatment in order to pass through the blood brain barrier. This study provides a possible treatment for traumatic brain injury that is relatively easy to implement in brain injured patients of all types avoiding the blood brain barrier problem.
Ketogenic diet is already known to improve the symptoms of epileptic patients, and have been found to improve some of the symptoms of neurodegenerative diseases such as Alzheimer's and Parkinson's. Our study sheds light on the beneficial effects of ketogenic diets on the spatial and visual memory, both of which are usually hampered following a brain-traumatic event, as well as the beneficial effects of the protein SIRT1 (an enzyme involved in a variety of physiological processes including cell life extension) on these impairments.
Following trauma to the brain, induced by using the weight drop model, mice were divided randomly into a ketogenic diet group and a standard diet group. While the standard diet is based upon carbohydrate, fat and protein, the main source of calories in the ketogenic diet's is fat. In each group, half of the mice received mild traumatic brain injury and the other half served as a control group (no brain trauma).
Cognitive and behavioral performances were assessed 7 and 30 days following the injury by comparing the performance of each groups. After receiving mild traumatic brain injury, visual memory (tested by the Novel Object Recognition test) and spatial memory (tested by the Y-maze test) deficits were significantly higher in the standard diet groups than in the ketogenic diet groups. Elevated Plus Maze analysis showed no difference between the groups, indicating that the injury did not cause any anxiety-like behavior in the mice. Mice fed the ketogenic diet (with or without injury) demonstrated a significant increase in ketone bodies in the blood compared with mice fed standard diet, at 3, 7 and 30 days after the diet was initiated, indicating that they were in a state of ketosis throughout the study. We also found that following traumatic brain injury, SIRT1 levels were significantly reduced in the standard diet group and increased in the ketogenic diet group. We are planning to investigate the underlying mechanism of neuroprotection that the ketogenic diet provides, whether indirectly through SIRT1, or directly through production of the ketone bodies. In addition, we plan to check in depth the behavioral changes following the ketogenic diet and its impact on other neurological conditions.
Unfortunately, a standardized treatment for traumatic brain injury has not yet been found. The effects of traumatic brain injury may range from nearly nonexistent to severely debilitating. That is why traumatic brain injury is categorized into three different levels, based on the symptoms and effects of the injury: Mild, moderate and severe. Mild traumatic brain injury consists of roughly 85% of reported head injuries. Traumatic brain injury persists to be a challenge for doctors, neuroscientists and patients around the world, and our findings suggest that using ketogenic diets may be a novel therapeutic approach in treating TBI.

Brain Development

TRANS-DIFFERENTIATION OF COCHLEAR OUTER HAIR CELLS INTO INNER HAIR CELLS IN THE ABSENCE OF INSM1

J. Garcia-Anoveros, T. Wiwatpanit, S. M. Lorenzen, J. A. Cantu, C. Z. Foo, J. C. Clancy, M. Cheatham, A. Duggan. Anesthesiology, Neurol. and Physiol., Northwestern Univ., Chicago, IL; Northwestern Univ., Evanston, IL; Anesthesiol., Northwestern Univ., Chicago, IL. (312) 503-4245. anoveros@northwestern.edu

Program Number: 010.02
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 2:45 PM
Room Number: Room S404
Board Number: N/A
Presentation Time: 1:15 PM - 1:30 PM
Session Title: In Vivo Studies of Stem Cell Fate

Our research reveals for the first time a mechanism by which the cochlea generates its two complementary types of mechanosensors, the outer (OHC) and inner (IHC) hair cells. Both types of hair cells are critical for normal hearing, but their roles are very different: IHCs transmit information from the airborne vibrations of sound to the brain; OHCs amplify these signals, allowing the detection of soft sounds, as well as the distinction between very similar tones. Cochlear hair cells are exclusively generated during development, and so their death due to loud noise, toxins or age results in irreversible deafness. OHCs are particularly vulnerable, and their death underlie the most common forms of hearing loss. Therefore, in order to guide and monitor the development of regenerative therapies, we must understand how to generate OHCs vs IHCs. There is an understanding for how hair cells are made to be different from supporting cells in the organ of Corti, the sensory epithelium of the cochlea. But how the hair cells develop into the two complementary types was unknown prior to this study. We found that the transcription factor INSM1, expressed in embryonic OHCs but not IHCs, is required during a critical period of development to consolidate the fate of OHCs. In the absence of INSM1, OHCs are born in the right place and with the expected molecular markers, but within a day or two many of them transdifferentiate, losing OHC features and completely transforming into IHCs. With transcriptomic technologies we found that INSM1 prevents expression in nascent OHCs of a core set of genes normally utilized by IHCs. In the absence of INSM1, OHCs begin to express these few IHC genes, and about half of them proceed to become IHCs. The transdifferentiated hair cells are distributed in a gradient, not randomly. This distribution implies the probable existence of an IHC-inducing morphogen in the developing cochlea. We propose that INSM1 prevents expression in OHCs of a few genes that allow IHCs to respond to a signal that promotes their differentiation. In the absence of INSM1, OHCs become responsive to this signal, so that those closer to its source have a higher probability of switching into IHCs. An implication of our finding is that the genes identified as de-regulated by INSM1 in transdifferentiating cells are likely to be critical during normal development for the formation of IHCs. Our study reveals for the first time a mechanism for the differential formation of OHCs, and at the same time it identifies the genes likely controlling the formation of IHCs. This provides an example for how complementary cell types are formed during development. In addition, the information here obtained will be useful for developing therapies aimed at the restoration of hearing through the regeneration of either OHCs or IHCs.

INTERNEURON TRANSPLANTATION CREATES NEW NETWORK STATES AND RESCUES SOCIAL BEHAVIOR DEFICITS IN A MOUSE MODEL OF AUTISM WITH EXCESSIVE SYNAPTIC INHIBITION

D. G. Southwell, H. Seifikar, R. Malik, K. Lavi, D. Vogt, J. L. Rubenstein, V. S. Sohal. Duke Univ., Durham, NC; Johns Hopkins, Baltimore, MD; Dept. of Psychiatry, Nina Ireland Lab. Dev Neurobiol, Univ. of California San Francisco, San Francisco, CA; Neurobio., Friedrich Miescher Inst., Basel, Switzerland; Dept. of Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI; Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA. dereksouthwell@gmail.com

Program Number: 039.12
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C9
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Structural Plasticity and Circuit Remodeling I

Our research demonstrates that neuronal transplantation may be used to modify abnormalities of brain activity and behavior that occur in autism.
Autism is a developmental disorder that is marked by three core symptoms: decreased social interaction, repetitive behaviors, and language deficits. It affects around one percent of the population, and it exerts significant personal, familial, and societal burdens. The economic impacts of autism, which includes direct costs, such as treatment expenses, and indirect costs, such as decreased caretaker productivity, are estimated to be 15 to 60 billion dollars annually.
Unfortunately there are no medical cures for autism, and no medications are currently approved for treating Autism core symptoms. Behavioral and communication therapies, which modify how persons with autism interact with their environment and with other people, currently represent the main form of autism therapy. A number of genetic factors have been linked to the development of autism. Additionally, some of the cellular and physiologic abnormalities that occur in autism have been identified in both animal and human studies. Recently, it has been hypothesized that the dysfunction of a population of brain cells (cortical inhibitory interneurons) may contribute to abnormal patterns of brain activity that occur during autism-like behaviors, such as reduced social interaction. It has been unknown, however, whether interneuron dysfunction can be therapeutically corrected, or compensated for, in a manner that improves the core features of autism. Through prior mouse studies, we and other researchers have developed a method for transplanting immature cortical interneurons into the recipient brain. When transplanted from developing mouse embryos or in vitro cell culture systems, immature interneurons disperse in the recipient brain, develop into mature and functional interneurons, and alter recipient brain plasticity. In the current study, we transplanted healthy, immature interneurons into a mouse model of autism in which interneuron function is altered by a genetic abnormality found in human autism. Surprisingly, we found that transplanted interneurons both corrected social interaction deficits in this autism model, and modified abnormal recipient brain patterns that occurred during social interaction. Our findings provide early evidence that interneuron transplantation may represent a cell-based therapy for autism. Future interneuron transplantation studies are likely to explore whether, and how, transplanted interneurons modify recipient brain function and behavior in other autism models. Additionally, for future clinical applications, there are ongoing efforts being made to generate donor interneuron populations from human stem cells.

CHRONIC SHORT SLEEP INITIATES GENDER-DEPENDENT LIMBIC SYSTEM NEURODEGENERATION

J. E. Owen, Y. Zhu, P. Fenik, K. Shulman, S. Veasey. Dept Med., Univ. of Pennsylvania, Philadelphia, PA. jessica.owen811@gmail.com

Program Number: 374.13
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C52
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Aging: Molecular Mechanisms II

Our research suggests that chronic short sleep in early adulthood could initiate neurodegeneration later in life that resembles the beginning stages of Alzheimer's disease, this effect seems to be more prominent in males than females.

Chronic short sleep is the common phenomenon of not getting sufficient sleep during the working/school week and then catching up on sleep over the weekend or days off. This pattern is prevalent in society and it was previously thought that catching up on lost sleep adequately reversed its adverse effects. It is now increasingly being recognized that sleep loss can lead to significant injury in the brain, particularly in neurodegeneration.

Alzheimer's disease (AD) is a common neurodegenerative disease that has three key pathologies; accumulation of amyloid beta plaques, tau neurofibrillary tangles and brain atrophy. Sleep disturbances are frequently found among AD patients. Animal models of AD demonstrate that sleep loss can accelerate the accumulation of amyloid beta plaques and tau tangles in the brain. Our research investigated whether sleep loss could initiate pathology resembling AD in non-genetically modified mice, a model more representative of the human AD disease.

Male and female mice were subjected to our chronic short sleep paradigm in which they were sleep deprived for 8 hrs a day, 3 days a week for 12 weeks during early adulthood. After a 12 month recovery period by which time the mice were 18 months of age, spatial memory was tested in sleep loss mice as well as age-matched control mice. Control mice had intact spatial memory, whereas sleep loss mice did not. Next, brain tissue was assessed for markers of AD. Sleep loss mice had significant atrophy in the hippocampus and entorhinal cortex, brain regions involved in memory processing and significantly affected in humans with AD. Further, increased accumulation of amyloid beta-42 peptide and phosphorylated tau were seen in sleep loss mice compared to controls. Microglial activation (the brains innate immune response) was also increased. While microglial activation alone is not a specific indicator of AD, it is known to increase with increasing AD pathology in the brain. When male and female mice were analyzed separately it was found that male sleep loss mice had more atrophy and more extensive tau pathology in the hippocampus. Female mice had no change in atrophy compared to control mice and had less extensive tau pathology than males. Female mice also had more microglial activation than male mice which may be acting to protect the hippocampus from sleep loss induced injury.

These results suggest that sleep loss early in life could initiate neurodegenerative changes in key brain regions affected in AD. It is important to acknowledge that these neurodegenerative changes are also seen in the ageing brain and thus the results could represent an accelerated ageing phenotype. Regardless, it is clear that sleep loss in early adulthood can lead to persistent injury in the brain.

The next steps involve investigating the molecular mechanisms by which sleep loss could be influencing neurodegenerative processes in order to develop targeted treatment to protect the brain from such injury.

THE ROLE OF GUT MICROBIOME IN AGE ASSOCIATED COGNITIVE IMPAIRMENT

J. Behera, J. Ison, K. Kelly, N. Tyagi. Physiol., Univ. of Louisville, Louisville, KY. jyotimaya.behera@louisville.edu

Program Number: 374.11
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C50
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Aging: Molecular Mechanisms II

Brain Wellness and Aging

VASCULAR AND NEUROGENIC REJUVENATION IN AGING MICE BY MODULATION OF ASM

J.-S. Bae, S. Han, J. Lee, K. Park, I. Jung, H.-J. Kim, H. Jin. Dept. of Physiology, Sch. of Med., Col. of Vet. Med., Kyungpook Natl. Univ., Daegu, Korea, Republic of; Dept. of Neurology, Col. of Med., Hanyang Univ., Seoul, Korea, Republic of. hkjin@knu.ac.kr

Program Number: 470.01
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: D2
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Brain Wellness and Aging: Mechanisms and Biomarkers

DUAL SEPARABLE FEEDBACK SYSTEMS GOVERN FIRING RATE HOMEOSTASIS

Y. Kulik, R. T. Jones, A. Moughamian, J. Whippen, G. W. Davis. Biochem. and Biophysics, UCSF, San Francisco, CA; Univ. of California, San Francisco, San Francisco, CA; Dept Biochem & Biophysics, Univ. California-SF, San Francisco, CA. yelena.kulik@ucsf.edu

Program Number: 040.08
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C23
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Neuronal Firing Properties: Modulation, Development, and Pathologies I

The nervous systems of all animals are endowed with powerful, self-corrective mechanisms that can counteract the deleterious effects of disease causing gene mutations, injury or environmental perturbation. Our research is unraveling the logic and the molecular mechanisms that enable this self-corrective stabilization of neural function, with broad implications for the treatment of neurological and psychiatric diseases ranging from epilepsy to autism and neurodegenerative disease.

Adaptive signaling systems that detect the presence and magnitude of a perturbation and actively restore normal neural function through counteractive measures are, collectively, referred to as '*homeostatic'*. How does the nervous system achieve self-corrective, homeostatic signaling? A widespread and compelling theory is that neural activity is continuously 'monitored' so that when the brain is confronted by a perturbation, a counteractive homeostatic response can be initiated. We set out to test this fundamental theory. This theory predicts that two distinct molecular perturbations with equivalent effects on neural activity should cause an equivalent homeostatic response. We prove that this simple prediction is inadequate and propose a new model that incorporates at least two separable regulatory systems.

Ion channels shape the activity of every nerve cell in the brain. We perturbed neural activity by generating two different molecular perturbations that affect a single ion channel gene, the Kv4.2 potassium channel. First, we generated animals that completely lack the Kv4.2 gene. Second, we used CRISPR technology to generate animals in which the Kv4.2 protein remains present, but is non-functional (termed a function blocking mutation). In both experimental conditions, the function of the Kv4.2 channel is erased, with equivalent effects on neural activity.

We find that these two experimental perturbations induce completely different types of self-corrective homeostatic signaling. Deleting the ion channel caused a compensatory, corrective change in the abundance of other ion channels, a process driven by a transcription factor named *Kruppel*. By contrast, the function-blocking mutation initiates a fundamentally different, *Kruppel*-independent, compensatory response. Since two perturbations with identical effects on neural activity induce different homeostatic responses, we conclude that neural activity cannot be the *only* source of information that drives homeostatic signaling in the nervous system. The existing model needs to be revised. We propose the existence of dual, separable homeostatic signaling systems, one capable of responding to changes in neural activity and another that responds to changes in the abundance of ion channel proteins, termed a '*proteostatic'* signaling system. Our current research continues to define how activity-dependent and proteostatic homeostatic signaling systems work at a genetic, molecular and cellular level. Our experiments, performed in *Drosophila*, may have implications for better understanding and treatment of neurological and psychiatric conditions. If we can understand the self-corrective, homeostatic mechanisms that stabilize brain function, it might be possible to promote these homeostatic systems, achieving a personalized approach to treatment of neurological and psychiatric disease. In addition, new therapeutics might be designed with an understanding of how the brain has already adapted to a disease causing gene mutation or injury, promoting drug efficacy and safety.

INVESTIGATING THE ROLE OF ASTROCYTES IN THE DEVELOPMENT OF SYNAPTIC CONNECTIVITY IN A RODENT MODEL OF NEONATAL ABSTINENCE SYNDROME

T. C. Boggess, A. Mazur, H. Sexton, W. C. Risher. Biomed. Sci., Marshall Univ., Huntington, WV. 1(937)572-3139. tboggess92@gmail.com

Program Number: 198.03
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: A41
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Synaptogenesis and Activity-Dependent Development II

Our research shows that prenatal drug exposure results in significant structural changes within addiction-associated regions in the brains of mice, and that the extent of this reorganization is dependent on the function of support cells within the central nervous system (CNS).

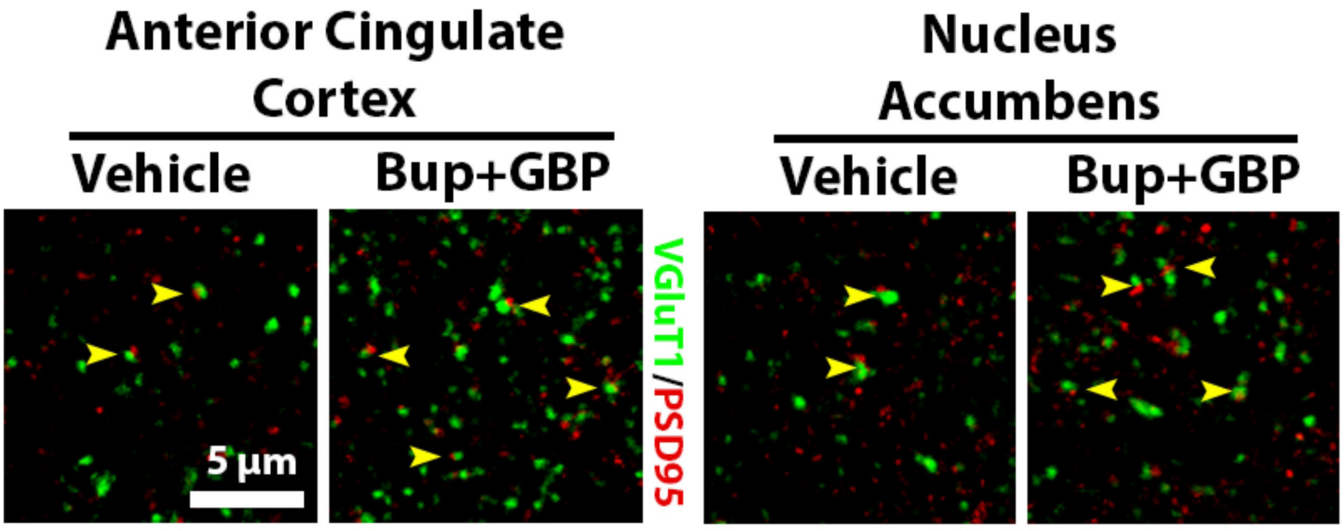
Rates of abuse of opioid drugs (e.g. heroin, morphine, oxycodone, buprenorphine, etc.) have risen dramatically in the United States in recent decades. One of the most significant outcomes of this opioid epidemic has been a rise in the incidence of neonatal abstinence syndrome. Neonatal abstinence syndrome (NAS) is the clinical diagnosis used to describe the collection of signs and symptoms commonly observed in the newborns of mothers who abused certain drugs, such as opioids, while pregnant. The fetus develops a physical dependence on these drugs during development. After being separated from the supply of drug during birth, the infant soon goes into withdrawal characterized by increased irritability, tremors, excessive crying, poor feeding, diarrhea, and seizures. Increased risk of sudden infant death syndrome (SIDS) has also been reported with NAS.

Substance abuse during pregnancy is rarely limited to just opioids. Gabapentin (brand name: Neurontin), an anti-epileptic drug also prescribed to treat neuropathic pain, has been increasingly co-abused with opioids in recent years, leading to its classification as a Schedule V drug in several states. Infants born to mothers who abused both opioids and gabapentin display behaviors unique to neonatal abstinence syndrome, including tongue thrusting, eye wandering, and back arching. Gabapentin is also significant for its role in inhibiting the development of neuronal networks. Specifically, gabapentin interferes with the ability of astrocytes, non-neuronal cells within the CNS that perform a variety of supportive functions, to promote the formation of synapses, which are the connections between neurons where electrochemical signals are passed from one neuron to the next. Taken together, these observations strongly suggest that astrocytes likely play an important role in the pathology of NAS.

In this study, we attempted to model the conditions of NAS in genetically modified mice bred to have impaired astrocyte-mediated synaptogenesis. Pregnant mothers were given daily access to either buprenorphine, gabapentin, or a combination of both drugs in a condensed milk mixture starting 7 days after conception until approximately 11 days following the birth of their litter. At 21 days old, the brains of these pups were extracted and examined for structural synaptic changes in regions associated with addiction, reward processing, and impulse control. The brains of drug-exposed mice showed significant synaptic reorganization compared to vehicle-treated (i.e. no drug) controls, and specific patterns of reorganization have been observed depending on the integrity of astrocyte synaptogenic signaling pathways. We speculate that this reorganization could allow for greater excitation in response to a rewarding stimulus and diminished capacity to control impulsive behavior in these mice.

These early results suggest that children born to mothers who abused drugs while pregnant may be inclined towards drug addiction themselves later in life. Future plans include behavioral testing to determine whether the structural changes observed in the brains of these mice lead to cognitive dysfunction as well as greater stimulus-seeking behavior and decreased impulse control later in life. Such behaviors would indicate a propensity towards developing drug addiction.

Although neonatal abstinence syndrome affects thousands of newborn infants every year, little is known about the long-term consequences of NAS long after the initial clinical symptoms have dissipated. Exploring the role of astrocyte-neuronal signaling in the pathology of NAS could lead to a better understanding of the effects of prenatal drug exposure on neurological development and inform future treatments.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Bup+GBP
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Cell Communication

INVESTIGATING THE ROLE OF A SYNAPTICALLY TARGETED INTRONIC LNCRNA IN MEMORY

E. Grinman, I. Espadas, Y. Avchalumov, S. Swarnkar, S. V. Puthanveettil. Neurosci., The Scripps Res. Inst., Jupiter, FL. (718)614-6601. egrinman@scripps.edu

Program Number: 284.04
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: B76
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Synaptic Transmission: Modulation and Mechanisms I

Our research has uncovered a noncoding RNA that is produced in hippocampal neurons during learning, transported to synapses, and regulates neuronal structure and signaling properties to mediate the learning process.

It has been known that in order to form long lasting memories, it is necessary for neurons, particularly in the hippocampus, to produce novel gene products. While previous research on the identity and function of these gene products has largely focused on genes that produce protein, far less attention has been paid to the vast majority of the genome which does not. Through advances in next generation sequencing, it is now known that these “dark regions” of the genome can also produce gene products. However, rather than protein, these gene products are in the form of RNA molecules, which have traditionally been thought to serve as messengers for protein production. However, these non-protein coding RNA molecules, or non-coding RNAs, have until recently been thought of as nothing more than transcriptional noise, with no significant function in neurons.

Recently, genome-wide association studies have identified mutations in these non-coding regions that are unique to patients with Alzheimer’s Disease and other forms of dementia. Yet, unlike much of the known proteins, non-coding RNAs have been largely uncharacterized. Revealing novel roles for these molecules in hippocampal neurons may aid in the comprehensive understanding of mechanisms of long term memory formation and disorders of memory.

Using next-generation sequencing, our lab has found hundreds of non-coding RNAs that are produced during learning in hippocampal neurons. Upon closer inspection, we have found that some of these RNAs can be transported to synaptic compartments: the sites of communication between neurons. We have focused on one specific non-coding RNA and characterized its mechanism of transport to synapses. We found that this RNA is immediately and robustly expressed during learning-related signaling pathways. We have also found that its expression is required for regulating neuronal structure and synaptic firing during a learning event. Importantly, we uncovered that this RNA is also activated in the hippocampus of mice undergoing a learning task.

These results suggest that this non-coding RNA can act as an immediate response element during learning, a characterization previously only held by protein-coding genes. This study is the first to show that a non-coding RNA behaves in such a manner during learning, and opens up a novel perspective for studying mechanisms of memory, as well as disorders of memory. Future work will delve into uncovering the interacting partners of this RNA at the synapse, as well as manipulating its expression in the hippocampus of live mice to assess their ability to form new memories.

The results obtained from studying the role of a specific synaptically targeted non-coding RNA can be applied to discovering novel mechanisms of memory. These studies will have broader implications in biology because the mechanisms and functions of non-coding RNAs are poorly understood in general. The ultimate goal for this research is to establish these molecules as suitable targets for development of therapeutics for disorders of memory.

DEVELOPMENT OF A NEXT GENERATION OF GABAERGIC COMPOUNDS FOR ANESTHESIA AND EPILEPSY

M. F. Davies, A. Jahangir, M. Shamloo, R. K. Lam, H. S. Mccarren, B. S. Barker, E. J. Bertaccini. Anesthesia, Stanford Univ. Sch. Med., Stanford, CA; Neurosurg., Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA; Neurosurg., Stanford Univ., Stanford, CA; Neurosci., US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; Neurosci., USAMRICD, Aberdeen Proving Ground, MD. (415) 493-5000 EXT 64854. fdavies@stanford.edu

Program Number: 645.02
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: B43
Presentation Time: 9:00 AM - 10:00 AM
Session Title: GABA(A) and Glycine Receptor Pharmacology

SUMMARY: We have developed a new anesthetic based on a novel chemical core that has potencies comparable to current standards while having little to no detrimental side effects on blood pressure or breathing. It can also be used to treat severe seizures.

There are currently three intravenous anesthetic agents that are available for clinical use to treat seizures as well as induce states ranging from sedation to general anesthesia. Each of these agents is associated with an entire spectrum of undesirable side effects, most of which result in lower systemic blood pressure. This is a side effect that is poorly tolerated in very young children who possess immature cardiovascular compensatory mechanisms, as well as in the elderly with confounding comorbidities and otherwise exhausted compensatory mechanisms. Such hypotension also presents extreme danger to any otherwise healthy individual when presenting after major traumatic injury (i.e. battlefield casualties, car accidents, etc.). Each of these agents has additional unique detriments. In particular, etomidate is the agent which comes closest to achieving ideal cardiovascular preservation while inducing dose-dependent alterations in consciousness. However, this is at the expense of clinically significant adrenal suppression via inhibited steroid biosynthesis. Propofol, the most widely used IV anesthetic, can produce profound hypotension. Ketamine is well known to induce delirium.

Sophisticated molecular computations, previously only available via supercomputing facilities, can now be achieved with advanced desktop workstations. Software development has taken full advantage of high-end 3D visualization as well as highly parallelized computational algorithms for efficient drug screening methodologies. Concurrent with this, our understanding of the molecular substrates for conscious states has also advanced in the form of robust models of the ion channels that mediate particular anesthetic actions, particularly the gamma amino butyric acid type A receptor ($GABA_AR$). The concept presented here is to leverage such modeling advances to now perform efficient lead refinement and drug design using our current state-of-the-art molecular models of the $GABA_AR$ to discover a new, safer anesthetic.

We have now identified a novel class of lead compounds which demonstrate overt anesthetic activity. The most potent of the series is KSEB 14-01 which anesthetizes both tadpoles and rats with a potency greater than that of propofol, the current intravenous anesthetic standard. KSEB 14-01 also amplifies the function of the $GABA_AR$ in the nervous system and is devoid of the chemical moiety known to produce adrenal suppression. *Of even greater importance is the fact that our new class of compounds shows minimal to no suppression of blood pressure and respiration or changes in blood gas concentration of CO_2, O_2 saturation or in blood pH at anesthetic concentrations, in stark contrast to the deleterious effects of propofol on these parameters.* Further, intramuscular administration suppresses seizures in rats exposed to chemical weapons. These compounds are derived from novel chemical structures not previously associated with or known to produce significant anesthetic effect. We have now refined our lead to enhance its solubility while maintaining the desirable characteristics of anesthesia and seizure suppression without significant hemodynamic or respiratory effects. This class of compound will have ready application as an anesthetic in any patient with the potential for such physiologic instabilities and, through its $GABA_AR$ mechanism, provide a stable means of acute and possibly chronic seizure suppression.

The next step is to investigate other members of this class of compounds that may have even more favorable pharmacokinetic or pharmacodynamic actions for either anesthesia or anticonvulsant action.

Circuit and Systems Techniques

OXYGEN PRODUCING ALGAE PERMIT FUNCTIONAL RECOVERY OF NEURONAL ACTIVITY IN *XENOPUS LAEVIS* UNDER HYPOXIA

S. Oezugur, M. N. Chávez, J. Nickelsen, L. Kunz, H. Straka. Dept. Biol. II, Ludwig-Maximilians-University Munich, Germany, Munich, Germany; Dept. Biol. I, Ludwig-Maximilians-University Munich, Germany, Munich, Germany. oezugur@biologie.uni-muenchen.de

Program Number: 433.18
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: DD63
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Novel Approaches in Neuromodulation II

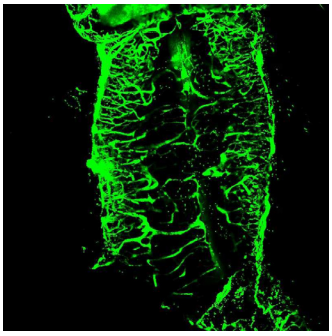
Our research demonstrates that green and blue algae can be inserted into blood vessels within the brain where photosynthesis of these single-celled plants can be activated upon illumination. Nerve cell activity which varies under oxygen-poor conditions, can be rescued by algal-produced oxygen.

Brain activity requires large amounts of energy, mostly provided by oxygen-dependent metabolic processes. In many animals and humans alike, oxygen derives from respiration through lungs or gills and is transported by red blood cells within the vascular system to all regions of the body. The brain of most animals and humans require a constant supply of oxygen through persistent blood flow. In contrast, frogs or salamanders are less oxygen-demanding and even allow experimental maintenance of the entire brain for several days in a supporting medium after isolation. If the medium contains at least air-equivalent oxygen levels, nerve cells in these isolated amphibian brains remain functional.However, it is so far unknown if additional oxygen, through ventilation, might facilitate the survival of such brains and potentially increases the functional capability. Another, rather exotic possibility to increase oxygen levels is the use of green plants, such as single-celled algae, which produce oxygen simply by illumination with visible light through a process called photosynthesis.

In this study, deeply anesthetized tadpoles of the clawed toad (*Xenopus laevis*) received an injection of green algae (*Chlamydomonas reinhardtii*) or cyanobacteria (*Synechocystis sp.*) into the vascular system, distributing these single-celled plants throughout the blood circulatory system of the body including the brain. After isolation, the amphibian brain was prepared for oxygen recordings in a small chamber during superfusion with supporting medium. In agreement with previous findings of a generally hypoxic state of brain tissue, measurements within the amphibian brain confirmed only negligible oxygen concentrations in the presence of air-saturated supporting medium. Illumination of the brains, which contained algae in the vascular system, increased the oxygen concentration up to a third of air-levels as long as the light was switched on. This indicates that the single-celled plants survive within the blood vessels, generate oxygen and thereby considerably augment the level in the brain.

To elucidate the impact of plant-derived oxygen on brain activity, the spike discharge of a nerve that controls eye movements was used as a proxy to monitor the activity of the isolated amphibian brain. At air-saturated oxygen levels of the supporting medium, the nerve activity remained robust for many hours. A decrease of the oxygen concentration in the supporting medium to negligible amounts by ventilation with nitrogen completely abolished the neuronal activity. Subsequent illumination of the brain - which contained algae - caused a restart of the neuronal activity after a few minutes along with a considerable increase of the brain oxygen level. This indicates that the single-celled plants provide sufficient oxygen upon illumination to generate the energy necessary to recover the function of nerve cells.

This is the first proof of principle report demonstrating that single-celled green plants in the brain of an animal can provide the brain with oxygen. This promising technique can be used in basic science to augment the oxygen level in any diffusion-limited preparation devoid of a functional blood circulation such as slice preparations of brain tissue or organoids for *in vitro* experimentation. Moreover, following further studies, photosynthetic oxygen production by plants might provide additional oxygen for regeneration or maintenance in particularly oxygen-demanding organs or during limited blood perfusion. While the experimental introduction of plant cells into animals sounds currently like science fiction, a temporary or permanent interaction between plant and animal tissue appears to yield in the future a number of benefits for basic scientific and medical applications.



PRECISELY-TIMED PHASIC DOPAMINE SIGNALING CREATES DISTINCT KINEMATIC REPRESENTATIONS OF SKILLED MOVEMENTS

A. Bova, A. Hurst, D. K. Leventhal. Neurosci. Grad. Dept., Neurol., Univ. of Michigan, Ann Arbor, MI. asbova@umich.edu

Program Number: 146.20
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: N8
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Basal Ganglia: Behavioral Control

We found that complex, dexterous skills, which require coordinated multi-joint movements, depend on both the history and current state of brain dopamine signaling. These findings, made possible by recent advances in neurophysiology and machine learning, reconcile two major theories of dopamine function and have important implications for Parkinson Disease and related movement disorders.

Parkinson Disease is a neurodegenerative movement disorder, whose motor symptoms are caused by the gradual loss of brain dopamine neurons. For the nearly 6 million people affected worldwide, Parkinson Disease causes significant impairment in motor function, including muscle stiffness, slowed movement, and tremor. The primary treatment, dopamine replacement therapy, only improves some aspects of motor function while dexterous skills remain impaired. Furthermore, with disease progression, clinical responses to dopamine replacement fluctuate rapidly between dyskinesias (excessive abnormal involuntary movements), “on” states with well-controlled motor symptoms, and “off” states in which movement is slow and difficult.

Broadly, there are two competing models of dopamine’s functional role in movement regulation. “Learning” models argue that dopamine signals the value of current actions, thereby influencing subsequent movement. In such models, dopamine loss gradually causes parkinsonism because the brain cannot recognize “successful” actions. Conversely, “performance” models suggest that dopamine exerts a rapid, real-time influence on movements. This model is analogous to a fuel tank, in which the motor state depends on whether the dopamine tank is empty (“off” state), full (“on” state), or overflowing (dyskinetic state). Evidence for “learning” models comes primarily from basic research using tasks that require no movement, simple movements (e.g., lever presses), or innate movements (e.g., locomotion). “Performance” models are supported by clinical observations that, in people with Parkinson Disease, motor function improves with dopamine replacement and declines as the drugs leave their system. Several clinical phenomena (for example, rapid motor fluctuations described above) cannot be explained by either model alone.

To study dopamine’s role in dexterous skill, rats were trained to reach for and grasp sugar pellets using action patterns similar to humans. We then selectively activated or suppressed dopamine neurons as rats performed the reaching movements. To analyze the effect of dopamine manipulations on reach kinematics (how the forelimb moves through space), we applied a machine learning algorithm to track individual digits to reconstruct their 3-dimensional trajectories. Dopamine neuron inhibition during reaching decreased the number of reaches performed, and gradually caused rats to extend their paws further than in the control condition. Conversely, stimulation of dopamine neurons during, but not between, reaches gradually impaired reach accuracy by shortening forepaw extension. These results are consistent with “learning” models, and demonstrate that skill execution depends on prior dopamine levels specifically at the time that it was previously performed. However, once stimulation-induced “bad reaches” were established, rats abruptly switched between “good” and “bad” reaches depending on current dopamine levels.

These results suggest that precisely-timed dopamine release during skilled movements drives gradual changes in motor circuits to establish multiple reaching strategies. Once established, dopamine levels during the current movement select which movement strategy to use. The next steps of this research are to: 1) determine the specific brain regions in which dopamine exerts different influences on motor skill learning and performance, and 2) record dopamine signals to determine how they naturally evolve as rats acquire dexterous skills.

Our results reconcile two major models - “learning” and “performance” - of dopamine’s role in motor control. They help to explain abrupt fluctuations between “off”, “on”, and “dyskinetic” states observed in people with Parkinson Disease after chronic dopamine replacement therapy. Finally, they have important implications for understanding normal motor skill acquisition, and could influence rehabilitation strategies (for example, after traumatic brain injury or stroke).

MODELING CATASTROPHIC CHILDHOOD GENETIC EPILEPSIES: THE EPILEPSY ZEBRAFISH PROJECT (EZP)

C. A. Carpenter, J. Liu, A. Griffin, B. Grone, K. Hamling, M. T. Dinday, M. Anvar, C. Ononuju, R. Paterno, S. C. Baraban. Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA; Gladstone Inst., Univ. California San Francisco, San Francisco, CA; NYU, New York, NY; Univ. of California, Berkeley, Berkeley, CA. 1(860)754-7669. colleen.carpenter@ucsf.edu

Program Number: 041.13
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C48
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Animal Models of Epilepsy I

One of the first steps in treating a disease is to obtain a better understanding of the disease in an experimental animal model. Next is to use this information to identify new therapies. Here, we report the use of gene editing to generate zebrafish models representing catastrophic childhood epilepsies.
Seizures represent abnormal activity along the electrical networks of the brain and may lead to sudden uncontrolled jerky movements, loss of consciousness, confusion and death. Epilepsy is a debilitating, chronic brain disorder, where individuals experience spontaneous, recurrent seizures. In children with epilepsy, debilitating neurodevelopmental, cognitive and behavioral problems are also common. Epilepsy currently affects ~1% of children in the US and although a variety of anti-epileptic drugs are available, about a third of these patients remain untreated. The drugs taken by these children can also have serious side effects on the developing brain. Thus, there remains an unmet need to improve the current drug discovery process for these catastrophic childhood epilepsies.
Many childhood epilepsies are genetic in origin. With increased recognition of this problem in recent years, almost 70 single-gene mutations have been identified in this patient population. Unfortunately, our understanding and treatment of these genetic epilepsies have been limited, in part, by the cost and time needed to model these genes in rodents. Zebrafish (*Danio rerio*) stand as a great alternative experimental model as they have a high degree of genetic similarity with humans (>80% for disease-causing genes). Additionally, their small size and low maintenance cost provide a unique opportunity for large-scale drug discovery efforts in zebrafish. As a disease of abnormal electrical activity, the 'gold standard' for studying epilepsy across all species, including humans, is to monitor brain electrical activity. The Baraban lab was the first to obtain recordings of electrical seizures from the brains of larval zebrafish some 15 years ago. Coupling this technique to behavioral assays, we have now screened more than 3500 drugs in a zebrafish model of Dravet Syndrome (DS), a severe genetic pediatric epilepsy associated with a sodium channel mutation. Drugs discovered only in our zebrafish DS model have already shown efficacy in DS patients and have moved into FDA-approved clinical trials. Here we hope to apply a similar 'aquarium-to-bedside' strategy targeted at a wide range of childhood epilepsies.
A gene editing technique known as CRISPR has led to a revolution in precision medicine. In this study, we have used CRISPR technology to create thirty-eight (38) new zebrafish lines with genetic mutations that mimic those observed in childhood epilepsies. We are screening these zebrafish lines for epilepsy phenotypes using recordings of electrical brain activity and a range of behavioral assays. To date, we have identified spontaneous recurrent electrographic seizure activity in approximately one third of these mutant zebrafish lines. Furthermore, many of these mutant zebrafish larvae display behavioral abnormalities or early life fatalities i.e., co-morbidities also associated with catastrophic childhood epilepsies.
This study represents the first large-scale effort to model genetic forms of epilepsy in zebrafish and gives us the opportunity to gain a better understanding of the functional consequences of these genetic mutations. Our strategy highlights zebrafish as a valuable experimental model and offers a roadmap for precision medicine in the epilepsies.

MODULATING PROMOTER ACTIVITY TO TREAT INTRACTABLE EPILEPSY

Y. Qiu, G. Colasante, C. Di Berardino, L. Massimino, J. Cornford, A. Snowball, S. Jones, S. Giannelli, M. Weston, A. Lieb, S. Schorge, D. Kullmann, V. Broccoli, G. Lignani. Clin. and Exptl. Epilepsy, UCL Inst. of Neurolgy, London, United Kingdom; Div. of Neurosci., San Raffaele Inst., Milan, Italy; Pharmacol., UCL Sch. of Pharm., London, United Kingdom. 39(10)71781. g.lignani@ucl.ac.uk

Program Number: 445.01
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 10:00 AM
Room Number: Room S403
Board Number: N/A
Presentation Time: 8:00 AM - 8:15 AM
Session Title: Mechanisms of Epilepsy

Our research raises the possibility that intractable epilepsy can be cured by modulating the expression of genes that regulate brain activity. Epilepsy is a devastating brain disorder characterised by recurrent seizures. Because epilepsy affects 1% of the global population, and one third of affected individuals have seizures which cannot be controlled by drugs, it represents a major health-care burden. In the majority of cases that do not respond to treatments, only one region of the brain, called epileptic focus, is responsible for the generation of seizures. In these cases, surgical removal of the epileptic focus is often the only treatment option, however, in many cases even surgery is not feasible because of risks to critical brain functions, such as language and movement. For these patients there is currently no hope for treatment. Our work is driven by the aim to provide new therapies for these patients.

Our research builds upon innovative CRISPR technologies that have been developed to generate precise genetic modifications in order cure human diseases. Whilst the potential for CRISPR technology to cure diseases caused by genetic mutations is well recognised (‘gene editing’), its potential applications for treating diseases that are not caused by gene mutation is less well known. For the treatment of epilepsy, an additional hurdle in the application of CRISPR is the challenge of delivering the treatment to the brain. We have developed and demonstrated in a pre-clinical study a CRISPR-based therapy to treat epilepsy. We have harnessed the ability of CRISPR to target specific genes in the genome, which normally leads to changes in the code, to instead activate the expression of a specific gene in order to change the level of protein produced (CRISPR activator or “CRISPRa”). We chose to use CRISPRa technology to change the level of an ion channel in the brain. Ion channels are important regulators of electrical activity in neurons of the brain and targeting ion channels in epilepsy (e.g. via anti-epileptic drugs) is currently the most effective approach to reducing seizures. We hypothesised that by fine-tuning the expression level of the channels that control the electrical activity of neurons, we could stop neurons from triggering seizure activity. We used CRISPRa technology to increase the amount the Kv1.1 ion channel in the mouse brain. Kv1.1 is widely expressed in healthy neurons, is important for regulating their excitability, and can dampen seizure activity when delivered via traditional gene therapy. In a blinded pre-clinical study, we used genetically engineered particles derived from viruses to deliver the CRISPRa tools to the seizure focus of epileptic mice. We found that increasing Kv1.1 in these mice led to a substantial decrease in pathologic electrical activity. This reduction in epileptiform activity not only decreased the number of seizures in the mouse, but also rescued cognitive impairment that is often also observed in patients with drug-resistant epilepsy (e.g. memory deficits). We also compared the expression of genes in the epileptic focus in healthy mice, epileptic mice treated with CRISPRa, and non-treated epileptic animals. We found that the efficacy of our treatment was associated with widespread changes in gene expression in the epileptic focus, which revealed a tendency to restore expression towards the profiles seen in non-epileptic mice. This study is an important proof-of-principle for a translational CRISPR-based approach in epilepsy. Furthermore, the versatility of CRISPRa means that a similar approach might be used in the future to treat other neurological diseases characterized by abnormal electrical activity. For example, the possibility to regulate the expression of any gene in the brain, could provide novel treatment avenues for devastating diseases such as Alzheimer’s and Parkinson’s.

Glia

ASTROCYTES REGULATE BRAINSTEM RESPIRATORY RHYTHM-GENERATING CIRCUITS

S. Sheikhbahaei, H. Koizumi, R. Zhang, A. V. Gourine, J. C. Smith. Cell. and Systems Neurobio. Section, NIH, Bethesda, MD; Univ. Col. London, London, United Kingdom. sheikhbahaeis@ninds.nih.gov

Program Number: 266.04
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 10:15 AM
Room Number: Room S103
Board Number: N/A
Presentation Time: 8:45 AM - 9:00 AM
Session Title: Astrocyte Networks Controlling Brain Function and Behavior

Our research results indicate that astrocytes, the star-shaped brain cells, can powerfully regulate activities of neural circuits in the brainstem that generate the rhythm of breathing in mammals. We also identified lactate as a novel signaling molecule that is released from astrocytes to activate this vital brainstem circuit.
Traditionally, neuroscientists assumed that astrocytes were passively supporting neurons by providing structural and metabolic support. However, accumulating data over the past two decades suggests that astrocytes play an active role in regulating the activity of neurons and circuits underlying complex behaviors. More than half of the brain is comprised of cells called glia, and astrocytes are believed to be the most abundant glia cells. Recently scientists have shown that astrocytes and neurons communicate by releasing chemical molecules, called transmitters.
Identifying the transmitter molecules used by astrocytes is a key step in understanding how these cells regulate neuron and neural circuit function. In general, more in-depth knowledge about the physiology of astrocytes and their chemical signals will expand our understanding of how these cells contribute to brain function. This understanding will also provide opportunities for identifying cell-specific therapeutic agents for brain disorders in which defects in astrocyte function underlie the pathophysiology.
Previously it has been suggested that astrocytes may function to regulate the activities of various neurons in the brainstem circuits that control breathing. These circuits include those within the preBötzinger complex (preBötC), which is a functionally specialized region that generates the basic rhythm of breathing. However, this function of astrocytes in the preBötC has not been directly demonstrated. In our study, we employed as an assay system living slices of brainstem tissue from neonatal mice that have rhythmically active preBötC circuits in vitro. This powerful experimental system provides direct access to preBötC circuits and astrocytes. This readily allows chemical signaling by astrocytes to be manipulated while observing the effects on the ongoing rhythmic activity. For this approach, we used a combination of methods including electrophysiology, pharmacology, and a technique named optogenetics, in which we genetically engineered mouse astrocytes to express the light-sensitive proteins channelrhodopsin or archaerhodopsin. For the first time, we showed that activation of channelrhodopsin with blue light caused preBötC astrocytes to release lactate, which activates preBötC neurons in vitro and powerfully augments their rhythmic activity. We also obtained striking experimental evidence that activation of archaerhodopsin in preBötC astrocytes with orange light stopped rhythmic activity in preBötC circuits. We have not yet identified the astrocyte transmitter molecule(s) responsible for this potent inhibition of circuit activity.
Our results indicate that astrocytes can play an active role in regulating neural circuit activity to affect critical neural functions such as control of the vital breathing rhythm. Astrocytes use many transmitter molecules to communicate with other cells in the brain, and lactate is just one of them. We plan to follow our experimental paradigm to identify other astrocyte signaling molecules and to understand how they regulate breathing. A deeper understanding of breathing control by astrocytes should provide general insights about how astrocyte signaling can regulate brain circuit activity and behavior. Failure of such signaling may adversely affect neural circuit function and contribute to the development of neurological disease(s).

PROGRESSION OF CORTICAL HYPERSYNCHRONY AND EPILEPTOGENESIS INDUCED BY BRAIN TUMORS

J. F. Meyer, K. Yu, A. Hatcher, I. Aiba, B. Deneen, J. L. Noebels. Neurol., Baylor Col. of Med., Houston, TX. (713)7987424. jfmeyer@bcm.edu

Program Number: 536.06
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 2:45 PM
Room Number: Room S401
Board Number: N/A
Presentation Time: 2:15 PM - 2:30 PM
Session Title: Molecular and Genetic Mechanisms Underlying Glia-Neuron Interactions

Our study utilizes novel tools for genetic manipulation and imaging to show that brain tumors can cause epileptic seizures and other abnormal brain activity patterns earlier and more widely in the course of the disease than previously described.

Epileptic seizures are the most common early symptom in several types of pediatric and adult brain tumors and they are often resistant to drug treatment and even removal of the tumor mass. At worst, seizures can be life-threatening events that require immediate medical intervention, and if intractable, have a profound negative impact on quality of life in brain tumor patients. Despite decades of research, brain tumors such as glioblastoma remain among the deadliest types of cancer, and better treatments for tumor-related seizures are essential to manage this disease.

Until now, brain tumors have been studied by implanting human tumor cells in the cortex of adult mice with a compromised immune system so the tumors could grow without being rejected. While many of these mice do develop seizures, the disadvantages of this approach are two-fold. First, we do not know if the lack of a normal immune system actually reveals the entire range of molecular trigger mechanisms that can serve as targets for better therapy. Second, by studying tumor cells grafted into an adult brain, we miss the early natural development of seizures when they arise in the central nervous system when tumor cells are present at birth.

Therefore, we chose to utilize CRISPR mediated genetic targeting to knockout key tumor suppressor genes associated with human glioma, allowing us to observe the progression of the disease from the earliest stages when malignant cells begin to proliferate and infiltrate the cortex. We also employed a specialized microscopic technique using a fluorescent calcium dye to image cellular activity in awake mice over long periods of time, in addition to simultaneously recording classic
EEG signals.

Many days before behavioral seizures occurred in mice with high-grade glioma, we observed abnormal cellular activity patterns, along with high-amplitude EEG waveforms indicating the simultaneous activation of large neuronal populations.

This coincided with a reduction, near the tumor, in a specific subset of inhibitory cells that normally dampen overall brain activity, and the appearance of high levels of microglial inflammation throughout the cortex. Over the following weeks, we monitored the spatial boundaries and progression of pathological synchronization by precisely visualizing a broad region of tumor cortex. At early stages, although tumor cells had invaded only one hemisphere, neuronal activation and EEG seizures were observed in both hemispheres of the brain simultaneously. As seizures developed, we also imaged slow waves that silence brain activity called spreading depolarization, similar to those described in migraine, which have not been previously described in brain tumor.

Our results indicate that malignant cells activate neurons, trigger inflammation, and initiate the development of seizures earlier and more widely than previously thought. In summary, our study reveals the spatial and temporal development of brain tumor-related epilepsy in a mouse model with a normal immune system, using novel genetic manipulation tools and chronic imaging with simultaneous EEG recordings. We detected early abnormalities in brain activity that are more subtle than seizures and, importantly, may signal the presence of tumor in patients at earlier stages. This model provides a more faithful representation of disease progression that may guide us toward more targeted treatments of tumor-related epilepsy.

DEFECTS OF MYELINATION ARE COMMON PATHOPHYSIOLOGY IN AUTISM SPECTRUM DISORDER

B. Phan, J. Bohlen, B. A. Davis, Z. Ze, H.-Y. Chen, B. Mayfield, S. C. Page, M. Campbell, H. L. Smith, D. Gallop, C. L. Thaxton, J. M. Simon, E. E. Burke, J. Shin, A. J. Kennedy, D. Sweatt, B. D. Philpot, A. E. Jaffe, B. J. Maher. Univ. of Pittsburgh, MSTP, Pittsburg, PA; Lieber Inst. For Brain Develop., Baltimore, MD; UNC Sch. of Med., Chapel Hill, NC; Bates Col., Lewiston, ME; Vanderbilt, Nashville, TN. joseph.bohlen@libd.org

Program Number: 556.03
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: B82
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Central and Peripheral Myelinating Cells I

Defects in myelination are an underlying disease mechanism leading to autism spectrum disorder (ASD).
ASD affects 1 in 160 children worldwide and refers to a range of conditions characterized by some degree of impaired social behavior, communication deficits, and repetitive behavior. Both genetic and environmental factors likely contribute to the development of ASD. Currently there are no pharmaceutical therapies that treat core ASD symptoms with behavioral therapy as the current best treatment strategy.
Myelination is a fundamental biological process where oligodendrocytes create concentric myelin wraps around the axons of neurons.
Myelin serves many important functions including regulation of the speed of electrical impulses that travel down axons, provides nutritional and energy support to neurons, and is critical for the proper progression of brain development. To date, myelination has not been implicated as a disease mechanism underlying ASD.
Targeting oligodendrocytes could provide a novel therapeutic avenue, as oligodendrocyte precursor cells which give rise to oligodendrocytes, are present throughout the lifespan and therefore could potentially be coaxed into making more oligodendrocytes and myelin.
In this study, we modeled ASD with mice harboring mutations in one copy of the transcription factor 4 (Tcf4) gene. In humans, mutations in TCF4 leads to a syndromic ASD called Pitt Hopkins Syndrome that is characterized by developmental delays, intellectual disability and autistic features. We analyzed gene expression in bulk brain tissue from mice with and without a mutation in Tcf4 using RNA sequencing to identify how disruption of TCF4 alters gene expression. In the adult mouse brain, mutations in Tcf4 led to altered expression of approximately 1800 different genes significantly enriched among oligodendrocytes and being involved in the biological process of myelination. We confirmed this transcriptional signature by showing that mouse brain with Tcf4 mutations had fewer oligodendrocytes, decreased proportions of myelinated axons, and altered electrical activity indicative of reduction in myelin. These results suggest that decreased myelination is associated with Tcf4 mutations and therefore may be present in humans with Pitt Hopkins Syndrome. Next we compared gene expression changes between our Tcf4 mouse model and two additional ASD mouse models that have harbor mutations in the genes Pten and Mecp2. We observed a significant overlap in differential gene expression between these three mouse models with 34 genes be common to all three mouse models. Remarkably, these 34 genes showed enrichment for biological processes associated with the process of myelination. Lastly, we used the expression levels of these 34 genes as biomarkers, and found significantly different patterns in human postmortem ASD cases compared to unaffected controls.
Together, these results suggest that defects in myelination may be a common disease mechanism underlying ASD in humans and could therefore be targeted for therapeutic intervention. We are currently determining if oligodendrocyte defects observed in our Tcf4 mouse model is recapitulated in humans using induced pluripotent stem cells (IPSCs) derived from Pitt Hopkins
Syndrome patients and neurotypical controls. We are differentiating IPSCs into oligodendrocytes to determine if patient-specific mutations in TCF4 alter oligodendrocyte survival with the ultimate goal of developing a platform to screen compounds for their ability to protect oligodendrocytes with mutations in TCF4.
The findings from this study indicate that defects in myelination may be one underlying pathophysiology in ASD and this opens the door to therapeutic targeting of oligodendrocytes and myelination as a treatment strategy.

KETAMINE ANESTHETIC TRIGGERS PERINEURONAL NET REMOVAL BY MICROGLIA

A. Venturino, R. Schulz, H. De Jesus-Cortes, F. Reilly-Andujar, B. Nagy, M. E. Maes, G. Colombo, F. E. Schoot Uiterkamp, M. Bear, S. Siegert. IST Austria, Klosterneuburg, Austria; Dept. of Brain and Cognitive Sci., The Picower Inst. for Learning and Memory, Cambridge, MA. +43224390003050. sandra.siegert@ist.ac.at

Program Number: 719.07
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 2:45 PM
Room Number: Room S405
Board Number: N/A
Presentation Time: 2:30 PM - 2:45 PM
Session Title: Microglial Activation in Disease States

In this study, we provide first indications that certain anesthesia drugs might have long-lasting impacts on brain function. General anesthesia is an important milestone in modern medicine as it induces unconsciousness, relaxation and analgesia during surgery. A general assumption of anesthesia action is that it only temporarily influences brain activity, and that brain function fully recovers afterwards.
Our research reveals how the anesthesia drug ketamine activates microglia—the immune cells of the brain—and, as a consequence, how this might influence the nervous system. The drug ketamine was classified as an essential drug for surgery by the World Health Organization (WHO). With humans, ketamine is the drug of choice for inducing and maintaining anesthesia, especially in pediatrics and with patients experiencing cardiopulmonary problems. Ketamine is also frequently used in veterinary surgery. To be effective in animals, it has to be combined with xylazine, which prevents ketamine-induced muscle rigidity, and the tranquilizer acepromazine.
In the last decades, studies have provided important insights into the pharmacokinetics and -dynamics of ketamine regarding the function of neurons. Yet, we are still lacking information on the interaction of the drug with the other half of cells in the brain, which are commonly described as non-neuronal glial cells. These cells provide a fine-tuning of the nervous system by supplying neurons with nutrients, disposing of their garbage, and ensuring fast signal transmission. Special glial cells called microglia are widely distributed across the brain. They function as immune cells and protect the brain from pathogens. In recent years, it has been shown that microglia also express a variety of neurotransmitter receptors, allowing them to survey and to respond to changes in neuronal activity. They are commonly found to act on neurons and their synaptic contacts especially during neurodevelopment, but also in the course of brain diseases such as neurodegeneration.
In our study, we were interested in whether the temporal alteration of physiological neuronal activity induced by ketamine leads to a microglia response. To answer this question, we anesthetized mice with ketamine-xylazine-acepromazine and investigated changes in the microglia branching complexity and their pro-phagocytic state. We found that ketamine-xylazine-acepromazine anesthesia turned microglia to be reactive. This then led microglia to remove extracellular matrix structures, which surround defined neurons and are supposed to protect them from activity-induced changes. We observed that repeated exposure of ketamine-xylazine-acepromazine seems to enhance this process, without inducing synapses loss nor neuronal cell death.
The activation of microglia through ketamine-xylazine-acepromazine in mice can have broad implications, not only with regard to potential and longer-lasting side effects in anesthesia recovery but also in the treatment of depression. Recently, the US Food and Drug Administration (FDA) approved lower concentrations of ketamine as a drug for treating depression. Yet, researchers still have only a minimal understanding of the interactions of the drug with non-neuronal glial cells, which might induce potential side effects of this treatment.
Our results indicate that changes in the neuronal activity induced by the anesthesia drug ketamine impacts non-neuronal microglia cells - with yet unknown short- and long-term consequences for the regeneration of the nervous system. Follow-up studies are required to provide insights into the details of the mechanisms behind this ketamine action and its implications for various brain functions.

ALTERED HUMAN OLIGODENDROCYTE HETEROGENEITY IN MULTIPLE SCLEROSIS

S. Jaekel, E. Agirre, A. Mendanha Falcao, D. Van Bruggen, K. Wai Lee, I. Knuesel, D. Malhotra, C. Ffrench-Constant, A. Williams, G. Castelo-Branco. Ctr. For Regenerative Med., Edinburgh, United Kingdom; Lab. of Mol. Neurobio., Department of Biochemistry and Biophysics, Sweden; F. Hoffmann La-Roche Ltd., Basel, Switzerland; Hoffmann-La Roche, Basel, Switzerland; Ctr. for Regenerative Med., Edinburgh, United Kingdom. sarah.jaekel@ed.ac.uk

Program Number: 740.01
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: C16
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Central and Peripheral Myelinating Cells II

With our research we analysed the pattern of gene expression in single cells from adult human brains post mortem and found first, that these patterns reveal multiple states of myelin-forming oligodendrocytes and second, that the distribution of these states changes in Multiple Sclerosis (MS).
MS is an inflammatory neurological disorder with about 2.5M affected people worldwide. It is characterized by the damage to a particular cell type in our brain, oligodendrocytes, and the fatty substance myelin they produce. This myelin is tightly wrapped around the long fibers (called axons) that connect our nerve cells in the brain and has two functions; it provides nutrients to the axons and also speeds up the conduction of nerve impulses along them. As a result, its loss leads to reduced function and then degeneration of the axon. This in turn causes the disability that characterizes progressive MS, for which there are currently no treatments.
Repair mechanisms within human brains initially cope with the damage caused by MS and replace the lost myelin– a process that we call remyelination. However as the years go by, the remyelination capacity of the brain decreases and persistent areas of myelin loss – called demyelinated lesions – remain. A key question for those trying to develop new treatments for progressive MS is therefore: What is the balance between inflammation-mediated damage and repair in the brain of each person affected with MS? Only by knowing this can treatments be targeted at the right process in the patient. However, up to now, MS lesions are usually characterized by pathological methods that examine the cells responsible for inflammation, and our knowledge about any changes associated with damage and repair in the oligodendrocytes themselves remains vague.
In order address this gap of knowledge, in our recent publication, we used a powerful technology called single-nuclei RNA-sequencing that allowed us to analyse the pattern of gene expression in thousands of individual brain cells within a frozen tissue sample from human brains post mortem. With this, we compared the oligodendrocytes of brains of people that were living with MS with normal brains.
We found (as we expected from previous studies in animal brains) that the human brain contains distinct oligodendrocyte states distinguished by the levels of expression of different genes. Most surprisingly, however, we found that although we could identify all these states in MS lesions the balance between them was substantially altered, with some being over- and some underrepresented. While we don't know yet whether each oligodendrocyte type has a distinct role in the brain, the fact we found examples of each being located in distinct regions of the brain strongly suggests this to be the case. We conclude therefore that the changing balance of oligodendrocyte types in MS may cause some of the symptoms of the disease.
By gaining a better knowledge about oligodendrocytes in our brain, our work is a key step towards understanding the cellular changes that are happening in this disease. The results are important for two reasons. First, this understanding will enable rational approaches to drug discovery based on targeting both the damage-causing mechanisms that are responsible for the selective loss of some oligodendrocyte states and the repair mechanisms required to restore an optimal balance of them. Second, the technology we have used will enable far greater accuracy in the pathological analysis of MS lesions that is possible by current microscopy based methods, an advance that will in turn lead to greatly improved knowledge as to the variation in MS between different patients.

CELLULAR REPRESENTATIONS OF HUMAN THEORY OF MIND

M. Jamali, B. Grannan, R. Báez-Mendoza, Z. Williams. Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA. 1(857)383-1966. mjamali@mgh.harvard.edu

Program Number: 249.03
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: AA6
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Human Social Cognition: Behavior, Mechanisms, and Disorders II

Our findings reveal neurons in the human prefrontal cortex that reflect variations in the thoughts of other individuals. The complex ability to represent the minds of others and predict others' beliefs is a major milestone and achievement in human development. The neurons detected in our study may support theory of mind in humans.

Humans have the ability to build a remarkably detailed understanding of the world and, based on this, reason about the beliefs or internal states of others. This capacity to form an implicit model of reality and, from this, reason about the beliefs of others is often referred to as theory-of-mind and is considered a core cognitive milestone in human ontogeny. Yet, how these computations are precisely formulated by individual neurons in the human brain is unknown.

In humans, functional imaging studies have provided a critical understanding of the network of brain regions that are likely involved in social cognition. The human prefrontal cortex has been implicated in inferential processing and the ability to reason about others, and is broadly connected with other brain areas thought to be involved in social behavior. Animal models of social behavior have also provided important evidence of neurons that respond to another's observed actions or receipt of reward. Yet, unlike the observed behavior of others, beliefs are inherently unobservable or unknown and are often distinct from the actions or events that drive them. They are also difficult to study explicitly in animal models.

A critical test for theory-of-mind is the false-belief task which aims to evaluate our ability to reason about the beliefs of others. In this task, a participant may be presented, for instance, with a narrative such as "You and Tom see an empty cup on a table. When Tom leaves the kitchen, you move the cup to the cupboard". The narrative would then be followed by the question "Where will Tom expect to find the cup?" This task therefore tests two core components thought to be necessary for theory-of-mind. First, it tests the participant's ability to form an understanding of the hidden causal state of events and, from this, reason about the beliefs of social agents within it. Second, it requires the participants to understand that another's beliefs may be false and potentially distinct from the participant's own.

Here, we aimed to study for the first time, how these processes may be represented by prefrontal neurons in human participants as they perform variations of the false-belief task. Across 11 participants, we recorded from a total of 212 neurons, of which 20% responded selectively to another's beliefs and differentiated another's beliefs from the participant's own. Whereas certain neurons reflect detailed information about the content of the social agent's beliefs, other neurons distinguish the other's beliefs from non-belief related events. Further, when processed collectively, these mixed populations accurately predict whether the other's beliefs are false.

Taken together, these findings represent a rare look into how humans form inferences about others. We speculate that neurons in our study may be good candidates for supporting theory-of-mind and a potential target of investigation for social behavioral conditions such as autism spectrum disorder. Further, integrating our findings with functional imaging and comparing recordings from different areas thought to be involved in theory of mind may allow for a more comprehensive understanding of how neuronal activity subserves theory of mind.

AN INTERBRAIN APPROACH FOR UNDERSTANDING EMPATHY

S. G. S. Shamay-Tsoory, P. Goldstein. Psychology, Univ. of Haifa, Haifa, Israel. simone.shamay@gmail.com

Program Number: 274.06
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 10:30 AM
Room Number: Room S401
Board Number: N/A
Presentation Time: 9:15 AM - 9:30 AM
Session Title: Social Cognition: Behavior and Neural Mechanisms II

INTRAOPERATIVE LARGE SCALE EXTRACELLULAR RECORDINGS FOR STUDYING THE CELLULAR AND MICROCIRCUIT BASIS OF HUMAN COGNITION

V. M. Eisenkolb, J. Gempt, B. Meyer, S. N. Jacob. Dept. of Neurosurg., Tech. Univ. of Munich, Munich, Germany. v.eisenkolb@tum.de

Program Number: 330.05
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: Z33
Presentation Time: 8:00 AM - 9:00 AM
Session Title: New Methods for Studying Cognition

Our interdisciplinary study describes technologies to investigate the basis of human cognitive functions at great detail, that is with a resolution of individual neurons.
Intelligent behavior is generated by specific areas of the human brain, in particular in the frontal and parietal lobes (fronto-parietal association cortex). These regions are necessary for all cognitive tasks, e.g. for processing and interpreting sensory events, for storage of information in working memory and for selecting the best responses amongst multiple alternatives. It is still unknown how these complex cognitive functions are implemented by the human brain. Commonly used non-invasive methods in cognitive neuroscience research such as electroencephalography (EEG) or functional magnetic resonance imaging (fMRI) do not have the spatial and temporal resolution to make statements about the mechanisms of cognitive functions at the level of individual neurons and their local networks.
To overcome these obstacles, recordings of neuronal activity directly inside the human cortex are necessary while subjects are engaged in cognitive tasks. In neurosurgical departments at large medical centers, clinicians regularly have direct access to the human brain. Some surgeries even require the patients to be awake, e.g. for neuropsychological testing during the removal of brain tumors that are close to the brain's language areas. This creates unique opportunities for collaborating neurosurgeons and neuroscientists to measure neuronal activity directly from the human cortex "in action".
For this study, we implanted miniature multi-channel electrode arrays (Utah arrays) into the association cortex of the left hemisphere in awake patients undergoing an operation to remove a left-sided brain mass. The array can be placed flexibly within the skull aperture (craniotomy), allowing us to record from various cortical locations. During anesthesia and later awake phases of the surgery, we recorded neuronal signals on up to 96 channels simultaneously. These included local field potentials (LFPs), generated by the summed electrical activity of large populations of single neurons, and importantly, also the activity of individual neurons. We were able to obtain high-quality signals in the demanding intraoperative setting with multiple sources of electrical noise. We combined these acute recordings with behavioral measurements in a working memory task where subjects had to remember numerical quantities presented either as Arabic numerals (symbolic, verbal format) or as dot patterns (non-symbolic, non-verbal format). We found that individual neurons have different encoding patterns, that is they respond maximally to one of the two formats. This result demonstrates how human language shapes brain activity. A second aspect of our work is that we can investigate disordered neuronal processing in brain diseases. For instance, we were able to monitor large synchronized brain waves emerging in the immediate vicinity of tumors, potentially representing epileptic micro-activity.
Currently we are further optimizing our implantation technique to achieve stable neuronal measurements throughout the entire surgery. In addition, we are working on lowering the associated costs and streamlining the recordings to promote dissemination to other neurosurgical and neuroscience centers.
The combination of multi-channel recordings directly from the human fronto-parietal cortex with controlled behavioral tasks opens up new avenues for the in-depth investigation of the neuronal principles that govern human-specific cognitive functions such as language, which cannot be studied in animals.

MODULATION OF HIPPOCAMPAL BRAIN NETWORKS PRODUCES CHANGES IN EPISODIC SIMULATION AND DIVERGENT THINKING

P. P. Thakral, K. P. Madore, S. E. Kalinowski, D. L. Schacter. Harvard Univ., Cambridge, MA; Stanford Univ., Stanford, CA. 1(617)347-2737. prestonthakral@fas.harvard.edu

Program Number: 195.03
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 3:15 PM
Room Number: Room S402
Board Number: N/A
Presentation Time: 1:30 PM - 1:45 PM
Session Title: Medial Temporal Lobe in Learning and Memory

Our research shows that the brain processes that support human memory also play a causal role in supporting our ability to imagine future experiences and to think creatively.

Episodic memory refers to memory for unique events that happened in a specific time and place, comprised of details concerning people, places, and actions. Scientists think of episodic memory not as a video camera that allows us to play a literal recording of a past experience, but instead as a constructive process: we remember bits and pieces of an event and link them together reconstructing the original episode. Recent work has shown that the same constructive processes that support episodic memory are adaptive in that they help to support other human abilities. For example, brain scans using functional magnetic resonance imaging (fMRI) have shown that the same brain regions active during the retrieval of episodic memories, including the hippocampus and parietal cortex, are also active when we imagine hypothetical future episodes (episodic simulation) and also when we generate creative ideas (creative thinking). These results suggest that we use our past episodic experiences flexibly both to simulate future events and to think creatively.

Here, we tested the causal role played by episodic memory in future imagining and creative thinking by using transcranial magnetic stimulation (TMS), a noninvasive procedure that uses a magnetic pulse to disrupt neural activity in specific parts of the brain. We aimed to disrupt neural activity in the parietal cortex and the hippocampus, two brain regions involved in episodic memory, and to provide the first test of whether they play a causal role in future imagining and creative thinking. It is not possible to directly stimulate the hippocampus because it is located deep in the brain. We thus used fMRI to identify a more accessible region on the surface of the brain in the left parietal cortex (specifically the left angular gyrus) that has strong connectivity to the hippocampus. When TMS is used to stimulate the left parietal cortex, other brain regions linked with the hippocampus become less synchronized with each other. Therefore, the less these regions work together (i.e., the less they are synchronously active), the less effectively participants should be able to think about the future or to think creatively.

TMS was applied to either a control site (the top of the head) or the target region in the parietal cortex: the left angular gyrus. Following application of TMS, participants underwent brain scanning and performed three tasks. In each task, participants were shown an object word and either imagined a novel future experience (episodic simulation task), generated creative and unusual uses of the object (divergent creative thinking task), or generated associated items and their definitions (control task). Results demonstrated that, compared with TMS to the control site, participants generated fewer episodic details (i.e., details concerning people, places, and actions) when describing their future events and also generated fewer creative uses for the divergent thinking task. By contrast, participants performed equally well in the control task after TMS to either the control or target region. Critically, the fMRI data showed that TMS caused the parietal cortex and the hippocampus to become less synchronized with each other, thus showing that reduced communication between these two brain regions led to reduced ability to think creatively and to imagine an episodic future event.

Our findings have implications for healthy age-related decline and neurological conditions, such as Alzheimer's disease, which are characterized by a decline in not only episodic memory but also imagination and creativity. Our experiment suggests two likely brain targets for appropriate interventions to alleviate declines in adaptive episodic functioning.

NEUROGENETIC AND EVOLUTIONARY MECHANISMS IN THE BENGALESE FINCH'S SONG: PARALLELS AND IMPLICATIONS FOR THE STUDY OF HUMAN SPEECH

M. Farias-Virgens, T. Deacon, K. Okanoya, S. A. White, E. Huerta-Sanchez. Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA; Univ. of California Berkeley, Berkeley, CA; Univ. of Tokyo and Riken Brain Sci. Inst., Tokyo, Japan; Brown Univ., Providence, RI. (510)421-2652. madzayasodara@ucla.edu

Program Number: 233.05
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: P30
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Vocalization and Social Behavior in Songbirds II

It is January 49 BC, confronted with the decision of whether to cross the Rubicon, Julius Caesar shouts "Alea iacta est"—the die is cast— and marches his legions through the river, unleashing a series of events that would lead to the fall of the republic and the rise of the Roman Empire. From then on "to cross the Rubicon" means to take a step from which there is no turning back. No doubt the rise of the Roman Empire was a major event in our history, but allegedly not the first time we have crossed the Rubicon. Fast-forward to Victorian London, German-born philologist Max Müller challenges, "Language is our Rubicon, and no brute will dare to cross it'. Let's render unto Müller the things that are Müller '. Language is a shared symbolic system that, in modern times, only humans possess. However, Müller was wrong to think that no other animal would dare to push that barrier. Now we know, despite not sufficient, certain components of spoken language are shared with communicative behaviors in other species. A robust body of evidence accrued over >100 years of research in birdsong demonstrates striking analogies between this exquisite behavior practiced by a much evolutionarily distant animal group and human speech. Both birdsong and speech depend upon vocal production learning, that is, the ability to learn to produce vocal sounds via imitation to vocal usage learning, the ability to associate innate sounds with specific elements and events. Like a child learning to speak, a young songbird must first hear the vocal sounds of adults and then imitate those sounds on its own. Initially, the bird will sing a faint, unstructured song, akin to babbling in human infants. By adulthood, this immature chirping progresses to a more consistent birdsong, just as babbling progresses to speaking. These parallel developmental trajectories are accompanied by striking similarities in the way brains and their underlying molecular apparatus function to produce speech and birdsong. All this resemblance has motivated the additional search for similar evolutionary pressures leading to vocal learning in songbirds and humans. Our research uses songbirds to identify evolutionary processes leading to increased complexity of learned vocal behavior, a key aspect in speech evolution. The Bengalese finch (BF) has a remarkably complex song, in which transitions between vocal syllables are less firmly fixed, introducing variability in song sequencing. This vocal complexity evolved during BF's domestication from the its ancestral species, the white-backed munia (WBM). We are using whole-genome sequencing of individuals within the two bird strains and analytical tools from comparative and population genomics to identify genes highly differentiated between the two strains. We are also inferring a demographic model shaping BF's genetic variation and estimating the impact of the population bottlenecks that happened during its domestication. We will access the relative contributions of different selection processes, such as female choice for more complex songs, and relaxation of sources of evolutionary constraints to song complexity that are commonly found in the wild but are absent in the domesticated scenario. These include the stress associated with finding food or defending from predators, or pressures to avoid confusion with cohabitating finch species. Our results will guide further comparative efforts towards identifying similar patterns of evolutionary change between humans and other primates or hominid lineages (e.g. Denisovans and Neanderthals). After all, if Caesar could cross the Rubicon, it was because the river was shallow enough in the first place, and so in a way it must have been for humans crossing the Rubicon of language.

Language

TRANSCRIPTIONAL REGULATORY DIVERGENCE UNDERPINNING SPECIES-SPECIFIC LEARNED VOCALIZATION IN SONGBIRDS

H. Wang, A. Sawai, N. Toji, R. Sugioka, Y. Ji, S. Hayase, S. Akama, J. Sese, K. Wada. Grad. Sch. of Life Science, Hokkaido Univ., Sapporo, Japan; Fac. of Science, Hokkaido Univ., Sapporo, Japan; Natl. Inst. of Advanced Industrial Sci. and Technol., Tokyo, Japan; Fac. of Sci., Hokkaido Univ., Sapporo, Hokkaido, Japan. wang-hongdi@hotmail.com

Program Number: 232.18
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: P21
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Vocalization and Social Behavior in Songbirds I

Species-specific behavior plays a role in a variety of inter- and intra-specific interactions, including reproduction and ecology, where species differences are thought to be an important factor in species co-occurrence. Such species-specific behaviors can arise via species differences in the structure and development of the neural circuits underlying behavior. Differences between closely related species are thought to be driven by differential expression and functional changes of orthologous genes in conserved neural circuits, which are often, in turn, driven by transcriptional regulatory divergence. Transcriptional regulatory divergence between species can arise due to species divergence in *cis*-regulatory elements that affect the transcriptional rate and stability, and/or in *trans*-regulatory factors that access cis-regulatory elements.

However, it remains largely unknown how transcriptional regulatory divergence contributes to the generation of species-specific behavior, especially in the case of learned behavior.

Songs produced by oscine birds are complex vocal signals acquired through vocal learning. Songs are species-specific and these species differences play an important role in mating interactions and territory defenses within and between species. In the songbird brain, a conserved neural circuit, called the song pathway, contributes to song learning and production. Birdsong is composed of two main traits associated with species specificity: the acoustic elements (syllables) and the temporal pattern (sequence) of song. The production of syllable acoustics and sequence are mainly regulated in the vocal motor song nuclei of the song pathway. The importance of these song nuclei in determining species-specific song traits suggests an underlying causative role of species differences in the structure and activity of these regions. Consistent with this, a variety of genes, including transcription factors and neuromodulator receptors, are differentially expressed in theses song nuclei between species even at a laboratory-controlled environment. However, a key gap in our knowledge is how species-specific patterns of gene expression in these regions arise via regulatory differences between species.

In this study, we used two closely related songbird species, zebra finch (*Taeniopygia guttata*), owl finch (*T. bichenovii*), and their interspecific first-generation F1 hybrids, to elucidate how transcriptional regulatory divergence is associated with species-specific song. These two species diverged about 6.5 million years ago and share overlapping habitats in the north and west of Australia. Using genome-wide transcriptional analyses with gene expression between the two species and allele-specific expression ratios in the F1 hybrids, we determined that the species-specific expression of 600-800 genes (approximately 10-15 % of all expressed genes) is regulated by *cis*- and/or *trans*-transcriptional divergence in the vocal motor song nuclei of the two songbird species. Furthermore, in the song nucleus RA, analogous to the mammalian laryngeal motor cortex, *trans*-, but not *cis*-, altered genes were significantly enriched, which was associated with the regulation of multiple neural functions. We identified BDNF as an upstream mediator of *trans*-regulated genes in RA, with a significant correlation between individual variation in BDNF expression level and species-specific song phenotypes in F1 hybrids. To support this, pharmacological overactivation of BDNF receptors altered the expression of its *trans*-regulated genes in RA and eliminated species-specific acoustic and sequence traits of adult zebra finch songs.

The current understanding of the evolution of behavioral differences even among closely related species is still limited. Specifically, very few studies have focused on “learned behaviors” at both the evolutionary and molecular genetic perspectives. To our knowledge, our study is the first to link transcriptional regulatory evolution with species differences in learned behavior. Songbirds and other vertebrates, including human, possess analogous neural networks for the learning and execution of motor patterns. Therefore, our findings are likely to make significant impacts on multiple research fields, including neuroscience, behavioral genetics, and evolutionary biology.

FINE-TUNING BIRDSONG: THE ROLE OF DELTA OPIOID RECEPTORS IN THE DEVELOPMENT OF SONG STRUCTURE

U. A. Singh, S. Iyengar. Natl. Brain Res. Ctr., Gurgaon, India. utkarsha@nbrc.ac.in

Program Number: 232.19
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: P22
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Vocalization and Social Behavior in Songbirds I

Our study suggests that blocking delta-opioid receptor signaling during the sensitive period for vocal learning affects the quality of vocalizations in adulthood.

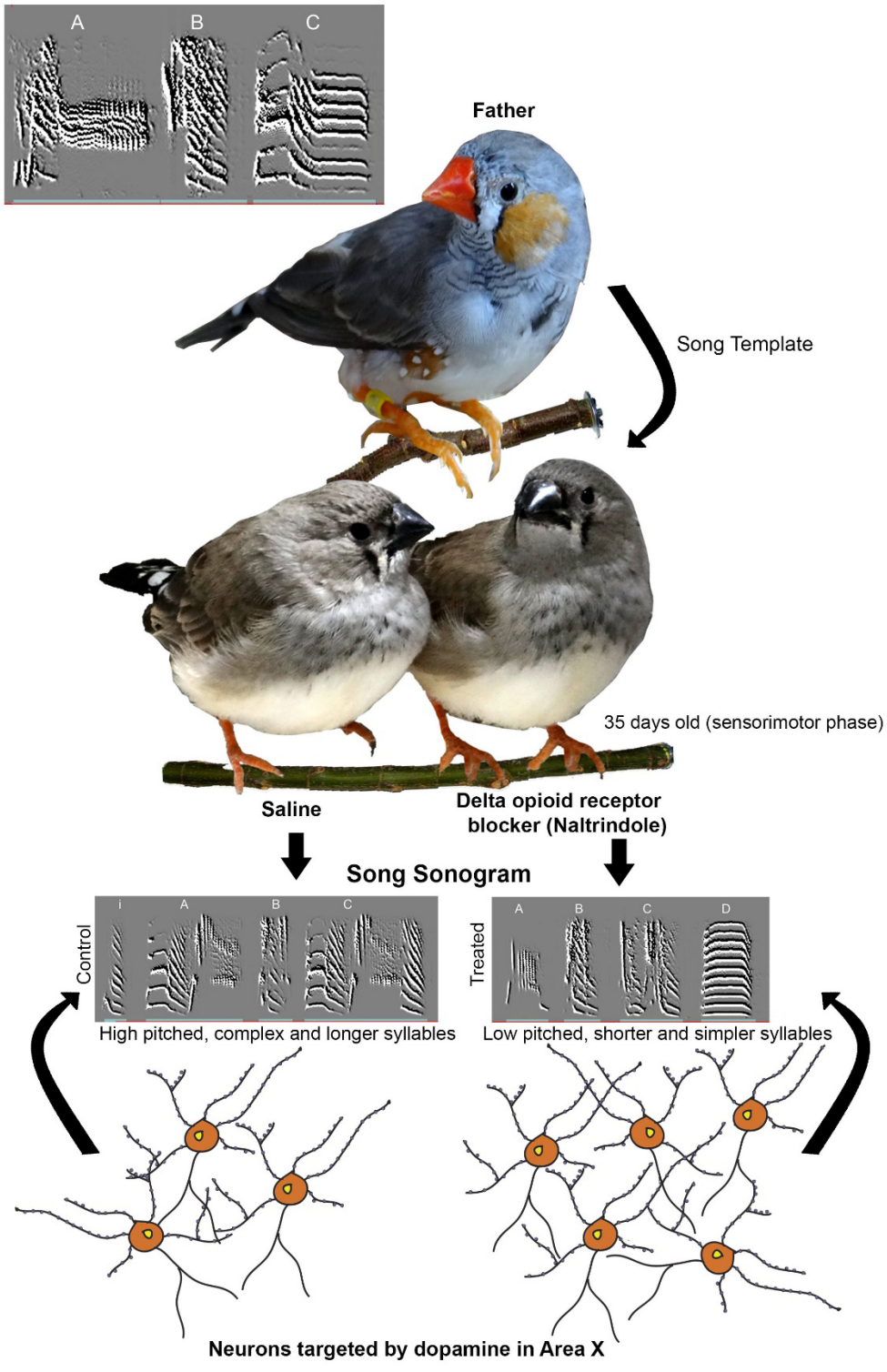
Opioids are the leading cause of substance abuse, affecting 27 million people worldwide. Over-prescription of opioid medication and the production of synthetic opioids have resulted in a surge in opioid abuse. Opioid abuse has overshadowed normal functions of these complex neurochemicals, which are endogenously manufactured by the brain. Endogenous opioids (enkephalin and endorphin) act as neuromodulators to alter neural circuits and can affect the production and maturation of new neurons in the brain. Well-known functions of endogenous opioids include modulating pain, hunger/satiety, motor functions, reward/motivation and social behaviors. Whereas opioid abuse is known to cause slurring of speech and verbal deficits, its role in speech and vocal learning remains unexplored.

Opioid ligands bind to opioid receptors (ORs) which are distributed across the brain. The three major types are mu, delta and kappa, of which mu and delta subtypes are involved in addictive behaviors. Studies have also shown that delta-ORs are involved in associative learning. However, there have been no reports on their role in vocal learning which occurs during early development. We decided to use zebra finches, an Australian songbird species to study the effects of the opioids on vocalization. Young males of this species learn their father's songs during the first 90 days after hatching and brain areas dedicated for vocalization have high concentrations of opioid receptors.

For our experiments, we used pairs of male siblings, wherein one bird received systemic injections of Naltrindole, a delta-OR blocker and the other received saline injections. The drug was administered every day for 10 days during the period when young birds are learning their father's songs and practicing their own. The songs of these birds which were directed towards females were recorded at 10-day intervals once they reached adulthood (from 80 -120 days post-hatch) and analyzed for quality. We found no difference in the number of songs between the drug-administered and control siblings. However, parameters including pitch, song complexity and time duration of individual elements of the song were significantly lower in the drug-administered sibling. Interestingly, neurons that serve as downstream targets of dopamine, a neurotransmitter of the reward pathway, and the number of synapses in a striatal song control area (Area X) were higher in birds treated with Naltrindole than in their control siblings.

These results suggest that altering opioid receptor signaling for a very short duration during the critical period resulted in long-lasting changes in brain architecture which were also manifested behaviorally. Currently, we are studying how delta-OR agonists can modulate song learning, vocalization and brain structure.

The alarming rise in opioid abuse has the unfortunate consequence of affecting future generations, since pregnant women suffering from opioid addiction expose the foetus to these powerful neuromodulators. This study is a step towards understanding the developmental effects of opioid imbalance in the young brain and to look at its long-term effects on learning and cognition.


A
B
C
Father
Song Template
35 days old (sensorimotor phase)
Saline
Delta opioid receptor blocker (Naltrindole)
Song Sonogram
Control
i
A
B
C
Treated
A
B
C
D
High pitched, complex and longer syllables
Low pitched, shorter and simpler syllables
Neurons targeted by dopamine in Area X

ELUCIDATING THE NEURAL MECHANISMS OF LEARNING-TO-LEARN

V. Goudar, B. Peysakhovich, E. A. Buffalo, D. J. Freedman, X.-J. Wang. New York Univ., New York, NY; Neurobio. and Computat. Neurosci., Univ. of Chicago, Chicago, IL; Physiol. and Biophysics, Univ. of Washington, Seattle, WA. vishwa.goudar@gmail.com

Program Number: 163.04
Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: X15
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Learning and Memory: Physiology I

THALAMUS-HIPPOCAMPAL DIRECT PATHWAY REGULATES SEX-DIFFERENCES IN MEMORY CONSOLIDATION

G. Torromino, V. Loffredo, F. Esposito, M. Colucci, M. De Risi, M. Gioffrè, E. De Leonibus. IBCN-CNR Inst. of Cell. Biol. and Neurobio., Rome, Italy; TIGEM - Telethon Inst. of Genet. and Med., Pozzuoli, Naples, Italy; PhD Program in Behavioral Neurosci. (XXXII cycle), Sapienza Univ. of Rome, Rome, Italy; IGB-CNR Inst. of Genet. and Biophysics, Naples, Italy; IMM-CNR Inst. of Microelectronic and Microsystems, Naples, Italy. torrominogiulia@gmail.com

Program Number: 360.07
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 2:45 PM
Room Number: Room N427
Board Number: N/A
Presentation Time: 2:30 PM - 2:45 PM
Session Title: Learning and Memory: Cortical-Hippocampal Interactions

We discovered a new sexual dimorphism in memory function due to different engagements of the same brain circuit.From science to literature, sex differences have historically been a crucial issue for humankind, faced from different perspectives. Beyond the fascinating aim of discovering how women and men are made different, studying sex-differences become relevant in the field of brain pathologies and disorders that have different incidence between the two sexes, such as Alzheimer' disease and Parkinson' disease. Memory capacity can be loaded by increasing the amount of information to retain for a short-time delay, or by increasing the retention interval. By singularly manipulating each of these variables we did not detect differences between male and female normal mice. However, in condition of high memory load (high number of items to remember) female, but not male mice, showed an accelerated forgetting of the information acquired. In a previous set of studies, our group had shown that when male mice are faced with high number of items to store for long-time interval they recruit the hippocampus to solve the task. Thus, we asked whether males and females were recruiting differentially the hippocampus to manage high memory load. We found that females had a lower activation of the hippocampus compared to males, but they had a higher activation of the thalamus. Since these two brain structures are anatomically connected, we artificially inhibited the thalamic-hippocampal circuit using optogenetics (a light-mediated manipulation of the pathway activation) and we were able to prevent the accelerated forgetting in conditions of high load in female mice. Although it doesn't sound like new that women and men use their brain differently, the novelty of our research lies in the discovery of the engagement of a totally new sexual dimorphic brain circuit and in the evidence that its manipulation can rescue subtle sex-specific deficits. Another important novel finding is the role of the thalamus, a subcortical brain region, in wiring the activity of the hippocampus, a cortical brain region, suggesting that sex-differences in the brain are controlled by bottom-up, rather than, top-down neuronal wiring.Understanding the neuropsychology of sex-differences in memory capacity is crucial to design training protocols tailored on the sex-specific neurocognitive strengths. In turn, knowing how we are made different allows the employment of sex-specific brain manipulation to modulate the efficiency of brain functions, both in normal and pathological conditions. We are trying to understand whether these sex-differences in memory capacity and in sex-regulated brain activation make women more prone to develop hippocampal-related pathologies during ageing and whether different behavioral and pharmacological strategies must be employed to treat these neurological dysfunctions in the two sexes. This places our research at the crossroad between basic neuroscience and clinical research.

DANIONELLA TRANSLUCIDA: A NOVEL FISH SPECIES FOR SYSTEMS NEUROSCIENCE

A. Penalva-Tena, A. D. Douglass, J. Bedke, J. P. Barrios, E. P. L. Bertram, W.-C. Wang, E. S. B. Cook. Univ. of Utah, Salt Lake City, UT. 3852592599. u1003286@utah.edu

Program Number: 601.24
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: Y42
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Neural Circuits for Learning and Memory

Our study establishes the optically transparent fish *Danionella translucida* as a new animal research model to investigate how neuronal activity shapes complex social behaviors.

A key goal of neuroscience research is to investigate how the brain regulates complex behaviors. The field aims to understand this question with both cellular and spatial specificity – that is, by determining where and how individual neurons within the brain coordinate their activity to shape a behavioral response. The extent to which this can be explored, however, is limited by the animal models currently used in neuroscience studies.

To fully explore the relationship between neuronal activity and behavior, it is necessary to do this in an organism that is both optically transparent (which allows individual neurons to be visualized throughout the brain of a living animal), and has a wide array of behaviors. The larval zebrafish (*Danio rerio)* is an attractive system for these studies, as it is nearly transparent throughout its first two weeks of life and can be easily manipulated due to its small size. Zebrafish are also easy to manipulate genetically, which further facilitates being able to identify specific sets of neurons and how they relate to a particular behavior. Unfortunately, the behavioral responses of larval zebrafish are limited to simple reflexes. As adults, zebrafish develop complex and interesting behaviors, but they lose their transparent features, grow considerably in size, and develop a layer of skull and scales around the brain, preventing its visualization without being highly invasive to the animal.

This project successfully establishes the closest relative of zebrafish, known as *Danionella translucida,* as a new animal model to explore the relationship between brain activity and behavior. This tropical fish is considered one of the smallest vertebrates on earth (reaching ~1 cm in length as an adult), and remains optically transparent its entire life. It also exhibits an array of complex behaviors during adulthood, including social preference and learning, that are not present in larval zebrafish.

The close relationship between *Danionella* and zebrafish allows for the successful adaptation of molecular and genetic tools from one system to the other. Using sophisticated microscopy techniques, we are able to image the whole brain of a living *Danionella* adult, as well as identify and record the activity of its individual neurons. We are also able to manipulate the genome of *Danionella* to the same extent as in zebrafish and have created several transgenic lines, introducing foreign genes into the *Danionella* genome

In this study, we also demonstrate that adult *Danionella* exhibits complex social behaviors. By adapting a social preference paradigm used in adult zebrafish, we show that adult *Danionella* spends significantly more time next to members of their same species as opposed to a neutral object. These innate social responses are mediated by visual and olfactory cues, as *Danionella* retain this preference even when exposed to a projection of a fish (purely visual input) or to water where fish of the same species were previously present (purely olfactory input). *Danionella* can also associate a neutral pattern with the presence of another fish, even when the fish is no longer present, indicating this novel species has the ability to learn.

Our findings establish *Danionella* as a novel tool for neuroscience studies seeking to understand the relationship between neuronal activity and complex behaviors in an adult system. The next step in our research is to combine the established advantages of *Danionella* to investigate which specific sets of neurons activate when encountering same-species visual and olfactory cues, and how the activity of these neurons coordinates to regulate social responses.

SIMILAR FIRING PROPERTIES OF NEURONS IN THE MEDIAL ENTORHINAL CORTEX IN MALE AND FEMALE MICE

M.-B. Moser, D. C. Rowland, E. I. Moser. Kavli Inst. Systems Neurosci, Trondheim, Norway; NTNU, Trondheim, Norway. 47 47-73-59-82. may-britt.moser@ntnu.no

Program Number: 604.12
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: AA8
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Cortical and Cortico-Hippocampal Circuits: Spatial Navigation III

The brain contains a network of interconnected regions that together form a mental map of the current spatial environment, akin to the GPS system in your mobile phone. In this study, we looked at one important structure within this network, the medial entorhinal cortex, and asked whether the spatial coding properties of the cells were similar in males and females. We found that the cells had strikingly similar properties in the two sexes.

Whether males and females navigate through their environments differently has been endlessly pondered in the general public, popular media, as well as in scientific studies. In humans, there is large corpus of data on the subject, but not always an agreement. Nevertheless, recent large-scale studies and meta analyses have shown a small but real male advantage in tasks that require way-finding or map-based navigation. Other studies have pointed to a small female advantage in landmark-based navigation. Although the results appear to be clear, most authors are quick to point out that a whole range of social and cultural factors are likely to play an important role in driving these differences in humans. Indeed, at least one recent global study found that the gap between males and females in a way-finding task was less in societies with high gender parity. Such findings raise suspicions about whether navigational differences in the sexes are biologically ingrained. Many studies have therefore turned to animal models, most notably rodents, for answers. Some have argued that males and females rodents should also show differences in spatial navigation. After all, male rodents typically journey farther away from their homes than females and defend their territory more the females. In addition, gonadal hormones might play an important role in spatial behaviors. Such factors could lead to an irreducible biological component to sex differences in spatial navigation.

In this study, we looked into the spatial coding properties of cells in the medial entorhinal cortex. We chose the medial entorhinal cortex because it has a collection of functionally specialized cell types that likely relate to different aspects of spatial cognition. For example, there are cells that measure distances travelled, cells that provide information about the geometry of the environment, cells the measure speed of movement, and other cells that measure the orientation of the animal's head. The medial entorhinal cortex therefore provides an interesting test case for examining sex differences in the brain.

Our analyses found that the firing properties of these were remarkably similar in male and female mice. Our results suggest that, at least in one key brain area, male and female mice have similar mental representations of space. We have not considered other brain areas nor can we answer whether male and female mice have similarly equivalent spatial behaviors, because other cognitive phenomena like attention might influence how these signals are used by the brain to produce behavior. Nevertheless, our study adds important new information to this debate. We further hope that these data will encourage more researchers in our field to use both sexes in their studies, which will reduce the number of animals used in experiments.

DEVELOPMENT OF A HIPPOCAMPUS-DEPENDENT MEMORY TASK FOR NEURAL RECORDINGS IN FOOD-CACHING BIRDS

M. C. Applegate, K. S. Gutnichenko, D. F. Scheck, D. Aronov. Columbia Univ., New York, NY. 1(908)487-8067. mca2179@columbia.edu

Program Number: 604.24
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: AA20
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Cortical and Cortico-Hippocampal Circuits: Spatial Navigation III

REPRESENTATION OF SPACE IN THE GOLDFISH BRAIN

E. Vinepinsky, L. Cohen, O. Ben-Shahar, O. Donchin, R. Segev. Ben Gurion Univ., Beer Sheva, Israel. ehpinsky@gmail.com

Program Number: 694.10
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: Z24
Presentation Time: 9:00 AM - 10:00 AM
Session Title: Cortical and Cortico-Hippocampal Circuits: Spatial Navigation IV

We report evidence for representation of space in the brain of freely swimming goldfish, in the form of cells which are active in specific locations in the environment. In addition, we observed border encoding cells, which are active mainly near the borders of the water tank.

Place and border encoding cells are believed to be part of the basic building blocks of the navigation system of mammals. Although there is a robust consensus regarding the inventory of cells that represent space in the mammalian brain, there is no accepted theory, based on empirical data, explaining how these components of spatial representations are integrated in the brain into a functioning navigation system. One solution can emerge from studying the neural mechanisms underlying navigation in other vertebrate classes, since elucidating the mechanisms of space representation in different vertebrate classes can help decipher how the elementary building blocks of navigation are integrated into a functional navigation system. Crucially, comparative studies can help determine whether the mammalian navigation system is unique or an instance of a more general biological design. Thus, this approach may help resolve the key question of the critical components making up a functioning navigation system.

Navigation is one of the fundamental cognitive capability in fish, which form the largest vertebrate class. This ability is important for finding food, shelter, and mates in order to survive. Behavioral studies have shown that Goldfish have the cognitive ability to navigate either by exploiting an allocentric or an egocentric frame of reference. This may imply that the goldfish has the ability to build an internal representation of space in the form of a cognitive map. This would include cognitive map-like navigation strategies to find a goal when starting from an unfamiliar initial position or taking shorter alternative routes (shortcuts) when possible. We show evidence of place and border encoding cells in the goldfish brain. These cells types can support the fish's ability to navigate using allocentric frame of reference. Our study is the first which shows these cell types in details.

In order to study the neural representation of space in the goldfish brain, we developed a novel wireless recording system for freely behaving fish. Our system is based on a set of electrodes connected to a neural data logger. The whole setup is waterproof and wireless, thus allowing the fish to swim freely, and anchored to the fish's skull. Using this system, we record extracellularly the spiking activity of single cells in the fish forebrain. More specifically, we implanted the electrodes into the goldfish lateral pallium, which is, by previous anatomical and lesions studies, suggested as a homologue to the mammalian hippocampal formation, and is crucial for allocentric navigation. After the surgery, we recorded the activity of single cells while the fish swam freely in a familiar shallow square water tank (0.6X0.6X0.2 meter) with visual cues available on the water tank walls.

In summary, we report evidence for representation of space in the form of cells that were active in specific locations in the environment. Those cells resemble cell types which are found in the mammalian hippocampal formation and believed to be the building blocks which drive the navigation system. Our study sheds light on how spatial information is encoded in the fish brain and whether the mechanisms of the neural navigation system are preserved across evolution.

NEURAL CORRELATES OF LOCOMOTION, CUES, AND CONTEXT IN THE INTERACTIONS BETWEEN HIPPOCAMPUS AND LATERAL SEPTUM

H. S. Wirtshafter, M. A. Wilson. Picower Inst. Learn/Memory, MIT, Cambridge, MA. hsw@mit.edu

Program Number: 694.07
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: Z21
Presentation Time: 10:00 AM - 11:00 AM
Session Title: Cortical and Cortico-Hippocampal Circuits: Spatial Navigation IV

Our everyday lives rely on planned movement through the environment to achieve most goals. Our recent research shows that the lateral septum (LS), a well-connected brain region considered integral to modulating behavior and implicated in many psychiatric disorders, directly encodes information about the movement of an animal as it navigates and learns how to obtain a reward in an environment. This finding provides evidence that the lateral septum may be a crucial link between circuits guiding goal-directed movement and motivated behavior.

Previous research has attributed important behavioral functions to the LS, such as modulating anxiety, aggression, and affect. It is also believed to be involved in addiction, psychosis, depression, and anxiety. Neuroscientists have traced its connections to the hippocampus, a crucial center for encoding spatial memories and associating them with context, and to the ventral tegmental area, a region that mediates goal-directed behaviors. But until now, no one had shown that the LS directly tracks movement or communicated with the hippocampus about movement and the spatial context of reward.

We were able to directly observe these interactions by simultaneously recording the electrical spiking activity of hundreds of neurons in the LS and hippocampus of rats both as they sought a reward in a T-shaped maze, and as they became conditioned to associate light and sound cues with a reward in an open box environment. We observed a speed and acceleration spiking code in the LS, and saw clear signs that an overlapping population of neurons were processing information based on signals from the hippocampus, including spiking activity locked to hippocampal brain rhythms, spatial tuning in the T-maze, and cue and reward responses during the conditioning task. This suggests that the septum may serve as a point of convergence of information about movement and spatial context.

We also showed that coordination of LS spiking with the hippocampal theta rhythm is selectively enhanced during choice behavior that relies on spatial working memory, suggesting that the LS may be a key relay of information about choice outcome during navigation.

These finding suggest that movement-related signaling in the LS, combined with the input that it receives from the hippocampus, may allow the LS to contribute to an animal's awareness of its own position in space, as well as its ability to evaluate task-relevant changes in context arising from the animal's movement, such as when it has reached a choice point.

This also suggests that the reported ability of the LS to modulate affect and behavior may result from its ability to evaluate how internal states change during movement, and the consequences and outcomes of these changes. For instance, the LS may contribute to directing movement toward or away from the location of a positive or negative stimulus.

Our work therefore offers new perspectives on the role of the lateral septum in directed behavior, and has implications for broader understanding of the mechanisms relating mood, motivation, and movement, and the neuropsychiatric basis of mental illnesses.

LARGE SCALE RECORDING OF POPULATION ACTIVITY DURING SOCIAL COGNITION IN FREELY MOVING NON-HUMAN PRIMATE

M. Franch, A. Wright, B. Aazhang, V. Dragoi. Neurobio. & Anat., UTHealth - McGovern Med. Sch., Houston, TX; ECE, Rice Univ., Houston, TX. (919)902-9217. melissa.franch@gmail.com

Program Number: 785.18
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: AA30
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Social Memory and Cognition II

Our research is transforming the way we study neural computations of complex behaviors. Our innovative experimental paradigm advances our field from traditional, restricted experimental designs to robust experiments that foster dynamic behaviors we have previously been unable to study in depth.
In order to understand how the brain processes social information, we've chosen to study the neural representations of cooperation. During cooperation, an individual must perceive and integrate socially relevant visual information from the environment in order to respond in a context appropriate manner.
Our lab developed and validated a novel, dynamic experimental paradigm where, for the first time, animals can freely move, view each other, and choose when to cooperate. In the experiment, two animals cooperate for a pellet reward by simultaneously pushing and holding down respective buttons, which move the trays containing their pellets. A trial starts when the pellets dispense and ends when the trays reach the animals. Our results confirm that nonhuman primates can use this apparatus to cooperate for a pellet reward, and their behaviors change when cooperating for unequal rewards.
Many studies, including our own, indicate that two brain areas highly involved in extracting visual features and decision making are the visual cortex (V4) and dorsolateral prefrontal cortex (dlPFC), respectively. By wirelessly recording from populations of neurons in V4 and dlPFC simultaneously, we are learning how salient visual social cues, such as the reward for cooperating and the actions of the partner, are integrated in these areas of the brain to give rise to social behavior. In order to know what the animal is viewing, the animal is wearing a wireless eye tracker. **For the first time, this eye tracking technology is being used on a freely moving nonhuman primate to understand social behavior**. Our results reveal that the animal attends to expected salient visual cues, particularly the actions of his partner, and this affects neural responses and the decision to cooperate.
The major limitation preventing our understanding of social cognition is the lack of a suitable framework to allow us to study how it emerges in real time from interactions among brain networks. Indeed, examining the neural bases of complex social interactions has been traditionally performed by studying the brain of nonhuman primates in a laboratory environment in which the head and body are restrained while synthetic stimuli are presented on a computer monitor. However, it has become increasingly understood that studying the brain in spatially confined, artificial laboratory rigs poses severe limits on our capacity to understand the function of brain circuits. Our project and novel experimental approach where animals can freely interact and choose when to make decisions overcomes these limitations.
Furthermore, the motivation and capacity to be social is a key component of human behavior. Unfortunately, this ability is impaired in many neurological disorders, such as autism spectrum disorder, which affects 1 in 59 individuals. Our research constitutes a paradigm shift by moving social neuroscience – from simply observing animal behavior and recording the responses of single cells – to a quantitative understanding of the distributed neuronal network encoding during social behavior in freely moving nonhuman primates performing rich naturalistic tasks. Thus, this project improves our understanding of the social brain, which can lead to therapeutic developments for individuals suffering from social dysfunction.

REPRESENTATION OF HEAD DIRECTION AND SPEED IN THE GOLDFISH BRAIN

L. Cohen, E. Vinepinsky, O. Donchin, O. Ben-Shahar, R. S. Segev. Ben Gurion Univ., Beer Sheva, Israel; Ben Gurion Univ., Be'er Sheva, Israel. 972 972-8-6. ronensgv@bgu.ac.il

Program Number: 789.06
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: BB33
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Intrinsic Hippocampal Circuits: Spatial Navigation

Navigation is one of the fundamental cognitive skills found in many animals across all of the animal kingdom, including fish. It is important for finding food, shelter and mates in order to survive. In mammals, head direction cells and speed cells were found in the hippocampal formation, which is crucial for navigation. Yet, there are only small number of studies that attempted at understanding the neural representation of self- kinematics outside of the mammalian kingdom. Our study is the first to show the existence of head-direction cells and velocity cells in fish. Furthermore, it is the first detailed description of speed cells in the fish brain, after previous studies have suggested their existence.

A critical component of the ability to navigate is a sense of direction and a sense of speed. However, almost nothing is known about the neural representation of direction and speed in the brain of vertebrates outside the mammalian class. The goldfish, which is a bony fish, the largest vertebrate family, have the cognitive ability to navigate using allocentric and egocentric cues. In addition, the lateral pallium in the goldfish brain is a possible homolog of the mammalian hippocampal formation and associated with allocentric navigation. Therefore, our study is a step forwards toward understanding the navigation system in non-mammalian vertebrates. This could lead to a better understanding of this system across all vertebrates.

Using a novel wireless recording system, we recorded single neurons activity from the goldfish brain while it is freely swimming and video tracked. Our findings include head direction cells, which are active when fish'hes ad is oriented at a specific direction, speed cells, which are active when the fish swims in a particular speed, and velocity cells, which encode both, i.e. are active when the fish swims relatively fast and towards a specific direction.

These cell types may constitute the basic building blocks of the goldfish navigation system and can thus shed new light on theories of navigation systems based on observations in the mammalian system alone. Further investigations are needed to obtain a complete functional map of this region and to understand whether it has a foundational role in the neural navigation system of the bony-fish brain.

NEURONAL ENSEMBLES DYNAMICS DURING SPATIAL LEARNING IN CA1 MOUSE HIPPOCAMPUS

M. Altamira, R. Olivares-Moreno, M. Lopez-Hidalgo, G. Rojas-Piloni. Dept. de Neurobiología del Desarrollo y Neurofisiología, Inst. de Neurobiología, Univesidad Nacional Autónoma de México, Queretaro, Mexico; Escuela Nacional de Estudios Superiores, Unidad Juriquilla, Univ. Nacional Autónoma de México, Queretaro, Mexico. moisesaltamiracamacho@hotmail.com

Program Number: 789.03
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: BB30
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Intrinsic Hippocampal Circuits: Spatial Navigation

PAVLOVIAN CONDITIONED APPROACH BEHAVIOR IS ENCODED BY CORTICO-ACCUMBENS ACTIVITY

M. G. Spring, J. R. Mcreynolds, A. J. Caccamise, K. Soni, E. A. Panther, B. M. Windsor, J. R. Mantsch, R. A. Wheeler. Marquette Univ., Milwaukee, WI. 1(978)578-3000. mitchell.spring@marquette.edu

Program Number: 075.03
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: U33
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Neural Mechanisms Underlying Motivated Behaviors and Addiction

A diverse array of mental disorders, ranging from major depressive disorder to schizophrenia, involve apparent problems in the way pleasure is experienced. Clinical descriptions of this shared symptom often focus on anhedonia (i.e. a reduced ability to experience pleasure) to the exclusion of other potential disruptions in reward processing. This narrow focus has likely limited research aimed at identifying overlapping neural circuits that are impaired in seemingly unrelated disorders. However, recent consideration of the complexity of reward processing recognizes anhedonia as only one component within a larger framework of reward processing deficits. During reward seeking and consumption, it is not only the hedonic impact of a reward (i.e. how much the reward is enjoyed) that is represented in the brain, but also the incentive value (i.e. how much effort the reward is worth). Consistent with this view, motivational and reward learning deficits have been repeatedly observed in disorders associated with anhedonia. In many cases, such deficits can be observed absent concurrent deficits in reward enjoyment. Therefore, it is unlikely that reduced motivation is simply a byproduct of reduced pleasure. This consideration requires the investigation of a broader scope of neural circuits that may be impaired to produce the reward-related deficits present in many psychiatric conditions.

One such circuit consists of neurons that project from the medial prefrontal cortex (mPFC) into the nucleus accumbens (NAc), a hub for collecting reward related information to guide behavior. This projection subserves an animal's ability to engage in goal directed action and has been well studied in instrumental learning, in which an animal must engage in a specific behavior (e.g. pressing a lever) to obtain a reward (e.g. a sugar pellet). Less is known about the role of this circuit in using reward related cues to direct approach behavior. Despite its simplicity, such behavior can be highly informative about an animal's general motivational state, as deficits can fundamentally change the way in which an animal interacts with its environment.

To study this latter form of appetitive learning, *in vivo* recording techniques were used to monitor either individual neuronal activity within the mPFC or the aggregate activity of a population of neurons within this subregion that project to the NAc during a Pavlovian conditioning task. In this task the presentation of a compound lever and light cue (collectively termed the CS+) was repeatedly paired with the subsequent delivery of a sugar pellet into a food cup. Animals in this task acquire either a learned interaction with the cue itself or a conditioned approach of the pellet delivery cup in response to the presence of the CS+. During these same training sessions, animals were also repeatedly exposed to a similar compound cue (the CS-) which did not predict a reward.

In one experiment, the activity patterns of individual mPFC neurons were monitored following training. The majority of recorded cells displayed a change in their firing rate in response to CS+ presentation but not the CS-, indicating that neurons in this region are tuned to reward relevant information in the environment. A separate experiment found that this activity change selectivity emerges in NAc-projecting mPFC neurons early in training and grows stronger concurrent with the development of conditioned approach toward either the CS+ or the cup. A final experiment showed that chronic stress, which causes anhedonia-like behaviors in rodents, also disrupts conditioned approach behavior. Together, these data suggest a crucial role for the projection pathway from the mPFC to the NAc in conditioned approach behavior and the deficits in approach motivation that are shared across mental disorders.

ACTIVATION OF THE M1 MUSCARINIC ACETYLCHOLINE RECEPTOR MODULATES NUCLEUS ACCUMBENS DOPAMINE RELEASE AND INCREASES MOTIVATIONAL RESPONDING

S. E. Yohn, J. Galbraith, E. G. Rieth, I. Wetzka, C. W. Lindsley, P. J. Conn. Pharmacol., Vanderbilt Univ., Nashville, TN. samantha.yohn@vanderbilt.edu

Program Number: 592.10
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: T17
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Subcortical Circuitry Motivation, Compulsive Behavior, and Psychostimulants

Motivational symptoms are debilitating features of several neuropsychiatric disorders and are highly correlated with problems in social function, employment and long-term treatment outcomes. Current neuropsychiatric medications do not have efficacy on motivational phenotypes, and, in many cases, can induce or exacerbate these symptoms. While the neural basis of motivational dysfunctions is still being characterized, it has been suggested that there may be common mechanisms across different disorders, and considerable evidence implicates central dopamine (DA) in the nucleus accumbens (NAc). A powerful modulator of DA signaling is the cholinergic system, which consists of nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors. mAChRs belong to the superfamily of G-protein coupled receptors (GPCRs) that either activate or inhibit signaling pathway systems through intracellular second messengers. The M1 mAChR subtype is densely expressed in the NAc, where they have long been suggested to influence DA release. Previous data suggests that M1 activation could regulate NAc DA release and reward-related dependent behaviors. However, the data are contradictory due to poor selectivity. To circumvent this problem, our laboratory has developed highly selective compounds that act at allosteric sites, which allow for activation of specific receptor subtypes. Through use of highly selective M1 preferring compounds, we now report that activation or potentiation of M1 increases motivated behavior and facilitates NAc DA release. Taken together, these findings suggest that activation of M1 may be therapeutic for the treatment of motivational dysfunctions.

CENTRAL EXENDIN-4 SELECTIVELY SUPPRESSES CUE-EVOKED PHASIC DOPAMINE SPIKES AND RESULTANT BEHAVIOR

V. R. Konanur, T. M. Hsu, M. F. Roitman. Dept. of Psychology, Univ. of Illinois at Chicago, Chicago, IL. vkonanur@gmail.com

Program Number: 773.04
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: U12
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Dopamine, Reward, and Reinforcement

BRAINSTEM COMMAND NEURONS THAT SPECIFY LOCOMOTOR DIRECTION

J. M. Cregg, R. Leiras, O. Kiehn. Univ. of Copenhagen, Copenhagen, Denmark. jared.cregg@sund.ku.dk

Program Number: 497.04
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: DP08/Q7
Presentation Time: 8:00 AM - 12:00 PM
Session Title: Control of Spinal Locomotion Circuits

We discovered a small population of hindbrain command neurons which control the ability of mice to move left or right.

Distinct hindbrain command neurons control basic movement parameters, including the type of gait and speed with which animals move. The diversity of these command neurons is beginning to be appreciated in detail. To-date, the description of command neurons in limbed animals is limited to those which have the ability to either completely arrest movement or increase movement speed. Nonetheless, animals also have an ability to move left or right—raising the question of how the nervous system allows for such directional movements?

Using anatomical tracing techniques, we found that a small population of brainstem neurons, *Chx10* neurons, project to the spinal cord and make synapses predominatly on the same side. We found that stimulation of *Chx10* neurons caused changes in spinal cord activity only on the same side, suggesting that they may control movements that are directed toward the side in which they are stimulated. We further probed the role of *Chx10* neurons in awake, freely moving animals: Stimulation of *Chx10* neurons abruptly changed the direction the animal moved; stimulation on the left side caused animals to move left. Mirroring this effect, we found that inhibition of *Chx10* neurons on the left side caused animals to move right. Finally, we found that *Chx10* neurons are actually required for all left/right movements—without them, animals cannot move in an intended direction.

How does this *Chx10* circuit control the muscular apparatus to make a left or right movement? Using high-speed video recording and machine learning algorithms to track movements, we found that stimulation of *Chx10* neurons caused trunk contraction while also shortening limb stride length on the same side. Mice thus use a two-component system for directional movements: a trunk system for controlling posture of the axial muscles combined with a limb system for differential control of speed on the left and right sides. Remarkably, this two-component biological turning system is a design principle adopted for steering four-wheeled vehicles millions of years after it was selected during evolution to controlling asymmetric movements in quadrupeds: turning in quadrupeds and four-wheeled vehicles is enabled by a dedicated steering/differential system for independent control of speed on the left and right sides.

To further understand how the brain controls left/right movements, we performed a screen for brain regions which interact with this dedicated *Chx10* steering system. We found a number of brain regions which exhibit hemisphere-specific innervation of *Chx10* neurons and demonstrated that a sensory brain hub—the superior colliculus—may funnel its activity through the *Chx10* steering system. These data deepen the mystery of how the brain actually controls the ability to move left or right, and provide a concrete platform for dissecting this fundamental question. Our future studies will focus on understanding how the brain organizes and distributes information concerning left/right movements between hemispheres.

The distinction between left and right is fundamental for most modes of animal behavior. Understanding how the brain controls asymmetric movements will not only shed light into normal brain function, but will likely also provide insight into neurological disease. Indeed, unilateral basal ganglia lesions—which model many aspects of Parkinson's Disease—cause asymmetric movements. The neural circuits which cause motor asymmetries in Parkinson's are unknown.

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STRIATAL PROJECTION NEURONS REQUIRE HUNTINGTIN FOR SYNAPTIC CONNECTIVITY AND LONGEVITY

C. Burrus, S. U. Mckinstry, N. Kim, F. Y. Fang, A. Santoki, H. H. Yin, C. Eroglu. Trinity Col. of Arts and Sci., Dept. of Psychology and Neurosci., Duke Univ., Durham, NC; Cell Biol Dept, Duke Univ, DUMC, Durham, NC. cjb53@duke.edu

Program Number: 016.01
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 2:45 PM
Room Number: Room S405
Board Number: N/A
Presentation Time: 1:00 PM - 1:15 PM
Session Title: Emerging Insights in Huntington's Disease Research: Pathological Mechanisms and Therapeutic Approaches

Huntington's Disease (HD) is an inherited neurodegenerative disorder which causes patients to develop progressive motor, cognitive, and psychiatric symptoms, typically beginning during the fourth or fifth decade of life. In contrast to many human brain disorders where the underlying cause is unknown, the genetic cause of HD has been known for over twenty years. However, there are currently no approved treatments to stop or delay progression of HD.

HD is caused by a mutation in one copy of the huntingtin gene, which alters the function of the huntingtin protein and renders it toxic to neurons. HD causes the death of neurons within the striatum, a brain region intimately involved in the regulation of motor function. Many HD researchers believe that the death of striatal neurons is due to toxic functions of the mutant huntingtin protein. However, in addition to its toxicity, the mutant huntingtin protein may also interfere with ability of the remaining normal huntingtin (produced by the non-mutated copy of the huntingtin gene) to perform its functions. Therapeutics currently in clinical trials aim to suppress the formation of mutated huntingtin, but these strategies also decrease the amount of normal huntingtin inside cells. Therefore, to properly treat HD, it is essential that we understand the normal role of huntingtin in striatal neurons, and what effect the loss of huntingtin from these neurons will have on their cellular health and function.

To determine the importance of normal huntingtin protein function in striatal neurons, we used a genetic mouse model to delete huntingtin protein specifically from striatal neurons. Interestingly, loss of huntingtin in striatal neurons caused extensive cell death with aging, similar to what is observed in HD mouse models, even though no mutated huntingtin is present in these cells. This result suggest that normal huntingtin protein is required for striatal neuron survival with aging.

The striatal neurons which die in HD are key components of the brain circuit controlling voluntary motor functions. In the healthy brain, striatal neurons control motor function by communicating with neurons in other brain regions through connections called synapses. Many HD patients have abnormal synaptic connectivity which may underlie HD symptoms. We found that deleting huntingtin from mouse striatal neurons altered the synaptic connections of striatal neurons, which corresponded with changes in motor behavior. Importantly, these synapse and behavioral changes occurred before the neurons began to degenerate. These findings suggest that addressing synaptic changes will be critical for future HD therapies. Collectively, our studies demonstrate that loss of huntingtin can recapitulate multiple aspects of HD.

While there is strong evidence that mutated huntingtin is toxic to neurons, our research shows that normal huntingtin must also be present for neurons to be fully healthy and functional. These findings raise several important questions. First, huntingtin was deleted in our mice very early during their development. Is removing huntingtin from neurons after neurodevelopment is complete still detrimental for neuronal health and function? Therapies currently in clinical trials that reduce huntingtin levels are administered to adults, so studies to clarify the effect of huntingtin loss on adult neurons later in life will be important. Second, we showed that huntingtin regulates synapse development, and abnormal synapse development is a common feature of many neurological and psychiatric conditions. We are currently investigating the precise mechanisms by which huntingtin controls synapse development, which will enhance our fundamental understanding of neurodevelopment and brain circuit formation.

PEPTIDE-DIRECTED SELECTIVE KNOCKDOWN OF MISFOLDED SOD1 AS A THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS

T. Zhou, T. Guan, Y. Wang, H. Marzban, J. Kong. Univ. of Manitoba, Winnipeg, MB, Canada; Univ. of British Columbia, Vancouver, BC, Canada. 1(431)335-5628. zhout346@myumanitoba.ca

Program Number: 475.17
Session Date/Time: Tuesday, October 22, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: F30
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Motor-Neuron Disease: Therapeutics

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disease that affects primarily a group of nerve cells that control movement of skeletal muscles in the body. Loss of these cells would result in paralysis and eventually death. Within a population of 100,000 people, there are 2 new ALS cases each year and 5 people living with ALS. Most people who develop ALS are between the ages of 40 and 70, with an average age of 55 at the time of diagnosis. About 90 percent of ALS cases occur without family history. The remaining 10 percent of ALS cases are inherited through a mutated gene. On average, it takes about one year before a final ALS diagnosis is made. About 70% of ALS patients die within 5 years after diagnosis. There is currently no cure for ALS.
In the last decades, a major discovery in the study of ALS is that mutations in the gene SOD1 are a cause of ALS. More than 180 ALS-linked mutations in the SOD1 gene are reported. The resulting mutant SOD1 proteins themselves initially appear not toxic to nerve cells but become more susceptible to chemical modifications such as oxidation. Then, these modified SOD1 proteins tend to form aggregates and become bad and toxic to nerve cells. Interestingly, normal SOD1, if exposed to oxidative stress conditions, can also be oxidatively modified, form aggregates and become bad to nerve cells. Aggregation of bad SOD1 has been found in brain and spinal cord of ALS patients with no mutations in their SOD1 gene. Therefore, misfolded (bad) SOD1 appears to be a therapeutic target for both familiar and sporadic ALS. What we report here is an approach to selectively clean up those misfolded SOD1 in cells and in animal models of ALS. We have designed a recombinant peptide named CT-4 that binds specifically to misfolded SOD1. The CT-4 peptide contains also a sequence that targets to lysosome, a structure for protein degradation in cells, In another word, the CT-4 peptide hijacks a cell's protein degradation system to selectively degrade bad SOD1. In cultured nerve cells, we found that treatment with the CT-4 resulted in a selective degradation of misfolded SOD1 in a dose-, time- and lysosomal activity-dependent manner, as expected. Intravenous injection of a single dose (10mg/kg) of the CT4 peptide in the G93A mouse model of ALS resulted in a 57% reduction of misfolded SOD1 in 24 hours and 70% in 48 hours in the brain and spinal cord. Levels of the misfolded SOD1 could recover in one week after a single administration. Daily injection of CT-4 peptide significantly delayed disease onset and extended lifespan of the G93A mice. We are working on to improve treatment outcomes and are cautiously optimistic that the peptide-direct protein degradation system might be developed into a cure for ALS.
The project is financially supported by Brain Canada and ALS Canada.

PERIPHERAL KNOCKDOWN OF ENDOCYTIC PROTEIN AP2A2 AMELIORATES ACUTE AND CHRONIC INFLAMMATORY PAIN-LIKE BEHAVIORS IN MICE

R. G. Powell, A. Bhattacharjee. SUNY Univ. At Buffalo, Buffalo, NY; Univ. at Buffalo - The State Univ. of New York, Buffalo, NY. rasheenp@buffalo.edu

Program Number: 056.02
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: J14
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Pain: Inflammatory Mechanisms

Endocytosis, the moving of membrane-bound proteins into the cell, is a basic cellular process that most of us studied in introductory biology courses and is often thought of as a process that simply terminates cellular signaling. While we now know the molecular players in endocytosis, surprisingly, we do not fully understand how endocytosis might impact broader signaling such as pain. Our studies sought to target the endocytosis process in peripheral pain sensing neurons and we discovered that endocytosis is obligatory for both initiating pain as well as maintaining chronic pain states. Moreover, we established that inhibiting endocytosis provides long-lasting analgesia (pain relief).

Chronic pain affects 116 million adults annually in the United States and has an estimated economic burden of nearly 635 billion dollars in lost productivity and direct medical costs. The number of adults living with chronic pain outnumber adults living with cancer, heart disease, and diabetes combined. There are many different types of chronic pain, however, chronic inflammatory pain, commonly associated with arthritis, is projected to worsen as the US population ages given current treatment regimens. In 2015, 11% - 33% of adults in the US living with arthritis also reported having severe joint pain. Additionally, there is a predicted increase in the number of adults living with arthritis from 54.4 million (2015) to 76 million in 2040; which does not bode well for existing therapy options. First-line treatment options for chronic inflammatory pain include opioids and non-steroidal anti-inflammatory drugs (NSAIDs). However, opioids carry a potential for addiction and NSAIDs can cause the development of life-threating ulcers in some patients. These adverse effects serve as a significant driving force for innovative analgesic development.

Previously, we showed that targeting adaptor protein complex 2 (AP2), a protein complex important for endocytosis, with a novel small molecule inhibitor blocked the inward movement of specific ion channels. Halting endocytosis decreased the electrical activity in sensitized pain neurons. One of the interesting characteristics of AP2 is that it is made up of smaller subunits (alpha; α, beta; β, sigma; σ, mu; μ), with the α subunit encoded by two independent genes: α-1 and α-2. It's unclear as to why there are two α genes but these are some of the first studies ever to address the functionality of the α-2 subunit.

To study the function of the AP2 α-2 subunit in pain, we pioneered a surgical technique in mice that allows for direct genetic down-regulation of specific protein targets within the sciatic nerve. After decreasing the AP2 α-2 subunit, we induced acute inflammation in mice and tested their resultant pain behavioral responses. When AP2 α-2 was genetically inhibited, we observed a significant decrease in thermal sensitivity and spontaneous pain behaviors. When testing these parameters in a chronic inflammatory pain model, we found similar results: diminished pain behavior. Our data suggests that the AP2 α-2 plays a substantial role in both the initiation and maintenance of acute and chronic inflammatory pain.

Finally, we pharmacologically targeted the AP2 complex with our endocytosis inhibitor to determine its effect on pain. We directly injected the inhibitor into the site of inflammation before or during established pain. In the acute inflammatory pain model, the molecule was able to decrease spontaneous pain and decrease thermal sensitivity. In established chronic inflammatory pain, the inhibitor generated a robust decrease in thermal and mechanical sensitivity 24-96 hours after only one injection/treatment.

Here we determined that endocytosis is a critical regulator of pain signaling and identified a novel analgesic target for the treatment of inflammatory pain.

INTERACTION AMONG COX2 ENZYME AND NMDA, P2X7 RECEPTORS IN DRG CONTRIBUTES TO INFLAMMATORY HYPERALGESIA IN THE PERIPHERAL TISSUE

N. S. Carvalho, F. H. Farias, G. F. Catroli, G. G. Dos Santos, A. F. Neves, S. F. Magalhães, J. B. P. Lemes, C. A. Parada. Univ. Estadual De Campinas - UNICAMP, Campinas, Brazil; Univ. of California, San Diego, CA. natyfallen@hotmail.com

Program Number: 056.23
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: J35
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Pain: Inflammatory Mechanisms

RESOLVING THE MOLECULAR IDENTITY AND CONNECTIVITY OF AMYGDALAR NEURAL ENSEMBLES ACTIVE DURING PAIN

D. J. Berg, C. Chen, B. Ahanonu, M. Chen, S. Quake, M. Schnitzer, G. Scherrer. Stanford Univ., Palo Alto, CA. (320)266-3375. bergd@stanford.edu

Program Number: 398.28
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: N17
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Central Nervous System Mechanisms in Pain

Our research identifies the neural circuits responsible for pain unpleasant emotions and novel targets in these circuits to treat pain.

A report from the Institute of Medicine has recently documented the exceptional magnitude of pain in the US, with more than 100 million Americans suffering from chronic pain. In parallel, the use of opioids for pain management has grown dramatically in the past two decades, driving an alarming increase in cases of transitions to addiction and deaths from opioid overdose. The identification of novel therapeutic targets within pain neural circuits is urgently needed to develop more efficient and safer pain medications.

Pain is a multidimensional experience with sensory and emotional components. The unpleasant quality of pain (i.e. pain negative emotions/affect) causes the majority of chronic pain patients' suffering, and often results in comorbid disorders, including anxiety and depression. We recently identified a group of neurons in the amygdala, a brain region of the temporal lobe important for emotions, that are responsible for the unpleasant emotions that characterize acute and chronic pain perception (Corder et al., 2019). However, it remains unclear how these pain neurons in the amygdala are connected with other regions of the nervous system to process incoming pain information from the nerves. Furthermore, the neurochemical characteristics of these neurons are unknown. By filling these gaps in knowledge, this study aims to localize the neurons throughout the brain that generate pain emotions and to identify novel molecules in these neurons to treat pain.

First, we identify the emotion-related brain circuits active during pain. We use different types of viruses that reveal which neurons are directly connected in the brain. Retrograde monosynaptic circuit tracing studies from the amygdala pain neurons reveal convergent synaptic input from several brain regions, including cortical regions such as the cingulate cortex and insula, that are thought to play an important role in affect. Additionally, in anterograde circuit tracing studies, we determine that outputs of the amygdala pain neurons include cortical and subcortical regions, such as the nucleus accumbens. These findings considerably expand our understanding of the neural networks that are responsible for pain unpleasantness.

Second, we investigate the neurochemistry and molecular identity of amygdala neurons that are responsible for pain emotions. We use deep sequencing of the transcriptome from individual amygdala neurons. This approach reveals a comprehensive catalog of surface proteins specifically expressed by amygdala neurons encoding pain unpleasantness for the development of therapeutics to treat pain affect. These surface proteins notably include many receptors belonging to the G protein-coupled receptor family that are commonly targeted in drug development. These findings suggest that drugs targeting these receptors could represent a novel class of analgesics to dampen negative affect during chronic pain.

Collectively, these neural circuit tracing and transcriptomic studies significantly advance our understanding of pain neurobiology and may uncover new receptor targets in the amygdala and connected regions for treating the unpleasantness associated with chronic pain.

Reference:

An amygdalar neural ensemble that encodes the unpleasantness of pain. Corder G, Ahanonu B, Grewe BF, Wang D, Schnitzer MJ, Scherrer G. Science. 2019 Jan 18;363(6424):276-281. doi: 10.1126/science.aap8586. PMID: 30655440

SELECTIVE NEURONAL SILENCING USING NOVEL SYNTHETIC BOTULINUM MOLECULES ALLEVIATES CHRONIC PAIN STATES

M. Maiaru, C. Leese, I. Echeverria-Altuna, J. Arsenault, B. Davletov, S. Hunt. Univ. Col. London, London, United Kingdom; Univ. of Sheffield, Sheffield, United Kingdom; The Hosp. for Sick Children, Toronto, ON, Canada. m.maiaru@ucl.ac.uk

Program Number: 748.06
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: F37
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Somatosensation: Treatments for Persistent Pain

Our research, funded by the Medical Research Council (MRC), demonstrated that a modified form of botulinum toxin gives long-lasting pain relief in mice without adverse effect and, in time, could replace opioid drugs as safe and effective way for treating chronic pain.

Chronic pain of 'moderate to severe' intensity is widespread affecting about 20% of adults worldwide and 10% are newly diagnosed with chronic pain each year.

It is a serious social and medical problem that negatively impacts quality of life.

Opioids like morphine and fentanyl are considered to be the gold standard for pain relief but there is little evidence that their long-term use is effective in treating chronic pain. This is because the body builds up a tolerance to repeated drug use over the long term. Paradoxically opioids can also increase the body's sensitivity to pain.

Opioid drugs can also activate brain reward area, causing addiction. Over 2 million of people in the US have 'opioid use disorders' with most starting with prescribed opioid pain killers and opioid overdose is now the second leading cause of death in the US.

To achieve long-lasting pain relief following just one injection we deconstructed the botulinum toxin molecule and reassembled it with an opioid called dermorphin to make Derm-BOT or the pain-related peptide substance P to make SP-BOT. Effectively these new compounds are taken up by just those neurons that express the opiate or substance P receptors allowing the botulinum toxin protease- the 'warhead' to block information transfer between neurons by silencing neurotransmitter release. This however does not kill neurons and the silencing process is thought to reverse within 100 days.

Previous pioneering studies in rat and companion dogs show that injection of toxic substance, such as 'substance P-saporin', over the spine kills neurons that 'sense' pain and send this information to the brain. While this was a very effective treatment to reduce both neuropathic and inflammatory pain the approach failed to reach the clinic primarily because clinicians were wary of killing spinal neurons. In contrast, Derm-BOT and SP-BOT are safe to produce, are non-toxic and do not kill neurons. When injected over the spinal cord of mice, Derm-BOT or SP-BOT relieve persistent pain, such as that caused by nerve injury, without the adverse effect of tolerance and addiction often associated with repeated opioid drug use.

In our study, mice were used to simulate human inflammatory and neuropathic pain state and were treated with a single spinal injection of BOT conjugate before or after establishing the pain state. Our compounds had a long-lasting effect in both inflammatory and neuropathic pain models, successfully silencing neurons without cell death. Furthermore, a single injection of Derm-BOT reduced mechanical hypersensitivity to the same extend as acute morphine while the specificity of our approach was demonstrated using knockout mice. Indeed the pain relieving effects of SP-BOT were lost in Substance P receptor knockout mice.

The results of this study clearly demonstrate that our compounds block pain due to nerve damage or inflammation for months without effecting normal pain response. It really could revolutionise how we treat chronic pain patients if we can translate it into clinic, removing the need of daily opioid intake.

SENSORIMOTOR FUNCTIONAL CONNECTIVITY TO THE SALIENCE NETWORK IS SPATIALLY HETEROGENEOUS IN HEALTHY HUMANS

A. K. Hegarty, M. S. Yani, A. M. Albishi, L. A. Michener, J. J. Kutch. Biokinesiology and Physical Therapy, USC, Los Angeles, CA. amyhegar@pt.usc.edu

Program Number: 750.06
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: G41
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Somatosensation: Pain, Imaging, and Perception

Chronic pain, a key driver of the opioid crisis, is a tremendous burden both for society and individuals. No one is sure where chronic pain comes from. We think that there may be clues to understand the development of chronic pain within the locations where pain tends to occur in the body - the low back, neck, and head. In this study, we took a first step to understand what might make these areas of the body especially prone to pain. By measuring brain activity in humans using magnetic resonance imaging, we are able to quantify how different regions of the brain cooperate even when the person is not performing a specific task (i.e. daydreaming or resting). We looked at how the representation of the body in the brain - the so-called homunculus - interacts with the brain regions that assess pain, which is called the salience network. We found that representations of the low back, neck, and head are particularly linked to the salience network. The connection between the brain regions studied in this analysis, the homunculus and salience network, are altered in people with chronic pain, as reported in previous studies from our lab and others. What is so intriguing about our current work is that we actually carried it out in healthy people without pain, suggesting there are fundamental differences in how these brain regions interact even without the presence of pain. The salience network is thought to provide the foundation to identify and respond to threats, and might explain why the connection between the salience network and homunculus is generally stronger in body regions that are critical for survival.

More research is needed to determine the relationship between altered brain activity and the development of chronic pain. However, our results lead us to speculate the source of chronic pain may be partially encoded within the fundamental wiring of the brain - when the body is injured, the chances of developing chronic pain would be higher in these body regions that have stronger pre-wiring to the salience network.

CYTOARCHITECTURAL CHANGES INDUCED BY CHRONIC CEPHALIC PAIN ARE REVERSED BY HDAC6 INHIBITION

Z. J. Bertels, H. Singh, I. Dripps, P. Shah, J. Chmura, S. M. Baca, M. M. Rasenick, A. A. Pradhan. Psychiatry, Univ. of Illinois at Chicago, Chicago, IL; Physiololgy & Biophysics, Univ. of Illinois at Chicago Col. of Med., Chicago, IL; Pharmaceut. Sci., Univ. of Colorado | Anschutz Med. Campus, Aurora, CO. 1(618)420-5433. zbertel2@uic.edu

Program Number: 748.03
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: F34
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Somatosensation: Treatments for Persistent Pain

Migraine is the third most prevalent disease worldwide and is estimated to affect upwards of 14% of the population. Especially debilitating is chronic migraine defined as 15 or more headache days/month. Despite the high incidence of migraine, the currently available therapeutic options are often poorly tolerated or only partially effective. To develop new treatments, it is important to gain a better understanding of the fundamental mechanisms that underlie the chronic migraine state. The aim of this study was to determine if chronic migraine showed altered neuronal plasticity, and if drugs that promote cellular flexibility could be promising migraine therapies.

A key way in which cells (including neurons) regulate their function is through their ability to change shape and make new connections. The size, shape, and mobility of the cell is determined by the cytoskeleton, which is a series of interconnected filaments and tubules. Microtubules are a major component of the cytoskeleton and is made up of α and β tubulin. To allow the cell to physically respond to its environment, microtubules are in a constant state of flux; and this movement is facilitated by various chemical modifications of the tubulin, including tubulin acetylation. When tubulin is acetylated it is more flexible and less prone to breakage, while deacetylated tubulin is more brittle and less-flexible. An abundance of deacetylate tubulin can therefore adversely affect cellular complexity as the cell is less able to respond or change its shape, and alterations in this dynamic process have been associated with other chronic disease states.

In order to investigate if changes in neuronal complexity regulated chronic migraine, we used two different animal models. In the first model, we induced a chronic migraine-like state by repeatedly administering nitroglycerin to mice. Nitroglycerin, is a known human migraine trigger that evokes a migraine attack in patients; and can produce head pain associated with migraine in rodents. Mice that were treated with nitroglycerin had severe chronic head pain. Using staining protocols that label the entire neuron, we also observed that these animals had decreased neuronal complexity in head pain-processing regions relative to control pain-free animals. We treated the animals with a histone deacetylase 6 inhibitor (HDAC6), ACY-738, which blocks tubulin deacetylation. Treatment with ACY-738 restored both the neuronal complexity induced by chronic nitroglycerin, and relieved the chronic migraine-associated pain.

The second migraine model we used examined cortical spreading depression which is a reflection of the brain events that can cause migraine aura, the visual disturbances that can accompany migraine. We found that cortical spreading depression caused a significant decrease in neuronal complexity in brain regions associated with aura; and that the HDAC6 inhibitor restored the complexity. ACY-738 also decreased the number of cortical spreading depression events, which is a characteristic of migraine preventive drugs.

Our research identifies a completely novel mechanism for chronic migraine that suggests that this state is associated with decreased neuronal plasticity. We show that HDAC6 inhibitors can increase tubulin dynamics thus restoring neuronal complexity, and results in alleviation of migraine-associated symptoms. These studies provide fundamental information on how microtubule dynamics are altered in chronic migraine, and form the basis for the development of HDAC6 inhibitors for migraine treatment.

MISREGULATION OF MITOCHONDRIA - LYSOSOME CONTACT SITES IN MUTANT GBA (BETA-GLUCOCEREBROSIDASE) PARKINSON'S PATIENT NEURONS

S. Kim, Y. C. Wong, L. F. Burbulla, D. Krainc. Dept. of Neurol., Northwestern Univ., Chicago, IL. 3124053422. sjkim@u.northwestern.edu

Program Number: 629.09
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 11:30 AM
Room Number: Room N426
Board Number: N/A
Presentation Time: 10:00 AM - 10:15 AM
Session Title: Parkinson's Disease: Cellular Mechanisms

Our research indicates that recently identified contact sites between mitochondria and lysosomes may contribute to disease pathogenesis in specific genetic forms of Parkinson's disease.Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease, and is clinically characterized by tremors, rigidity, postural instability and slowness of movement. While Parkinson's disease is known to be caused by the death of dopamine neurons in patient brains, the pathways involved in neuronal death is still not well understood. Consequently, the generation of therapies to target these pathways and prevent Parkinson's disease progression remains highly elusive. Importantly, Parkinson's disease pathogenesis has been linked both functionally and genetically to two critical organelles - mitochondria and lysosomes. Indeed, mutations in the lysosomal enzyme GBA (β-glucocerebrosidase) can cause genetic forms of Parkinson's disease, and also represent the most common risk factor for developing Parkinson's disease. Thus, further understanding the crosstalk between mitochondria and lysosomes may provide important insight into Parkinson's etiology and therapeutic drug development. We recently identified a new pathway for mitochondria and lysosomes to bidirectionally interact with one another via inter-organelle mitochondria-lysosome contact sites, which dynamically form throughout the cell (Wong et al., Nature 2018). We further found that mitochondria-lysosome contact sites are essential for regulating the overall network dynamics of both mitochondria and lysosomes, and also identified several proteins which regulate the formation and dynamics of these contact sites. Importantly, these findings highlighted mitochondria-lysosome contact sites as a significant contributor to organelle dynamics and modulator of cellular health. However, whether and how mitochondria-lysosome contact sites form in human neurons, as well as whether they might contribute to Parkinson's disease pathogenesis was previously unknown.In this study, we used human-derived dopamine neurons and found that mitochondria-lysosome contact sites dynamically form in the cell body, axon and dendrites of human neurons. Next, we examined Parkinson's disease patient neurons with heterozygous mutations in GBA and which exhibited decreased GBA lysosomal enzyme activity. Surprisingly, we found that these Parkinson's patient neurons exhibited defective mitochondria-lysosome contact site dynamics, compared to control neurons. Moreover, these defects further contributed to dysfunctional mitochondrial dynamics in both the axon and cell body of patient neurons. Our results suggest that mitochondria-lysosome contact sites may play a role in Parkinson's disease pathogenesis which has not been previously studied. We are currently in the process of examining whether other Parkinson's disease mutations may also disrupt mitochondria-lysosome contact sites and additional roles of these contact sites. In addition, we are also interested in whether targeting mitochondria-lysosome contact sites may help prevent neuronal dysfunction in Parkinson's patient neurons. Together, the findings from this study point to a novel pathway of mitochondria-lysosome contact sites in regulating organelle dynamics in human neurons, and will help advance our understanding of the fundamental biology underlying the neuronal interplay between mitochondria and lysosomes. In addition, our results further highlight a role for both mitochondrial and lysosomal dysfunction in Parkinson's disease, and suggest that this dysfunction may become further exacerbated at and converge at mitochondria-lysosome contact sites. Ultimately, further study of this novel pathway will help provide important insight into Parkinson's disease pathogenesis and future therapeutic approaches.

Senses and Perception

SENSORIMOTOR PROCESSING IN FREELY-MOVING HYDRA VULGARIS

W. Yamamoto, R. Yuste. Columbia Univ., New York, NY. wy2303@columbia.edu

Program Number: 067.11
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: DP09/O37
Presentation Time: 1:00 PM - 5:00 PM
Session Title: Invertebrate Sensory-Motor Integration

Cnidarians can internally anticipate the generation of complex motor behaviors.
Hydra, a freshwater relative of corals, jellyfish, and sea anemones, has a simple nervous system consisting of a net of neurons distributed across the body. Despite its simple nervous system, *Hydra* can sense the outside world, integrate information, capture prey, and even move by somersaulting. However, the sensorimotor mechanisms used by the *Hydra* nervous system to generate these behaviors have remained unknown. Our lab has previously generated a *Hydra* strain that produces a protein that fluoresces when the calcium level rises in neurons as they fire. With this strain we can image the activity of every neuron in a freely-moving *Hydra*. Using computational methods, we can analyze the relationship between the neuron firing patterns and the generation of motor behaviors.
We have focused our studies on two types of behavior: one is a simple contraction that does not lead to locomotion of the animal, and the other is contraction that leads to locomotion by somersaulting. By computationally extracting two types of neuronal firing activity called CB and RP1, we found that simple contractions can occur in the absence from preceding RP1 firing. In contrast, contractions that lead to somersaulting is preceded by a long gradual increase in the frequency of RP1 firing. Somersaulting only happens if the RP1 frequency reaches a high frequency. This suggests that somersaulting is triggered by an increase in RP1 firing frequency, and that this can happen minutes before locomotion is initiated. Thus the internal state of the ongoing neuronal activity of the animal (through the RP1 frequency) can predict whether or not it will initiate a somersault. These results indicate that the RP1 endogenous neuronal oscillation serves to gate motor programs. Future studies will attempt to decipher the neural mechanisms by which simple nerve nets generate motor behaviors.

DIFFERENTIAL EFFECTS OF PHASIC AND TONIC LOCUS COERULEUS ACTIVATIONS ON ODOR DISCRIMINATION AND VALENCE LEARNING

S. Torraville, A. Ghosh, T. Omoluabi, F. Massaeli, V. Strong, K. Power, O. Audu, X. Chen, G. M. Martin, C. W. Harley, Q. Yuan. BMS-Neuroscience, Psychology, Mem. Univ. of Newfoundland, St. John's, NL, Canada. 1(709)422-2461. set260@mun.ca

Program Number: 058.06
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: K23
Presentation Time: 2:00 PM - 3:00 PM
Session Title: Chemosensory Processing I

Our research shows that phasic activation of the locus coeruleus dramatically accelerates learning while tonic activation at the same frequency does not change the normal learning rate.
This is consistent with earlier recording studies showing novelty and interesting environmental changes, as well as painful events, produce phasic activation of the locus coeruleus, a nucleus in the brainstem that produces noradrenaline and is proposed to help us adapt our brain and behavior to changes in our world. Since the locus coeruleus has been implicated as the origin of the spread of Alzheimer's Disease pathology understanding its role in learning and memory is ever more pressing. The patterns of locus coeruleus activity offer one link to its potent modulation of memory and learning.
Activation of the locus coeruleus helps to wake us up and its tonic activity is lower when we are relaxed and higher as we become stressed. Firing rates for the locus coeruleus neurons range from nearly silent to ~20 times/sec in stressful settings, or for brief phasic bursts. To understand how locus coeruleus neuron firing helps us learn and remember, researchers have inserted light sensitive channels into locus coeruleus cells so they can control its activation using pulses of blue light. Previous studies using these methods confirmed the importance of the locus coeruleus for normal waking and arousal. Experiments in mice also showed that mice became more anxious as tonic frequencies of activity increased beyond 3 times/sec, while only higher frequency phasic bursts have been effective in improving spatial learning and memory in mice. Thus, locus coeruleus activity can be either brain enhancing or stress-promoting. What determines this duality?
Mouse studies had given the same number of locus coeruleus activation pulses in a given period of time, so tonic activity was always slower while phasic bursts were at a higher frequency but spaced apart. One explanation for the learning effect could have been higher amounts of locus coeruleus norepinephrine were released by higher frequencies of activity. Here we kept the frequency constant at 10 times/sec and to produce a phasic profile we did not activate the locus coeruleus for either 20 sec after 10 sec of activity or for 2 sec after .3 seconds of activity, this very short burst pattern is most like the normal firing associated with interesting changes in the environment.
Rats did not become anxious at the 10 Hz tonic frequency of activation, unlike mice, possibly due to species differences in response to arousing events. On the other hand, both phasic patterns were accompanied by rapid learning of a difficult discrimination between two highly similar odors. A higher tonic activation frequency of 25 times/ sec did produce anxiety in the rat and was aversive. The 10 Hz phasic pattern, on the other hand, was a positive experience. The timing is critical.
Thus, pauses in neuronal activity may be as important as neuronal activity itself in enhancing the support of learning and memory processes.

RECIPROCAL ANTAGONISM BETWEEN HUNGER AND MATERNAL CARE

X. Xu, X.-Y. Li, Y. Han, W. Zhang. Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China. xiaohong.xu@ion.ac.cn

Program Number: 068.19
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: P24
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Vertebrate Sensory-Motor Integration

There are so many things to pursue in life that one seems to never have enough time. Learning to prioritize among different activities is an essential skill for a grownup in the modern times, especially for those with kids and busy work schedules. Evidently, such conflicts are not unique to humans, as animals also need to coordinate different behavioral outputs. For example, a hungry mother mouse or hen may need to balance between offspring-care and food-seeking/eating, or more broadly speaking, balance between behaviors that benefit the propagation of the species and those that serve immediate individual needs. Such behavioral coordination can potentially impact an animal's survival or reproductive fitness, therefore is of grave importance. So how does a female animal accomplish it?

It is known that caloric insufficiency elicits a sense of hunger, which in turn drives food forage and feeding behaviors via activation of a group of hypothalamic neurons that express the neuropeptide Agouti- related peptide (AGRP). It has also been shown that hunger or activation of Agrp neurons competes with other motivated behaviors and suppresses non-feeding-related behaviors. Thus, it seems that a female individual may prioritize feeding and self- preservation over pup care during period of caloric insufficiency.

On the other hand, in species such as mouth-breeding cichlid fish or domestic chicken, females actually undergo lengthy voluntary anorexia during brood care. Over a three-week egg incubation period a hen directs majority of its activities sitting in the nest and spends very little time on feeding, which leads to loss of body weight despite the ready availability of food. These observations demonstrate that females of some species do prioritize care of offspring over feeding.

Inspired by these observations, we examined relationship between hunger-induced feeding and pup-induced maternal care in female mice. Surprisingly, we found that presence of pups strongly delays and suppresses food consumption. To our knowledge, these results provide the first evidence for a possible anorexigenic effect of pups in mammals. In additon, we find that AGRP neurons send inhibitory inputs to □30% neurons in the medial preoptic area (mPOA), a region critical for maternal care in mammals and birds. Remarkably, activation of AGRP neurons affect a specific component of maternal care, dramatically decreasing maternal nest-building while minimally affecting other maternal behaviors, which partly recapitulates the effects of food restriction on maternal care in female mice. In parallel, stimulating AGRP projections to the mPOA similarly decreases maternal nest-building. Importantly, nest-building during low temperature thermal challenge is not affected by stimulation of AGRP→mPOA projections. Together, these results demonstrate a reciprocal antagonism between neural substrates that regulate hunger behaviors and maternal care. In summary, it seems that "the wisdom of life" may be embedded in the brain to allow a female animal to juggle between being a mother and being hungry.

CONTEXT DEPENDENT SENSORY PROCESSING ACROSS PRIMARY AND SECONDARY SOMATOSENSORY CORTEX DURING A TACTILE WORKING MEMORY TASK

C. Condylis, E. Lowet, J. Ni, K. Bistrong, T. Ouellette, N. Josephs, E. D. Kolaczyk, J. L. Chen. Biomed. Engin., Biol., Mathematics and Statistics, Boston Univ., Boston, MA. 41(617)3531734. jerry@chen-lab.org

Program Number: 057.24
Session Date/Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: K17
Presentation Time: 4:00 PM - 5:00 PM
Session Title: Touch: Barrel Cortex Coding

In order think of new ideas and the world around us, we need to bind together past and ongoing experiences to form increasingly abstract thoughts. An example of this is processing a series of spoken words. Each new word builds upon the prior words to ultimately for a sentence which conveys an idea. How such computations are carried out in the brain has been a longstanding question in neuroscience.
The cortex is critically involved in the generation of abstract thought. The cortex is thought to be organized into specific sub-regions with dedicated functions such as processing sensory information or executing motor actions. Some regions are believed to be involved in processing more current experiences while others are more involved in building abstract ideas by combining the current information with prior information. These different regions need to be able to interact to send their respective bits of information back and forth to each other.
To discern whether these theories hold true, it is necessary to measure the activity patterns in multiple cortical areas and track how activity flows across these areas. Recently, new microscope technology has been developed that have enabled scientists to track the activity patterns of thousands of individual neurons located across multiple cortical areas in rodents. We trained mice in a working memory task in which they received recent and new tactile sensory information and had to decide whether or not the pieces of information were the same or different. In this case, ‘same’ and ‘different’ reflect two abstract concepts that are generated by the comparison of recent and new information. We imaged neuronal activity in the cortex at the first and second stages of sensory processing. We observed that even in these early cortical regions, neurons were able to signal whether the stimuli that the animals were experience were same or different. Information about past experiences flowed from the higher order areas back to the first order areas. Our results provide definitive evidence for how cortical areas are organized to evaluate sensory information to general more abstract thoughts.
In summary, this study brings us one step closer towards understanding how our brain is able to carry out complex mental processes that enables us to understand the world around us.

GENETIC DISSECTION OF ASCENDING SPINAL PATHWAYS FOR AFFECTIVE TOUCH AND PAIN

S. Choi, J. Hachisuka, M. A. Brett, H. R. Koerber, S. E. Ross, D. D. Ginty. Neurobio., Harvard Med. School/HHMI, Boston, MA; Neurobio., Univ. of Pittsburgh/Pittsburgh Ctr. for Pain Res., Pittsburgh, PA. seung-won_choi@hms.harvard.edu

Program Number: 190.08
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 3:15 PM
Room Number: Room S106
Board Number: N/A
Presentation Time: 2:45 PM - 3:00 PM
Session Title: Pain and Itch Behavior, Circuitry, and Novel Techniques

Our research defines parallel ascending spinal pathways that mediate pleasant feelings associated with gentle touch and negative feelings associated with pain. These ascending pathways may be ideal therapeutic targets for treating disorders associated with affective touch and pain.

Approximately 100 million adults in the United States suffer from chronic pain each year. Opioids are currently the most commonly used pain medicines because of their potency and availability. However, misuse of and addiction to opioids pose serious public health problems and have led to the "opioid crisis"; in the United States alone, more than 130 people die every day from overdosing on opioids. On the other hand, autism is a highly prevalent and complex, heterogeneous neurodevelopmental disorder. One in 59 children is diagnosed with autism spectrum disorder (ASD), according to a 2018 CDC report. ASD patients often report altered touch and/or pain sensitivity. Moreover, our lab recently reported that over-reactivity to light touch may contribute to the development of a subset of aberrant behaviors associated with ASDs in mouse models. A long-term goal of our work is to define the neurobiological mechanisms underlying pain and touch so that new medications can be developed to treat disorders including pain in chronic pain patients and altered touch sensitivity in patients with ASDs and related disorders.

Ascending spinal pathways are attractive therapeutic targets for treating disorders associated with alterations in somatosensory function because all tactile, thermal, and noxious signals emanating from the body channel through these pathways *en route* to the brain. However, therapeutic strategies targeting a previously identified ascending spinal pathway, the NK1R projection pathway, to treat pain have been minimally successful, suggesting the existence of additional, non-NK1R ascending spinal pathways that convey pain signals to the brain. In the present study, we identified a novel ascending spinal pathway - the GPR83 projection pathway - that cooperates with the NK1R pathway to convey pain signals to the brain in mice. This pathway is responsive to painful stimuli such as noxious heat or cold temperatures. Strong activation of the GPR83 pathway induces behaviors indicative of pain sensation, including escape behaviors and place avoidance. Moreover, simultaneous inhibition of both NK1R and GPR83 pathways reduced pain behaviors in response to noxious stimuli, suggesting that the GPR83 pathway cooperates with the NK1R pathway to mediate painful feelings.

Interestingly, while strong activation of the GPR83 pathway conveys negative feelings associated with painful stimuli, weak activation of this pathway leads to a positive association with gentle touch. We found that the GPR83 pathway is highly responsive to mechanical stimuli including a gentle indentation of the skin. Moreover, when mice were placed in a chamber in which lever-pressing was coupled with low-intensity stimulation of the GPR83 pathway, they increased their lever-pressing behavior, suggesting that weak stimulation of the GPR83 pathway is appetitive. Therefore, the GPR83 spinal pathway to the brain mediates either pleasant feelings associated with gentle touch or negative feelings associated with pain, depending on the stimulus intensity.

Together, our results suggest that pain may be relieved by inhibiting both NK1R and GPR83 pathways, whereas touch over-reactivity associated with ASDs and other disorders may be treated by targeting the GPR83 pathway. Intriguingly, NK1R and GPR83 are structurally highly related "druggable" proteins. Future studies of the NK1R and GPR83 ascending spinal pathways, and the NK1R and GPR83 proteins, may provide new insights into therapeutic approaches for treating disorders associated with pain and affective touch.

THE NEURAL BASIS OF OLFACTORY-TRIGEMINAL INTERACTION

P. Karunanayaka, L. Jiaming, Q. X. Yang, K. Sathian. Ctr. for NMR Research, Dept. of Radiology, Penn State Col. of Med., Hershey, PA; Drum Tower Hospital, Med. Sch. of Nanjing Univ., Nanjing, China; Dept. of Neurol., Milton S. Hershey Med. Ctr. & Penn State COM, Hershey, PA. pkarunanayaka@pennstatehealth.psu.edu

Program Number: 665.10
Session Date/Time: Wednesday, October 23, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: J36
Presentation Time: 9:00 AM - 10:00 AM
Session Title: Cross-Modal Processing in Humans I

Multisensory integration binds different sensory signals and defines how we make sense of the world. It is particularly important for the human olfactory system, which is highly dependent on non-olfactory cues. For example, what is commonly known as the sense of smell is, in fact, composed of multiple sensations, predominantly mediated by two highly overlapping neural systems: the olfactory and the trigeminal systems. They influence each other's performance by mutually suppressing and/or enhancing interactions. However, the dynamic mechanisms of this interdependence remain unclear. One potential region of interest for olfactory–trigeminal interaction is the primary olfactory cortex (POC) which receives direct and indirect input from the olfactory bulb (OB) and from several higher-order cortical areas. The experiment described in this research is novel in that it will allow us to explain how information from these two systems are integrated in the POC via super-additive interactions, defined as multisensory integration that is most robust when unisensory inputs are weak. We used a localization task to test the sensitivity of the trigeminal system in which a trigeminal stimulus is presented to one or the other nostril and the subject is required to idicate the stimulated nostril.
Two recent behavioral studies [Tremblay et al. (2018), Chem Senses (43):611-616 and Karunanayaka et al. (2018), AChems 2019] reported enhanced trigeminal sensitivity during co-stimulation with pure and mixed olfactory stimuli. In this study, we used functional magnetic resonance imaging (fMRI) to investigate the neural basis of trigeminal sensitivity enhancement of intranasal somatosensory stimulation during olfactory co-stimulation. We hypothesized that olfactory-trigeminal integration is mediated by the POC via superadditive interactions. Additionally, we also hypothesized that olfactory-trigeminal interactions will be mediated by dynamic connectivity changes in the olfactory and trigeminal systems. Fifteen healthy human subjects, all with normal olfactory function, performed a localization task for weak air-puff stimuli in the presence or absence of the pure odorant, phenyl ethyl alcohol (PEA; rose odor). During this task, a weak air-puff stimulation was presented to one or the other nostril and subjects were asked to indicate the stimulated nostril. These air puffs, with a flow rate of 2L/min and 100ms duration, were randomly delivered either to the left or right nostril, with or without concurrent PEA, with an inter-stimulus interval of 10s. Visual cues informed subjects to hold their breath to localize incoming weak air-puffs embedded in a constant flow of odorless air, delivered bilaterally at a rate of 1 L/min. It is known that PEA cannot be localized to a nostril above chance. There was a significant improvement in localization accuracy of weak air-puffs in the presence of PEA when both stimuli were delivered to the same nostril. This improvement was absent when air-puffs and PEA were delivered to different nostrils. Results revealed that olfactory-trigeminal integration is mediated by the POC, insula and bilateral superior temporal sulcus (STS) via superadditive interactions. This activity was significantly correlated with the behavioral enhancement of weak air-puff localization in the presence of PEA. Our results provide strong functional evidence for integration of olfactory and trigeminal sensory inputs. Previously, multisensory integration in the olfactory system has been studied using univariate methods and focused mostly on localized responses in highly specialized brain regions. Our experiment shifts the focus to a network based approach in which the olfactory and trigeminal interactions are hypothesized to be supported via the modification of olfactory and intranasal trigeminal network connectivity. Furthermore, our work will provide a solid basis to reconcile existing univariate fMRI literature with the network perspective of olfactory-trigeminal interaction.

Tools and Techniques

MULTIMODAL CELL AND FIBER MAPPING IN FULL VERVET BRAIN SECTIONS

R. N. Kooijmans, M. Axer, M. Schober, D. Gräßel, P. Schlömer, K. Zilles, P. R. Roelfsema, K. M. Amunts. Vision & Cognition, Netherlands Inst. for Neurosci., Amsterdam, Netherlands; Inst. of Neurosci. and Med., Res. Ctr. Jülich, Jülich, Germany. r.kooijmans@nin.knaw.nl

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Session Date/Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: BB53
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Anatomic Methods: Image Acquisition II

QUANTITATIVE MCELL MODEL SHOWS COMPETITION AND DYNAMIC EQUILIBRIUM WITHIN PDZ DOMAIN OF PSD-95 AT THE POSTSYNAPTIC DENSITY

S. Sameni, T. M. Bartol, Jr, M. B. Kennedy, T. J. Sejnowski. Salk Inst. For Biol. Studies, La Jolla, CA; Caltech, Pasadena, CA. (949)648-6390. ssameni@ucsd.edu

Program Number: 794.01
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: CC45
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Novel Techniques of Biochemical Analysis

Our research shows that a shift in the competitive interactions among 3 key proteins located at synapses in the brain could explain changes that occur during learning and memory formation in the brain.
Information processing, including learning and memory formation, depends upon the ability of neurons to communicate with one another at synapses — specialized points of contact between neurons. Changes in the strength of synaptic contacts create new networks of neurons in the brain that encode new information. An important feature of synapses is an assembly of scaffold proteins and signaling proteins known as the postsynaptic density (PSD). PSDs are protein-rich sub-compartments, a few hundred nanometers in width and 50 nm in thickness that are attached to the postsynaptic membrane at excitatory synapses. Scaffold proteins form the basic structure of the PSD and bind appropriate signaling enzymes to position them near the membrane where new biochemical signaling is triggered by synaptic activity. The signaling initiated by PSD proteins regulates adjustments in the structure and function of synapses and enables formation of memories. However, we don't yet fully understand how it is assembled and how changes in the PSD regulate memory storage and information processing. It is known that mutations in certain PSD proteins can confer susceptibility to mental disorders such as schizophrenia and autism. In particular, we know that mutations in synGAP, which binds to the major scaffold protein PSD-95, cause severe intellectual disability with some features of autism.
We have found that synGAP binds tightly to three major sites on PSD-95. Two of these sites also bind glutamate receptor complexes to immobilize them at the postsynaptic membrane. These receptors sense the neurotransmitter glutamate when it is released at the synapse and, in response, they create electrical signals that can be passed along the neuronal network. The "strength" of a synapse refers to the size of the electrical signal the synapse produces. It is determined by the number of receptor complexes immobilized at the postsynaptic membrane. We found that synGAP is abundant in the PSD and can therefore compete with receptor complexes for binding to PSD-95 and reduce the number of receptors that can be immobilized at the synapse. We also found that repeated synaptic activity causes an enzyme in the PSD to modify synGAP such that its binding affinity for PSD-95 is reduced. We hypothesize that this reduction in binding of synGAP allows more receptors to be immobilized at the synapse, strengthening the synapse and contributing to formation of a memory.
Our team has made progress by making a computer simulation of the dynamic interaction that occurs between these molecules in response to synaptic activity. The simulation incorporates experimental measurements of the interactions of PSD-95, synGAP, and receptor complexes, and predicts the shift in the balance between different molecular states that can be tested experimentally. To create the simulation, we used software called MCell which was developed to study the binding interactions among multiple proteins. We are using this to understand the rules for competition between synGAP and receptor complexes for binding sites on PSD-95 as they establish a dynamic equilibrium within the PSD scaffold. We will measure how biochemical modifications that are initiated by synaptic activity can shift this equilibrium as memories are formed. We expect that our simulation will be a foundation for future studies that incorporate more of the proteins that contribute to the dynamic structure of the PSD. The quantitative understanding that we will gain through simulation will improve our understanding of the complexity of synaptic disease mechanisms and guide the development of new therapeutics.

THE EFFECTS OF NADPH OXIDASE INHIBITOR, DIAPOCYNIN, TREATMENT IN DIISOPROPYLFLUOROPHOSPHATE (DFP)- INDUCED LONG-TERM NEUROTOXICITY

M. Putra, M. Gage, G. Gasser, S. Sharma, V. Anantharam, T. Thippeswamy. Biomed. Sci., Iowa State Univ., Ames, IA; PK Biosci., Ames, IA. 1(515)231-9475. mrputra@iastate.edu

Program Number: 389.03
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: I31
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Our research demonstrates the long-term brain-protective effects of an herbal-derivative, a natural antioxidant, in an organophosphate intoxicated experimental model.

Organophosphate chemicals from agriculture and industries are impacting public health globally. Currently, about 40 organophosphorus insecticides registered for use in the US. In a study, the organophosphorus residues found in human urine samples were greater than 20 fold than the average levels in the US population. Incidentally, these higher levels were suggested to be associated with neurological deficits. Research suggests a close association between organophosphorus chemical exposure and an increased risk of developing neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and epilepsy. Despite advancement in scientific research, there is no cure for these neurological conditions, and they continue to increase the financial burden of governments and individuals. Adding to these problems, organophosphate derivatives of nerve agents (sarin and VX) are emerging as global threat agents. Organophosphorus pesticides/insecticides consumed in suicidal attempts, in some parts of the world, the survivors suffer from long-term neuropsychiatric problems such as difficulty in learning and memory, motor dysfunction, anxiety, and depression-like behavior.

One of the most common signs of acute organophosphate toxicity, soon after the exposure, are seizures or tremors. Currently, medications are available to treat such symptoms. However, the response depends on how early treated. If delayed (greater than 30 minutes), seizures can cause brain damage even after administering the prescribed medication. Such prolonged seizures permanently change the behavior of nerve cells, and their supporting cells (neighbors) called "glial cell," in the brain. Now the brain is also no longer protected, for at least 2-3 days, from the circulating cells and residual poison in the blood since the so-called "blood-brain-barrier" is broken allowing immune cells to migrate into the brain. Misbehaving resident neighboring cells and migrated immune cells produce nitro-oxidative stress and inflammation-causing molecules/chemicals, and begin to attack the nerve cells' network in the brain and nerve cells begin to die (the process is called "neurodegeneration"). All these changes gradually progress over months to years before an individual experience a spontaneous seizure (meaning becoming an "epileptic patient") or a significant functional deficit such as loss of memory (AD), motor incoordination (PD), anxiety, depression and so on. None of the medications administered earlier can prevent the changes that occur in the brain during the first 2-3 days or a week or later. Therefore, there is an urgent need for the development of supplementary or a follow on therapy to prevent long-term brain damage.

Our research focused on identifying a therapeutic agent called "diapocynin." It is a novel natural antioxidant derived from a vanilla-flavored herb, which has been used as an anti-inflammatory medicine for many years in Europe and Asia. We used a chemically synthesized drug in a rat model exposed to an organophosphate, which showed similar clinical signs as in humans. As in humans, we first treated the animals with symptomatic drugs (atropine and diazepam) after seizures, and two hours later, one group received diapocynin and the other group placebo twice daily for first three days only. Six weeks later, we conducted motor function tests and euthanized. The brains were processed to evaluate inflammation and neurodegeneration. Diapocynin treated rats showed significantly improved motor function and significantly less brain inflammation and nerve damage when compared to the placebo-treated group. Though further research is needed, overall, these findings suggest beneficial effects of an antioxidant in treating organophosphate toxicity in addition to the current medication.

ASSESSMENT OF ADVERSE NEUROTOXICITY OF BONT/A BY USING AN ENGINEERED HUMAN BRAIN MODEL

G. Ambrin, B. R. Singh, H. Cho. Biomed. Engin. and Biotech., Univ. of Massachusetts, Dartmouth, N. Dartmouth, MA; 2Institute of Advanced Sciences,, N. Dartmouth, MA; UNC Charlotte, Charlotte, NC. ghuncha@gmail.com

Program Number: 539.02
Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 4:00 PM
Room Number: Room S103
Board Number: N/A
Presentation Time: 1:15 PM - 1:30 PM
Session Title: Neurotoxicity, Inflammation, and Neuroprotection

Our research focuses on the detrimental synaptic impairment caused by the prolonged use of Botulinum Neurotoxin (BoNT) mediated by gliosis. The alternate pathways that mediate the inter-neuronal exchange of several biomolecules demonstrates that elements of CNS interact with host cells in a more complex manner than was originally envisioned, suggesting need for further re-evaluation of the clinical uses of BoNT/A. The study evaluates the potential side effects of continual use of BoNT/A as a potential risk factor for synaptic impairment eventually leading to dementia.

BoNT based therapeutics and cosmetics are being used by millions of patients throughout the world, with branded names as Botox™, Dysport™, and others for treatment of over 800 disorders, and the list is slowly expanding to chronic conditions that respond only partially to medical treatment. As the effect of peripherally injected BoNT/A lasts for about 3 months, the injections are repeated at 3-month intervals to maintain ongoing benefits. A critical and perhaps one of the most intriguing aspects of BoNT-mediated blockage of the acetylcholine release is the long-lasting poisoning of the nerves. In such applications, the toxin is used frequently in small doses over a long period of time. Its long-term unintended effects on neuronal system, including brain, especially in view of its retrograde transportation to the central nervous system could be substantial as we have started seeing cases of depressive adaptive disorder, complete amnesia, paranoia and short-term memory loss. With our research, it is the first time that the effect of prolonged use of BoNT, in the CNS is being investigated, which has so far gone unnoticed. We have confirmed one of the pathways which leads to extreme neurological dysfunction.

BoNT (A-G) interferes with the neural transmission by blocking the release of acetylcholine at the cholinergic nerve endings of the peripheral nervous system, resulting in paralysis. Initially it was believed that the BoNT/A confers its medicinal effects by inhibiting synaptic transmission near the site of injection, but recent studies suggest that it can spread through axonal retrograde transport within networks of neurons to have distal effects while retaining its enzymatic properties, although its detailed pathway still remains obscure.

In this study we are investigating the neurodegenerative effects of BoNT/A at the synapse activated by reactive astrocytes and mediated by microglial inflammation. BoNT/A is introduced in a dose dependent manner in an engineered microfluidic device on a tripartite culture medium of neuron, astrocytes and microglia. Acetylcholine (Ach) appears to act as a neuromodulator in the brain that changes the state of an ensemble of neurons in response to changing environmental conditions. Astrocytes are activated into expressing soluble factors in response to the decrease in Ach, stimulating the neurons to express C1q. We observed that the complement proteins were extremely upregulated prior to signs of neuronal loss. The initiation of the complement pathway and downstream tagging of receptors lead to elimination of nerve terminals by the phagocytic microglia, co-functioning with astrocytes. A significant increase in chemokine release and pro-inflammatory secretions from the microglia was quantitively analyzed. The plethora of evidence implicates the microglia as a key element in impairing synaptic connectivity and inducing neuroinflammation.

The outcomes from this study evaluated the utility of the platform in the model system of side effects of continual BoNT/A use leading to synaptic impairment and neuroinflammation as a potential risk factor for other types of neurodegenerative diseases. In the future study we will be investigating if the prolonged exposure of BoNT/A has the potential to trigger early onset and/ or exacerbate neurodegenerative diseases like Alzhmiers, Parkinsons etc.

ASPIRIN UPREGULATES IL-1RA IN GLIAL CELLS VIA PPAR-ALPHA

K. Pahan, S. Chakrabarti, A. Roy, T. Prorok, D. R. Patel, S. Dasarathy. Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL. (312) 563-3592. kalipada_pahan@rush.edu

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Session Date/Time: Tuesday, October 22, 2019, 1:00 PM - 4:00 PM
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Board Number: N/A
Presentation Time: 2:30 PM - 2:45 PM
Session Title: Neurotoxicity, Inflammation, and Neuroprotection

Baby aspirin may be beneficial for Alzheimer's disease

Our body is endowed with various beneficial molecules that are needed to protect us from injuries and insults. However, the pathways by which such protective molecules are regulated and the drugs that can be used to augment these molecules in the brain are poorly understood. Aspirin (acetyl salicylic acid) is one of the oldest and most widely-used medicines in the world. It is often used as an antipyretic to reduce fever and as an analgesic to relieve minor aches and pains. Scientists at the Rush University Medical Center and Jesse Brown VA Medical Center (Chicago) have shown that low-dose aspirin may upregulate one such protective molecule interleukin-1 receptor antagonist (IL-1Ra) in brain cells to protect against neuroinflammation in a mouse model of Alzheimer's disease.

Alzheimer's disease is a fatal form of dementia that affects up to 1 in 10 Americans age 65 or older. It affects a small area within our brain known as hippocampus. Gradual degeneration of hippocampal neurons causes loss of vital connections leading to memory loss. To date, the FDA has approved very few drugs for the treatment of Alzheimer's disease that can only provide limited symptomatic relief.

Neuroinflammation is becoming a hallmark of different neurodegenerative disorders including Alzheimer's disease. Therefore, understanding how the commencement of neuroinflammation occurs is important to developing effective drugs that protect the brain and stop the progression of Alzheimer's disease.

The proinflammatory cytokine IL-1 and its receptor IL-1R play an important role in propagating neuroinflammation. At low doses, aspirin specifically upregulated IL-1Ra, but neither the receptor IL-1R nor the ligand (IL-1) of the receptor, in brain cells. Therefore, by increasing IL-1Ra-mediated anti-inflammatory signaling, low-dose aspirin should be able to attenuate neuroinflammatory responses observed in neurodegenerative disorders.

Mechanism by which aspirin increases IL-1Ra is also exciting. Peroxisome proliferator-activated receptor alpha (PPARα) is known to control fat metabolism in the liver. Accordingly, PPARα is highly expressed in liver. Our recent studies have identified high level of PPARα in the hippocampus and other parts of the brain. Interestingly, aspirin requires this fat-lowering factor PPARα to increase IL-1Ra in brain cells as well as in the hippocampus of a mouse model of Alzheimer's disease.

Accordingly, we found that low-dose aspirin also improved memory and learning in Alzheimer's disease mouse model in a PPARα-dependent manner. Our results suggest that in the absence of a basal level of PPARα, aspirin may not inhibit neuroinflammation and protect cognitive abilities.

Moreover, since PPARα is involved in fatty acid metabolism, overweight people are deficient in PPARα. Our study also indicates that aspirin may not exhibit anti-inflammatory and neuroprotective effect in overweight people when PPARα level is compromised. In fact, one recent study (Lancet, 2018, 392: 387-399) suggests that low-dose aspirin is ineffective in preventing cardiovascular events in overweight patients. Here, it is important to mention that inflammation aggravates cardiovascular problems and IL-1Ra-mediated anti-inflammation is also beneficial for the heart.

Therefore, the findings of our study have major potential implications for the therapeutic use of aspirin in Alzheimer's disease and other human disorders in which inflammation plays an important role.

This study was funded by U.S. Department of Veterans Affairs, Alzheimer's Association and National Institutes of Health.

HIGH-RESOLUTION ARTIFICIAL VISION WITH SHAPE PERCEPTION VIA A CHRONICALLY IMPLANTABLE 1024-CHANNEL NEUROPROSTHESIS IN MONKEY VISUAL CORTEX

X. Chen, F. Wang, B. Li, P. Roelfsema. Netherlands Inst. For Neurosci., Amsterdam, Netherlands; Dept. of Integrative Neurophysiol., Vrije Univ., Amsterdam, Netherlands; Dept. of Psychiatry, Academic Med. Ctr., Amsterdam, Netherlands. axienjii@hotmail.com

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Room Number: Hall A
Board Number: L25
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Spatial and Chromatic Vision

Globally, 40 million people across the world are blind. Our research lays the foundations for a neuroprosthetic device that could allow profoundly blind people to regain functional vision through a brain interface, significantly improving their independence levels and quality of life.

How does the technology work? We implanted electrodes into the visual cortex (the part of the brain that allows us to see) in monkeys. When electrical stimulation is delivered to the brain via an individual electrode, it generates the percept of a dot of light at a particular location in visual space, known as a 'phosphene.' To create interpretable images, electrical stimulation is delivered via multiple electrodes, thereby generating a percept that is composed of multiple phosphenes.

What have we achieved? We created high-resolution implants consisting of over 1000 electrodes (Blackrock Microsystems), and tested them in two sighted monkeys. Our subjects successfully recognised shapes and percepts using their artificial vision, including lines, moving dots, and letters. This proof-of-concept demonstrates that a human version of this device would allow profoundly blind people to recognise objects, navigate in unfamiliar surroundings, interact more easily in social settings, and live more independently.

Details of the research Our visual cortical neuroprosthesis consisted of 1024 electrodes (sixteen 8x8 Utah arrays) that were chronically implanted in the left visual cortex, in each of two monkeys. First, the monkeys performed a simple behavioural task in which they made eye movements to report the location of a phosphene that was elicited during electrical stimulation via individual electrodes. The visual cortex contains a map of visual space. We found that the locations of the reported phosphenes closely matched our predictions, based on the positions of the electrodes within this map. Next, the monkeys were tested on more complex tasks: a line orientation discrimination task, in which microstimulation was delivered on several electrodes simultaneously, creating the percept of either a horizontally or a vertically oriented line; a direction-of-motion task, in which microstimulation was delivered on a sequence of electrodes; and a letter discrimination task, in which microstimulation was delivered on 10-15 electrodes simultaneously, creating a percept in the form of a letter. The animals successfully reported the identity of these artificially generated percepts, performing above chance levels even with novel combinations of electrodes.

What are the novel aspects? We have achieved breakthroughs in a number of aspects: 1) Due to the unprecedentedly high number of electrodes implanted, we were able to generate numerous artificial pixels and create high-resolution artificial images. 2) When stimulation is delivered to our chosen site of implantation (the primary visual cortex), the individual phosphenes produced are small, allowing for the creation of high-density artificial images. 3) The implant interfaces with large areas of the visual cortex, yielding images that span a large portion of the visual field. 4) The amount of current needed for electrical stimulation is low, reducing the risk of epileptic attacks (as is sometimes seen with surface electrodes). 5) Our system allowed for chronic stimulation of the visual cortex in a primate over a long time period. 6) The ability to immediately recognize shapes during electrical stimulation of visual cortex has not been documented in the peer-reviewed literature-only in anecdotal reports.

Summary The quest of restoring vision in blind patients through a brain implant is underway and on the verge of becoming reality. In the future, this technology could be used to restore low vision in people who have lost their sight due to injury or degeneration of the retina, eye, or optic nerve, but where the visual cortex remains intact.

Vision

SEEING IN 3D WITHOUT A CORTEX - NEURONS FOR STEREOPSIS IN AN INSECT BRAIN

R. Rosner, G. Tarawneh, V. Nityananda, J. Von Hadeln, J. Read. Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; Philipps-University Marburg, Marburg, Germany. ronny.rosner@ncl.ac.uk

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Room Number: Room S505
Board Number: N/A
Presentation Time: 1:15 PM - 1:30 PM
Session Title: Neuronal Circuits Underlying Binocular Vision and Stereopsis

By putting 3D glasses on praying mantids, we research how insects see in 3D. Our work is showing that insect 3D is surprisingly sophisticated– much more similar to humans than had been expected. We're hoping that this could help engineers to develop depth perception in autonomous devices, such as drones or self-driving cars.

3D vision, or stereopsis, is the ability to judge distance based on the different views in both eyes. Try holding a finger at arm's length and closing first one then the other eye – you'll see your finger jump left and then right. Human brains detect these differences and use them to generate depth perception.

In humans, many areas of visual cortex are involved in this process, contributing to the view that stereopsis is particularly challenging. So scientists were surprised in the 1980s when Samuel Rossel demonstrated that the praying mantis – despite having only a million or so neurons compared to our hundred billion – uses stereopsis to judge distances to prey.

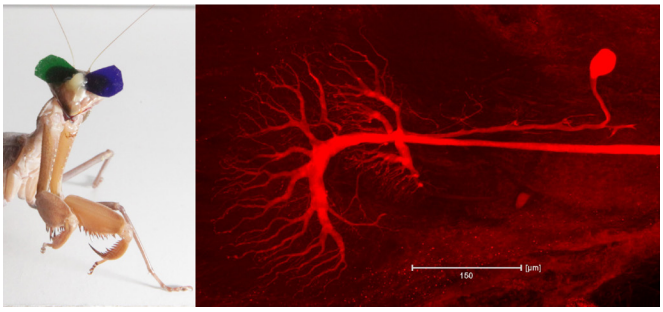
We've now used electrode recordings in mantis brains to learn more about how insect stereopsis works. We've found surprisingly complex circuitry, with at least four well-defined types of stereoscopic neuron. Individual neurons are tuned to specific distances and directions, i.e. to specific locations in 3D space. This is similar to what we see in primates and other mammals, and much more complex than how we thought insect stereopsis worked.

Previously, we thought that information from a mantid's two eyes might be processed independently, and not combined until just before the signal is sent to muscles to strike. But our work rules out such a simple form of stereopsis. Rather, we show that information from left and right eyes is combined very early in visual processing, with "commissural" neurons linking the optic lobes on either side of the head. Just as in humans, mantis stereopsis involves many different areas of the brain.

For these experiments, we treated our mantids to their very own 3D film screening: they viewed a computer screen through coloured filters, like the 3D glasses used in vintage 3D cinemas. We know the mantids experience the 3D effect because when we show them virtual prey in 3D, seeming to float in front of the screen, they strike out and try to catch it. We recorded the electrical activity in visual neurons while mantids viewed these virtual prey and other stimuli. We also stained the neurons to reveal their shape, location and potential connectivity.

This anatomical information revealed feedback loops within the stereo vision circuit: neurons which transmit 3D information from the central brain to early visual processing areas, as well as the other way around. We speculate that these could help guide visual attention in 3D space. We don't yet know whether primate brains contain similar 3D feedback. However, finding it in a tiny insect brain suggests it might be a useful approach for engineers programming robot 3D.

This first glimpse at invertebrate stereopsis reveals the sophisticated vision of these tiny predators – and suggests that humans have much to learn from them.



MICE DISCRIMINATE STEREOSCOPIC SURFACES WITHOUT FIXATING IN DEPTH

J. M. Samonds, V. Choi, N. J. Priebe. Neurosci., Univ. of Texas at Austin, Austin, TX. (412) 748-0484. samondjm@gmail.com

Program Number: 191.03
Session Date/Time: Sunday, October 20, 2019, 1:00 PM - 3:15 PM
Room Number: Room S505
Board Number: N/A
Presentation Time: 1:30 PM - 1:45 PM
Session Title: Neuronal Circuits Underlying Binocular Vision and Stereopsis

Our research demonstrates that mice estimate information about depth from their binocular vision in a distinct manner from humans.

By comparing differences between the images projected onto our left and right eyes, we are able to determine how far away objects are in space. Although this computation is based on a simple geometric calculation, the process becomes complicated as it requires matching features from the two eyes. How we solve this stereo matching or correspondence problem is one of the fundamental questions in vision science. Stereo correspondence has been studied extensively in humans and other primates, but has received little attention in animals such as mice. The mouse is one of the most widely used animal models in neuroscience because of the genetic and imaging techniques that are available to study neural circuitry. Therefore, we sought to characterize stereoscopic vision behavior in the mouse as a first step in using the mouse as an experimental model for understanding how neural structures solve the stereo correspondence problem.

We first had the mice successfully perform a task where they had to run for surfaces that were relatively nearer and stop for surfaces that were relatively farther away compared to a background reference surface. These surfaces were rendered with random dot patterns and presented using the same technology that is commonly used in 3D movie theaters. The mice had no other depth cues about these surfaces and had to match dots between the eyes in able to distinguish near from far surfaces. We measured responses of neurons in their visual cortex to surfaces of different depths and found that these responses matched their behavior.

Humans fixate on different objects of interest in space. This fixation happens in depth as well. Our eyes rotate inward or outward so that the object is aligned on the two eyes. This alignment allows us to use a very narrow and fine range of depth processing and focus resources on where we are looking. In contrast to primates, we found no relationship between the relative positions of the eyes and the depth of a surface in mice. Instead of fixating on surfaces in depth, based on their neural responses, it appears that mice encode depth information over a large portion of their field of depth. Although mice did not fixate on surfaces in depth, there were still significant inward and outward rotations. These eye movements may be related to running behavior and other visual functions, but these are still open questions.

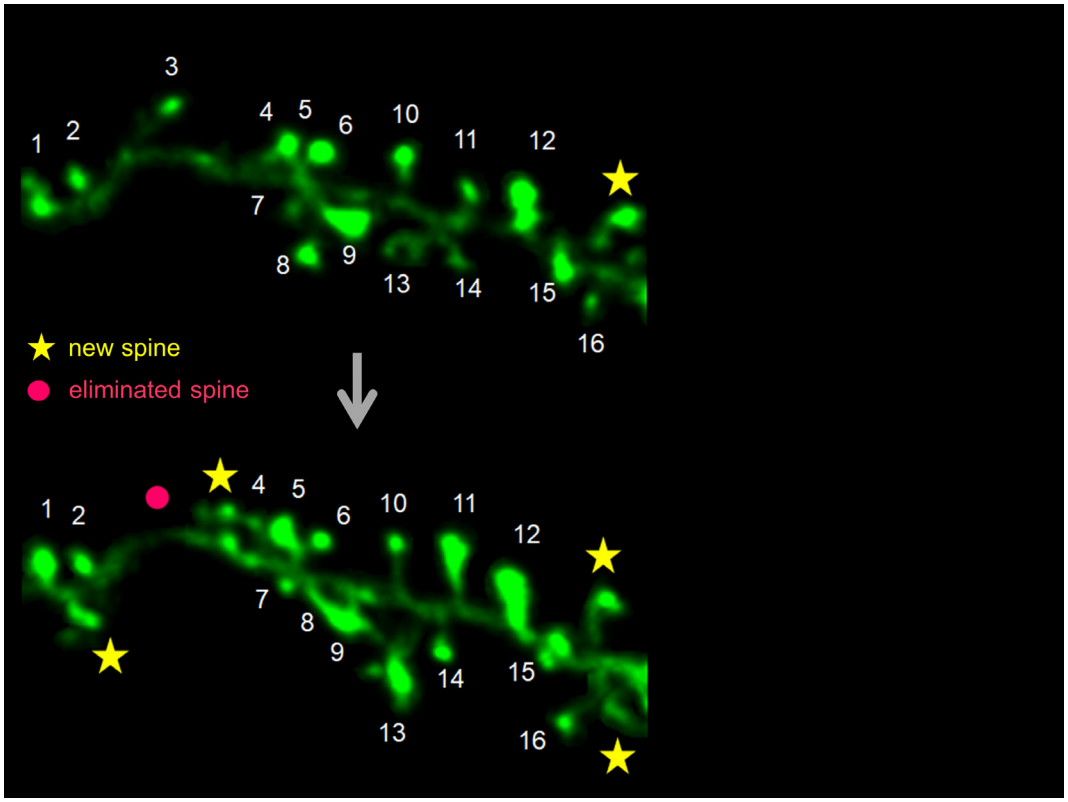
Even though mice do not use eye movements to refine their estimates of the depth of stereoscopic surfaces, we found that mouse behavior and neural responses still shared several characteristics with primates. Mice use disparity cues to solve the stereo correspondence problem indicating that the mouse model is likely to offer promising insights into how neural circuits are employed to construct a sketch of our environment in three dimensions.

INCREASED SPINE DYNAMICS IN THE VISUAL CORTEX OF PSD-95 KNOCKOUT MICE: CHRONIC TWO-PHOTON IMAGING OF NEURONAL MORPHOLOGY IN THE AWAKE BRAIN

A. Tippmann, B. Joachimsthaler, S. Treue, C. Schwarz, O. Schlüter, S. Löwel. Dept. of Systems Neurosci., Univ. of Goettingen, Goettingen, Germany; Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; CIN Systems Neurophysiol., Univ. of Tuebingen, Tuebingen, Germany; Collaborative Res. Ctr. 889, Goettingen, Germany; Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; Dept. of Psychiatry and Psychotherapy, Univ. Med. Ctr., Goettingen, Germany; Campus Inst. for Dynamics of Biol. Networks, Goettingen, Germany. 49(551)39 Ext 20161. sloewel@gwdg.de

Program Number: 308.01
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: L23
Presentation Time: 8:00 AM - 9:00 AM
Session Title: Visual Cortex: Plasticity

Nerve cell circuits are more dynamic when a signalling scaffold protein, postsynaptic density 95 (PSD-95) of excitatory synapses, is missing.
We performed high-resolution imaging in the brain of awake mice to monitor dynamic changes of neuron protrusions that represent synapses, contact points between excitatory neurons. It is known that during “critical periods” of the developing brain, neuronal circuits are highly plastic, and thus synapses are more dynamic. These dynamics were now observed in *adult* mice lacking PSD-95 and resemble the state of young wildtype mice during the „critical period“. Thus the loss of a single protein can maintain the plasticity of the young brain and might facilitate reorganization to “correct” problems in neuronal circuits caused by neurodevelopmental disorders. In the long run, these new insights might pave the way for additional therapeutic options for treating brain lesions and psychiatric disorders. By artificially reopening juvenile critical periods, intact brain regions may be boosted to learn to take over functions of lesioned regions, or otherwise compensate missing functions.
Interactions between billions of our brains’ neurons are at the basis of all our capabilities and behaviors. Therefore anything influencing the dynamics of these interactions may exert a huge influence not only on brain wiring and functioning, but also on brain plasticity and possibly also regeneration after brain lesions.
We have previously shown that a single protein, PSD-95, is essential both for maturing and stabilizing excitatory synapses. When PSD-95 is missing in a particular brain region, excitatory neuronal circuits cannot stabilize and remain in a juvenile state of increased plasticity even during adulthood and ageing, and after lesions. Since excitatory neurons constitute about 80% of all neurons in our brains, manipulating this protein impacts the vast majority of the brain’s neuronal circuits.
We hypothesized that the observed increased neuronal plasticity must be due to increased morphological changes at the level of single synapses onto individual neurons. To test this idea we reasoned that we essentially need to film a movie of the wiring changes happening in the awake living brain. Since synapses are tiny and measure only about 1/1000 of a millimeter we had to use a high-resolution (2-photon) microscope. In addition, we labelled the dendritic spines, the contact sites of individual neurons for incoming information from other neurons, with a green fluorescent dye that the microscope can capture. This was done in the visual cortex, the brain region analysing visual information. Finally, we trained the mice extensively to sit quietly under the 2-photon microscope while we visualized neuron morphology repeatedly. The challenge was to relocate these tiny structures every day. But we were successful and then could count how many of the spines disappeared or were built new between the daily imaging sessions. To our knowledge this is the first study that managed to repeatedly image dendritic spines in the visual cortex of an awake brain. Our data clearly reveal that spine dynamics and turnover was much higher in the cortical circuits missing PSD-95 compared to wildtype animals. In fact, spine dynamics of adult brain circuits without PSD-95 was rather similar to dynamics previously observed in young wildtype mice. Thus, modifications of PSD-95 in adult brain circuits may constitute a completely new therapeutic avenue to boost brain plasticity in the ageing, diseased or lesioned brain.
Supported by the Deutsche Forschungsgemeinschaft via CRC 889 (projects B03 to OS and B05 to SL)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CIRCUIT-SPECIFIC CHANGES IN THE VISUAL CORTICAL EXCITATION/INHIBITION RATIO ACROSS THE LIGHT/DARK CYCLE

M. C. D. Bridi, F.-J. Zong, X.-T. Zhang, D. Severin, K.-W. He, A. Kirkwood. Zanvyl Krieger Mind/Brain Inst., Mind Brain Inst., Johns Hopkins Univ., Baltimore, MD; Shanghai Insititute of Organic Chemistry, Chinese Acad. of Sci., Shanghai, China; IRCBC, Chinese Acad. of Sci., Shanghai, China. (215)589-9933. michelle.bridi@jhu.edu

Program Number: 308.07
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: L29
Presentation Time: 10:00 AM - 11:00 AM
Session Title: Visual Cortex: Plasticity

Our research indicates that the balance between excitation and inhibition in certain brain circuits varies over the course of the day, which has many implications for how the brain functions in health and disease.
The brain contains excitatory and inhibitory neurons, which send synaptic signals to other neurons to increase or decrease their activity, respectively. It is thought that the strength of these excitatory and inhibitory signals must maintained at the correct ratio for the brain to function properly. Disruption of this balance has been implicated in a number of neurological diseases and disorders, including autism spectrum disorder.
Previous research has suggested that the ratio between excitatory and inhibitory synaptic transmission is maintained within a narrow range in the brain. However, the excitation side of this equation is labile: excitation can change over the course of the sleep/wake cycle, such that it is higher after an animal has spent more time awake. This raises the question of whether inhibition undergoes the same changes in order to maintain a constant excitation/inhibition balance.
To address this question, we obtained brain slices from mice at different points of the 24-hour day and measured inhibition using whole-cell patch clamp recordings of spontaneous inhibitory synaptic events. We found that inhibition changes over the course of the day but, surprisingly, it changes in the opposite direction of excitation. We also tested whether this was due to sleep or circadian factors by measuring inhibition after a brief period of sleep deprivation. We found that sleep was responsible for these changes in inhibition, such that inhibition is lower after the animal has spent more time awake. Together with previous studies, these findings indicate that the ratio between excitation and inhibition varies greatly over the course of the day, and is likely modulated by sleep/wake states.
Recording spontaneous inhibitory synaptic events allowed us to measure the overall amount of synaptic inhibition that a neuron receives. However, these measurements cannot identify the origin of these inhibitory inputs. In order to pinpoint the circuits in which the excitation/inhibition balance changes throughout the day, we stimulated different portions of the slice to evoke excitatory and inhibitory responses in the recorded cell. First, we stimulated visual cortical layer 2/3 while recording laterally in the same layer. Consistent with the spontaneous synaptic measurements, the ratio between excitatory and inhibitory responses varies with time of day this circuit. We found that these changes have functional consequences for the cell: when the excitation/inhibition ratio is high, less excitation is required to evoke an action potential, since there is less inhibition to overcome. Next, we measured excitation and inhibition in a second circuit. We stimulated visual cortical layer 4 while recording in layer 2/3. However, the excitation/inhibition ratio did not change over the course of the day in this circuit.
These novel findings suggest that the regulation of excitation and inhibition in the brain is more complex than previously believed. Our results indicate that the excitation/inhibition ratio is highly labile over the 24h day in certain brain circuits, and is likely modulated by sleep and wake states, providing insight into how cognitive function may vary across the day.
These results have clinical implications, since the excitation/inhibition ratio is thought to be altered in many neurological diseases and disorders. This raises the possibility that the dynamic regulation of the excitation/inhibition balance described here may be disrupted in these conditions. We are currently investigating whether this may be the case in by measuring the excitation/inhibition ratio at different times of day in mouse models of autism spectrum disorder.

OPTICAL ACTIVATION OF TRKB RECEPTORS IN PARVALBUMIN INTERNEURONS REGULATES PLASTICITY MODES IN CORTICAL NETWORKS

F. Winkel, G. Didio, M. Llach Pou, A. Steinzeig, M. Ryazantseva, J. Harkki, J. Englund, T. Taira, S. Lauri, J. Umemori, E. Castren. Neurosci. Ctr., Mol. and Integrative Biosci. Res. Programme, Dept. of Vet. Biosci. and Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland. frederike.winkel@helsinki.fi

Program Number: 308.08
Session Date/Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Room Number: Hall A
Board Number: L30
Presentation Time: 11:00 AM - 12:00 PM
Session Title: Visual Cortex: Plasticity

We have developed a light-controllable tool that can induce the rewiring of neuronal networks in the adult brain, when combined with training or rehabilitation.
Information in the brain is processed in neuronal networks and dysfunctional networks may underlie several brain disorders. Neuronal networks are tuned to optimally represent the environment through neuronal plasticity. The guiding principle in neuronal plasticity is synaptic activity between neurons: active connections are maintained and strengthened, while inactive connections are weakened and pruned away. Neuronal plasticity peaks during critical periods of early postnatal life, when changes in the environment bring about large changes in the structure of neuronal networks; after the closure of the critical periods, plasticity is much more limited. Visual cortex has become the best characterized experimental tool for the investigation of neuronal network plasticity. If vision of one eye is weakened by a refraction error or strabismus during the critical period before school age, the more active healthy eye takes over the innervation of the visual cortex at the expense of the weaker eye, which leads to a permanent reduction in the vision of that eye, a condition known as lazy eye or amblyopia. After the closure of the critical period, blockade of vision in one eye no longer leads to a lazy eye. It is known that closure of the critical period is associated with increase in the activity of inhibitory interneurons, particularly of those containing parvalbumin (PV).
We have previously shown that critical period-like plasticity can be reactivated in the adult brain by the treatment with antidepressant drugs. When one eye of an adult mouse is patched for a week during antidepressant treatment, vision of that eye is weakened in the same way that normally only happens during the critical period. We have also found that antidepressants promote plasticity by activating a neurotrophic factor signaling pathway. In this study, we have created optoTrkB, a light activated version of the neurotrophic factor receptor called TrkB. We found that when we express optoTrkB in mouse brain specifically in the PV containing interneurons, a short exposure of the visual cortex to a beam of light, which activates optoTrkB in the PV neurons, reactivates a critical period-like plasticity and leads to a weakening of a visual response if one eye is patched for a week. We further found that optoTrkB activation decreased the activity of the PV inhibitory cells in the visual cortex, which in turn resulted in disinhibition of excitatory neurons in the visual cortex. This increase in excitatory transmission then allowed the rewiring of the visual cortical networks. We observed the same effects after treatment with an antidepressant for a month, however, when we deleted TrkB receptors from the PV inhibitory cells, the antidepressant-induced effects were abolished. These results demonstrate that activating the TrkB pathway in PV inhibitory cells is sufficient and necessary to allow the structural rewiring underlying amblyopia when combined with eye patching. It is important to note, however, that the induction of the TrkB pathway alone is not sufficient to restructure brain connections but it needs to be combined with training or rehabilitation. OptoTrkB is an exciting new tool that can rapidly reactivate a juvenile-like state of plasticity in the visual cortex. The activity of optoTrkB can be precisely controlled temporally (through exposure to light), spatially (through expression in specific brain regions and placement of the light beam) and cell-type specific manner. We are now investigating whether activating the
TrkB pathway in PV inhibitory cells or other cell types can also induce the structural rewiring in other brain circuits, such as the circuits underlying depression, anxiety or addiction.

MORPHOLOGICAL DIVERSITY WITHIN AND ACROSS TRANSCRIPTOMICALLY-DEFINED INTERNEURON TYPES IN MOUSE PRIMARY VISUAL CORTEX

S. A. Sorensen, R. Dalley, C. Lee, S. Kebede, A. Mukora, G. Williams, D. Sandman, K. E. Link, C. Gamlin, J. Berg, N. W. Gouwens, B. R. Lee, J. A. Miller, R. Nicovich, H. Peng, K. Smith, B. Tasic, Z. Yao, J. T. Ting, G. J. Murphy, E. Lein, C. Koch, H. Zeng. Cell and Circuit Genet., Human Cell Types, Structured Sci., Allen Inst. for Brain Sci., Seattle, WA. 1(206)548-7096. stacis@alleninstitute.org

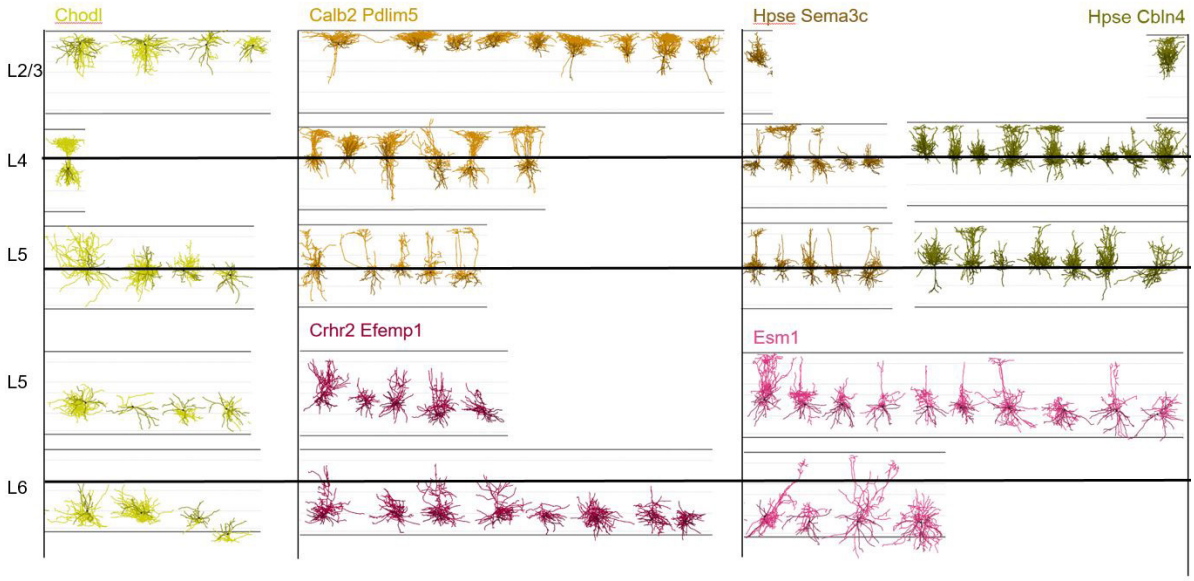
Program Number: 403.07
Session Date/Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: P11
Presentation Time: 3:00 PM - 4:00 PM
Session Title: Visual Cortex: Circuits

Our research provides the first direct, comprehensive description of the relationship between brain cell types defined using genes, shape and electrical responses of several major subclasses of neurons (brain cells) in the cortex, the outermost part of the brain.

Cells are the fundamental building blocks of the brain. Understanding the cell type landscape will help us better understand the cells that contribute to sensation and behavior in health and disease. For this reason, there is a major effort in the field of neuroscience to understand and describe the diversity of cell types in the brain. Recently, a new technique called single cell transcriptomics, which allows us to identify thousands of genes expressed in individual neurons, has massively accelerated this effort, and transformed the way that we approach cell type classification. Instead of relying on more classical cellular properties such as cell shape (morphology), electrical responses (electrophysiology) and/or function to define cell types, transcriptomic studies rely largely on gene expression pattern. Much less work has been done to understand the relationship between gene-defined cell types and these other important cellular properties.

To make that connection directly, we employed the 'Patch-Seq' method. This involves using a tiny glass pipette to form an electrical seal with a cell enabling access to expressed genes, morphology, and electrophysiology – all from the same cell. We used this method to perform a systematic, cell type characterization of hundreds of inhibitory neurons in the mouse brain. Using the gene expression data, we mapped each cell to an existing gene-expression (transcriptome)-based cell type taxonomy (Tasic et al., Nature, 2018), and created detailed drawings of a representative set of neurons from multiple inhibitory transcriptomic types. Our analyses of these data revealed a complex relationship between morphological properties and transcriptomic cell types. For some transcriptomic types, there is a clear consistency with morphological types, particularly for cells located in the same sub-region of cortex, indicating a tight relationship between these two properties. For other transcriptomic types, morphology varies in terms of features such as the amount of axon, while other features, such as axon location are consistent within a type. Importantly, the location of the axon likely reflects where the cell is sending information. This work is essential in helping us to understand what properties of a cell can be understood or predicted from gene expression patterns.

With genetic access to these cell types we can study these neurons in a functional context, and provide a means for targeted intervention (e.g., gene therapy) in disease. Knowing their shape and electrical properties under normal conditions will give us insight into how disease alters brain circuitry and how it is affected by treatment.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L6

THE SENSORY REPRESENTATION OF CAUSALLY CONTROLLED OBJECTS

K. Clancy, T. D. Mrsic-Flogel. Sainsbury Wellcome Ctr. for Neural Circuits and Behavior, UCL, London, United Kingdom; Univ. Col. London, London, United Kingdom. clancykelly@gmail.com

Program Number: 752.05
Session Date/Time: Wednesday, October 23, 2019, 1:00 PM - 5:00 PM
Room Number: Hall A
Board Number: H39
Presentation Time: 1:00 PM - 2:00 PM
Session Title: Active Vision and Context Modulation

An organism's ability to exert intentional control over external objects is informed by their sensory experience, in a continuous dialog between action and perception. How such control is represented at the sensory level, or its efficacy judged, however, is not understood. In this work, we devised a brain machine interface (BMI) task that enabled mice to control a visual feedback cursor using neural activity alone (a "mouse-brain-controlled-mouse"). Transgenic mice expressing a fluorescent calcium activity indicator in cortical pyramidal cells were head-fixed under a widefield microscope and trained to control a visual feedback cursor using areal calcium signals. The activity of two small regions, usually over primary or secondary motor cortex, were arbitrarily assigned control of this visual feedback cursor. Fluorescence from these regions was read-out in real time and transmuted into the position of the onscreen cursor, and animals learned over the course of several days to use their brain activity to guide this cursor to a target area for reward.

We found evidence that parietal and higher visual cortices were engaged when expert animals controlled the BMI for reward, but not in naïve mice still learning the task. Parietal cortex has been previously implicated in human studies in mediating a sense of agency or intention in body movements. In this work, single-cell recordings from parietal cortex indicated that the same visual cursor stimulus elicited larger responses when an animal was controlling the cursor than when passively viewing it. This increase was sensitive to the cursor's trajectory — responses were greater when the cursor was moving towards the target than away from it. Thus, the sensory representation of a causally controlled object is sensitive to the subject's intention, as well as the object's instantaneous trajectory towards or away from its goal. The heightened sensory representation at the target position might serve as a fixed goalpost for downstream evaluation, given the animal must relearn a changing sensorimotor contingency on the fly, potentially strengthening the signal to adjudicating areas for informing fluent, expert control.

The BMI control areas were changed from day to day, so animals had to learn a different mapping between brain activity and cursor control every training session. Early in a session, as animals were exploring the contingency between their brain activity and cursor position, their brain activity was erratic and high in entropy, until a successful strategy was discovered and activity patterns became stereotyped as animals 'exploited' the successful pattern—suggestive of a kind of 'exploration' and 'exploitation' in neural activity space.

In motor learning, the relationship between an animal's action and its consequence in the world can be learned and re-learned throughout adulthood as animals acquire new motor skills. Brain machine interfaces (BMI) are a novel method for investigating how subjects learn such arbitrary action-outcome relationships. Unlike motor learning, whereby animals learn a stereotyped movement and researchers must search for correlates of the behaviour in the patterns of neural activity, BMIs allow the experimenter to prescribe the requisite activity patterns for task performance, which can be changed day to day. Thus, animals learning neuroprosthetic control must engage in continuous self-monitoring to assess the contingency between their activity and its outcome, preventing them from executing a habitual or fixed motor pattern. This enabled us to investigate what areas were implicated in this kind of intentional control over brain activity patterns, and how animals explored neural activity space to settle on successful activity patterns to achieve reward.