Studying the Physiological Role of Connexin Proteins in Developing Human Neurons - Silencing of the Connexin Genes by Antisense Oligonucleotides.

Anzhela M. Moskalik, Mandakini B. Singh, Pallavi V. Limaye, Jesse A. White, Nada Zecevic and Srdjan D. Antic

UConn Health, Stem Cell Institute, Institute for Systems Genomics, Department of Neuroscience, Farmington, CT 06030

Introduction: Spontaneous electrical activity plays an important role in newborn neurons by supporting their differentiation, maturation and synaptic connectivity. Hypothesis: We hypothesize that connexin hemichannels provide depolarizing currents in this process. Methods: Experiments were designed to: [1] Proliferate human fetal cells in vitro; [2] Record spontaneous physiological activity; and [3] Silence individual connexin genes using antisense oligonucleotides (ASOs); and [4] Determine the effect of this gene silencing on the physiological activity. In the first series of experiments, an ASO designed against the housekeeping gene *HPRT*, was tested at different time-points (days in vitro, DIV 10 - 13) to establish a time point which produced the most robust gene knock down. "ASO-HPRT" was compared to the control (no ASO treatment) by real-time PCR. Based on the above experiment, a 48-hour time point was chosen. Next, two different ASO sequences, targeting gene *Cx26*, were tested. Immunolabeling against Cx 26 was used to test for the presence of protein in control human cultures. Real time PCR were performed to determine the knock down of the four connexin genes of interest: *Cx26*, *Cx36*, *Cx43*, and *Cx45*. Beta-actin (*ACTB*) was used as a housekeeping gene. Results: The expression of *Cx26* could not be reliably calculated in some of the samples due to highly varying *ACTB* levels. Expression of *Cx26* was delayed by 5 cycles in the stable *ACTB* expression group, which indicates a small knockdown. The expression of *HPRT* in the stable *ACTB* expression group showed a 12% knockdown, while 60%-70% knockdown has been reported when cationic lipids were added to the *HPRT* ASO cell lines. Conclusions: However, a small knockdown in connexin gene *Cx26* was observed at 48 hours compared to the control ASO sequence (scrambled ASO). We continue to search for a robust method for silencing human connexin genes in vitro. Significance: Our results indicate that adding ASO on its own to neuronal progenitor cells is not an efficient method of gene knockdown at 48 and 72 hours (because cells are rapidly proliferating).

Research Support:

[1] Connecticut Innovations grant 09-SCA-UCHC-13,

[2] NIH grant MH104775,

[3] HCRAC Institutional grant. Electrophysiological and optical measurements were performed in the Stem Cell Physiology and Chemistry Core, which was supported by Connecticut Innovations grant SCD-01-2009 to SDA.

Mechanisms of Spontaneous Electrical Activity in the Developing Cerebral Cortex – Subplate Zone

Mandakini B. Singh and Srdjan D. Antic
Dept. of Neuroscience, UConn Health, Farmington, CT

During cortical development, elemental processes such as neurogenesis, migration, neurodifferentiation and synapse formation are regulated by spontaneous calcium and electrical activity. Environmental cues affect electrical activity at the early stages of brain development, which then controls gene expression, which in turn provides feedback loops to fine-tune steps of development. The aim of this work is to investigate the cellular mechanisms contributing towards the generation of spontaneous electrical activity in the mammalian cortical mantle. We are focused on a group of neurons located in the subplate zone (SP). SP neurons govern the path-finding of incoming axonal projections and establishment of cortical connections and cortical columns. The selective disruption of SP neurons has been implicated in mental retardation and schizophrenia. SP neurons, in both rodent and human cerebral cortex, exhibit spontaneous electrical activity comprising of highly irregular plateau depolarizations crowned with action potentials. We performed whole-cell recordings, immunolabeling and calcium imaging from positively identified SP neurons in acute brain slices obtained from the newborn mice pups (P01 – P06). The spontaneously-occurring outbursts of electrical activity were challenged with drugs that block voltage-gated and ligand-gated membrane conductances. These experiments established that the spontaneous activity in SP neurons was not solely mediated by glutamatergic, GABAergic, or glycinergic synaptic transmission. Also, a significant portion of spontaneous depolarizing currents is linked to intact functioning of connexin-based pores and purinergic receptors. Immunostaining performed on brain sections from young mice (P1-P6) detected connexins 26 and 45, but not connexins 32 and 36. Using multi-site calcium imaging, application of glial toxin DL-Fluorocitrate and purinergic antagonist PPADS, we found that spontaneous calcium events in SP zone are, in part, mediated through release of ATP. Three observations from our study: [1] high sensitivity of SP neurons to lanthanum, [2] decrease of activity upon blockade of purinergic receptors; and [3] decrease in spontaneous calcium events upon blockade of glial metabolism by DL-Fluorocitrate, prompted a working hypothesis that spontaneous flickering of connexin hemichannels on both neuronal and glial populations causes initial depolarizations along with release of metabolites (e.g. ATP) that stimulate spontaneous electrical activity in cortical SP zone.

Source of financial support: NIH Grants 1 MH104775 and MH109091, as well as the Institutional HCRAC Grant.
Keywords: Cortical development, Subplate, Electrophysiology, Spontaneous activity

Nicotine enhances responding for chocolate rewards

Alexandra Palmisano[1], Ellie Hudd[1], Courtney McQuade[1], Harriet de Wit[2], & Robert Astur[1]
[1] Department of Psychological Sciences, University of Connecticut, Storrs, CT 06269
[2] Department of Psychiatry, University of Chicago, Chicago, IL 60637

Introduction: In addition to its primary addictive properties, nicotine is believed to enhance the rewarding effects of other stimuli. We aimed to determine whether nicotine enhances the value of food rewards in a virtual reality conditioned place preference paradigm in humans. Methods: Undergraduate participants with varying levels of nicotine dependence were recruited for a 2-day study. On day 1, participants explored two virtual rooms where they received multiple pairings of real M&M rewards in one room, and no rewards in the other room, followed by a free-access test session with no rewards. On the second day, participants received multiple test sessions to assess extinction. Subsequently, participants received M&Ms in a novel context and were then tested for reinstatement. Prior to testing on each day, subjects were administered either nicotine (4 mg) or placebo lozenges. Results: For participants who demonstrate some level of nicotine dependence, the previously-paired M&M room was rated as significantly more enjoyable for the nicotine group compared to the placebo group on Day 1. Those who received nicotine on Day 1 spent significantly more time in the previously-paired M&M room than did the placebo group during the last extinction session on Day 2. Moreover, participants who received nicotine on Day 2 demonstrated significant reinstatement compared to placebo-treated participants. Conclusions: These data show that nicotine increases an explicit place preference in humans with mild nicotine dependence, promotes the return of a previously extinguished conditioned place preference, and promotes reinstatement. Significance: These findings provide insight into how nicotine dependence can be particularly resistant to treatment and also demonstrate the efficacy of utilizing a virtual conditioned place preference paradigm to help understand the behavioral mechanisms of substance dependence.

Transcriptional profiling of naïve and inflamed GFAP+ satellite glial cells in the mouse dorsal root ganglion

Knight, B.E.[1], Yeung, S.T[5], Young, E.E.[2, 3, 4, 5], Baumbauer, K.M.[1, 2, 4, 5]

[1]Department of Neuroscience, UConn Health,[2]School of Nursing, Center for Advancements in Managing Pain, University of Connecticut,[3]Genetics and Genome Sciences, UConn Health,[4]Institute for Systems Genomics, [5]Department of Immunology, UConn Health

Peripheral inflammation and tissue injury causes alterations in nociceptor activity and contributes to the development of normal and pathological pain states. Primary afferent nociceptors have been extensively characterized, and like central neurons, neurons that exist within the DRG do not appear to act in isolation in the peripheral pain circuitry. A resident population of peripheral glial cells, satellite glial cells (SGCs), appears to influence nociceptive activity, but the precise mechanisms by which this occurs is not well understood. SGCs appear to be biochemically diverse, and in order to understand how they contribute to inflammatory pain processing, we have started developing cellular profiles based on patterns of protein and gene expression. SGCs express a variety of identifying proteins, including GFAP, and we focused on these cells to limit our initial analysis. To first visualize the relationship between neurons and SGCs in naive conditions, we used Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging (CLARITY) on GFAP-CreERT-tdTomato mice to fluorescently tag SGCs and later stain neurons for NeuN. This allowed us to 3D construct the proximal relationship between SGCs and neurons in whole tissue. We next wanted to know what percentage of SGCs in lumbar DRG express GFAP, we used fluorescence activated cell sorting (FACS) to identify neuronal, glial, and immune cell populations in mouse L2 and L3 DRG. Using this gating strategy we identified putative neurons in the neuronal population, in the second population putative SGCs, and then immune related cells. We found that in naïve conditions 60% of GFAP+ cells, putative SGCs, express purinergic channel P2RX7 and inward rectifying channel Kir4.1, but not NeuN or CD45. To determine how expression of these proteins is regulated by peripheral inflammation, we again FACS sorted L2 and L3 DRG 1, 3, and 7 days following subcutaneous hindpaw injections of the inflammatory substance, complete Freund's adjuvant (CFA). We found that inflammation decreased the expression of inward rectifying potassium channel kir4.1 by 30% in GFAP+ cells 7 days following inflammation. No significant changes were observed in protein expression of P2RX7 over time. To gain further insight into the expression profile of GFAP+ SGCs, we subjected individual fluorescently labeled SGCs collected from GFAP-CreERT-tdTomato mice to single cell real-time RT-PCR. We found that naïve GFAP+ SGCs expressed the truncated version of the TrkB receptor, TrkB.T1, the purinergic receptor, P2Y1, glutamine synthetase (GS), and the Ca2+ sensing protein S100β, but not purinergic receptors P2X3 and P2RX7. This suggests a potential switch in cellular phenotype in GFAP expressing SGCs when challenged with inflammation. Ongoing experiments are examining how inflammation impacts the profile of gene expression in GFAP-expressing DRGs, and how this expression profile compares to other known populations of SGCs. Transcriptional profiling will provide more detailed regarding SGCs heterogeneity, how inflammation alters SGC population-specific patterns of gene expression, and, ultimately, how inflammation-induced alterations in SGC-specific genes influence sensory neuron function.

Decreased Methylation of Pain Sensitivity Genes Differentiates a Chronic Pain Trajectory in Patients at the Onset of Low Back Pain

Mallory Perry[1]; Jessica Yasko[2], Brittany Knight[2], Angela Starkweather[1], Erin Young[1,3], & Kyle Baumbauer[1,2]

1 – University of Connecticut School of Nursing; 2- University of Connecticut Department of Neuroscience; 3 - University of Connecticut Department of Genetics and Genome Sciences

Introduction: Chronic low back pain is one of the most common and costly pain conditions in the United States. The genetic contributions of transitioning from acute to chronic low back pain have not been fully explicated but may assist in early identification and intervention for patients at risk of a chronic low back pain trajectory.

Methods: In this study we evaluated mRNA expression levels, global methylation and acetylation of 84 genes involved in the pain signaling among participants with acute nonspecific low back pain. Participants enrolled in the study during an acute phase of low back pain and were categorized as either “recovered” if their pain resolved in the first six weeks after onset or as “chronic” if their pain continued for six months. Blood samples collected via venipuncture were assayed at baseline, during the acute phase of low back pain, to examine mRNA expression, global methylation and acetylation of pain sensitivity genes.

Results: Differential mRNA expression of pain sensitivity genes was identified among the chronic, recovered and no-pain groups. Decreased global methylation of pain sensitivity genes was found in the chronic low back pain group compared to the resolved comparison group and no-pain control group. There was a lack of difference in acetylation among groups.

Conclusions: The findings suggest that methylation as opposed to acetylation is a prominent mechanism in the transition to chronic pain. Decreased methylation is potentially linked with increased mRNA expression of pain sensitivity genes in the chronic low back pain group. The results will be used to identify specific genes that are upregulated at baseline in the chronic low back pain and to evaluate the influence on pain outcomes over time.

Significance: The study results suggest that decreased methylation of pain sensitivity genes contributes to the transition from acute to chronic low back pain.

Research/Grant Support: The study was supported by the National Institute of Nursing Research (R013932; PI, Starkweather), the Young Lab & Baumbauer Lab.

Disclosures: The authors have no conflicts of interest to disclose.

Alterations in afferent pathway signaling and neurogenic inflammation following spinal cord injury

Jessica R. Yasko, Erin E. Young, Kyle M. Baumbauer

Spinal cord injury (SCI) can lead to a devastating loss of function and intractable chronic pain. Estimates of the prevalence of SCI-induced pain ranges from 60-90%, with 25-30% of those patients rating their pain as severe. Pain is consistently ranked as one of the most significant health concerns among patients, and many report receiving inadequate pain relief. Pain following SCI is unique because it is one of few instances where pain is generated centrally. However, evidence has supported that the cells within the spinal cord are not the only cells in the nociceptive system impacted by SCI and that primary afferent nociceptors also play an integral role in the development and maintenance of chronic SCI-induced pain. Studies examining the contribution of primary afferents to SCI pain have shown that sensory neurons exhibit spontaneous activity following SCI. Most research concerning SCI pain mechanisms emphasize shifts in neuronal responses associated with inflammation and glial activation within the spinal cord. However, these inflammatory responses may also impact primary sensory neurons, prompting changes in the excitability state and spontaneous activity (SA) of nociceptors. Studies have demonstrated that rodents receiving SCI show a greater SA incidence in lumbar DRG neurons, particularly capsaicin sensitive and express the TRPV1 receptor, and to a lesser degree those that bind the non-peptidergic, nociceptive marker, isolectin B4 (IB4). Here we examine how SCI impacts gene expression in sensory neurons, as well as the skin and muscle in which they innervate. Mice were placed under an impactor to receive a contusion injury (70kD impact, 10s dwell time) at the level of T10-11, producing complete paralysis below the level of the forelimbs. To determine the time course of alterations in targets of interest, we injected WGA-488 or IB4-488 into the saphenous nerve to backlabel peptidergic and nonpeptidergic cutaneous afferents, respectively. Mice were then sacrificed 24 hr and 7 days following SCI and individual fluorescently labeled neurons were collected, along with skin and muscle. Tissue was then processed and analyzed for changes in gene expression using real-time RT-PCR. Analysis of WGA-labeled peptidergic neurons revealed significant increases in the expression TRPV1, calcα (CGRP), TRPA1, GFRα3, P2X3, and ASIC3 mRNA, and no change in expression of ASIC1, ASIC2, or P2Y1 mRNA. No significant changes in mRNA expression were observed in IB-4-labeled nonpeptidergic afferents. Analysis of quadriceps femoris muscle showed significant increases in the expression of acid sensing ion channel-2 (ASIC2), ASIC3, TRPA1, P2X3, TrkA, and TrkB mRNA. Analysis of hindlimb hairy skin revealed increased expression of GFRα2 as early as 24 hr following injury. Ongoing experiments are examining the time course of additional changes in gene and protein expression, alterations within individual afferents, and how severity of injury impacts these changes. Our data suggest that SCI results in early sensitization of afferent pathways and the development of neurogenic inflammation in a temporally specific manner.

Modeling Memory Encoding via a Convolutional Neural Network

Aaron Palmer*, Chi-Ming Chen⋆, Jason Johannesen†,‡, Josh Kenney†, Jinbo Bi*

*Department of Computer Science and Engineering, University of Connecticut, Storrs, CT 06269
⋆Department of Psychological Sciences, University of Connecticut, Storrs, CT 06269
†Psychiatry, Yale School of Medicine, West Haven, CT 06517
‡Psychology Service, VACHS, West Haven, CT 06516

Introduction: Traditional analysis of electroencephalogram (EEG) data involves *a priori* selection of the features submitted to statistical analysis, in which oscillatory signals recorded over many trials are averaged and scored according to conventional frequency bands (α, β, θ, δ, and γ). However, these pre-specified components may exclude other relevant frequency activity, while temporal averaging reduces sensitivity to the evolving nature of signal change in response to mental effort and behavioral feedback over the course of the experiment. A convolutional neural network (CNN) approach is presented in which Morlet wavelet transformed EEG data is represented as a series of single-trial time-frequency plots at each of three EEG electrodes: Fz, Cz, and Oz. Hypothesis: We model the encoding stage of a working memory task performed by subjects diagnosed with schizophrenia and a healthy comparison group. Prior work has shown that traditional machine learning models can effectively classify trials by accuracy (correct vs incorrect) based on the averaged EEG data. CNN may further elucidate spatio-temporal patterns representing network-level coordination of distributed brain areas during memory encoding. Accordingly, we hypothesize that effective encoding will be associated with synchronized responses to stimulus presentation and evidence of coordinated (i.e., phase-locked) activity across electrodes while ineffective encoding will lack coherent structure of brain activity. Methods: A fine-tuned CNN is employed to take stacked time-frequency maps and produce classifications of single-trial EEG data labeled by accuracy (correct vs incorrect). To gain insight into what the network is responsive to, without a priori/arbitrary frequency-band selections, node deconvolutions are used to maximally activate a neutral input image of features that commonly represent a given class probability. Results: A deconvolved representation of data reveals evidence of theta-range synchrony at periods of stimulus presentation, as well as phase-locking across electrodes in beta-to-gamma range, preceding correct responses. Overall, no consistent organization of EEG across electrodes or frequencies was evident in trials preceding incorrect responses. Conclusions: The CNN allows more complex spatio-temporal features to be considered when modeling memory encoding, while deconvolutions provide a format for interpreting network structure. Significance: The modified data representation combined with feature extraction may offer new insights into complexities, reflective of network-level behavior, which cannot be detected effectively using traditional machine learning classification methods.

Research support: This work was supported by NSF IIS-1320586 and DBI-1356655 and NIH R01DA037349 to Jinbo Bi, NARSAD YIA to Chi-Ming Chen, and VA D7008-W (CDA-II) and l21 RX001731-01A1 (SPiRE) to Jason Johannesen.

Directed Evolution of a High Affinity and Specificity Antibody Targeting Phosphorylated Tau

Dan Li[1] and Yong Ku Cho[2]

[1]Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269
[2]Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT 06269

Introduction: Phospho-specific antibodies are defined by their specific interaction with an epitope containing a specific sequence of amino acids, one or more of which are phosphorylated. Phospho-specific antibodies are important in the detection of site-specific phosphorylation in tau, because the initial phospho-oligomer can be a key indicator of tau-mediated neurodegeneration. However, the poor specificity and low affinity of antibodies result in irreproducibility in cell or tissue labeling experiments, due to the transient and heterogeneous nature of protein phosphorylation. We aim to develop a high throughput approach to discover and engineer antibodies against phosphorylated tau with high affinity and specificity. Methods: We developed a new approach applying the yeast surface display system to quantify the specificity of phospho-specific antibodies using multiple structurally related but distinctly labeled epitopes. We applied directed evolution strategies to optimize a phospho-tau specific antibody with high affinity and specificity. Results: The engineered single-chain antibody fragment (scFv) has picomolar monovalent affinity against a phospho-epitope (pT231) but no detectable binding to non-phospho-epitopes and irrelevant phospho-epitopes. Conclusions: The results show that our approach enables the optimization of antibodies specificity and affinity against phosphorylated tau while performing high-throughput screening of large number of antibody clones. Significance: Our approach enables the validation and improvement of the quality of post-translational modification (PTM) specific antibodies, and potentially antibodies in general.

Research Support: National Institutes of Health, Office of Extramural Research

In vitro multichannel single-unit recordings of action potentials from mouse sciatic nerve

L. Chen, A. Abbiati, T.J. Gallerani, C.S. Kneeshaw, C. Gonzales, B. Feng
Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269 USA

Introduction: Multichannel electrode arrays have been widely used to interface with the central nervous system for simultaneous signal-unit recordings from multiple neurons. In contrast, electrode arrays interfacing with peripheral nerves have not been extensively studied but are becoming the focus of research in the context of next-generation neuromodulation devices that target peripheral organs to relieve symptoms. Particularly, the ability to modulate (i.e., single-unit recording and stimulating) individual nerve axon, although with significant clinical and pre-clinical implications, remains a technical challenge. In this preliminary study, we report the feasibility of simultaneous single-unit recordings by interfacing state-of-the-art multichannel electrode arrays with mouse sciatic nerve axons *in vitro*. Materials and Methods: All procedures were approved by the University of Connecticut IACUC. Male C57BL/6 mice were anesthetized, euthanized by exsanguination and immediately transferred to a dissection chamber circulated with oxygenated ice-cold Krebs solution. The whole length of bilateral sciatic nerves (~30 mm) was harvested from their proximal projection to L4 spinal cord to their distal branches innervating gastrocnemius muscles. The nerve was transferred to a custom-built tissue perfusion chamber circulated with oxygenated Krebs solution at room temperature. The ~5mm distal end of the sciatic nerve was gently pulled into an adjacent recording chamber filled with paraffin oil to enhance the signal-to-noise ratio (SNR) of single-unit recordings. Action potentials were evoked by electrically stimulating the proximal end of the sciatic nerve. To minimize stimulus artifact, a suction electrode fabricated with quartz glass capillary was used to deliver the stimulus pulse of 0.2 mSec duration. In the recording chamber, microelectrode arrays from NeuroNexus (tetrode), BlackRock (ICS 96) and Microprobes (4ch MEA) were utilized to interface with sciatic nerve axons. To enhance the signal-to-noise ratio of the recording, the epineurium and perineurium were carefully dissected away to allow splitting of individual nerve fascicle into filaments of ~10 microns thick. Single-units from multiple electrodes were recorded simultaneously, digitized at 24 kHz and stored using a Tucker-Davis Technologies system (RZ5D, PZ5-32). Data were processed off-line using customized MATLAB programs (Mathworks R2016b). Results and Discussion: The stimulus artifact was significantly reduced (< 6 mSec) in the record when implementing a tight seal of the suction stimulus electrode onto the nerve. In addition, the long nerve length (25mm) in the *in vitro* setup permitted single-unit recordings from A-type myelinated nerve fibers with conduction velocities up to 4 m/sec. Mechanical splitting of the nerve fascicles combined with the use of paraffin oil allowed single-unit recordings from fine unmyelinated nerve fibers which are usually elusive to direct extracellular recordings. In addition, our *in vitro* setup allowed stable, robust and repeated recordings for up to 2 hours. Conclusions: This study demonstrated the feasibility of simultaneous single-unit recordings from multiple peripheral nerve fibers including both A- and C-type axons. This *in vitro* setup can be used as a test bench to objectively assess the design of next-generation electrode arrays for interfacing with peripheral nerves. This setup can also function as an objective and convenient platform to study a variety of neuromodulation strategies that target peripheral nerves, including electrical, infra-red, ultrasonic and pharmacological manipulations of peripheral nerve axons.

Acknowledgements: supported by NIH grant DK100460 awarded to BF.

Effects of caffeine and hypothermia on neuropathology in P6 rats with experimentally induced hypoxic ischemic brain injury

*M. POTTER[1], A. L. SMITH[1], H. M. CONTRERAS-MORA[1], T. S. ROSENKRANTZ[2], R. H. FITCH[1]

Department of Psychological Sciences: Behavioral Neuroscience, University of Connecticut, Storrs, CT 06269

Abstract:

Introduction: Neonatal hypoxic ischemic (HI) injury reflects reductions in blood and/or oxygen supply, as often seen preterm/low-birthweight infants. Neural HI injuries in this populations stem from hemorrhagic or ischemic vascular events, and/or or systemic hypoxia, and typically manifest as white matter damage (with more subtle gray matter damage in older preterms). HI injuries are associated with later deficits in cognition and behavior, including motor skills, memory, and language. To improve long-term outcomes, various neuroprotective interventions have been explored. In term infants with HI stemming from birth complications, the primary intervention is hypothermia (aka "cooling"). Mechanistically, hypothermia hinders aspects of apoptosis and thus reduces downstream tissue loss. We recently reported beneficial effects of even brief and subtle intra-insult hypothermia (i.e., during insult) in a postnatal day 7 (P7) HI rat model. More recently, putative benefits of extended hypothermia (3 hours) in a late preterm HI model (P6 HI) were also examined, to assess whether benefits of hypothermia might transfer to a late preterm population. We were surprised to find that hypothermia failed to rescue behavior in P6 HI subjects, and even appeared detrimental to shams (Contreras-Mora et al., 2015). Other preterm HI interventions under study include caffeine, a non-selective adenosine antagonist used as a respiratory stimulant in ventilated infants. Previous studies in our lab showed a protective behavioral benefit from caffeine when HI injury was induced on postnatal (P) day 7 in rats (Alexander et al., 2013), and more recently, on P6 (Contreras-Mora et al., 2015). Importantly, this protection was optimal when caffeine was administered immediately following the HI insult. Hypothesis: The current study sought to investigate the potential beneficial effect of caffeine and hypothermia on neuropathology in a P6 HI model. Methods: On P6, rats received sham or HI surgery, plus (1) caffeine treatment, (2) 2 hours hypothermia, or (3) no treatment. Various HI groups were then compared to shams using measures of behavior and *post mortem* neuroanatomy. For the latter, we assessed volumes of the cortex, hippocampus, and corpus callosum. Measures were quantified *post-mortem* to determine whether caffeine and/or hypothermia acted to reduce HI-mediated tissue loss. Volumetric measures were also correlated with behavioral measures to determine whether gross neuropathology related to long-term behavioral outcomes. Results: We report a trend for caffeine to rescue some HI-induced tissue loss relative to HI-plus-saline, although not to sham values. We also found that hypothermic treatment did not rescue any tissue loss, and in fact HI hypothermic brains showed tissue damage comparable to HI normothermic brains. Conclusion: Our neuroanatomical measurements parallel prior behavioral results – specifically that caffeine but not prolonged hypothermia yields some protection in a preterm HI model. Results suggest treatment for the preterm population suffering HI insults should avoid hypothermia – at least at the temperature and duration used here – but support ongoing use of caffeine for optimal outcomes.

Research Support: UConn Research Excellence Proposal (REP) to RHF and TR.

Electrophysiological phenotype and optogenetic silencing of histaminergic neurons of the hypothalamic tuberomammillary nucleus in a transgenic mouse line

A. FUJITA[1,2], P. BONNAVION[3], M.H. WILSON[1], L.E. MICKELSEN[1], L. DE LECEA[4], A.C. JACKSON[1]

[1]Department of Physiology and Neurobiology and [2]Department of Biomedical Engineering, University of Connecticut, Storrs, Connecticut 06269, USA, [3]Laboratory of Neurophysiology, Université Libre de Bruxelles (ULB)-UNI, 1050 Brussels, Belgium. [4]Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305, USA.

Introduction: Neurons that synthesize the monoamine neurotransmitter histamine (HA) exclusively reside in the tuberomammillary nucleus (TMN) of the posterior hypothalamus, extend fibers throughout the central nervous system and are interconnected with other neuromodulatory systems. Multiple lines of evidence suggest that HA neurons are well-positioned to drive transitions between global brain states and influence arousal and attention. *In vivo* single-unit recordings during the sleep-wake cycle have shown that putative HA neurons display slow pacemaking activity (1-5 Hz), which is tightly coupled to behavioral arousal, and exhibit a wake-specific firing profile. Hypothesis: We hypothesize that 1) the Hdc-Cre (GENSAT) mouse reports Hdc expression with high fidelity to visualize and manipulate HA neurons, 2) HA neurons exhibit a unique repertoire of membrane properties, distinguishing it from nearby cells, and 3) HA neurons can be targeted for optogenetic silencing for *in vivo* and *in vitro* studies. Methods: In the present study, we undertook a characterization of HA neurons, identified in a transgenic mouse line, Tg(*Hdc*-Cre)IM1Gsat/Mmucd (GENSAT), that expresses cre recombinase in cells that express histidine decarboxylase (Hdc), the synthetic enzyme and defining marker for HA neurons. We conducted an anatomical analysis of the specificity of this mouse line by crossing *Hdc*-Cre mice with a cre-dependent ROSA26-tdTomato reporter line to visualize expression of cre recombinase. Hdc-Cre;tdTom neurons were then observed via fluorescent and confocal microscopy and the specificity of this line was confirmed by immunohistochemistry using an anti-HDC antibody. We then carried out a systematic analysis of the passive and active membrane properties of both HA (tdTom+) and non-HA (td-Tom-) neurons in the TMN through cell-attached and whole-cell recordings at physiological temperature. Additionally, optogenetic silencing through the selective, cre-dependent expression of archaerhodopsin (Arch) in HA neurons was examined both *in vitro* and *in vivo* as a first step in probing the role of the HA system in arousal. Results: Analysis of these data suggest that the Hdc-Cre (GENSAT) line specifically expresses cre recombinase in the TMN. Electrically, the membrane properties of HA cells are relatively uniform while neighboring non-HA neurons displayed a heterogeneous range of membrane properties. Finally, HA neurons were manipulated and silenced through optogenetics. Conclusions/Significance: The Hdc-Cre (GENSAT) mouse line may be a valuable transgenic tool for both the identification of HA neurons for electrophysiological recording and for the expression of cre-dependent viral reagents for the optogenetic interrogation of HA neurons and their role in physiology and behavior.

Research/Grant Support: NIMH (R00MH097792)

The neurochemical phenotype of lateral hypothalamic hypocretin/orexin and melanin-concentrating hormone neurons identified through single-cell transcriptional profiling

***L.E. MICKELSEN[1]**, F.W. KOLLING IV[1,2], B. CHIMILESKI[1,2], C. NORRIS[2], C.E., NELSON[2], A.C. JACKSON[1]

[1]Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT; [2]Dept. of Molecular and Cellular Biology, University of Connecticut, Storrs, CT

Introduction: The lateral hypothalamic area (LHA) orchestrates fundamental aspects of behavior including arousal, feeding, metabolism, stress and reward. Through its unique position at the intersection of multiple neural and humoral systems, the LHA drives essential behavioral programs that maintain homeostatic balance in physiology and behavior. Underlying the diverse functions of the LHA is an exceptionally heterogeneous population of neuronal cell types, typically defined by their neuropeptide expression. Two well-described neuropeptidergic neuronal populations, exclusively found in the LHA, are defined by their expression of hypocretin/orexin (Hcrt/Ox) or melanin-concentrating hormone (MCH). Hcrt/Ox and MCH neuronal populations are both important regulators of the sleep-wake cycle and metabolic function. Mounting evidence indicates that these cell populations may be diverse and that single markers may not adequately capture the complexity of their signaling repertoire. Outstanding questions remain concerning the neurochemical phenotype of Hcrt/Ox and MCH neurons and, in particular, their co-expression of other neuropeptides and the machinery for the synthesis and release of the fast amino acid neurotransmitters GABA and glutamate. Hypothesis: We hypothesize that the neurochemical identity of LHA Hcrt/Ox and MCH neurons will be distinct based on their unique expression profiles of neuropeptides and fast neurotransmitter components. Methods: In the present study, we undertook a single-cell transcriptional profiling approach to further our understanding of the neurochemical phenotype of Hcrt/Ox and MCH neurons. Using transgenic mouse lines that label each cell population, we optimized methods for the microdissection of brain slices, followed by fluorescence-activated cell sorting (FACS) into 96-well plates for the isolation of individual labeled cells. We then carried out gene-specific reverse transcription to convert mRNA into cDNA followed by qPCR to quantify the expression of 48 key genes at single cell resolution. These include housekeeping genes, neuronal and glial markers, neuropeptides, fast neurotransmitter components, transcription factors, receptors and calcium-binding proteins. Results/Conclusions: Our single-cell transcriptional analysis of Hcrt/Ox and MCH neurons revealed unique expression patterns of neuropeptides and fast neurotransmitter components, demonstrating distinct neurochemical phenotypes. The transcriptional profiles of Hcrt/Ox and MCH neurons are consistent with known markers of each cell population while also exhibiting novel expression patterns. Significance: Identifying the underlying neurochemical phenotype of Hcrt/Ox and MCH neurons, and diversity within these cell populations, will further our understanding of how these neurons modulate postsynaptic excitability at their targets and participate in diverse behavioral outputs.

The Oncogenic Mutation B-RAF V600E Transforms the Physiology of Neocortical Pyramidal Neurons.

Goz. RU, LoTurco JJ.

University of Connecticut Physiology and Neurobiology Department Storrs, CT.

Introduction: The mechanisms by which long-term epilepsy associated tumors (LEATs) cause hyper-excitability of cortical tissue remain poorly understood. B-RAF V600E somatic mutation has been found in 20-58% gangliogliomas (GG), the most common LEAT found in resected brain tissue from young patients undergoing surgical treatment for drug refractory epilepsy. Hypothesis: One hypothesis proposed is that the somatic mutations associated with LEATs such as GG, may transform the physiology of neurons carrying those mutations into hyper-excitable states. Which prompted us to test whether or not induced BRAFV600E expression in cortical neurons *in vivo* would lead to epileptogenic phenotypes and hyper-excitable neurons in mouse neocortex. Methods: *In-Utero* Electroporation (*IUE*) with PiggyBac transposon system was utilized to transfect layer 2/3 pyramidal neurons of mouse neocortex with human pPB-BRAFV600E plasmid together with pPB-EGFP and, the donor plasmid – GLAST-PBase, with transposase element. For control experiments pPB-mRFP was used as a substitution of the mutated gene in additional animals. To investigate whether transfected with BRAF V600E gene mice has epileptiform activity – ElectroCorticoGraphic (ECoG) recordings were done on P45 mice. Immunohistochemical investigation was used to assess changes in neuronal and cortical morphology. *Ex-vivo* whole-cell patch-clamp experiments were performed on cortical slices and transfected as well as untransfected, neighbor, pyramidal neurons were recorded for the assessment of electrophysiological changes. Results: We found that expression of BRAF V600E resulted in focal cortical disruptions and electrographic seizures in mice. In whole cell patch clamp recordings of cortical neurons we found that BRAF V600E transfected neurons showed marked changes in intrinsic excitability relative to control pyramidal neurons including depolarized resting membrane potential (RMP), decreased Action Potential (AP) threshold, decreased rheobase, and increased AP kinetics at the negative phase (dV/dt). Neurons expressing BRAF V600E had high spontaneous spiking rates, and generated high frequency non-adapting AP patterns to depolarizing current pulses. Some BRAF V600E neurons also showed a distinct type of bursting behavior that was not observed in control pyramidal neurons. In addition BRAF V600E expressing neurons had increased voltage hyperpolarization SAG. We found an increase, relative to control-transfected cells, in the hyperpolarization and cyclic nucleotide activated current (I_h). Moreover, the elevated Ih in BRAF V600E expressing neurons could be significantly reduced to control cell levels by pre-incubation with the B-RAF inhibitor Vemurafenib suggesting that B-RAF overactivation is responsible for elevated Ih. Conclusion: Based on these results we propose that the same somatic mutation that may drive the formation of ganglioglioma, BRAF V600E, may also have a direct effect on pyramidal neuron excitability, and that this transformation in physiology is at least partially due to an elevation in Ih. Significance: These results suggest a possible mechanism for neuronal hyperexcitability in one of the most common LEATs, GG with the most frequent somatic mutation, BRAF V600E, and that this hyperexcitability can be decreased upon acute inhibition of this mutation with specific inhibitor - Vemurafenib.

Research Support: This work is supported by Grants from NIH/NICHD, UConn/IBACS, and the Munson Family foundation.

Inactivation of medial prefrontal cortex, dorsal, or ventral hippocampus during a temporal sequence task in a radial arm water maze

Shang Lin (Tommy) Lee, John Riley Pflomm, Mahathi Kumar, Etan J. Markus

Department of Psychological Sciences, University of Connecticut, Storrs, CT 06269

Introduction: The hippocampus and medial prefrontal cortex have been linked to memory formation, specifically working and episodic memories. These types of memories both have a temporal component. To further examine temporal processing we developed a temporal sequence order task in an 8-arm radial water maze. Rats experienced multiple maze sessions in the same room. Each session had a different fixed correct goal arm; however the room and maze were kept identical. Therefore to identify the correct goal for a given session one would need to remember the number of previous sessions already experienced that day. We have previously shown that rats can learn up to seven locations in this task, and these seem to be learned as a sequence with more errors in the middle sessions than in the beginning and end sessions.

Hypothesis: The current experiment used Muscimol to temporarily inactivate the dorsal/ventral hippocampus or medial prefrontal cortex in a within-animal repeated design. Dorsal hippocampus has been shown to be primarily responsible for spatial navigation, whereas ventral hippocampus is suggested to be more important for the processing of emotional memories. Medial prefrontal cortex has been shown to play a role in temporal sequences and rule learning.

Methods: Male F-344 rats (N=9), approximately six months old, were trained in an eight-arm radial water maze with a removable escape platform. Rats were taught the first arm in the sequence, and additional arms were added when rats mastered the previous arm(s). Prior to surgery, rats were trained on a three-arm sequence. Post surgery after their performance stabilized, the animals were tested under muscimol and control conditions. Animal testing concluded with infusions of fluorescent muscimol to visualize the spread of inactivation.

Results: After initial exposure to the maze and task, swim latencies stabilized to five seconds for a rat to select their first arm choice. Rats successfully selected the correct goal location in less than one-minute. Initial results indicate that medial prefrontal cortex inactivation impaired performance.

Conclusions: The complete finding and implications will be presented and discussed within the context of temporal processing in medial prefrontal cortex, dorsal, or ventral hippocampus.

Significance: These results suggest medial prefrontal cortex plays a crucial role for spatial navigation in a temporal sequence task.

Research support: UCRF, PCLB Foundation

Differential proliferative response of white and gray matter NG2 cells to PDGF-AA is partly regulated by microglia Neuropilin-1

Authors: Mohammad Amin Sherafat, William Wood, Eeman Abbasi, and Akiko Nishiyama

Affiliation: Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT.

Introduction- NG2 cells or polydendrocytes comprise a major cell type in the developing and mature central nervous system (CNS), which have essential role to provide myelin sheaths for neural network by giving rise to oligodendrocytes. The gray and white matter have uniform distribution of NG2 cells in a tiled fashion, and their proliferation and differentiation are tightly regulated to maintain homeostasis of oligodendrocyte density. However, NG2 cells in the white matter proliferate and differentiate into oligodendrocytes at a greater rate than those in the gray matter with unclear mechanism. We have shown that NG2 cells in the white matter proliferate significantly more in response to platelet-derived growth factor AA (PDGF) when added to slice cultures, despite similar levels of platelet-derived growth factor receptor alpha (PDGFRa) expressed on their surface. Neuropilin-1 (Nrp1) has been shown to interact with Pdgfra on vascular smooth muscle cells. Microglia are resident immune cells in the CNS that actively participate in neuronal homeostasis and express Nrp1. In this study we investigated the role of microglia Nrp1 in mediating the differential response of NG2 cells in white and gray matter to PDGF.

Method- To examine the role of microglial Nrp1 on PDGF-dependent NG2 cells proliferation, slice cultures were prepared from Nrp1 inducible conditional knockout mice ($Nrp1^{flfl}$) crossed to a microglial Cre driver mouse line (Cx3CR1-creERT2).

Result- Incubating slice cultures with function-blocking antibodies to Nrp1 resulted in a dose-dependent reduction of PDGF-dependent proliferation of NG2 cells in white but not gray matter. Nrp1 is detected on endothelial cells in both gray and white matter and is also colocalized with F4/80+ microglia in the corpus callosum of postnatal day 8 mice. Knocking out of Nrp1 at P5 mg-$Nrp1^{flfl}$ mice showed a significant decrease in corpus callosum endogenous NG2 cell proliferation. Moreover, slice cultures prepared from 4-hydroxytamoxifen-treated mice exhibited reduced PDGF-mediated proliferation of NG2 cells in the corpus callosum but not in the neocortex.

Conclusion- Our data suggests that microglia Nrp1 activates Pdgfra in a cell non-autonomous manner in the white matter to induce NG2 cell proliferation.

Research/ Grant Support- NIH (PI: Professor Nishiyama)

Spike-timing neural discrimination of sound envelope shape in primary and non-primary auditory cortices

A. F. OSMAN[1] Monty Escabi[1,2] and *H. L. READ[1,3]

(1) Biomedical Engineering. Univ. of Connecticut, Storrs, CT
(2) Electrical Engineering. Univ. of Connecticut, Storrs, CT
(3) Psychology, Behavioral Neuroscience. Univ. of Connecticut, Storrs, CT

Changes in the sound envelope amplitude over time provide perceptual shape cues to identify and discriminate sounds (Iverson and Krumhansl 1993; Irino and Patterson 1996, Drullman, Festen et al., 1994; Geffen et al., 2011). Mammals need cortex to detect many temporal sound cues; however, the underlying thalamo-cortical circuit and neural coding mechanisms for this ability remain unknown. Here we ask whether these spiking patterns can be used to discriminate different sound shapes and whether primary (A1) and non-primary auditory cortices discriminate similarly. Indeed, we find spike-timing patterns can be used to discriminate sound in A1 and non-primary cortices. Furthermore, non-primary cortices have sustained spiking that contributes to discrimination; whereas, A1 does not. This suggests parallel and distinct mechanisms for discriminating sound shape in A1 and non-primary cortices.

State-Feedback Regulation of Deep Brain Stimulation in a Computational Model of Parkinson's Disease

Stefan L. Sumsky and Sabato Santaniello

Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269

Introduction: Recent evidence indicates that evoked compound action potentials (ECAP) and local field potentials are viable feedback variables for closed-loop deep brain stimulation (DBS). Current closed-loop DBS schemes, however, either follow a binary paradigm (i.e., the feedback variable determines when to switch the stimulation ON, while the DBS settings are fixed a priori) or periodically adjust the stimulation parameters based on known rhythms in the feedback variable, which may limit the overall performance of the closed-loop. Hypothesis: ECAPs in thalamus reflect synchronized neural activation due to Parkinson's disease and DBS pulses. A more normal activity is restored in thalamus as a consequence of therapeutic DBS. We hypothesize thalamic LFPs may be a viable feedback variable for real-time adaptive DBS. Methods: We propose a model-based closed-loop scheme for real-time adaptation of the DBS settings. A state-space model of the neural activity is estimated from post-stimulus ECAP recordings and the model parameters are used to formulate a linear-quadratic regulation problem. The solution is a state-feedback control strategy that adjusts the DBS signal based on past ECAP measurements and stabilizes the control loop while minimizing the magnitude of the DBS signal. Results: Applied to a computational model of the ventral intermediate thalamus under tremulous Parkinson's disease, the proposed strategy decreased the required DBS frequency, while still decreasing the neural activity in the band [4-7] Hz (which is a signature of parkinsonian tremor), and restored a more normal spiking pattern in thalamocortical neurons, while the DBS signal remained charge-balanced and low-amplitude. Conclusions: Decreased DBS frequency can produce effective reductions in characteristic tremor frequency when under adaptive closed-loop control. Significance: These results show the viability of thalamic LFP as a potential effective feedback variable for closed-loop control in Parkinson's disease.