2021 Visiting Student Symposium

Book of Abstracts

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Program

SESSION I

Neuronal substrates of Rapid Eye Movement sleep disruption after sevoflurane anesthesia

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Background: Sleep disturbances are a common clinical problem after anesthesia and surgery. In particular, disruption of Rapid Eye Movement sleep (REMS) promotes hyperalgesia, hypoxemia, and hypoventilation in postoperative patients, and is a risk factor for myocardial infarction, stroke and death. Given REMS central role in executive cognition and memory, postoperative REMS disturbances may also be implicated in the pathogenesis of delirium. Recent experimental evidence shows that sevoflurane (SEVO) alone - in the absence of surgery - causes REMS rebound in rodents, suggesting that SEVO may impact endogenous neuronal networks that govern REMS. However, the specific REMS circuits affected by SEVO remain elusive. Thus, we aimed to resolve which REMS-associated neuronal populations are targeted by SEVO.

Approach: Adult male and female rats were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes, and randomized to receive 2.8% SEVO in 75% O_2 for 3 h or control conditions (75% O_2 for 3h) after a minimum of 12 recovery days. Twenty-four hour-long EEG/EMG recordings were obtained after SEVO, and one week later. Signals were scored automatically, then manually by two independent investigators. Next, a Targeted Recombination in Active Populations (TRAP) approach and conventional c-Fos immunohistochemistry (IHC) were used to identify regions of the brain that were active during SEVO-induced REMS rebound. Brains were processed 8-10 h after SEVO. TRAP brains were treated with CLARITY, while IHC sections were subjected to free-floating c-Fos labelling. Fos+ neurons were quantified using ImageJ and Imaris software. Investigators were blinded to experimental conditions.

Results: SEVO-exposed animals exhibited a 30% increase in REMS after SEVO, and decreased REMS latency. No differences in REMS quantity or latency were found one week later. The sublaterodorsal (SLD) nucleus, i.e., the midbrain REMS-generator, exhibited a robust increase in Fos+ neurons in SEVO-exposed animals compared to controls. Conversely, Fos+ neurons were markedly reduced after SEVO in the ventrolateral periaqueductal gray (vlPAG), a pontine nucleus that tonically suppresses REMS. The pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei, i.e., two basal forebrain regions that are critical for REMS maintenance, also exhibited a significant increase in the number of Fos+ neurons after SEVO.

Conclusions: We found hypo-activity of the vlPAG REMS-suppressing neurons, and activation of the SLD REMS-generating neurons, in rodents displaying REMS rebound after SEVO. Our findings are consistent with existing literature implicating the vlPAG and SLD brain regions in the control of REMS and with the notion that a REMS debt accrues in rodents anesthetized with SEVO.

Metabolic regulation of seizures

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Background: Epilepsy is a common neurological disorder, which affects more than 50 million people worldwide. An especially life-threatening condition is status epilepticus, characterized by abnormally prolonged seizures. Unfortunately, almost 1/3 of patients are or become resistant to current treatments.

High-fat, low carbohydrate metabolic therapy, commonly called ketogenic diet (KD), reduces seizures in patients with refractory drug epilepsy and showed encouraging results in status epilepticus. However, the mechanism of stopping seizures remains elusive. KD leads to the production of ketone bodies (KB): β-hydroxybutyrate (BHB), acetoacetate (ACA), and acetone, from free fatty acids in the liver. Few mechanistic hypotheses about how KD might work in epilepsy include the role of KB as direct anti-seizures agents, changes in ion channel regulation, and neurotransmitter systems. Hydroxy-carboxylic acid receptor 2 HCA_2 (also known as GPR109A), G_i protein-coupled receptor which BHB activates, is known to be expressed on adipocytes, neutrophils, and in the brain. It might be a possible mechanism that allowed BHB to act as a signaling molecule in the brain.

Objective: I investigated the effect of BHB on status epilepticus duration, EEG signal power spectrum, and behavior.

Methods: We used continuous hippocampal stimulation (CHS) model in C57/Bl6 mice to induce status epilepticus. Fifteen minutes after the end of stimulation, we intraperitoneally (IP) injected either 1mg/kg BHB or saline.

Results: Our preliminary data suggest that there is a trend towards shorter SE after BHB administration. EEG signal recordings, together with behavior data analysis, suggest that after BHB injection, mice tend to have less severe seizures compared to saline controls.

Conclusions: Our data imply that BHB might have a suppressive effect on seizures. Further studies should involve experiments with HCA_2-receptor knock-out mice. This strategy might help to understand the mechanism underlying KD anticonvulsant efficacy.

Development of synapses in motor cortex

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Background: Hypoxic ischemic encephalopathy (HIE) is a disease that affects 3/1000 newborns annually. It is a neurologic injury caused by acute loss of blood and oxygen flow to a baby's brain and is the most common cause of death and disability in neonates. HIE can lead to seizures, cognitive impairment, and motor deficits, such as cerebral palsy. HIE occurs during a very critical period when synapses are forming. We proposed that HIE disrupts the formation and maturation of motor cortex synapses which can lead to permanent motor impairment. There are no previous studies of the development of excitatory and inhibitory synapses in the motor cortex.

Objective: The aim of this study was to: 1) compare synaptic development in injured and normally developing brains between postnatal day (p)10 (neonatal) and p30 (adults) and 2) examine differences in motor learning in injured and uninjured adult mice.

Methods: There were three groups of mice: normally developing (control), neonatal HIE model (unilateral carotid artery ligation+60 min of 8% O_2 on p10), and sham mice (anesthesia+neck incision only). We used immunohistochemistry for pre and postsynaptic markers to quantify and localize GABAergic and glutamatergic synapses. Western blots to examine differences in protein production in the motor cortex. Patch-clamp biotinylated filled cells to assess their growth and length of dendrites. Lastly, mice underwent a complex running wheel task to test motor learning.

Results: We observed an increase in pre and postsynaptic markers from p10 to p30 by immunohistochemistry and Western blots. There was more intense staining, differences in the distribution within layers of the motor cortex, and differences in dendrite length and number. Motor cortex layers were not as apparent in p10 brains as in p30 mice. Western blot data shows that there's more production of pre and postsynaptic receptors in p30. Dendrites in p30 were more abundant and more prolonged compared to p10.

Conclusion: Preliminary data suggest differences in synaptic maturation between neonatal and young adult mice in normal developmental conditions. Ongoing work is to quantify measures of synaptic development of the motor cortex and motor learning following neonatal HIE.

SESSION II

Biomolecular condensation of cancer mutants of an epigenetic regulator

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Permeability of Pannexin1 ion channel

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Background: Pannexin channels are newly found transmembrane ion channels topologically similar to connexins. They are widely expressed, including in neuronal cells. Although they are capable of forming gap junctions under some circumstances, they are believed to function in native settings as hemi-channels. They are most renowned for supporting ATP release and purinergic signaling in various physiological contexts. It has been claimed that Pannexin 1 (Panx1) is an anion-selective channel based on electrophysiological experiments and, more recently, cryo-EM structural studies. However, other studies have demonstrated large molecule permeation through purified Panx1 (e.g, fluorescent dyes up to 980 Da), including large cationic dyes. The possible permeability of smaller Ca^{2+} ions via Panx1 has only been investigated in cell experiments, where other permeation paths could not be excluded. It was proposed that Panx1 can be activated by elevated intracellular Ca^{2+} although the mechanism is unclear: Panx1 has no canonical Ca^{2+} binding domains and its activation is unaffected by physiological Ca^{2+} concentrations.

Objective: The aim of this project is to determine Ca^{2+} permeability (and maybe gating) of purified *Xenopus* Panx1 (fPanx1) reconstituted in proteoliposomes; this controlled experimental environment will test fPanx1 Ca^{2+} permeability and gating while eliminating uncertainities with cell experiments.

Methods: A fPanx-eGFP construct was expressed from pFastBac in Sf9 cells, membranes were isolated by Dounce homogenisation, and the protein was purified in Econo-column and Size-Exclusion Chromatoghraphy. Creation of membrane, creation of liposomes, sonication, protein incorporation into liposomes, dialysis. Dye loading: Freeze - thaw, extrusion, purifying liposomes from extraliposomal dye by G-25 Sephadex column and centrifugation. Casp-3 activation. Fluorescent assay, Western Blot, gel electrophoresis, Nycodenz float assay, silver staining.

Results: Fluorescence measurements have given confounding results. We have been able to eliminate non-specific fluorescence from liposomes and buffers, and buffered trace amounts of Ca^{2+} with EDTA. This is moving us towards greater confidence that our fluorescent measurements will faithfully report Ca^{2+} flux via fPanx1

Conclusions: Additional controls and repetitions are required to determine whether fPanx1 is permeable to, or activated by Ca^{2+}. In light of the key role Ca^{2+} plays as a cellular chemical messenger, these results will provide physiologically relevant information regarding Panx1 and Ca^{2+} signaling.

The role of G4R1 helicase and DNA G-quadruplex structures in the formation of DNA double-strand breaks

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Background: Genomic regions with the potential to form highly stable DNA secondary structures are enriched for intrinsic DNA double-strand breaks (DSBs) in human cells. Unrepaired DSBs can contribute to the formation of gene rearrangements or amplifications leading to genome instability. G-quadruplexes (G4s) are one of the noncanonical four-stranded secondary structures formed by particular G-rich nucleic acids, that lead to the DNA breakage through their role in DNA replication and transcription. G4s can occur in the promoter regions of oncogenes, such as *MYC*, which make them an attractive therapeutic target.

Objective: G4R1/DHX36 helicase is one of the major enzyme that resolves array of G4s. The aim of our study is to characterize the role of DNA G-quadruplex structures in the formation of DSBs through this enzyme.

Methods: Using genome-wide break mapping and sequencing protocol at the single-nucleotide resolution, we examined two Jurkat G4R1 knockout (KO) cell lines along with the unmanipulated control. Sequencing reads were then processed and analyzed with the pipeline developed in the Wang lab and also with BEDtools and standard Linux commands.

Results: Comparing to the control, KO of G4R1 helicase in Jurkat cell lines results in the accumulation of DSBs at transcription start sites (TSSs) in a expression-dependent manner and at regions that form G4 structures.

Conclusions: KO of G4R1 affects stability of G4s resulting in higher number of DSBs at TSSs, suggesting a role of this structures in the regulation of gene expression. Unresolved G4 structures can promote or repress the transcription. This preliminary data can utilize the path to design a G4s-interacting small molecules that can control gene expression in therapeutic fields such as cancer.

SESSION III

Identification of HULLK binding partners to determine mechanism of HULLK oncogenicity in prostate cancer

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Background: Prostate cancer (PCa) is the most commonly diagnosed cancer among men worldwide. Treatment options for PCa patients with aggressive disease include androgen deprivation therapy (ADT) which takes advantage of the dependency of prostate cancer cells for androgens. However, as a result of ADT therapy, castration-resistant prostate cancer (CRPC) will inevitably arise. Thus, searching for new therapeutic options is necessary. Recently, a new long-non coding RNA within LCK (HULLK) was discovered. Expression of HULLK is upregulated by androgen and HULLK overexpression increased proliferation of PCa cell lines whereas, HULLK knock down decreased proliferation. Expression of HULLK positively correlates with Gleason score of PCa patients. All these observations suggest a significant role of HULLK in oncogenesis of PCa.

Objective: Aim of this study is to identify HULLK binding partners to determine the mechanism of HULLK driving prostate cancer oncogenesis.

Methods: In order to identify HULLK binding partners, the MS2 trap method was used. Constructs carrying HULLK tagged with either 2x or 12x MS2 loops (HULLK-MS2) or MS2 protein tagged with HA (MS2-HA) were generated and verified by sequencing. Next, the prostate cancer cell line LNCaP was cotransfected with both constructs. HULLK expression was measured by qPCR and MS2 protein presence was examined by Western Blot. HULLK pull-down was performed using anti-HA magnetic beads which allow for capturing the HULLK-MS2 loop interacting with MS2-HA protein. The efficacy of the method was estimated by qPCR.

Results: Coexpression of HULLK-MS2 loops and MS2-HA was confirmed by qPCR and Western Blot, respectively. MS2 trap method is applicable for exogenous HULLK pull down, no significant RNA degradation was observed.

Conclusions: MS2 trap method can be used for exogenous HULLK pull down to identify HULLK binding partners.

Nuclear Cytoplasmic trafficking of the SARS-CoV-2 Nucleocapsid protein

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Background: SARS-CoV-2 is a novel coronavirus that emerged in 2019, causing a serious pandemic of acute respiratory disease (COVID-19). As in other coronaviruses, nucleocapsid (N) is one of the most significant structural components of the SARS-CoV-2 virus particle. It also has an essential role in virus replication and assembly, which takes place entirely in the cytoplasm of infected cells. However, unexpectedly, some coronavirus N proteins have been shown to traffic to the nucleus.

Objective: To investigate if the SARS-CoV-2 N protein can traffic between the nucleus and cytoplasm.

Methods: To study N trafficking, we made lentiviral vectors expressing N mutants that individually had changes in 3 amino acids in two potential nuclear export signals (NES1 and NES2) or in both signals (NES1/2). Vector stocks expressing the mutant and wild type proteins were transduced into 293T and HeLa cells. N protein localization was then analyzed using fluorescent microscopy, image flow cytometry, and western blotting. Interactions between either the wild type or mutant protein N and host cell proteins were also analyzed by immunoprecipitation of tagged N proteins and LCMS.

Results: Our data suggest that the mutations in either NES1 or NES2 increase the steady-state levels of N protein in the nucleus by about 40%. We also identified multiple host cell protein binding partners of both the mutant and wild-type proteins.

Conclusions: Our findings support the hypothesis that SARS-CoV-2 N can shuttle between the nucleus and cytoplasm of infected cells. Further understanding of this and how it might affect host cell gene expression has the potential to lead to future novel therapeutic strategies.

SESSION IV

Multi-ethnic genome-wide meta-analysis in type 1 diabetes

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Background: Type 1 diabetes (T1D) is a chronic autoimmune disorder in which the pancreatic □-cells have been destroyed, requiring exogenous insulin for survival. Multiple genetic and environmental risk factors are involved in development of T1D. To date over 50 loci associated with the risk of developing T1D have been identified, yet limited genome-wide analyses have been completed over the last decade.

Objective: The aim of this study is to use genome-wide analyses to identify genomic regions and candidate genes that influence risk of T1D.

Methods: A total of 3,222 families (affected sib-pairs and trios, 11,476 individuals), the majority of European ancestry, 891 individuals of African ancestry (AFR, 409 T1D cases, 482 controls) and 308 individuals from Admixed populations (AMR, 153 T1D cases, 155 controls) were genotyped on the Illumina HumanCoreExome Beadarray. A total of 430,930 single nucleotide polymorphisms (SNPs) passed quality control metrics. Additional SNPs and insertion/deletion (InDel) polymorphisms were obtained using imputation (TOPMed reference panel). The Generalized Disequlibrium Test (GDT) was used to analyze the family data, and logistic regression models were used to analyze the case-control data. Meta-analysis (Metal software) was used to combine results and to identify SNPs and InDels associated with T1D, using a genome-wide significance threshold of $P < 5.0 \times 10^{-8}$.

Results: Following imputation, 8,711,967 common variants (minor allele frequency, MAF, > 0.01) were used in meta-analysis. There were 8 genomic regions (loci) with SNPs/InDels significantly associated with T1D. As expected, the most significantly associated SNP with T1D (rs9273364, $P = 2.94 \times 10^{-501}$) resides in the MHC region (*HLA-DQB1*) on 6p21.32. Several known genomic locations associated with T1D have been confirmed, including *INS* (rs3842753, $P = 1.35 \times 10^{-49}$, in complete LD with known *INS* SNP rs689) on 11p15.5, *PTPN22* (rs2476601, $P = 3.45 \times 10^{-26}$) on 1p13.2, *SH2B3* (rs3184504, $P = 1.05 \times 10^{-18}$) on 12q24.12, *ERBB3* (rs705708, $P = 1.60 \times 10^{-14}$) on 12q13.2, *RNLS* (rs3781197, $P = 4.01 \times 10^{-12}$) on 10q23.31 and *CTLA4* (rs3087243, $P = 1.82 \times 10^{-9}$) on 2q33.2. Two regions (*FAM43A*, 3q29, novel; *IL2RA*, 10p15.1, known) have suggestive ($P < 1.00 \times 10^{-7}$) evidence of association with T1D.

Conclusions: These findings suggest that inclusion of subjects with diverse genetic ancestry can identify new and refine known regions of the genome contributing to risk of T1D, and the results can aid in understanding the biology underlying type 1 diabetes.

A Disulfide chaperone knockout facilitates *in-vivo* spin-labeling and EPR spectroscopy of the Escherichia coli ferric citrate transporter, FecA

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Background: TonB-dependent transporters are an important group of outer-membrane proteins that transport trace nutrients into Gram-negative bacteria, with high affinity and specificity. They have been studied extensively over the last few decades but the exact mechanism of transport is still not well understood. Previous research from the Cafiso lab has shown that it is possible to examine the dynamics and structure of double spin labeled mutants of the cobalamin transporter BtuB in cells, utilizing a DsbA knockout strain. It has also been shown that BtuB behaves differently *in-vivo* than it does in a purified, reconstituted membrane system, yielding information on the transport mechanism. Analyzing TBDTs and other outer-membrane proteins in a whole cell system can give us valuable insight into how these proteins function in their natural environment.

Objective: The aim of this work was to determine whether measurements in cells can be extended onto other TBDTs, in this case the ferric citrate transporter FecA, and used to characterize conformational differences between apo and holo states *in-vivo*.

Methods: Mutants of FecA were constructed and a spin labeled side chain was attached to pairs of cysteine residues on both the extracellular and periplasmic surfaces of the protein. Differences between apo and holo states were then examined by Continuous Wave EPR and Double Electron-Electron Resonance for distance measurements. FecA expression was controlled through arabinose induced expression of the T7 RNA polymerase from the pTARA plasmid.

Results: The *dsbA*$^{-}$ strain facilitates in-vivo spin labeling of FecA double mutant sites, otherwise impossible in a WT strain. Arabinose controlled pTARA system enables regulation of mutant overexpression.

Conclusions: The DsbA knockout intact cell system can be extended to studies of other TBDTs and outer membrane proteins.

Biochemical studies on Nup358 and its interactions with HIV-1 capsid proteins

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Background: Human Immunodeficiency Virus (HIV) is one of the most extensively studied retroviruses, both because of its interesting biological properties and the fact, that it imposes danger to millions of people (~38 million people infected, ~69 thousand died from AIDS-related illnesses; according to UN statistics from 2020). Like other lentiviruses, it has the ability to infect non-dividing cells which requires hijacking of nucleopores being large protein complexes responsible for the trafficking of various biomolecules through nuclear envelope. Despite years of studies, many questions about HIV-1 nuclear transport remain unanswered but the fact that it is one of the most crucial points of the HIV lifecycle is not debatable. Nup358 is one of the nucleoporins building the nucleopore complex and was identified as an important factor for HIV infectivity. Characterization of two C-terminal domains of Nup358, namely, RAN Binding Domain IV (RBD4) and Cyclophilin-like Domain (Cyp) and their interactions with HIV-1 capsid protein (CA) seems to be helpful for a deeper understanding of HIV-1 nuclear transport mechanism.

Objective: The aim of this study is to characterize interactions between RBD4 and Cyp domains of Nup358 with HIV-1 capsid protein (CA) and its mutants.

Methods: Various biochemical and biophysical methods were used to characterize studied proteins and their interactions, such as Isothermal Titration Calorimetry (ITC), Bio-layer Interferometry (BLI), analytical Size Exclusion Chromatography (SEC), Nano Differential Scanning Fluorimetry (nanoDSF), Pull Down experiments and Electron Microscopy (EM). Basic molecular biology techniques were used to obtain desired mutants and protein domains used for studies.

Results: Studies have shown that properties of RBD4/Cyp construct in the context of interactions with CA differ from Cyp domain alone. The most interesting result is that, unlike Cyp and Cyclophilin A, Nup358-RBD4/Cyp does not show binding to unassembled CA protein and its individual N-terminal domain. However, experiments showed, that the construct being the topic of the study can bind to CA penta- and hexamers as well as to CA assemblies such as tubes.

Conclusions: Behavior of Cyp domain coupled with RBD4 seems to be less similar to cyclophilin A then independent Cyp. However, it is still able to interact with CA multimers and assemblies. Further investigation on a structural level will be helpful to understand this phenomenon.