האגודה הישראלית לפיזיולוגיה ופרמקולוגיה
ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY

THE ISPP 2019 ANNUAL MEETING

ABSTRACT BOOK

Sponsored by:

JBC Jerusalem Brain Community

המרכז לחקר הכאב
האוניברסיטה העברית בירושלים
THE HEBREW UNIVERSITY OF JERUSALEM
CENTER FOR RESEARCH ON PAIN

INSTITUTE FOR DRUG RESEARCH
המכון למדעי התרופה
The Hebrew University of Jerusalem
האוניברסיטה העברית בירושלים
School of Pharmacy
בית הספר לרוקחות

BLAVATNIK CENTER
for Drug Discovery
מרכז בלווטניק
לפיתוח תרופות

DanyelBIOTECH

alomone labs
Your partner in ion channel research

Eisenberg Bros. Ltd.
אחים איזנברג בע"מ

NBT
New Biotechnology Ltd.

February 14th, 2019, Mishkenot Sha'ananim, Jerusalem

Meeting Program

Throughout the meeting, each session will consist of three short (25 minutes) presentations by PIs and one short lecture (20 minutes) presented by research students and postdocs.

8:00-9:30 – Registration

9:30-11:10 – Parallel sessions

Session 1 (big hall)

Physiology of cellular signaling

Chairs: Prof. Sivan Korenblit (Bar-Ilan University), Prof. Michal Hershfinkel (Ben-Gurion University)

9:30-9:35 – Introduction

9:35-10:00 – Meital Oren (Weizmann Institute of Science): Dimorphic connectivity is established by sex-specific synapse pruning during development

10:00-10:25 – Tami Geiger (Tel-Aviv University): Proteomic analysis of melanoma response to immunotherapy

10:25-10:50 – Adi Kimchi (Weizmann Institute of Science): The protein interaction map of programmed cell death: from basic principles to a therapeutic vision

10:50-11:10 – Eran Schmukler (Tel-Aviv University): Altered autophagy and mitochondrial dynamics in APOE4-expressing astrocytes

Session 2 - In Memory of Prof. Abraham Haim (University of Haifa) (small hall)

Environmental Stress: preconditioning and adaptive memory

Chair: Prof. Michal Horowitz (The Hebrew University)

9:30-9:35 – Introduction

9:35-10:00 – Noam Meiri (Volcani Center): Early-life heat exposure influence resilience or vulnerability to heat stress later in life by an epigenetic mechanism

10:00-10:25 – Mirit Eynan (Israel Naval Med Institute): Day night activity at the underwater environment

10:25-10:50 – Oren Froy (The Hebrew University): Timing of macronutrient intake: underlying mechanisms and endocrine outcomes

10:50-11:10 – Oded Rechavi and Itamar Lev (Tel-Aviv University): Transgenerational inheritance of small RNAs in C. elegans

11:10-11:30 – Coffee break

11:30-12:30 – Plenary Lecture 1

ANALYSIS OF THE FIRST HUMAN LOSS OF FUNCTION MUTATION IN TRPV1: IMPLICATIONS ON THE ROLE OF TRPV1 IN INFLAMMATORY PAIN

Prof. Baruch Minke (The Hebrew University)

12:30-13:30 – Lunch

13:00-13:30 – General (business) meeting of the society members:

1) ISPP financial report and approval of changes in the bylaws.

2) Report from Prof. John Finberg, Chairman of the Inter-University Forum for Biomedical Research in Israel

13:15-14:30 – Poster sessions

Every poster presenter will give a 5-min oral presentation at the poster, to a committee composed of senior scientists; 3 prizes (500$, 300$, 200$) will be awarded to best posters presented by students.

14:30-16:10 – Parallel sessions

Session 3 (big hall)

PHYSIOLOGY AND PATHOPHYSIOLOGY OF EXCITABLE TISSUES

Chair: Dr. Moran Rubinstein (Tel-Aviv University)

14:30-14:35 – Introduction

14:35-15:00 – Ilya Fleidervish (Ben Gurion University): Mechanism of persistent Na^+ current generation in axon initial segment of layer 5 pyramidal neurons.

15:00-15:25 – Bernard Attali (Tel Aviv University): Intrinsic and synaptic homeostatic interplay triggered at different temporal scales by M-current inhibition in hippocampal neurons

15:25-15:50 – Firas Mawase (Technion): Improving hand dexterity in chronic stroke through training out of abnormal hand synergy

15:50-16:10 – Ana Turchetti-Maia (Tel Aviv University; Moran Rubinstein lab): Visual system dysfunction in Dravet Syndrome epilepsy

Session 4 (small hall)

AGING

Chair: Prof. Mickey Scheinowitz (TAU) and Prof. Yael Yaniv (Technion)

14:30-14:35 – Introduction

14:35-15:00 – Sivan Henis-Korenblit (Bar-Ilan University): Endo-siRNA induced inactivation of a neddylation suppressor promotes longevity and HSF-1 activation in germline-less animals

15:00-15:25 – Itamar Harel (Hebrew University): The African turquoise killifish: a model for exploring vertebrate aging and diseases in the fast lane

15:25-15:50 – Mickey Scheinowitz (Tel Aviv University): Physiological effects of exercise with aging

15:50-16:10 – Joachim Behar (Technion): Pacemaker from aged perspective

16:10-16:30 – Coffee break

16:30-16:45 – Announcement of the Garty Student Prizes for best posters.

16:45-17:45 – Magnes Plenary Lecture

Introduction: Prof. Nathan Dascal

NEURONAL CALCIUM CHANNEL TRAFFICKING AND FUNCTION: RELEVANCE TO CHRONIC PAIN

Prof. Annette Dolphin (University College London)

ISPP ORAL PRESENTATIONS

SESSION 1

Physiology of cellular signaling

Chairs: Sivan Korenblit (BIU) and Michal Hershfinkel (BGU)

DIMORPHIC NEURONAL CONNECTIVITY IS ESTABLISHED BY SEX-SPECIFIC SYNAPSE PRUNING DURING DEVELOPMENT

Oren-Suissa M.

Weizmann Institute, Department of Neurobiology, Israel.

meital.oren@weizmann.ac.il

In sexually reproducing species, males and females respond to environmental sensory cues and transform the input into sexually dimorphic traits. These dimorphisms are the basis for sex-biased phenotypes in many neurological diseases. Yet, complete understanding of the underlying mechanism is still missing. How does the sexual identity impose molecular changes to individual neurons and circuits? What are the sex-specific synaptic changes that occur during development in these circuits? We recently demonstrated a sexually dimorphic dimension of neuronal connectivity: neurons belonging to the shared nervous system in C. elegans rewire in a sex-specific manner to generate sexually dimorphic behaviors. Sex-shared neurons initially connect in a neutral manner to form a similar synaptic pattern in both the male and the hermaphrodite, but upon sexual maturation each sex prunes a specific subset of connections, resulting in sex-specific connectivity patterns and dimorphic behaviors. We will demonstrate the involvement of the ubiquitin-proteasome system (UPS) in sex-specific synapse pruning, and discuss how the sexual identity intersects with signaling pathways to confer spatial and temporal specificity for synapse elimination.

PROTEOMIC ANALYSIS OF MELANOMA RESPONSE TO IMMUNOTHERAPY

Geiger T.[1], Harel M.[1], Ortenberg R.[2], Mardamshina M.[1], Markovits E.[2], Baruch E.N.[2], Markel G.[2]

[1]Department of Human Molecular Genetics and Biochemistry, The Sackler School of Medicine, Tel Aviv University, Israel. [2]Ella Lemelbaum Institute of Immuno-Oncology, Sheba Medical Center, Israel.

geiger@tauex.tau.ac.il

Immunotherapy has revolutionized cancer treatment, nevertheless most patients do not respond to treatment. Here, we investigated mechanisms of response by deeply profiling the proteome of clinical samples from stage IV melanoma patients that either responded or did not respond to treatment with checkpoint inhibitors targeting PD1, or treatment with tumor infiltrating lymphocytes (TIL)-based immunotherapy. Using high-resolution mass spectrometry, we quantified over 9,500 proteins with high accuracy. Statistical analyses revealed higher oxidative phosphorylation and lipid metabolism in responders to both treatments. Interestingly these signals highly correlated with interferon signaling and pathways of antigen presentation. Aiming to elucidate the effects of the metabolic state on the immune response, we examined the proteome of a melanoma cell line upon metabolic perturbations and upon Crisp-Cas9 knock out of beta-oxidation genes. These analyses showed that lipid metabolism and oxidative phosphorylation affect cancer cell immunogenicity by regulating antigen presentation. Co-culture with T-cells showed that these pathways are important for T-cell mediated anti-tumor effect. Last, in-vivo analyses showed that knock-out of lipid metabolism proteins increases tumor growth due to reduced anti-tumor immune response. Altogether, melanoma proteomics revealed a novel mechanism that regulates tumor immunogenicity and may serve as the basis for better tumor targeting.

THE PROTEIN INTERACTION MAP OF PROGRAMMED CELL DEATH: FROM BASIC PRINCIPLES TO A THERAPEUTIC VISION

Kimchi A., Dekel N., and Gilad G.

Department of Molecular Genetics, Weizmann Institute of Science, Israel.

adi.kimchi@weizmann.ac.il

Apoptosis, necroptosis and autophagy are distinct biological processes which control life/death decisions of the cell, each driven by a different set of protein-protein interactions. We delineated a protein connectivity map of programmed cell (PCD) comprising 80 proteins from these three modules. To assess the dynamics of the global profile of protein interactions in cells, we have adapted the Protein fragment Complementation Assay (PCA), which monitors binding between proteins fused to complementary fragments of a luciferase reporter. A library encompassing the proteins from these three modules, and some of their regulatory proteins, was constructed for the analysis of ~4000 protein-pair combinations. This generated a detailed landscape of the global protein connectivity in the cells, identifying 46 previously unknown sets of direct protein-protein interactions within and between the PCD modules. Next, the emerging landscape of the global map was used for planning strategies which measure the 'functional cell death signature' of tumor cells by identifying points of vulnerability (soft spots) in individual patient's tumor. It comprises a sub library of 80 siRNAs directed against the components of the PCD map, trained to identify hits in the network that significantly increase cell death in tumors exposed to targeted therapy. We analyzed a cohort of metastatic melanoma tumors carrying the BRAF mutation treated in culture with the approved BRAF inhibitory drug. Results point at the heterogeneity in the 'functional death signature' of each tumor and at our success to identify promising targets in one or more of the different PCD modules for getting efficient cell killing and reduced probability of getting acquired drug resistance. This opens a new direction in the field of precision cancer therapy.

ALTERED AUTOPHAGY AND MITOCHONDRIAL DYNAMICS IN APOE4-EXPRESSING ASTROCYTES

Schmukler E.[1], Simonovitch S.[1], Solomon S.[1], Goldshmit Y.[2], Michaelson D.[1] and Pinkas-Kramarski R.[1]

[1]Department of Neurobiology, Tel-Aviv University, Israel. [2]Steyer School of Health Professions, Sackler School of Medicine, Tel-Aviv University, Israel.

eran.schmukler@gmail.com

The Apolipoprotein ε4 allele (*APOE4*) is the most prevalent genetic risk factor for sporadic Alzheimer's disease (AD); however, the mechanisms by which it affects disease progression and exerts its pathological effects are still not clear. In the present study, we investigated the effect of *APOE4* expression in astrocytes on two closely related cellular processes known to be involved in AD development, namely, autophagy and mitochondrial dynamics. We found that basal and induced autophagy is impaired in *APOE4* compared to *APOE3*-expressing astrocytes. The results further demonstrate that *APOE4* astrocytes differ in their mitochondrial dynamics, suggesting that under basal and stress conditions, the mitochondria of *APOE4* astrocytes exhibit reduced fission and mitophagy (autophagic degradation of mitochondria). Accordingly, the mitochondrial network of these astrocytes is hyperfused. Interestingly, we have also discovered that *APOE4* astrocytes exhibit deficient removal of amyloid-beta and mitochondrial dysfunction, which are related to AD pathology. Importantly, the autophagy inducer rapamycin alleviated these *APOE4*-associated impairments. Collectively, the results demonstrate that *APOE4* expression is associated with altered autophagy and mitochondrial dynamics, which might lead to impaired removal of amyloid-beta and mitochondrial dysfunction in astrocytes. This, in turn, may contribute to the pathological effects of *APOE4* in Alzheimer's disease.

SESSION 2

Environmental Stress: preconditioning and adaptive memory

Chair: Michal Horowitz (The Hebrew University)

EARLY-LIFE HEAT EXPOSURE INFLUENCE RESILIENCE OR VULNERABILITY TO HEAT STRESS LATER IN LIFE BY AN EPIGENETIC MECHANISM

Meiri N[1]., Cramer T[1,2]., Rosenberg T[1,2], Kisliouk T[1]

[1]Institute of Animal Science, ARO, The Volcani Center, Israel. [2]The Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Israel.

noam.meiri@mail.huji.ac.il

Background; Altering the future reaction of an organism, in a manner that will determine whether a stressful encounter will lead to future resilience or vulnerability to similar encounters depends on a delicate balance of a probably adjustable stress response set-point, which is most flexible during sensory postnatal development and involves the HPA axis. Here we demonstrate that heat stress during the critical period of thermal control establishment in chicks, renders habituated or sensitized response, depending on the ambient temperature and is governed by epigenetic modifications, specifically DNA-methylation and demethylation. **Results;** CRH protein levels and mRNA expression of CRH in the hypothalamic PVN, alongside the plasma corticosterone level, were elevated a week after heat conditioning in chicks which were trained to be vulnerable to heat, while they declined in chicks that were trained to be resilient, demonstrating correlative changes in the HPA axis. Interestingly, specific CpG methylation (5mC) and hydroxymethylation (5hmC) analysis of the CRH gene between the different heat conditioned groups, a week after their conditioning, displayed low 5mc% alongside high 5hmc% in resilient chicks, while vulnerable chicks displayed an opposite pattern of high 5mc% and low 5hmc%. Furthermore, we found evidence indicating that the CRH intron serves as a repressing element of the CRH gene; RE1-silencing-factor (REST), which has a binding site on this area of the CRH gene, binds abundantly during acute heat stress and is nearly absent during moderate heat stress. Hence the direct binding of REST restricts the repression of the repressor element thus activating the transcription of the CRH gene. **Conclusion;** These dynamic changes contribute to the differences in the expression level of CRH, emphasising that early life epigenetic changes along the CRH gene determine long term stress resilience or vulnerability.

DAY-NIGHT ACTIVITY IN THE UNDERWATER ENVIRONMENT

Eynan M., Danino Y., Aviner B., Rabinovitz R., Kronfeld-Schor N., Menajem D. and Arieli Y.

Israel Naval Medical Institute, Israel.

emirit@netvision.net.il

Combat divers, who use closed-circuit oxygen diving apparatus, undergo part of their training during the night hours. The greatest risk of diving with an oxygen rebreather is central nervous system oxygen toxicity (CNS-OT), which may lead to seizures underwater and eventually drowning. We conducted a two-arm study, in a diurnal animal model, the fat sand rat and in a cohort of combat divers, to investigate whether the switch from day to night activity may be a risk factor for the development of CNS-OT. The animal model study comprised two groups: 1. Control group: Animals were kept awake and active during daylight hours, between 09:00 and 15:00. 2. Experimental group: Animals were kept awake and active during the night hours, between 21:00 and 03:00. After 3 weeks, melatonin derivative in urine (6-SMT levels) was determined over a period of 24 hours. Animals were then exposed to hyperbaric oxygen (HBO), after which biochemical indicators of oxidative stress were measured in the cortex and hippocampus. In the diver arm, we accompanied subjects throughout a combat diving course, during which urine samples were taken and measured at different stages in their training using the same design as in the animal study. Latency to CNS-OT was significantly reduced in the sand rat after the transition from day to night activity. This was associated with alterations in the levels of melatonin metabolites secreted in the urine. In the divers, alterations in melatonin metabolite levels were also observed after 3 weeks of diving with an oxygen rebreather during the night hours. On the basis of these findings, we propose that a switch from day to night diving using oxygen apparatus may represent an additional risk factor for the development of CNS-OT.

TIMING OF MACRONUTRIENT INTAKE: UNDERLYING MECHANISMS AND ENDOCRINE OUTCOMES

Froy O.

Institute of Biochemistry, Food Science and Nutrition, The Hebrew University, Israel.

oren.froy@mail.huji.ac.il

Disruption of circadian rhythms leads to obesity and metabolic disorders. Time restricted feeding (RF) provides a time cue and resets the circadian clock leading to better health. In contrast, high-fat (HF) diet leads to disrupted circadian expression of metabolic factors and obesity. We tested whether long-term clock resetting by RF can attenuate the disruptive effects of diet-induced obesity. Analyses included liver clock gene expression, locomotor activity, blood glucose, metabolic markers, lipids and hormones around the circadian cycle for a more accurate assessment. Compared with mice fed HF diet *ad libitum*, timed HF diet restored the expression phase of some clock genes and phase-advanced others. Although timed HF-fed mice consumed the same amount of calories as *ad libitum* low fat-fed mice, they showed reduced body weight, reduced cholesterol levels and increased insulin sensitivity. Compared with mice fed HF diet *ad libitum*, timed HF diet led to lower body weight, decreased cholesterol levels, reduced TNFα levels and improved insulin sensitivity. Timed HF-fed mice exhibited a better-satiated and less stressed phenotype of lower ghrelin and corticosterone levels compared with mice fed timed low-fat diet. Altogether, our findings suggest that timing can prevent obesity and rectify the harmful effects of HF diet. Our clinical studies in humans support these findings.

TRANSGENERATIONAL INHERITANCE OF SMALL RNAS IN C. ELEGANS

Lev I. and Rechavi O.

Department of Neurobiology, Wise Faculty of Life Sciences & Sagol School of Neuroscience, Tel Aviv University, Israel.

Itamai.et@gmail.com

Small RNA-mediated gene regulatory responses are inherited in *Caenorhabditis elegans* nematodes across multiple generations. In addition, exposure to a number of environmental challenges, such as viral infection, starvation, and heat induces heritable physiological responses in *C. elegans*. In certain cases, the inheritance of such responses can be linked to inheritance of small RNAs or chromatin modifications and was suggested to protect and prepare the progeny for the environmental challenges that the ancestors met. A feed-forward loop between heritable small RNAs and repressive histone marks maintains the heritable information in various organisms. Here we untangle the roles of repressive histone marks in the maintenance of heritable small RNA, present a new environmental challenge that induces an intergenerational response independent of small RNAs and histone marks, and discuss how such short-term heritable responses may affect the slow process of evolution.

SESSION 3

Physiology and pathophysiology of excitable tissues

Chair: Dr. Moran Rubinstein (Tel-Aviv University)

MECHANISM OF PERSISTENT Na+ CURRENT GENERATION IN AXON INITIAL SEGMENT OF LAYER 5 PYRAMIDAL NEURONS

Fleidervish I.

Department of Physiology and Cell Biology, Faculty of Health Sciences and Zlotowski Center for Neuroscience, Ben-Gurion University, Israel.

ilya@bgu.ac.il

In neocortical pyramidal neurons, the persistent Na^+ current (I_{NaP}), which is only a small fraction of the total Na^+ current, plays a crucial role in synaptic integration, by amplifying excitatory and inhibitory synaptic potentials and determining spike threshold. We previously showed that, at functionally critical subthreshold voltages, most of the I_{NaP} is generated in the axon initial segment (AIS). Two mutually not exclusive mechanisms have been offered to explain the AIS I_{NaP} generation: 1) the “window current”, which is an integral feature of the Hodgkin Huxley formalism, and 2) “modal gating”, which entails periodic failure of individual channels to inactivate. In AIS, the window current contribution is expected to be particularly large since activation of the underlying channels is shifted in hyperpolarizing direction by about 15 mV. We now measured the AIS Na^+ transients during the depolarizing voltage ramps of variable speed and amplitude. Our detailed analysis of these transients revealed that in AIS, as in other neuronal compartments, the persistent current reflects “modal gating” of Na^+ channels.

M-CURRENT INHIBITION IN HIPPOCAMPAL NEURONS TRIGGERS AT DIFFERENT TEMPORAL SCALES INTRINSIC AND SYNAPTIC HOMEOSTASIS INTERACTIONS

Attali B., Lezmy J., Styr B., Katsenelson M., Gelman H., Tikochinsky E., Lipinsky M., Peretz A., Slutsky I.

Department of Physiology & Pharmacology, Sackler Faculty of Medicine and Sagol School of Neurosciences, Tel Aviv University, Israel.

battali@post.tau.ac.il

Alterations in synaptic input, persisting for minutes to days, elicit homeostatic plastic changes, including synaptic scaling and/or alterations in intrinsic excitability. However, the possible interaction between these two forms of homeostatic plasticity remains largely unknown. Here, chronic M-channel blockade in hippocampal pyramidal neurons triggers homeostasis of intrinsic excitability (1-4 hours) that precedes a slow (≈ 2 days) homeostatic synaptic downscaling reflected by a decrease in mEPSCs amplitude and in GluA2-positive spine density. The fast homeostatic intrinsic plasticity tightly depends on network synaptic activity and is concomitant with a fast adaptation to bursting firing pattern. This indicates that M-channel inhibition triggers at different temporal scales intrinsic and synaptic homeostatic plasticity and that the two processes are interacting. Although the hierarchical nature of their interactions is not clear yet, there is a functional dependence between the homeostasis of intrinsic excitability and the network firing activity, more specifically the bursting firing pattern.

BRAIN AND MUSIC: PIANO-LIKE SKILL LEARNING FOR STROKE REHABILITATION

Mawase F.

Faculty of Biomedical Engineering, Technion, Israel

mawasef@technion.ac.il

Stroke is one of the most common causes of physical disability worldwide and the majority of people with stroke experience impairment of movement. Lesions to the motor cortex and the corticospinal tract areas following a stroke cause deficits in generating isolated finger movements and thus limiting basic daily functions. Unfortunately, conventional rehabilitation strategies fail to improve hand dexterity in the chronic stage of the stroke. In my talk, I'll show and discuss how novel motor skill training might help restore the ability to improve finger dexterity in people with chronic stroke. People with chronic stroke first underwent baseline assessments of hand function, impairment and hand dexterity. Then, participants were trained for 5 consecutive days on a *piano chord-like* task in which they simultaneously pressed two or three digits while keeping all other fingers at rest. Task's difficulty was *individualized* for each patient based on the baseline performance. We used *reinforcement* strategies to insure high level of motivation. To test retention and generalization following training, participants underwent post-training assessments one *day* and one *week* after finishing training. Our data show that chronic strokes could significantly improve finger individuation. We have found enhanced clinical hand function, and that this improvement was correlated with improved finger individuation. The generalization to motor hand functions, like pinch precision, suggests that training improved movement quality rather than task-specific improvement. Our finding suggests a novel training protocol that could be used clinically to help improve finger dexterity and reduce abnormal flexion synergy in people with chronic stroke.

EARLY VISUAL AND MOTOR DEFICITS IN DRAVET SYNDROME

Rubinstein M., Turchetti-Maia A., Fadila S., Almog Y., Brusel M., Anderson K.

Goldschleger Eye Research Institute, Sackler School of Medicine, Tel Aviv University, Israel.

moranrub@post.tau.ac.il

Dravet syndrome (Dravet) is an infantile epileptic encephalopathy with ominous course. Children develop normally during the first year of life, but subsequently exhibit unusually severe febrile seizures that progress to prolonged refractory seizures and frequent episodes of status epilepticus. Following the onset of epilepsy, developmental delay becomes evident with cognitive decline, motor deficits and visual symptoms. The onset of Dravet-associated comorbidities and its relationship to the recurrent seizure are unclear. Here, we conducted behavioral studies and electrophysiological measurements in Dravet mouse model in order to characterize developmental changes in motor and visual symptoms. Our behavioral experiments demonstrated motor problems even before the onset of spontaneous seizures, with lower performances on the rotarod and reduced coordination in the CatWalk test. These deficits persisted also after the onset of epilepsy. In contrast, visual symptoms were detected at the onset of epilepsy, but not before. In the cliff avoidance test Dravet mice jumped many more times over the ledge, in the visual cliff test Dravet mice took longer to get to the safe side of the arena and in the looming stimulus test, while the vast majority of wild-type mice escaped to the refuge, most of the Dravet mice had no response. Moreover, in vivo electrophysiological recording of visual evoked potential demonstrated slower conduction of visual information in Dravet mice. Together, our data demonstrate evidence for early motor and visual system alterations in Dravet mice. Notably, these deficits are present before or at the onset of epilepsy, indicating that these deficits are core symptoms in Dravet and are not caused by the recurrent seizures. Furthermore, better characterization of early presented symptoms can facilitate prompt and correct diagnosis of Dravet.

SESSION 4

Aging

Chairs: Prof. Mickey Scheinowitz (TAU) and Prof. Yael Yaniv (Technion)

ENDO SIRNA INACTIVATES A NEDDYLATION SUPPRESSOR TO PROMOTE LONGEVITY AND PROTEOSTASIS IN GERMLINE-LESS ANIMALS

Henis-Korenblit S. and Cohen-Berkman M

The Mina and Everard Faculty of Life Sciences, Bar-Ilan University, Israel.

sivan.korenblit@biu.ac.il

One way to slow aging in *C. elegans* is removing its germline. Small RNA molecules are putative signaling molecules that can spread and affect gene expression throughout the animal. In this study, we demonstrate for the first time the contribution of endogenous siRNA to longevity determination. Specifically, we find that mutations that disrupt the processing of endogenous siRNA molecules abrogate the lifespan extension and heat shock resistance of germlineless animals compared to animals with an intact reproductive system; implicating endogenous siRNA signaling in the reproductive longevity pathway. Insights into the underlying mechanism of this regulation will be discussed. Altogether, our data suggest that a novel siRNA signal can regulate proteostasis and aging throughout the animal; coupling them to the reproductive state of the animal.

ITAMAR HAREL – TBA

EXERCISE INDUCES BONE MARROW STIMULATION AND TISSUE REGENERATION IN AGED RATS

Scheinowitz M., Klo M. and Binderman I.

Neufeld Cardiac Research Institute and Department of Biomedical Engineering, Tel Aviv University.Israel.

mickeys@post.tau.ac.il

Background: Exercise is an important factor for optimizing overall health and preventing chronic diseases. Aim: To examine the effects of voluntary exercise on old rat's bone marrow by measuring physiological and molecular variables following exercise stimulation. Methods: SD male rats (young, 3 months; and old, 12 months old) had been assigned to 14 days of voluntary exercise (Exe) or remained sedentary (n=6 per group). The running speed (revolution per minute; RPM) and the total distance (meters) were monitored. At the end of sedentary/exercise period, rats were sacrificed, and their bone marrow was aspirated for in vitro CFU's assays. In another group of animals, the young exercise (YX) stimulated marrow was transplanted into demineralized bone matrix (DBM) ectopic model in order to observe its angiogenic possessions. Finally, bone marrow of all groups underwent molecular profiling. Results: We found that voluntary exercise speed and endurance are reduced with ageing. The in vitro results displayed enhanced proliferating and differentiating rate of bone marrow cells. When transplanting the exercise stimulated marrow of old rats into an ectopic model of DBM, we found newly formed blood vessels and bone formation. Tissue repair and perfusion were improved when exercise marrow was transplanted to ischemic limb of donor rat. The number of transplanted cells survived in the ischemic limb was higher when previously stimulated by exercise. Exercise stimulated bone marrow exhibited differential gene expression that are involved in glucose and lipids metabolism, bone formation, angiogenesis and inflammation. Conclusion: Our data highlight the importance of exercise in ageing animals.

EFFECTS OF AGING ON PACEMAKER CELLS FUNCTION: INSIGHTS FROM A NEW MATHEMATICAL MODEL

Behar J.

Faculty of Biomedical Engineering, Technion, Israel.

joachim.a.behar@gmail.com

Age-related deterioration of pacemaker function has been documented in mammals, including humans. In aged isolated sinoatrial node tissues and cells, reduction in the spontaneous action potential (AP) firing rate was associated with deterioration of intracellular and membrane mechanisms; however, their relative contribution to age-associated deficient pacemaker function is not known. Interestingly, pharmacological interventions that increase posttranslation modification signaling activities can restore the basal and maximal AP firing rate, but the identities of the protein targets responsible for AP firing rate restoration are not known. Here, we developed a numerical model that simulates the function of a single mouse pacemaker cell. In addition to describing membrane and intracellular mechanisms, the model includes descriptions of autonomic receptor activation pathways and posttranslation modification signaling cascades. The numerical model shows that age-related deterioration of pacemaker function is related to impaired intracellular and membrane mechanisms: HCN4, T-type channels, and phospholamban functions, as well as the node connecting these mechanisms, i.e., intracellular Ca2+ and posttranslation modification signaling. To explain the restored maximal beating rate in response to maximal phosphodiesterase (PDE) inhibition, autonomic receptor stimulation, or infused cyclic adenosine monophosphate (cAMP), the model predicts that phospholamban phosphorylation by protein kinase A (PKA) and HCN4 sensitivity to cAMP are altered in advanced age. Moreover, alteration in PKA and cAMP sensitivity can also explain age-reduced sensitivity to PDE inhibition and autonomic receptor stimulation. Finally, the numerical model suggests two pharmacological approaches and one gene manipulation method to restore the basal beating rate of aged pacemaker cells to that of normal adult cells. In conclusion, our numerical model shows that impaired membrane and intracellular mechanisms and the nodes that couple them can lead to deteriorated pacemaker function. By increasing posttranslation modification signaling, the deteriorated basal and maximal age-associated beating rate can be restored to adult levels.

ISPP POSTERS

#1

SLC13-MEDATED CITRATE/SUCCINATE TRANSPORT FUNCTION IS CONTROLLED BY POSITIVELY CHARGED RESIDUES ON AN INTRACELLULAR

Khamaysi A., Aharon S. and Ohana E.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Slc13 transporters mediate the transport of citrate and succinate which are intermediates of the Krebs cycle. In our previous study we reported a reciprocal regulation between the citrate transporter, NaDC-1 and members of the slc26 family of transporters. The interaction is mediated by the intracellular STAS domain of Slc26a6 and the H4c region of the Slc13 co-transporter NaDC-1. More specifically, we found that STAS/H4c interaction is mediated by the negatively charged amino acid glutamate in the STAS domain and two positively charged amino acids (lysine and arginine) in H4c region, that are highly conserved. Here, we aimed to identify the intracellular binding site between the slc13 and Slc26 transporters and to understand how it regulates ion transport. To this end we predicted the structure of mammalian slc13 transporters and based on that we have generated point mutations in two different slc13-citrate/succinate transporters. Next, we monitored slc13/slc26 interaction by coIP and succinate/citrate transport function using electrophysiologic measurements in *Xenopus* oocytes. Our results show that a negatively charged residue on slc26 is crucial for binding and regulation of slc13-mediated succinate transport. Moreover, we show that two adjacent positively charged residues (K107 and R108) have different function. K107 appears to be crucial for binding while R108 is essential for slc13 function. Taken together, our results suggest that an electrostatic interaction may occur between a negatively charged glutamate in slc26 and a positively charge Lysine and arginine in slc13 to control slc13-mediated succinate/citrate transport. This molecular mechanism controls transepithelial metabolite absorption and, when impaired, may lead to several diseases including hypertension, kidney stones and inflammation.

#2

DEVELOPMENTAL CHANGES IN THE ACTIVITY OF INHIBITORY NEURONS IN DRAVET SYNDROME

Almog Y., Anderson K. and Rubinstein M.

Goldschleger Eye Research Institute, Sackler School of Medicine, Tel Aviv University, Israel.

Dravet syndrome (DS) is an epileptic encephalopathy caused by loss-of-function mutations in the *SCN1A* gene, which encodes for the voltage-gated sodium channel $Na_V1.1$. Initial symptoms are febrile seizures occurring around six months of age. Within a few months, the epilepsy progresses to recurrent refractory seizures with frequent episodes of status epilepticus. However, around the age of six, seizure frequency decreases and clinical symptoms improve. DS mice recapitulate the developmental changes seen in patients. Spontaneous seizures start at postnatal day 21 (P21), followed by profound premature death during the fourth week of life and improvement of the clinical symptoms toward the fifth week. Previous electrophysiological studies in DS mice revealed reduced excitability in multiple types of GABAergic inhibitory interneurons at the onset of spontaneous seizures. However, the neuronal changes preceding both the onset and later improvement of epileptic symptoms are largely unknown. We examined the excitability of murine CA1 hippocampal stratum oriens interneurons (O-LM) at three age points: P14 – before the onset of epilepsy; P21 – at the onset of spontaneous seizures; P35 – following the improvement of epilepsy. Measurements of intrinsic firing properties demonstrated a transient reduction in excitability at P21, yet normal firing at P14 and P35. In contrast, examination of synaptically evoked excitatory post-synaptic potentials (EPSPs) and synaptically evoked action potentials (AP) revealed more complex developmental changes, with increased EPSP amplitudes and AP threshold at P14 and P21, followed by improvement of these parameters by P35. Together, our electrophysiological data demonstrate alteration in the activity of O-LM inhibitory neurons at different developmental stages, as well as correlation between the severity of epilepsy and the level of functional impairment in inhibitory neurons.

#3

MECHANO SIGNAL TRANSDUCTION BY CALCIUM AND PHOSPHORYLATION IN HEALTHY AND DYSFUNCTIONAL HEART PACEMAKER TISSUE

Arbel Ganon L.[1], Behar J.[1], Gomez A.M.[2] and Yaniv Y.[1]

[1]Biomedical Engineering Faculty, Technion, Israel. [2]Inserm UMR-S 1180, France.

Normal automaticity of the sinoatrial node (SAN) pacemaker cells is regulated by integrated functions within a system of two coupled clocks. Stretch of the SAN leads to tachycardia under normal conditions. However, acute SAN stretch was associated with arrhythmias in patients with mutations in the cardiac ryanodine receptor (RyR2), such as in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). Irregularities in SAN cells Ca^{2+} handling have been reported; however, the mechanistic explanation for this phenomenon is unknown. We hypothesize that mechanical overload can modulate SAN function through mechanisms involving modifications in Ca^{2+} and post-translational signaling in different cell compartments and can lead to arrhythmias in patients with RyR2 mutations. We aim to design tools that investigate the molecular mechanisms associated with stretch, using experimental and computational models. We developed a system to stretch mice SAN tissue and measured in parallel the beating rate using optic flow techniques. The system was tested on tissues dissected from control mice at different stretch levels. Stretching the SAN tissue from healthy mice led to an increase in the beating rate and a decrease in its variability around the mean. To understand the internal mechanisms that connect between the mechanical load and pacemaker function in RyR2 mutant pacemaker cells, we developed a MATLAB computational model. The model describes autonomic-nervous receptors, post-translational signaling cascades, membrane molecules, and internal pacemaker mechanisms. The model predicted that in a RyR2 mutant cell, the reduction in Ca^{2+} transients and phosphorylation activity mediate a reduction in L-type and NCX currents, which reduces the spontaneous beating rate. In conclusion, an increase in load by mechanical stretch of healthy mice SAN leads to tachycardia with no rhythm disturbance. However, alterations in internal pacemaker mechanisms, such as Ca^{2+} and phosphorylation signaling, lead to a rhythm disturbance in RyR^{R4496C} SAN cells in response to an increased load.

#4

Functional Role of ZIP1 and ZIP3 in the hippocampus

Bogdanovic M.[1], Aizenman E.[1, 2] and Hershfinkel M.[1]

[1]Department of Physiology and Cell Biology and Zlotowski Center of Neuroscience, Ben-Gurion University of the Negev, Israel. [2]Department of Neurobiology, University of Pittsburgh School of Medicine, USA.

Two zinc transporter families regulate zinc homeostasis, ZIPs, which are responsible for transport of zinc into the cytosol; and ZnTs, which function opposite to ZIPs. ZIP1 and ZIP3 were shown to play a role in neuronal zinc toxicity, however, their localization in the brain and function in neurons is poorly understood. We therefore sought to determine how expression level of ZIP1 and ZIP3 changes during normal brain development and what is their role in regulating neuronal zinc accumulation. Hippocampal tissue was isolated from WT mice of different ages and quantitative mRNA and western blot analysis were performed to determine gene expression and protein levels of ZIP1 and ZIP3. Our results show that gene expression levels for both transporters increase during the first postnatal days, reaching a peak on day 14. Immunofluorescence labeling of the hippocampus revealed that ZIP1 is predominantly expressed in the granular layer of the CA3 region, as well as in the granule layer of the dentate gyrus. Lower expression level of ZIP3 is found in the granular layer of CA3 compared to the stratum radiatum and stratum lucidum. We then studied intracellular Zn^{2+} accumulation using live cell imaging of primary hippocampal neuronal cell cultures after siRNA silencing of each transporter. Our results demonstrate that silencing of either ZIP1 or ZIP3 reduces the rate of cytoplasmic Zn^{2+} accumulation by approximately 50%. Our data indicate that ZIP1 and ZIP3 are essential for Zn^{2+} uptake in neurons and thereby may play a role in zinc neurotoxicity.

#5

A *DROSOPHILA* MODEL FOR A DEHYDRODOLICHYL DIPHOSPHATE SYNTHASE (DHDDS) MUTATION CAUSING RETINITIS PIGMENTOSA IN HUMANS

Ifrah R.[1], Brandwine T.[1], Mizrahi-Meissonnier L.[2], Sharon D.[2], Katanaev V.[3] and Minke B.[1]

[1]Department of Medical Neurobiology, Faculty of Medicine and the Edmond and Lily Safra Center for Brain Sciences (ELSC), Hebrew University, Israel. [2]Department of Ophthalmology, Hadassah- Hebrew University Medical Center, Jerusalem. [3]Department of Pharmacology and Toxicology, University of Lausanne, Switzerland.

A founder mutation in the Dehydrodolichyl Diphosphate Synthase gene (*DHDDS*) was reported in 2011. This mutation induced non-syndromic retinitis pigmentosa (RP) in Ashkenazi Jews. The DHDDS enzyme is known to be involved in dolichol synthesize, a lipid molecule that is important for various biological functions, including N-glycosylation and membrane fluidity. The fact that patients with a DHDDS mutation do not suffer from other clinical problems is surprising because genetic defects in related enzymes cause a systemic phenotype with involvement of multiple organs. (Zelinger et al., 2011). We hypothesize that the genetic defect in *DHDDS* partially affects N-glycosylation, causing stress in the photoreceptor cells, resulting in their gradual degeneration. Aiming to shed light on DHDDS function in retinal cells, we propose to analyze a *Drosophila* transgene with knockdown of DHDDS. Therefore, we generated a stable *Drosophila* line in which the *CG10778* gene, the *Drosophila* orthologue of human *DHDDS*, is knocked down by RNAi in the retina (DHDDS-RNAi). Analysis of these transgenic flies using electron microscopy (EM) and functional analysis by electroretinography (ERG) revealed a unique pattern of retinal degeneration. Genetic analysis showed that knockdown of the *CG10778* gene in total body or specific tissues caused a significant damage to the fly development. Electrophysiological assay of the fly rhodopsin level pointed to a drastic reduction of rhodopsin level in the DHDDS-RNAi flies when the RNAi driver was specifically directed to the retina. This conclusion was strongly supported by Western blot analysis showing a pronounced reduction in rhodopsin expression in these specific DHDDS RNAi flies. This study is expected to give a better understanding of DHDDS function in the retina. This information might be useful when trying to slow-down or even halt the retinal degeneration process in relevant patients.

#6

CHARACTERIZNG EARLY PHENOTYPES IN DRAVET SNYDROME MICE

Fadila S., Turchetti Maia A., Anderson K. and Rubinstein M.

Goldschleger Eye Research Institute, Sackler School of Medicine, Tel Aviv University, Israel.

Dravet Syndrome (DS) is an epileptic encephalopathy of early childhood caused by severe loss of function mutation in the SCN1A gene, encoding the voltage gated sodium channel NaV1.1. Children with DS develop normally during their first months of life. However, between six to nine months, they begin to experience febrile seizures, which progress, towards the end of the first year of life, to refractory spontaneous seizures and frequent episodes of status epilepticus. Following the onset of epilepsy, additional comorbidities are presented — developmental delay, cognitive decline, autistic features and motor deficits. Importantly, at the onset of epilepsy, DS is indistinguishable from other milder forms of SCN1A related epilepsies, and quantitative analysis of background electroencephalogram (EEG) activity of DS patients fails to detect any alterations, so DS diagnosis is confirmed only after a clear presentation of developmental delay. Quantitative analysis of the frequency distribution of background EEG in DS mice and age- matched wild-type (WT) controls did not reveal any significant changes, similar to the clinical data. In contrast, analyses of the amplitude distribution and the power of non-normalized background EEG revealed a marked reduction in DS mice with profound differences at the onset of spontaneous seizures. In addition, we used multiple behavioral tests (RotaRod, CatWalk, and home-cage running wheel) to trace the onset of motor deficits in DS. Our results demonstrate a clear motor deficit starting early in life, even before the progression to spontaneous seizures. These data suggest that motor dysfunctions in DS are not caused by recurrent uncontrolled seizures, but rather a direct cause of SCN1A mutations. Better characterization of early DS phenotypes will facilitate DS diagnosis. Prompt and correct diagnosis, together with aggressive anti-epileptic treatment might ameliorate DS epilepsy and its associated comorbidities.

#7

HOW CELLULAR TRANSPORT MECHANISMS REGULATE SUCCINATE HOMEOSTASIS?

Fremder M., Rachamim R., Khamaysi A. and Ohana E.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Succinate is a TCA-cycle intermediate which is gaining attention as a signaling molecule that plays a major role in inflammation and ischemia-reperfusion. Recent studies delineate an important role of succinate in promoting and maintaining the pro-inflammatory response of macrophages trough metabolic and signaling pathways. Since succinate is a charged molecule, it is crucial to identify the mechanisms which facilitate and regulate its transport across membranes. Here, we focused on two families of transport proteins that mediate succinate transport, namely, the slc13 and slc22 families. We utilized western blot analysis and radiolabeled ^{14}C-succinate flux assays to monitor protein expression and function in *Xenopus* oocytes, mammalian epithelial cells and primary macrophages. We found that slc26 transporters regulate apical succinate uptake, while succinate signaling via the IRBIT/IP3 pathway regulates and orchestrates transepithelial transport via slc13 and slc22 transporters. Finally, we show that succinate influx into pro-inflammatory macrophages is elevated compared to other macrophage populations. Our findings suggest that a senso-regulatory mechanism controls transepithelial succinate absorption and maintain succinate homeostasis in epithelia and macrophages. Our findings may have important implications for the understanding and treatment of inflammatory diseases.

#8

PORE-MODULATING TOXINS EXPLOIT INHERENT SLOW INACTIVATION TO BLOCK K^+ CHANNELS

Karbat I.[1], Altman-Gueta H.[2], Fine S.[1], Gordon D.[1], Gurevitz M.[2] and Reuveny E.[1]

[1]Department of Biological Chemistry, Weizmann Institute of Science, Israel. [2]Department of Plant Molecular Biology and Ecology, Tel-Aviv University, Israel.

Voltage dependent potassium channels (K_vs) gate in response to changes in electrical membrane potential by coupling a voltage-sensing module with a K^+-selective pore. Animal toxins targeting K_vs are classified to “pore-blockers” that physically plug the ion conduction pathway and “gating modifiers” that disrupt voltage sensor movements. A third group of toxins blocks K^+ conduction by an unknown mechanism via binding to the channel turrets. Here we show that Cs1, a peptide toxin isolated from cone snail venom, binds at the turrets of K_v1.2 and targets a network of hydrogen bonds that govern water access to the peripheral cavities that surround the central pore. The resulting ectopic water flow triggers an asymmetric collapse of the pore by a process resembling that of inherent slow inactivation. Pore modulation by animal toxins exposes the peripheral cavity of K^+ channels as a novel pharmacological target and provides a rational framework for drug design.

#9

ALLOSTERIC REGULATION OF NCLX BY MITOCHONDRIAL MEMBRANE POTENTIAL LINKS THE METABOLIC STATE AND CA^{2+} SIGNALING IN THE MITOCHONDRIA

Kostic M.*, Katoshevski T.* and Sekler I.

*Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel. *These authors contributed equally.*

Calcium is a key regulator of mitochondrial function under both normal and pathological conditions. The mechanisms linking metabolic activity to mitochondrial Ca^{2+} signaling remain elusive, however. Here, by monitoring mitochondrial Ca^{2+}transients while manipulating mitochondrial membrane potential ($\Delta\Psi_m$), we found that mild fluctuations in $\Delta\Psi_m$, which do not affect Ca^{2+} influx, are sufficient to strongly regulate NCLX, the major efflux pathway of Ca^{2+} from the mitochondria. Phosphorylation of NCLX or expression of phosphomimicking mutant (S258D) rescued NCLX activity from $\Delta\Psi_m$- driven allosteric inhibition. By screening $\Delta\Psi_m$ sensitivity of NCLX mutants, we also identified amino acid residues that, through functional interaction with Ser258, control NCLX regulation. Finally, we find that glucose-driven $\Delta\Psi_m$ changes in pancreatic β- cells control mitochondrial Ca^{2+} signaling primarily via NCLX regulation. Our results identify a feedback control between metabolic activity and mitochondrial Ca^{2+} signaling and the “safety valve” NCLX phosphorylation that can rescue Ca^{2+} efflux in depolarized mitochondria.

#10

PKA SIGNALING IN ELECTRICALLY STIMULATED ATRIAL CELLS

Kirschner Peretz N.[1], Segal S.[1], Arbel-Ganon L.[1], Ben Jehuda R.[2,3], Shemer Y.[2,3], Eisen B.[2,3], Davoodi M.[1], Binah O.[2,3] and Yaniv Y.[1]

[1]Biomedical Engineering Faculty, Technion, Israel. [2]Department of Physiology, Biophysics and Systems Biology, Technion, Israel. [3]The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Israel.

cAMP/PKA signaling regulates atrial cell function by affecting the kinetics of membranal and internal molecules. Atrial cells have a high cAMP/PKA level due to Ca2+-activated calmodulin. However, their spatial and temporal kinetics have not been quantified under physiological conditions. A culture method is essential to characterize atrial cAMP/PKA activity. Unfortunately, previous cultured rabbit atrial cell studies were unable to maintain cell function. Therefore, any phosphorylation measurements were not physiologically relevant. Our study has two aims: (1) establishing an atrial culture method capable of maintaining cell function and ability to be electrically stimulated at a physiological rate (rabbit atrial cells beat at 3Hz) and (2) characterizing cAMP/PKA activity in different cell compartments. The essence of the culture method is to use 2, 3-Butanedione monoxime (BDM), a myofilament contraction inhibitor, for 24h and wash it from the cells before the experiments. The cells maintained their morphology, their ability to contract under 1-3 Hz stimulation, and their global and local Ca2+ release characteristics in comparison to freshly isolated cells. Quantification of atrial physiological PKA activity required stimulating the cells at 1-3 Hz. PKA was measured in three cellular compartments: the cytosol, the mitochondrial matrix, and the outer mitochondrial membrane (OMM). PKA activity was normalized to its maximum and minimum using PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) and PKA inhibitor H-89 dihydrochloride-hydrate, respectively. PKA activity was significantly different in stimulated cells than in quiescent ones. In all compartments, increased stimulation frequency elevated PKA activity (for 3Hz stimulation: 5.49±0.47 for the cytosol, 5.14±0.45 for the OMM, and 2.79±0.22 for the mitochondrial matrix; all increases were fold-increases from quiescent PKA activity). Higher relative increases were found in the cytosol and the OMM. Therefore, when modeling PKA activity, different compartment kinetics should be considered. In conclusion, PKA signaling has different spatial resolution that depends on the electrical stimulation rate.

#11

K_{ir} CHANNELS HETROMERIZATION : PRINCIPLES OF ASSEMBLY AND PHYSIOLOGICAL SIGNIFICANCE

Koren S., Mett A., Karbat I. and Reuveny E.

Department of Biological Chemistry, Weizmann Institute of Science, Israel.

Inwardly rectifying K^+ channels (Kir) are a superfamily of K^+ selective channels, divided between seven sub-families termed Kir1.x-Kir7.x. Under physiological conditions Kir channels allows small outward K^+ fluxes that hyperpolarize the cell membrane potential towards the K^+ battery. Individual Kir subunits may assemble into homo- and hetero-tetramers. Hetero-assembly, which is a potential source of physiological variability, takes place mainly between members of the same sub-family. A Recent research conducted in our lab indicated the assembly of Kir3.1 with Kir2.1, Kir2.3 and Kir4.1 *in vivo*. These novel associations were revealed during a co-immuno-precipitation assay followed by a mass spectrometry analysis of membrane lysate from mice brains. Analyses of cellular contiguity using proximity ligation assay and TIRF microscopy (by measuring FRET) supported these results. In electrophysiological experiments, co-expressing the dominant negative form of Kir3.1 with either of the candidate subunits Kir2.1, Kir2.3 and Kir4.1 in *Xenopus* oocytes, demonstrated a significant decrease in current. The results indicate that Kir3.1 forms heteromeric channels with Kir2.1, Kir2.3 and Kir4.1 *in vivo*.

#12

ACUTE EFFECTS OF SUMOATION ON CORTICAL NEURONAL EXCITABILITY

Kotler O. and Fleidervish I.A.

Faculty of Health Sciences and Zlotowski Center for Neuroscience, Ben-Gurion University, Israel.

Background: The activity of proteins can be modulated by covalent binding of molecules to specific sites in that protein; Small Ubiquitin-like Modifier (SUMO) is one such modulator. SUMO has been shown to modify the activity of the K2P, Kv and Nav channels and thereby change the activity of cultured neuron. Here, using current clamp, whole cell recording from L5 cortical pyramidal neurons in murine brain slices, we examined the effects of SUMOation on excitable properties of their membrane. Exogenous SUMO1 (1 nM) or catalytic domain of deSUMOylating enzyme, SENP1 (1 nM), were applied intracellularly via the somatic pipette. **Results:** We found that changes in SUMOation levels had little effect on passive neuronal properties including resting potential, apparent input resistance and membrane time constant. By contrast, SUMOation rapidly affected the parameters of individual spike generation as well as the frequency of repetitive firing at a given current input. An examination of the dV/dt-V_m relationship of somatic action potentials elicited by brief current pulses revealed a leftward shift in threshold, slowing of the somatic phase of the spike upstroke and slowing of spike repolarization following SUMO application, whereas application of SENP had opposite effect. Neuronal gain was significantly increased by SUMO and decreased when applying the SENP enzyme. These changes are consistent with hypothesis that, in cortical neurons, SUMO directly affects kinetics of voltage-gated Na^+ and K^+ channels. **Conclusion:** We show that activation of the SUMOation cascade could elicit a rapid neuromodulatory response in cortical pyramidal neurons. These functional changes could be a "missing" element in understanding of neuronal dysfunction in stroke and epilepsy.

#13

ZnT10 DEPENDENT EXTRUSION OF CELLULAR Mn^{2+} DRIVEN BY AN ACTIVE Ca^{2+} COUPLED EXCHANGE

Levy M.[1*], Elkoshi N.[1*], Barber-Zucker S.[3], Hoch E.[2], Zarivatch R.[3], Hershfinkel M.[1] and Sekler I.

[1]Department of Physiology and Cell Biology, Ben-Gurion University of the Negev, Israel. [2]Broad Institute of Harvard and MIT, USA. [3]Department of Life sciences and The National Institute for Biotechnology in the Negev and Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Israel.

Manganese (Mn^{2+}) is extruded from the cell by the metal transporter ZnT10. Loss of expression, by autosomal mutations of this transporter, leads to hypermagnesemia in multiple organs. Here we show that ZnT10 is exploiting the trans-membranal Ca^{2+} inward gradient for active cellular exchange of Mn^{2+} based on the following findings: Application of Mn^{2+}-containing Ca^{2+}-free solution to ZnT10 expressing cells triggered an influx of Mn^{2+}. Reintroduction of Ca^{2+} led to cellular Mn^{2+} extrusion against an inward Mn^{2+} gradient. The cellular transport of Mn^{2+} by ZnT10 was coupled to a reciprocal movement of Ca^{2+}. Remarkably, replacing a single aspargine (ZnT10 N43 residue) with threonine (ZnT10 N43T) converted the Mn^{2+}/Ca^{2+} exchange to an uncoupled channel mode, permeable to both Ca^{2+} and Mn^{2+}. This study identifies the first transporter that utilizes the Ca^{2+} gradient for active counter ion exchange. It further shows a remarkable versatility in metal selectivity and mode of transport controlled by the tetrahedral metal transport site of ZnT proteins.

#14

SCREENING OF NEGATIVE CHARGES BY Ca^{2+} IN THE TURRET REGION CONTROLS KV7.1 INACTIVATION GATING AND IS REGULATED BY PIP_2 AND CALMODULIN

Lipinsky M.[1], Tobelaim W.[1], Peretz A.[1], Yakubovitch D.[1], Paas Y.[2] and Attali B[1]

[1]Sackler Faculty of Medicine, Tel Aviv University, Israel. [2]The Mina and Everard Goodman Faculty of Life Sciences Bar-Ilan University, Israel.

Inactivation is an intrinsic property of numerous voltage-gated K^+ (Kv) channels and can occur by N-type or/and C-type mechanisms. While fast N-type inactivation involves the inner pore occlusion by N-terminal peptide domains of α and β subunits, C-type inactivation is suggested to involve structural rearrangements in the outer pore leading to a loss of K^+ coordination sites in the selectivity filter. In Kv7.1 channels, inactivation is invisible macroscopically and does not exhibit the hallmarks of N- and C-type mechanisms. However, Kv7.1 inactivation is revealed by hooked tail currents, which reflect the recovery from an inactivation state. We show that removal of external Ca^{2+} increased the activation kinetics and produced a large voltage-dependent inactivation of Kv7.1 channels. Increasing external Ca^{2+} suppresses inactivation gating with an EC_{50} of 1.5 μM. While Sr^{2+} and Cd^{2+} mimicked the effects of Ca^{2+}, other divalent cations like Mg^{2+} and Mn^{2+} were ineffective. External K^+ (50 mM) did not prevent the inactivation evoked in Ca^{2+}-free external solutions suggesting a mechanism different from C-type inactivation. External acidification or introduction in the pipet solution of calcified calmodulin or PIP_2 slowed down the activation kinetics and precluded inactivation gating evoked in Ca^{2+}-free external solutions. Experimental data and kinetic modeling indicated that Kv7.1 channels exhibit two distinct inactivation states. Mutagenesis studies and structural modeling suggest that external Ca^{2+} ions act to screen the negative charges of neighboring glutamate and aspartate residues located respectively, in the turret and filter entrance of the channel pore. Our results reveal a new mechanism whereby external Ca^{2+} exquisitely controls inactivation gating of a Kv channel that is allosterically modulated by PIP_2 and calcified calmodulin at the inner face of the channel transmembrane core.

#15

STRUCTURE-FUNCTION INVESTIGATIONS OF THE INTERPLAY BETWEEN REDUCED GGPPS ACTIVITY AND BISPHOSPHONATES-INDUCED ATYPICAL FRACTURES

Lisnyansky M.[1], Kapelushnik N.[1], Ben-Bassat A.[1], Marom M.[1], Loewenstein A.[2], Khananshvili D.[1], Giladi M.[1,3] and Haitin Y.[1]

[1]Sackler Faculty of Medicine, Tel Aviv University, Israel. [2]Division of Ophthalmology, Tel Aviv Sourasky Medical Center, Israel. [3]Tel Aviv Sourasky Medical Center, Israel.

Bisphosphonates are widely used for treating osteoporosis, a common disorder in which bone strength is reduced, increasing the risk for fractures. Rarely, bisphosphonates can paradoxically lead to atypical fractures occurring spontaneously or with trivial trauma. Recently, a novel missense mutation (D188Y) in the *GGPS1* gene, encoding for geranylgeranyl diphosphate synthase (GGPPS), was associated with bisphosphonates-induced atypical fractures. However, the molecular basis for GGPPS involvement in this devastating condition remains elusive. Here, we show that while maintaining an overall unperturbed global enzyme structure, the D188Y mutation leads to a ~4-fold catalytic activity decrease. Furthermore, GGPPS-D188Y is unable to support cross-species complementation, highlighting the functional significance of the reduced catalytic activity observed *in vitro*. We next determined the crystal structure of apo GGPPS-D188Y, revealing that while Y188 does not alter the protein fold, its bulky side-chain sterically interferes with substrate binding. In agreement, we show that GGPPS-D188Y exhibits ~3-fold reduction in the binding affinity of zoledronate, a commonly used bisphosphonate. However, inhibition of the mutated enzyme by zoledronate, in pharmacologically-relevant concentrations, is maintained. Finally, we determined the crystal structure of zoledronate-bound GGPPS-D188Y, revealing large ligand-induced binding pocket rearrangements, revising the previous model for GGPPS-bisphosphonates interaction. In conclusion, we propose that among heterozygotes residual GGPPS activity is sufficient to support physiological cellular function, concealing any pathologic phenotype. However, under bisphosphonates treatment, GGPPS activity is reduced below a crucial threshold for osteoclasts function, leading to impaired bone remodeling and increased susceptibility to atypical fractures.

#16

THE ROLE OF ZNR/GPR39 IN REGULATION OF Na^+/K^+ ATPASE IN SALIVARY GLAND DUCT CELLS

Melamed M., Asraf H. and Hershfinkel M.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Human tissues and fluids contain large concentrations of zinc, which fulfills crucial roles in cell functionality, growth, proliferation and survival. Zinc may also be found as a free ion, Zn^{2+}, in organelles. Particularly, Zn^{2+} is selectively concentrated in secretory granules of the salivary glands. In the salivary duct cell line, HSY, extracellular Zn^{2+} activates a Zn^{2+} sensing receptor, ZnR/GPR39. Based on the role of Zn^{2+} in regulating salivary contents, we hypothesized that ZnR/GPR39 may regulate ion transport in these cells. Cellular pH imaging studies, using NH_4^+ as a surrogate to K^+, suggest that ZnR/GPR39 signaling leads to enhanced K^+ transport. We therefore studied the role of the Na^+/K^+ ATPase pump, which is present on basolateral plasma membrane and regulates the osmolarity of the cytosol by controlling intracellular solute concentration. The ZnR/GPR39-dependent ion transporter activity is reduced by using a specific inhibitor of the pump Ouabain. Silencing the α1 subunit of Na^+/K^+ ATPase using siRNA constructs abolished the enhanced ion transporter activity. To study the link between the ZnR/GPR39 and Na^+/K^+ ATPase pump activity we used cyclosporine A, an inhibitor of calcineurin. Zn^{2+}-dependent upregulation of the ion transporter activity was partially attenuated suggesting that FXYD-dependent activation may regulate the pump activity. Thus we conclude that ZnR/GPR39 activates the Na^+/K^+ ATPase, and may regulate duct secretory activity, that is important for regulation of salivary contents.

#17

THE MOLECULAR BASIS FOR SOCE REGULATION BY SARAF

Meshcheriakova A.[1], Saikia C.[1], Palty R.[1], Karbat I.[1], Kimberlin C.[2], Minor M.[2] and Reuveny E.[1]

[1]Weizmann Institute of Science, Israel. [2]Cardiovascular research Institute, University of California San Francisco, USA.

Calcium signaling, an essential component of many cellular processes, employs numerous proteins for its appropriate regulation. SARAF, an endoplasmic reticulum (ER) resident transmembrane protein and a negative regulator of store-operated calcium entry (SOCE), prevents cell overfilling with calcium in a calcium-sensitive manner. Its calcium sensing mechanism and mode of operation are currently enigmatic. We have recently solved the crystal structure of SARAF ER luminal domain, from which a putative redox-dependent regulation mechanism was inferred. Biochemical studies involving SARAF cytosolic domain indicate an interaction with the ER resident single pass protein STIM1. By deciphering the yet unknown structure of SARAF cytoplasmic domain we aim to elucidate the mechanism by which it regulates SOCE.

#18

EFFECT OF SITE-1 PROTEASE INHIBITOR ON VIABILITY OF MULTIPLE MYELOMA CELLS

Rai S., Tal S. and Barki Harrington L.

Department of Human Biology, University of Haifa, Israel.

Multiple myeloma (MM) is a B-cell bone marrow neoplasm accounting for 10-15% of all hematologic malignancies. It is characterized by anemia, compromised immune function, bone lesions, hypercalcemia and renal dysfunction. While scientific advances have prolonged the life expectancy of patients, development of drug resistance continues to present a major problem in MM treatment. Therefore, there is a great need for the discovery of new efficient treatments. Recent studies have shown that inhibition of site-1 protease (S1P) during the transition of healthy activated B-cell to plasmabalsts reduces cell number and generates two distinct populations $CD38^{high}$ and $CD38^{low}$, the latter with an altered secretory potential. Here we examined the efficacy of the S1P inhibitor PF-429242 (PF herein) in halting proliferation and inducing apoptosis in three MM cell lines (KMS-11, RPMI-8226 and U266), alone or in conjunction with clinically used drugs. Initial analysis showed that only RPMI-8226 were $CD38^{high}$ while the other lines lacked CD38 expression altogether. Treatment of the cells with PF resulted in significant time and dose-dependent effect with an IC_{50} of 10 μM for KMS-11 and RPMI-8226 and 20 μM for U266, and reached >99% cell death in all lines within 72h. Combinations of treatment with currently used drugs showed that PF treatment potentiated the killing effect of the corticosteroid Dexamethasone, as well as the effect of the immunomodulatory drug Lenalidomide. In contrast, no synergism was observed with treatments of proteasome inhibitors (Bortezomib and Carfilzomib), suggesting separate killing pathway. Finally, exposure of all three lines to the anti-CD38 drug Daratumumab was largely ineffective in inducing direct cell death, and pre-exposure of the cells to PF did not elevate CD38 levels. Together our data suggest that inhibition of S1P may serve as a potential new treatment for MM.

#19

REGULATION OF MITOCHONDRIAL NCLX - EXCHANGER IN NEURONS BY CAFFEINE AND NEUROTRANSMITERS NOREPINEPHRIN AND VASOPRESSIN

Rozenfeld M., Savic Azoulay I. and Sekler I.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Mitochondrial Ca^{2+} transients are mediated via the mitochondrial Ca^{2+} uniporter (MCU) followed by Ca^{2+} efflux by an electrogenic Na^{+}/Ca^{2+} exchanger (NCLX). The NCLX ($Na^{+}/Ca^{2+}/Li^{+}$ exchanger) belongs to the NCX super family, a major known pathway for mitochondrial Ca^{2+} extrusion. NCLX-exchanger is activated in response to rise in mitochondrial Ca^{2+} levels and upregulated when phosphorylated by PKA. The Phosphodiesterase 2 (PDE-2) is a regulator of neuronal cAMP levels and when inhibited, cAMP accumulates in the cell and leads to enhanced activity of PKA. To study the physiological link between PDE-2 and NCLX in neurons, we used Bay 60-7550, a PDE-2-specific blocker and Caffeine, a non-specific PDE-2 inhibitor that mediates Ca^{2+} fluxes from the ER to the mitochondria. In addition, we asked if the interaction of PDE-2 and NCLX is linked to neurotransmitters dependent regulation of mitochondrial Ca^{2+}. We first investigated the link between Caffeine, NE and VP via PDE-2 on mitochondrial Ca^{2+} signaling focusing on the mitochondrial NCLX-exchanger. Our results show that caffeine activates mitochondrial Ca^{2+} efflux in WT but not in NCLX-KO hippocampal neurons. The effect of caffeine was replicated upon application of the selective PDE-2 blockers Bay 60-7550 indicating that both block PDE-2. Bay-60-7550 also increased mitochondrial Ca^{2+} efflux when co-applied with neurotransmitters. We found a stronger effect of NE, VP when combined with the application of Bay 60-7550 on upregulation of NCLX activity in neurons. The results highlight the importance of PDE-2-dependent regulation of NCLX, in concert with neurotransmitters on mitochondrial Ca^{2+} homeostasis.

#20

LACTATE AS A NOVEL METABOLIC MEDIATOR BETWEEN ASIC1A AND MITOCHONDRIA

Savic Azoulay I[1], Fan L.[2], Xu T.L.[2] and Sekler I.[1]

[1]Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel. [2]Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, China

A major hallmark of neuronal metabolic activity is the buildup of lactic acid resulting in mild drop in pH. The decrease in extracellular pH can be sensed by acid-sensing ion channels (ASIC). ASIC1a, which is main ASIC subunit in neurons, is predominantly Na^+ conducting channel and is expressed on both plasma membrane and inner mitochondrial membrane. Previous studies linked ASIC1a to pathophysiological insults in particular brain ischemia, which is followed by a very large pH drop. Here we ask how lactate affects mitochondrial Na^+ and Ca^{2+} signals as well as mitochondrial metabolic activity and if its extra and intra cellular presence is related to ASIC activation. We show that racemic D/L-lactate triggers robust increase in both cytosolic and mitochondrial Ca^{2+} influx in WT neurons, and not in ASIC1a inhibited or ASIC KO neurons. Mitochondrial Ca^{2+} transients are evoked exclusively when L-lactate is present in the extracellular space, which indicates that there is a mechanism of ASIC1a activation by lactate other than chelating divalent ions, as shown in previous studies. We next show that acidification of intracellular space follows extracellular acidification caused by L-lactate, only when L-lactate is being transported into the cell. Furthermore, mitochondrial Ca^{2+} signal is L-lactate dependent, as we demonstrate that when lactate transport is blocked, mitochondrial Ca^{2+} transients are lower. More importantly, PcTx1 strongly reduces mitochondrial Ca^{2+} signal triggered by L-lactate, suggesting ASIC1a channel via L-lactate propagation of and Ca^{2+} signals, plays significant role in the modulation of mitochondrial activity.

#21

PKA AS A REGULATOR OF ATP SUPPLY TO DEMAND MATCHING IN PACEMAKER CELLS: INSIGHTS FROM COMPUTATIONAL AND EXPERIMENTAL RESULTS

Mazgaoker S., Behar J. and Yaniv Y.

Biomedical Engineering Faculty, Technion, Israel.

Introduction: Cardiac pacemaker cells within the sinoatrial node (SAN) initiate the cardiac rhythmic impulse by generating a spontaneous action potential (AP) that triggers other excitable cells in the heart. SAN cell function is determined by a coupled system of two periodic oscillators: cell membrane electrogenic proteins (the "M-clock") and intracellular Ca^{2+} cycling proteins (the "Ca^{2+}-clock"). The two clocks are connected through Ca^{2+} and post-translational modification signaling. The ATP, the energy to generate the heartbeat, is generated by the mitochondria in the SAN. Interestingly, in other cell types, Ca^{2+} and post-translational modification signaling match ATP supply to demand. However, whether these mechanisms match between energy production to demand in SAN cells is unknown. **Methods:** Our coupled-clock model that includes a description of ionic channels and internal signaling was modified to include mitochondrial energetics in SAN cells. Results were experimentally validated with oxygen consumption measurements in spontaneously beating rabbit SAN cells. The major mechanisms that consume ATP in the cell were examined, together with model response to different interventions. When different interventions are used, the model predicts changes in PKA activation, ATP production, and oxygen consumption, as well as changes in other outputs. **Results:** In SAN cells ATP is mainly consumed to pump Ca^{2+} to the sarcoplasmic reticulum and to maintain cell ionic homeostasis, as opposed to ventricular cells where most of the ATP is consumed to support contraction. The model also suggests that activation of F1F0-ATPase by calcium and phosphorylation of complexes I, IV and V are essential for normal automaticity. Finally, the latter is essential to increase the AP firing rate in response to different interventions. **Conclusion:** Our novel integrative model predicts that in SAN cells both phosphorylation and Ca^{2+} signaling control ATP supply to demand matching.

#22

THE PHYSIOZOO WORLD: INTEGRATING IN VIVO AND IN VITRO DATA FROM DIFFERENT MAMMALS

Weiser-Bitoun I., Shemla O., Rosenberg A.A., Yaniv Y. and Behar J.

Biomedical Engineering Faculty, Technion, Israel.

Heart rate variability (HRV) analysis tools have been mainly available for analysis of human electrocardiographic derived heart rate. We explore extending HRV analysis to two additional dimensions: (1) analysis across multiple mammalian species and (2) analysis across different levels of integration for example sinoatrial tissue. We analyzed the beating rate variability (BRV) across the two additional dimensions using the PhysioZoo computer program that we recently introduced. We used published databases of electrocardiograms from four mammal types: human (n=18), dog (n=17), rabbit (n=4) and mouse (n=8). We computed the BRV measures for each. We also show how the PhysioZoo program can be used for the analysis of sinoatrial node tissue BRV. The study of typical mammalian heart and respiration rates (obtained from the dominant high frequency peak) revealed a linear relationship between these two quantities. Analysis of the rabbit sinoatrial node tissue BRV showed that it had reduced overall variability when compared to in vivo heart BRV.

#23

CAV1.2 MEDIATED NUCLEAR SIGNALING VIA THE H-RAS-ERK-CREB PATHWAY

Servili E., Trus M. and Atlas D.

Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel.

Voltage-gated calcium channels are transmembrane cell surface proteins responsible for multifunctional signals. The mechanisms linking neuronal activity to the excitation-transcription (ET) coupling is not fully understood. The L-type Cav1.2 channel has been shown to activate many different neuronal-specific genes. The prevailing view of L-type Ca^{2+} channels activation of the downstream signaling is via mobilization of calmodulin (CaM) and the CaM-dependent protein kinase kinase (CaMKK) cascade responsible for the CREB phosphorylation. Other proposed pathways involve the calcium-regulated phosphatase calcineurin and the activation of the Ras/MAPK signaling pathways. To investigate the underlying mechanism of ET coupling, we transiently transfected HEK293 cells with wild-type Cav1.2 channel and monitored phosphorylation of ERK1/2, RSK, and CREB. We found that expressing dominant-negative H-Ras^{S17N} mutant together with wild-type Cav1.2 channel significantly inhibited ERK phosphorylation, while the dominant negative Ras^{S17N} only partially inhibited CREB. This negative impact suggests a functional role of H-Ras in mediating nuclear signaling. We tested the role of Ca^{2+} and showed that Ca^{2+}-impermeable mutant channel $\alpha_1 1.2^{L745P}$/α2δ/β2b expressed in HEK293 cells triggers ERK1/2 and CREB phosphorylation, albeit smaller compared to wt channel. These results indicate that there is an additional ERK-independent nuclear activation pathway. The Fluo-4 fluorescence assay showed elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) under depolarizing conditions in HEK293 cells expressing wt channel, while cells expressing the Ca^{2+}-impermeable mutant channel exhibited no $[Ca^{2+}]_i$ rise. Taken together, our data suggest a specific mechanism by which Cav1.2 can activate nuclear signaling independently of Ca^{2+} entry, and provide insight into the role of the Cav1.2 Ca^{2+}-bound pore in triggering ET coupling.

#24

INHIBITION OF GIRK CHANNELS BY ANTIEPILEPTIC DRUG ETHOSUXIMIDE IS SUBUNIT COMPOSITION DEPENDENT

Shalomov B., Dabbah S. and Dascal N.

Sackler Faculty of Medicine, Tel Aviv University, Israel.

Ethosuximide is a well-known drug in the treatment of absence epilepsy. Ethosuximide is believed to be a T-type Ca^{2+} channel blocker ($Ca_v3.1$, $Ca_v3.2$, and Cav3.3). A decade ago, Kobayashi et al (2009) showed that ethosuximide also blocks G-protein inwardly rectifying K^+ (GIRK) channels. Recently it was discovered that Ethosuximide reduces epileptic-like seizures in a mouse model with mutation in *GNB1* gene that causes a severe neurological disorder in humans. *GNB1* encodes for $G\beta_1$ subunit of heterotrimeric G proteins. Gβγ dimer is the main gating factor of GIRK channels. Here, we show that inhibition of GIRK channels by ethosuximide is dependent on subunit composition; while GIRK2 homotetrameric channels are strongly inhibited when activated by Gβγ (K_d = 31.13 μM), GIRK1/2 and GIRK1/3 are less sensitive (K_d = 1235 μM and K_d = 3803 μM, respectively). Moreover, we show that application of Ethosuximide within its therapeutic range inhibits GIRK channels with higher affinity compared to T-type Ca^{2+} channels, particularly homotetrameric GIRK2 channels, and to a lesser extent the heterotetrameric GIRK1/X channels. Therefore, we suggest that the effect of ethosuximide in the regulation of absence seizures occurs mainly via GIRK channels.

#25

FUNCTIONAL CHARACTERIZATION OF HUMAN SLC26A6 POLYMORPHISMS FROM CALCIUM OXALATE KIDNEY STONE PATIENTS

Shimshilashvili L. and Ohana E.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Citrate is a metabolic intermediate of the TCA cycle which also functions as an endogenous chelator of calcium. Hence, urinary citrate protects against the formation of calcareous kidney stones. Urinary citrate concentrations are strictly controlled by a coplex of transporters in the proximal tubule epithelia, NaDC-1 and slc26a6. We previously reported that the major citrate transporter, NaDC-1, interacts with SLC26A6 via the H4c domain and the STAS domain, respectively. The STAS domain is the cytoplasmatic unit of the SLC26 superfamily which is required for proper trafficking to the membrane and interaction with NaDC-1. Here, we studied two SLC26A6 polymorphisms found in kidney stone patients. Our results suggest that although the surface expression of R621G mutant was lower compared WT SLC26A6, the Cl^-/HCO_3^- exchange activity and the interaction with NaDC-1 is retained. On the other hand, the membrane trafficking of slc26a6(D674N) is impauired and its Cl^-/HCO_3^- exchange activity is abolished. Our results suggest that two slc26a6 polymorphisms impair slc26a6 function and trafficking. This is expected to result in low urinary citrate concentrations impaired oxalate clearance in the intestine and the onset of kidney stones.

#26

KINETICS OF PERSISTENT Na^+ CURRENT IN AXON INITIAL SEGMENT OF LAYER 5 PYRAMIDAL NEURONS

Shvartsman A., Khrapunsky Y., and Fleidervish I.A.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Background: In addition to the well-described, fast-inactivating component of the Na^+ current, neocortical neurons also exhibit a slowly inactivating, persistent Na^+ current (I_{NaP}), that plays a role in determining AP threshold and in synaptic integration. By imaging Na^+ influx during slow voltage ramps, we previously showed that, at functionally critical subthreshold voltages, most of the I_{NaP} is generated in the axon initial segment (AIS). We now measured the AIS Na^+ influx during the depolarizing and hyperpolarizing voltage ramps of variable speed and amplitude in order to characterize the I_{NaP} kinetics. **Results:** The voltage dependence of the AIS Na^+ flux was shifted in hyperpolarizing direction by about 15 mV as compared to the somatic I_{NaP}. The conductance-V_m relationship for the axonal I_{NaP} derived from the Na^+ flux measurements was bell-shaped with peak at about -43 mV suggesting that the axonal I_{NaP} is generated predominantly by “window conductance”, as predicted by the Hodgkin Huxley formalism. Moreover, uncontrolled axonal spikes were frequently observed during hyperpolarizing voltage ramps, indicating the presence of wide window of voltages between the steady state activation and inactivation curves for the axonal Na^+ channels. The slow inactivation kinetics of the AIS I_{NaP} assessed by delivering depolarizing voltage steps of varying ampliude and duration before the ramp, were not significantly different from those of somatic Na^+ channels. **Conclusions:** Kinetic properties of the persistent Na^+ conductance in the axon differ significantly from those in other neuronal compartments. The quantitative characterization of the axonal I_{NaP} would be helpful for modeling of neuronal synaptic integration and spike generation.

#27

THE ROLE OF NCLX – EXCHANGER IN MITOCHONDRIAL CALCIUM REGULATION IN THE LIVER

Taha M, Kostic M and Sekler I.

Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.

Mitochondria are the energy hub of the cell, but also have an important role in calcium (Ca^{2+}) signaling. Calcium ions flow into the mitochondria via the mitochondrial Ca^{2+} uniporter (MCU), powered by the steep mitochondrial membrane potential. In excitatory cells Ca^{2+} is primarily extruded by the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX), but the role of this exchanger in non-excitatory cells, such as hepatocytes is still not fully understood. Previous studies proposed that mitochondrial Ca^{2+} efflux in the liver is primarily mediated by a H^{+}/Ca^{2+} exchanger, and that Na^{+}/ Ca^{2+} exchange has a minimal role. To determine the role of NCLX in the liver, we recorded mitochondrial Ca^{2+} transients triggered by purinergic activation by ATP in Rhod-2 pre-loaded primary hepatocytes from WT and NCLX KO mice. We observed a ~two-fold higher rate of mitochondrial Ca^{2+} efflux in NCLX expressing cells. Next, we asked whether NCLX KO would have an effect on oscillatory Ca^{2+} responses in hepatocytes triggered by low, physiological concentrations of hormones (1nM vasopressin and 1μM epinephrine). Consistent with previous studies hormone application triggered mitochondrial Ca^{2+} oscillations in WT. In contrast, oscillations were absent in NCLX KO hepatocytes. Taken together, our results indicate that NCLX is the primary Ca^{2+} extruder in hepatocytes and is essential for mediating the hormone-dependent Ca^{2+} oscillations in these cells.

#28

LIGAND-INDEPENDENT NUCLEOLIN-MEDIATED ERBB2 ACTIVATION IN BREAST CANCER: ONCOGENIC SIGNIFICANCE AND POTENTIAL FOR TARGETED THERAPY

Wolfson E.[1], Goldenberg M.[1], Solomon S.[1], Goldshmit Y.[1], Frishberg A.[2] and Pinkas-Kramarski R.[1]

[1]Department of Neurobiology and [2]Department of Cell Research and Immunology, Faculty of Life Sciences, Tel-Aviv University, Israel.

Global breast cancer incidence and mortality rates are second only to lung cancer, with drug resistance of ErbB2$^{+/+}$ breast cancer (~30% of cases) being a major challenge in patient treatment and therapeutic development. ErbB2 is a member of the ErbB family of receptor tyrosine-kinases, which are major participants in key cellular signaling processes. Thus, it is an excellent target for both cancer research and drugs development. Previously, we have reported that nucleolin, a multifunctional protein, is able to bind and activate ErbB1/EGFR. Yet, the nucleolin-ErbB2 interaction, and its implications in malignant transformation and therapeutic potential are yet to be fully examined. Recently, we have also shown that nucleolin binding by ErbB2 causes receptor activation and increases cell tumorigenicity. Bioinformatical analysis of clinical and transcriptome data from ErbB2$^{+/+}$ breast cancer patients revealed that high nucleolin levels correlate with poor prognosis and increased tumor growth. In addition, similar correlation was evident in an *in vivo* xenograft model of ErbB2$^{+/+}$ breast cancer. Accordingly, monotherapy with the anti-nucleolin G-rich oligonucleotide GroA (AS1411) significantly inhibited viability and growth of ErbB2$^{+/+}$ breast cancer cells *in vitro* and *in vivo*, and appears to specifically target and affect cancer cells. Moreover, combination of GroA with ErbB2 inhibitors reduced breast cancer cells tumorigenicity more efficiently than GroA alone. Interestingly, we have found that ErbB2-nucleolin complexes reside not only adjacent to the plasma membrane, as expected given the known localization of ErbB receptors, but also in the nuclei of mammalian cells; however, the exact role and significance of this finding are yet to be studied, though recent reports suggest nuclear ErbB2 is responsible for acquired tumor resistance to tyrosine-kinase inhibitors. Given the fact that both ErbB2 and nucleolin are major oncoproteins, such findings could provide new insight into the development of novel, previously untested, therapeutic approaches, based on specific inhibition of ErbB-nucleolin complexes.

#29

HILL EQUATION UNDERESTIMATES AFFINITY FOR HIGH DENSITY PROTEIN-PROTEIN INTERACTIONS

Yakubovich D. and Dascal N.

Sackler School of Medicine, Tel Aviv University, Israel.

Dose-response relationship is a powerful pharmacological method for analysis of receptor-ligand systems. Mathematical apparatus of dose-response fitting is based on hyperbolic or sigmoidal functions such as Michaelis-Menten kinetics, Briggs-Haldane kinetics and Hill plot. All 3 mechanisms are based on free ligand approximation assumption. It is assumed that free ligand concentration can be approximated by total ligand concentration. This assumption is valid as long as receptor concentration is below the dissociation constant of the whole system. If this condition is not met, Morrison equation must be used instead. This equation is valid for one-binding site systems. For systems, which have more than binding site, derivation of analytic equations for fitting dose-response relationship is becoming more and more complex and general solution does not exist for systems above 2 binding sites (resulting in 3rd power algebraic equation). For these cases it is recommended to conduct kinetic simulation in order to estimate affinity. GIRK channels are activated by direct interaction with Gβγ subunits of G proteins. Expression in *Xenopus laevis* oocytes is a well-established method to study GIRK channels. This method enables rigorous control of GIRK channel and G protein densities. In the current work we utilize simulated GIRK dose responses to Gβγ proteins according to 4 site cooperative scheme recently proposed by MacKinnon's group, and demonstrate that implementation of straightforward Hill equation fitting can underestimate channels affinity to Gβγ, especially in the case of high channel expression density.

#30

SIGLEC-7 ON PERIPHERAL BLOOD EOSINOPHILS: SURFACE EXPRESSION AND FUNCTION

Zaffran I., Landolina N. and Levi-Schaffer F.

School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel.

Inhibitory receptors (IRs) are surface expressed molecules on both normal immune cells and on neoplastic cells that once activated, inhibit cell functions, regulating inflammatory processes. Siglec-7 is an inhibitory receptor (IR) expressed on human blood eosinophils. Whereas activation of other IRs, including Siglec-8 and CD300a, has been shown to downregulate eosinophil function, little is known about the role of Siglec-7 on human eosinophils. In this work we evaluated Siglec-7 expression and function on peripheral blood eosinophils. Eosinophil expression of Siglec-7 was quantified by flow cytometry, the effect of Siglec-7 on eosinophil viability and degranulation was assessed *in vitro* by measuring cleaved-Caspase3 and by measuring GM-CSF-induced mediator release in culture supernatants. Signal transduction was studied by Western Blot. Here, we show that Siglec-7 was expressed *ex vivo* on peripheral blood eosinophils and using a specific activating monoclonal antibody (anti-Siglec-7 mAb), eosinophils degranulation and release of mediators (eosinophil peroxidase EPX and pro-inflammatory cytokines TNFα and IL-8) was inhibited. Differently from Anti-Siglec-8 mAb, Siglec-7 crosslinking with mAb did not induce eosinophil apoptosis. Finally, Siglec-7 crosslinked with GM-CSF-activated eosinophils induced phosphorylation of SHP-1 and de-phosphorylation of MAPKs. Siglec-7 is constitutively expressed on human eosinophils and downmodulates their activation. Targeting of Siglec-7 on eosinophils might enhance treatment efficacy in eosinophil-driven disorders and allergic diseases. Conversely, therapeutic interventions that inhibit Siglec-7 could have unanticipated consequences and promote eosinophilic inflammation.

#31

INVESTIGATING A HUMAN SUBJECT HARBORING A NOVEL LOSS-OF-FUNCTION MUTATION IN THE TRPV1 CHANNEL

Zaguri R., Katz B., Binshtok A. and Minke B.

Faculty of Medicine and the Edmond and Lily Safra Center for Brain Sciences (ELSC), Hebrew University, Israel.

The Transient Receptor Potential Vanilloid 1 (TRPV1) channel is one of the most researched and targeted protein for the development of novel analgesics for inflammatory pain. It is predominately expressed in nociceptors and localized in both pre- and post-synaptic neurons in spinal dorsal horn, as well as in glial cells. To date, only three studies have been reported on human subjects with deficiency in TRPV1 expression and activity. However, it is unclear what are the effects of theses mutations on the overall expression and activity of the channel *in-vivo*. We have recently examined a patient showing no oral sensitivity to hot chili pepper. Taste examination of this patient revealed normal sensitivity to the four taste modalities, but total insensitivity to capsaicin. Whole exome sequencing revealed a single nucleotide alteration in the TRPV1 gene, causing a novel homozygote missense mutation in a conserved residue located at the Ankyrin-repeat-domain (ARD) of the TRPV1 channel. To examine the effect of this mutation on TRPV1 function, we generated inducible HEK293 cell line expressing either the WT ($hTRPV1^{WT}$) or the mutant ($hTRPV1^{mut}$) channel. Application of known TRPV1 activators resulted in robust $hTRPV1^{WT}$ activation but had no effect on the expressed $hTRPV1^{mut}$ channel. These results demonstrate that a single point mutation in the ARD causes complete inactivation of the TRPV1 channel, which is not due to abnormal assembly or mislocalization of the channel at the plasma membrane. To investigate the effects of a non-functional TRPV1 channel in humans, we performed series of psychophysical sensory test examinations. General physical examination showed that the patient fits the normal growing curves with no apparent health problems. Quantitative sensory testing revealed elevated temperature of noxious heat tolerance (the temperature at which noxious heat becomes unbearable), an elevated sensitivity to cold and an abnormal reaction to topical skin application of mustard oil (AITC), which mimics neurogenic inflammation. This study is thus expected to promote our understanding on the participation of TRPV1 in human pain physiology and the involvement of the ARD in channel activation.

#32

EXPLORING THE METAMORPHIC BEHAVIOR OF HUMAN CLIC5A USING STRUCTURAL AND BIOCHEMICAL ANALYSES

Ferofontov A., Marom M., Giladi M. and Haitin Y.

Sackler Faculty of Medicine, Tel Aviv University, Israel.

Chloride intracellular channels (CLICs) are a family of unique metamorphic proteins. While exhibiting both soluble and transmembrane states, the molecular basis of their inherent flexibility remains largely unknown. Here, we present the high-resolution crystal structure of the human CLIC5A. The structure reveals a monomeric conformational arrangement and shows a high degree of structural conservation with other CLIC proteins. Moreover, Small Angle X-ray Scattering (SAXS) analysis under reducing conditions demonstrates monomeric distribution in solution, reminiscent of the crystallized form. Importantly, Ensemble Optimization Method (EOM) analysis of the SAXS data revealed that hCLIC5A can sample multiple conformations in solution, supporting the metamorphic classification of the family. CLICs were previously shown to incorporate into membranes and mediate ion conduction *in vitro*, suggesting multimerization upon membrane insertion. Here, we provide evidence for the ability of CLIC5A to undergo oxidation-dependent oligomerization in solution. Using Size Exclusion Multi Angle Laser Scattering (SEC-MALS) we demonstrate the ability of purified wild-type hCLIC5A to oligomerize in solution and that it is cysteine-dependent. CLIC5A was suggested to play an important role in membrane remodeling processes. Interestingly, using both dynamic light scattering and fluorescence measurements, we demonstrate the ability of CLIC5A to mediate liposomal fusion *in vitro*, which is greatly enhanced by oxidative conditions. Together, these results provide insights into the structural conservation and inherent flexibility of CLICs and shed light on their regulation by changes in cellular redox potential as well as the molecular mechanisms associated with their possible roles during membrane remodeling.