



Cellular and Molecular Neuroscience: From Generation to Degeneration

April 5-6, 2017

Mishkenot Sha'ananim, Jerusalem

Abstracts:

Eric Kandel, Columbia University, USA

The biology of memory and age related memory loss

Johan Ericson, Karolinka Institute, Sweden

Composition of a timer regulating temporal identity and fate of neural stem cells

Neural stem cells often produce distinct neural progeny in an ordered temporal sequence during development, but the molecular composition of timers or clocks underlying temporal transitions of neural stem cell behavior have not been resolved. By examining a temporal lineage in the brainstem in which a defined population of neural stem cells sequentially generate motor neurons (MNs), serotonergic neurons (5HTNs) and oligodendrocyte precursors, we have characterized the activity of transcription factors and switch signals in this temporal patterning process. In my presentation I will provide evidence for a three-node timer network topology that determines the time period of MN-production and sets the time at which cells undergo a MN-to-5HTN fate switch.

Orly Reiner, Weizmann Institute of Science, Israel

Brain development in health and disease

Aberrant neuronal migration may result in devastating consequences, such as severe brain malformation, mental retardation, epileptic seizures and early death. Many of our studies have been devoted to a severe form of brain malformation, known as lissencephaly, which means "smooth brain". Our presentation will be focused on new findings regarding the mechanisms underlying the pathophysiology of this disease.



Avihu Klar, The Hebrew University of Jerusalem, Israel

Characterization of neuronal circuits for coordinated limb movements in avian

Adaptation to powered flight in birds, that evolved from striding *quadrupedal* reptiles, was achieved by the evolution of two main features: morphological changes that patterned the wings from limbs, and the transition from alternate gait to synchronous flapping. Comparative genomics analysis revealed that dozens of syntenic gene clusters were deleted in the evolution of birds from reptiles. We hypothesize that the evolution of wing flapping is a direct consequence of the genome size reduction, either loss of genes or enhancer elements. We found that the gene encoding the axon guidance molecule Ephrin-B3, that serves as a midline barrier for midline axonal-crossing of excitatory interneurons in the mouse spinal cord, is missing in chick. Notably, mice null for *Ephrin-B3* hop by synchronous gait. Neuronal circuits, at the lumbar (legs) and brachial (wings) levels of the chick spinal cord, were revealed by utilizing targeted expression of reporter genes in specific spinal interneurons and via limb-injected trans-synaptic viruses. We report axonal decussation of excitatory and pre-motor interneurons at the brachial, but not lumbar, spinal cord. Midline crossing at the lumbar spinal cord is obstructed by an avian-specific ovoid gelatinous mass, termed glycogen body, inserted at the dorsal midline of the lumbar spinal cord.

To challenge the role of the *loss of Ephrin-B3* in decussation of axons at the brachial level, the mouse *Ephrin-B3* was expressed at the dorsal midline of the chick spinal cord. Dorsal midline axonal crossing was impeded following *Ephrin-B3* expression. Hence, supporting a role for gene-loss in shaping the circuitry at the wing level in avian spinal cord. The consequence of circuitry alteration on wing flapping is currently being tested.

Joanna Wysocka, Stanford School of Medicine, USA

Gene regulatory principles in human development, disease and evolution

Oren Schuldiner, Weizmann Institute of Science, Israel

From genetics to system, and back: A systematic exploration of neuronal remodeling reveals a transcription factor hierarchy

In our laboratory, we study the molecular mechanisms that regulate, control and execute developmental neuronal remodeling in *Drosophila melanogaster*. Remodeling refines neural circuits by a combination of degenerative processes, such as axon and synapse pruning, as well as regenerative processes, such as regrowth to form adult specific connections. Neuronal remodeling is essential for sculpting the mature nervous systems



of both vertebrates and invertebrates during development. Our knowledge of the mechanisms that regulate remodeling are far from being complete. We believe that understanding the mechanisms of developmental remodeling should shed light on the mechanisms that regulate axon degeneration and growth during development, injury and disease. In addition, defects in neuronal remodeling has been linked to human diseases such as schizophrenia and autism.

The *Drosophila* mushroom body (MB), a central nervous system (CNS) structure involved in learning and memory, is comprised of three types of neurons (γ , α'/β' , and α/β), sequentially born from identical neuroblasts. MB γ neurons undergo stereotypic remodeling such that their larval connections undergo pruning during early metamorphosis and later regrow to adult specific lobes. The genetic power of *Drosophila*, and especially the ability to perform detailed mosaic analyses, together with the stereotypy of the MB development make this a unique system to study the mechanisms of remodeling. In my talk, I will describe our efforts to uncover the transcriptional landscape of MB γ neurons in unprecedented developmental resolution. Using state of the art RNA-seq, we have uncovered a temporal sequence of transcription factor hierarchy that regulate various aspects of neuronal remodeling. Finally, by resequencing mutant MB γ neurons, we have identified specific developmental programs that are regulated by the transcription factor network in order to execute neuronal remodeling.

Li-Huei Tsai, Massachusetts Institute of Technology, USA

Bringing gamma back — using noninvasive sensory stimulation to modify Alzheimer's disease

Gamma oscillations arise from the resonating activation of local circuits comprised of excitatory and fast spiking inhibitory neurons. Impaired gamma oscillations have been observed in both mouse models and human subjects with Alzheimer's disease. Using a noninvasive visual flicker stimulation protocol, we enhanced gamma oscillations and found that it ameliorated Alzheimer's disease pathology. I will discuss the potential mechanisms and effects of gamma oscillations on cognitive behavior.

Uri Ashery, Tel Aviv University, Israel.

Multifaceted mitigating effects of hyperbaric oxygen therapy in Alzheimer's Disease mouse models

Alzheimer's disease (AD) is the most common form of dementia in the elderly. In addition to its main pathological signatures like accumulation of extracellular amyloid



plaques and intracellular neurofibrillary tangles, AD is associated with hypoxia, neuroinflammation and diminished blood flow. Hyperbaric Oxygen Therapy (HBOT), the medical administration of 100% oxygen at environmental pressures greater than 1 atmosphere absolute (ATA), has been used successfully in the treatment of other neurological conditions. Here, we investigated the effects of HBOT on two different mouse models of AD pathology, the 3xTg-AD and 5xFAD mouse models. 17 month old 3xTg-AD mice were exposed to HBOT at 2 ATA for 60 minutes per day for 14 days while 6 month old 5xFAD and wt mice were exposed to HBOT at 2 ATA for 60 minutes per day, 5 days a week for 1 month (20 treatments). Subsequently to HBO treatment, a behavioral test battery was performed and histological analysis of hippocampal slices was conducted. HBOT ameliorated cognitive deficits of both 3xTg-AD mice and 5xFAD mice. Moreover, HBOT reduced significantly the presence of hypoxia and amyloid burden in the hippocampal area in both models. In the 3xTg-AD mice, HBOT attenuated neuroinflammation by reducing astrogliosis, microgliosis and secretion of proinflammatory cytokines. In the 5xFAD, HBOT induced neurogenesis and enhanced survival of adult-born granule cells. These findings demonstrate that HBOT acts via multifaceted mechanisms including increase in neurogenesis and reduction of inflammation to affect brain functionality and may present a novel therapeutic intervention of AD.

Ohad Birk, Ben Gurion University of the Negev, Israel

Monogenic diseases: From phenotypes to genes and novel molecular pathways

Sebastian Kadener, The Hebrew University of Jerusalem, Israel

In vivo functions of circRNAs

Circular RNAs (circRNAs) are highly abundant and evolutionary conserved RNAs of mostly unknown functions. We recently investigated their mechanism of production and determined that some of them can encode proteins. Here, we report the generation of *Drosophila* lines in which specific circRNAs were targeted for degradation using shRNAs. We observed that some circRNAs are essential for proper fly development, whereas others are involved in behavioral, neural, or muscular functions. Interestingly, downregulation of circMbl, the most abundant circRNA in flies, leads to partial male embryonic lethality and a characteristic wing posture defect. Moreover, the wing posture phenotype was partially rescued by introduction of a circMbl transgene, demonstrating that the observed phenotype is due to the lack of this circRNA. We also determined that downregulation of circMbl in the fly central nervous system leads to abnormal wing posture and synaptic function. Together, our results constitute the first



proof of functionality of circRNAs at the organismal level and provide a methodological approach to tackle this issue comprehensively.

Jens Schwamborn, University of Luxemburg, Luxemburg

Brain-on-a-Chip technology and brain organoids for in vitro modeling of neurodegenerative diseases

Deborah Toiber, Ben-Gurion University of the Negev, Israel

Neuroprotective functions for the histone deacetylase SIRT6

The histone deacetylase SIRT6 promotes DNA repair, while its activity declines with age, with a concomitant accumulation of DNA damage. Furthermore, SIRT6KO mice exhibit an accelerated aging phenotype and die prematurely. Here, we report that brain-specific SIRT6-deficient mice survive, but present behavioral defects with major learning impairments by 4 months of age. Moreover, the brains of these mice present increased signs of DNA damage, cell death and hyperphosphorylated Tau – a critical mark in several neurodegenerative diseases. Mechanistically, SIRT6 regulates Tau protein stability and phosphorylation through increased activation of the kinase GSK3 α/β . Finally, we found SIRT6 mRNA and protein levels to be reduced in patients with Alzheimer's disease. Together, our results suggest that SIRT6 is critical to maintain genomic stability in the brain and its failure leads to toxic Tau stability and phosphorylation. Therefore, SIRT6 and its downstream signaling could be targeted in Alzheimer's disease and age related neurodegeneration.

Bart De Strooper, University of Leuven, Belgium

The cellular phase of Alzheimer Disease

Inna Slutsky, Tel-Aviv University, Israel

Maintaining the balance between stability and plasticity in hippocampal circuits

How neuronal circuits maintain the balance between stability and plasticity in a constantly changing environment remains a fundamental question in neuroscience. Empirical and theoretical studies suggest that homeostatic negative feedback mechanisms operate to stabilize the function of a system at a set point level of activity.



While extensive research uncovered diverse homeostatic mechanisms that maintain activity of neural circuits at extended timescales, several key questions remain open. First, what are the basic principles and the molecular machinery underlying invariant population dynamics of neural circuits, composed from intrinsically unstable activity patterns of individual neurons? Second, does aberrant brain activity, associated with distinct types of neurodegenerative disorders, result from failures of homeostatic control system? To target these questions, we have developed an integrative approach to study the relationships between ongoing spiking activity of individual neurons and neuronal populations, inhibition-excitation balance, intrinsic excitability of neurons and signaling processes at the level of individual hippocampal synapses *ex vivo*. I will describe the basic relationships between ongoing spiking properties of individual neurons, population dynamics and neuronal adaptive mechanisms.

Giovanna Mallucci, University of Cambridge, USA

Cool synapses: The mechanistic basis of neuroprotection

Sudha Seshadri, Boston University, USA

Emerging novel approaches to dementia prevention and therapy: Population neuroscience and personalized care in the Omics Era

Moussa B.H. Youdim, Technion-Israel Institute of Technology, Israel

Anti alzheimer multi target drugs possessing neuroprotective, neurorestorative and mitochondrial biogenesis activities via activation of hif

Novel therapeutic approaches for the treatment of Alzheimer's disease (AD) comprise drug candidates designed specifically to act on multiple CNS targets, rather than a single "receptor" as has been done with cholinesterase inhibitors. Major pathology of AD is the accumulation of iron in nucleus basalis, dentate gyrus, amyloid plaques, and tangles and increase in monoamine oxidase (MAO). The iron contributes to the onset of oxidative stress and glutaminergic excitotoxicity via interaction with hydrogen peroxide generated by the reaction of MAO. We have synthesized several multi target non-toxic, brain permeable iron chelator drugs, M-30 and HLA-20, possessing propargyl MAO inhibitory moiety, with neuroprotective and neurorestorative activities. These drugs possess antiapoptotic, pro-survival neurorescue effects, induction of neuronal differentiation and regulation of amyloid precursor protein (APP) and β -amyloid ($A\beta$) levels. They induce the outgrowth of neurites in neuronal cell



cultures, trigger cell cycle arrest in G0/G1 phase and enhance the expression of growth associated protein-43, HIF (Hypoxia Inducing Factor) and increase brain levels BDNF, GDNF, VEGF and erythropoietin. This has been shown to be associated with the inhibition of iron dependent prolyl-4-hydroxylase that regulates HIF. Both M30 and HLA-20 process APP via activation of alpha secretase. They possess neurorestorative activity in in vivo models of Parkinson's disease and restore the cognitive deficit in APP/PSI double transgenic mice and the streptozotocin (STZ) models of AD. The dual control of mitochondrial biogenesis and energy metabolism is regulated by silent information regulator-1 and -3 (SIRT1 and SIRT3). The peroxisome proliferator activated receptor γ co-activator 1 α (PGC-1 α) is a transcriptional co-activator that is a central inducer of mitochondrial biogenesis in cells. SIRT1 is necessary for HIF-1 α protein accumulation and activation of HIF-1 target genes and activates PGC-1 α -mediated transcription of nuclear factor (Tfam) and mitochondrial genes encoding for proteins promoting mitochondria proliferation, We have shown that M30 and HLA-20 activate SIRT1, PGC-1 α , and Tfam in cell cultures and consider them as a novel therapeutic approach for neurodegenerative disorders.

Sagiv Shifman, The Hebrew University of Jerusalem, Israel

Systematic comparison of genes implicated across neuropsychiatric disorders

Genetic susceptibility to intellectual disability (ID), autism spectrum disorder (ASD), and schizophrenia (SCZ) often arises from mutations in the same genes, suggesting that they share common mechanisms. We studied genes with de novo mutations in the three disorders and genes implicated in SCZ by genome-wide association study (GWAS). Using biological annotations and brain gene expression, we show that mutation class explains enrichment patterns more than specific disorder. Genes with loss of-function mutations and genes with missense mutations were associated with different pathways across disorders. Conversely, gene expression patterns were specific for each disorder. ID genes were preferentially expressed in the cortex; ASD genes were expressed in the fetal cortex, cerebellum, and striatum; and genes associated with SCZ were expressed in the adolescent cortex. Our study suggests that convergence across neuropsychiatric disorders stems from common pathways that are consistently vulnerable to genetic variations but that spatiotemporal activity of genes contributes to specific phenotypes.



Tamir Ben-Hur The Hebrew University Medical Center, Israel

Basic aspects of translational issues in cell therapy for Multiple Sclerosis

The field of regenerative cell therapy in neurological diseases has undergone a major shift from the concept of "cell replacement" approach to that of utilizing the bystander immune-modulatory, neuro-trophic and neuro-protective properties of stem / precursor cells.

When considering the clinical translation of cell therapy and utilizing these therapeutic properties we need to deal with several crucial issues, as will be discussed for multiple sclerosis (MS). We will discuss the relevance of experimental models to clinical translation. We will present our findings on the therapeutic properties of candidate cellular platforms, their impact on transplanted cell fate, the role of cell migration in their therapeutic effect, the need to target therapy in a compartmentalized disease situation of chronic-progressive MS, and its influence on choice of optimal route of cell delivery.

Finally, we will discuss the challenges in the roadmap to true regenerative, cell-replacement therapy for MS.

Nissim Benvensity, The Hebrew University of Jerusalem, Israel

Modeling and treating neurological disorders using human pluripotent stem cells

Martyn Goulding, The Salk Institute for Biological Studies, USA

Circuits in the Spinal Cord for Touch and Movement

Animals use a variety of sensory modalities to interact with and explore the environment in which they move. Of particular importance is the somatosensory system, which monitors the internal and external state of the body during movement. However, very little is currently known about how somatosensory information is processed and gated by the spinal cord. Using a sophisticated suite of genetic tools, we have begun to functionally dissect the cutaneous arm of the somatosensory system, which plays a central role in generating many of the protective and affective behaviors animals display. Our studies have led to the identification of a number of excitatory and inhibitory cell types that play key roles in processing and gating noxious and innocuous mechanosensory stimuli. This knowledge is now being used to determine how sensory afferent feedback interfaces with the spinal motor system to control movement and generate stimulus-specific motor reflexes.



Yechiel Elkabetz, Tel-Aviv University, Israel

Reliable modeling of cortical development and microcephaly in rosettes and organoids by combined pathway inhibition

Gou-li Ming, Johns Hopkins University, USA

Modeling human brain development and developmental diseases using hiPSCs

Three dimensional (3D) cerebral organoid cultures from human iPSCs have been recently developed to recapitulate the cytoarchitecture of the developing brain. This system offers unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in modeling congenital neurodevelopmental disorders, such as microcephaly. We have improved the organoid technology and developed a protocol to produce forebrain-specific organoids derived from human iPSCs using a novel miniaturized spinning bioreactor that recapitulate the human embryonic cortical development. ZIKV, a mosquito-borne flavivirus, has re-emerged as a major public health concern globally because ZIKV causes congenital defects, including microcephaly, and is also associated with Guillain-Barré syndrome in infected adults. We found that ZIKV exhibit specific tropism towards human neural progenitor cells and results in cell death and defects in neural development. I will discuss our recent work in further dissecting the molecular mechanisms underlying the ZIKV pathogenesis and microcephaly.