ESN
EUROPEAN SOCIETY FOR NEUROCHEMISTRY

FENS Forum 2022

9-13 July 2022 | Paris, France

Mini-Conference

"Molecular basis for synaptic function highlighting disease mechanisms"

Programme
and
Abstract Book

Organized by

European Society for Neurochemistry (ESN)

In Collaboration with

Federation of European Neuroscience Societies (FENS)

Mini-Conference Organizers:

Illana Gozes (Secretary, Chair),
Johannes Hirrlinger (President),
Carlos Duarte (Treasurer),
Natalia Nalivaeva (Past-President)

DESCRIPTION OF THE TOPIC

There are currently hardly any disease modifying treatments for neurodevelopmental or neurodegenerative diseases presenting malfunctioning synaptic connections. Our Mini-Conference will highlight novel aspects of genes and behavior toward better therapeutics.

Last year, the Autism Sequencing Consortium published the largest autism spectrum disorder (ASD) exome sequencing to date, revealing 102 candidate genes and suggesting that ASD must arise by phenotypic convergence among diverse origins. In other words, common molecular pathways may explain the apparently diverse genomic landscape behind ASD. In line with this hypothesis, our Mini-Conference addresses recent work to bridge the nature of this convergence and brings together a group of scientists who are studying the molecular, cellular and synaptic disruptions caused by mutations in genes associated with ASD. Additional disease mechanisms inflicting synapse function, such as protein mis-folding, mitochondrial activity, lipid rafts and gene regulation will be addressed with converging and disparate mechanisms.

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VENUE

Pavillion 7, Paris Expo Porte de Versailles
Hall C

PROGRAMME

Saturday, 9th of July 2022

9:00 am to 3:00 pm CEST

9:00-9:15 **Opening remarks: Illana Gozes (Secretary, ESN)**

9:15-9:45 L1

L1 Patricia Monteiro (Portugal) "SHANK proteins: roles at the synapse and in autism spectrum disorder"

9:45-10:15 L2

L2 Illana Gozes (Israel) "Essential for the synapse: ADNP a major regulator of development and aging"

10:15-10:45 L3

L3 Julien Courchet (France) "Fine-tuning of cortical circuits development through a local regulation of mitochondrial metabolism"

10:45-11:15 **Coffee Break, Poster Viewing**

11:15-11:45 L4

L4 Natalia Nalivaeva (UK/Russia) "Prenatal stress and neuronal gene regulation"

11:45-12:15 L5

L5 Angelo Poletti (Italy, ESN Council Member) "Protein misfolding in motor neuron diseases"

12:15-12:45 L6

L6 Sara Grassi (Italy, ESN Council Member, Chair YSSC) "Lipid rafts in neurodegeneration and neuroprotection"

12:45-13:00 **General Discussion – Organizing Committee/ESN Council Members**

13:005-14:00 **Lunch Bag, Poster Viewing**

14:00-14:30 **Closing Remarks:**

Illana Gozes (Secretary, ESN), "Best Poster Prize (Free Registration to the ISN-ESN 2023 Meeting and ESN travel scholarship awards for FENS 2022)".

Carlos Duarte (Treasurer, ESN) "Our next meeting" ISN-ESN 2023, Porto, Portugal

14:30-15:00

Informal ESN Council Meeting and General Assembly, ESN Members Only.

Posters

P1
Ellouze, Salma “Alterations of cortical connectivity in a mouse model of premature brain injury”

P2
Gomez de Salazar, Macarena “Reverse engineering of the synaptic tagging and capture mechanisms”

P3
Lam, Jacqueline CK “Designing a Protocol Adopting an Artificial Intelligence (AI)–Driven Approach for Early Diagnosis of Late-Onset Alzheimer’s Disease”

P4
Li, Victor O. K. “Somatic Mutations and Alzheimer’s Dementia”

P5
Meyer-Dilhet, Géraldine “Alteration of mouse cognition and neural circuits formation resulting from mutations on the autism-linked gene Nuak1”

P6
Monteiro, Laloe “A cell-based model to validate the pathogenic nature of epilepsy-associated variants of the RORB gene”

P7
Paes-de-Carvalho, Roberto “Differential Regulation of CREB and AKT by cyclic AMP in the developing retina: The role of A1 adenosine receptors”

P8
Schwarz, Alexander “Timing and Region-Specific Reference Gene Selection within the Rat Brain in the Lithium Pilocarpine Epilepsy Model”

P9
Shapira, Guy “Hippocampal differential expression underlying the neuroprotective effect of Δ9-tetrahydrocannabinol (THC) microdose on old mice”


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ABSTRACTS

L1

SHANK proteins: roles at the synapse and in autism spectrum disorder

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The SHANK3 gene is currently one of the best characterized autism risk genes. Studies by us and others have been revealing the biological extent to which mutations in Shank3 impact brain function and development in zebrafish, mice, rats, and even primates, leading to reduced social interaction and repetitive behavioural patterns in all species. To investigate the in vivo function of Shank3 and to elucidate how its disruption may lead to atypical social behaviour, we are using a transgenic mouse line harbouring a patient-linked ASD mutation. These Shank3 mutant mice exhibit compulsive/repetitive behaviour and impaired social interaction, together with structural and functional brain changes. Furthermore, we have now found that Shank3 mutant mice tend to withdraw from noisy environments and have functional deficits in the brain auditory cortex, providing the first mechanistic link between auditory sensitivity, autism and atypical social behaviour. Taking advantage from this unique animal model, we are now applying neuroproteomics and brain electrophysiology recordings to reveal downstream molecular alterations that can be used as future therapeutic targets.

Acknowledgements: This work has been funded by Fundacao para a Ciencia e a Tecnologia (FCT; grant number PTDC/MED-NEU/28073/2017 and POCI-01-0145-FEDER-028073) and FEBS (Federation of European Biochemical Societies) Excellence Awards 2021.

L2

Essential for the synapse: ADNP a major regulator of development and aging

Illana Gozes

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Activity-dependent neuroprotective protein (ADNP) and its smallest active fragment, drug candidate NAP (davunetide) were discovered and first characterized in our laboratory. *De novo* mutations in ADNP cause the autistic intellectual disability (ID) ADNP syndrome. Now, we showed that ADNP and related genes are somatically mutated in post-mortem aged Alzheimer's disease (AD) brains correlating with increasing AD tau pathology (Ivashko-Pachima, Hadar et al., Molecular Psychiatry, 2021). We then revealed tauopathy in post-mortem 7-year-old autistic ADNP syndrome boy (Grigg et al., Translational Psychiatry, 2020). Using CRISPR-Cas9 genome editing, we developed a mouse model carrying the most abundant ADNP syndrome mutation, showing early tauopathy and reversal by NAP treatment (Karmon et al., Biological Psychiatry 2022). Mechanistically, ADNP/NAP fortify microtubules through NAP (amino acid sequence: NAPVSIPQ), containing a microtubule end binding proteins (EB1, EB3) domain SxIP (Ivashko-Pachima et al., J. Mol. Neurosci. 2021) and an SH3 binding site interacting with other autism syndrome genes, like SHANK3 (Ivashko-Pachima et al., Molecular Psychiatry, 2022). NAP enhances microtubule dynamics, augmenting Tau-microtubule association and protecting against tauopathy. ADNP mutations, disrupt Tau-microtubule interaction, which is ameliorated by NAP treatment. We have further demonstrated NAP enhancement of Tau/sirtuin1(SIRT1)-microtubule interaction in human induced pluripotent stem cell-derived neural cells, with SIRT1 and its partner Forkhead Box O3 (FOXO3, also regulated by ADNP/NAP) being major controllers of healthy aging (Hadar, Kapitansky et al., Molecular Psychiatry, 2021). Lastly, STOP codon mutations in ADNP, implicated in the ADNP syndrome and in AD represent sites of natural caspase cleavage, hallmarking apoptosis (Gozes and Shazman, Frontiers in Endocrinology, 2022). Thus, NAP (Davunetide) fortification of ADNP activity is predicted to be beneficial in autism and neurodegenerative diseases.

Conflict of interest: NAP (Davunetide), under patent protection is exclusively licensed for development to ATED Therapeutics Ltd. Professor Illana Gozes is ATED's Chief Scientific Officer.

Support: I thank all my collaborators and students cited above for their excellent contributions. My laboratory is supported in part by the 2021 US National Academy of Medicine Catalyst Award on Healthy Longevity, awarded to a team led by Principal Investigator VOK Li, and Co-Investigators JCK Lam, J Downey, and I Gozes. The laboratory is also supported by ERA-NET neuron ADNPinMED, as well as Drs. Ronith and Armand Stemmer (French Friends of Tel Aviv University), Holly and Jonathan Strelzik (American Friends of Tel Aviv University) and - Anne and Alex Cohen (Canadian Friends of Tel Aviv University).

L3

Fine-tuning of cortical circuits development through a local regulation of mitochondrial metabolism

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The proper function of neuronal circuits in the adult brain relies on glucose metabolism to ensure energy-demanding neuronal functions such as synaptic activity or long-distance axonal transport. Deregulation of the energy metabolism is strongly associated to many neurodegenerative diseases and has been linked to some neuropsychiatric diseases such as schizophrenia. However, our current understanding of metabolic regulation in the developing brain and in particular in rapidly growing neurons is still fragmental.
We previously identified a signaling pathways involving two kinases LKB1 and NUAK1, and controlling cortical axons outgrowth and terminal branching through a novel mechanism involving the regulation of mitochondria trafficking and clustering in the developing axon. My presentation will review the latter findings, as well as our use of molecular tools to visualize mitochondria trafficking and function in cultured neuron.

L4

Prenatal stress and neuronal gene regulation

Natalia Nalivaeva[1,2], Dimitrii Vasilev[2], Nadezhda Dubrovskaya[2], Daria Kozlova[1], Ekaterina Kochkina[2], Natalia Tumanova[2], Anthony Turner[1]

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Adverse conditions during pregnancy have significant impact on brain development via epigenetic regulation of neuronal gene expression. Such changes can affect brain metabolism and functions during the entire postnatal life of individuals and underlie the processes leading to various neurological disorders, which range from autism to Alzheimer's disease (AD). Among the factors of prenatal stress maternal and fetal hypoxia due to complications of pregnancy are the most common. Hypoxic stress affects regulation of various genes involved in metabolism of amyloid Aβ peptide among which are amyloid-precursor protein (APP), proteolytic enzymes participating in its processing, amyloid degrading enzymes (ADE) and transport proteins which maintain physiologically balanced levels of Aβ. An endopeptidase, neprilysin (NEP), cleaves a variety of regulatory peptides in the brain including somatostatin and Aβ. NEP expression and activity in rat cortical structures and hippocampus are particularly reduced after prenatal hypoxia (PH) which correlates with impaired memory and olfaction of the animals during their postnatal development. A transport protein transthyretin (TTR) is another protein whose gene expression is affected by PH. Taking into account the mechanisms of epigenetic regulation of NEP gene expression we have demonstrated that treatment of rats with a histone-deacetylase inhibitor valproic acid or a caspase inhibitor Ac-DEVD-CHO restored decreased levels of NEP expression and cognitive and olfactory functions of rats subjected to PH. Epigallocatechin gallate treatment of animals also resulted in increased NEP expression and activity and improved memory of PH animals. These results suggest that the model of PH in rats can be used as a valid tool for testing the efficacy of the compounds which affect expression of target genes. It also helps to decipher the epigenetic mechanisms through which prenatal stress affects brain development allowing the design of new therapeutic strategies. Supported: Russian Foundation for Basic Research (RFFI-19-015-00232), Russian state budget (assignment 075-00408-21-00).

L5

Protein misfolding in motor neuron diseases

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Motor neuron disease are a class of neurodegenerative diseases in which selective populations of cortical, bulbar and/or spinal cord motor neurons are selectively affected and die. While the molecular alterations responsible for cell death may take place directly in motoneurons, non-cell autonomous mechanisms have been clearly identified. These include alterations of the dialogs between motoneurons and the surrounding glial cells or with the target muscle cells, as well as defects at the levels of the synaptic connection forming the neuromuscular junction (NMJ). Based on the clinical and experimental findings motor neuron diseases may be associated to developmental defects (as in the case of prenatal and juvenile forms) and/or neurotoxic mechanisms (as frequently cause of adult-onset diseases). One of the main mechanisms of neurotoxicity is associated to the generation of aberrant proteins characterized by misfolded conformations. A misfolded protein may either loose its function(s) or acquire novel neurotoxic activities (gain-of-function), which may affect several motoneuronal functions, both at the soma levels (e.g.: organelles dysfunctions, nuclear alterations, etc.) or in its very long axonal processes (e.g. axonal transport alteration, synaptic dysfunctions, etc.). Misfolded proteins generally tend to aggregate forming intracellular and axonal inclusions that may perturb motor proteins activities, and thus have to be rapidly eliminate from cells with the assistance of molecular chaperones and the involvement of specific degradative systems (the ubiquitin proteasome system (UPS) and autophagy). We recently focused of mechanisms that control the delivery of misfolded proteins to their clearance. In particular, we have evaluated the therapeutic potential of the pharmacological stimulation of the chaperone assisted selective autophagy (CASA) pathways as a way to counteract protein misfolding associated damages at the level of motoneuronal cells. Our data have demonstrated that the induction of one of the crucial CASA complex component, the small heat shock protein B8 (HSPB8) is beneficial against accumulation of several type of mutant misfolded protein that cause different forms of adult onset motor neuron diseases.

L6

Lipid rafts in neurodegeneration and neuroprotection

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Lipids in the brain are major components, involved in processes such as neurogenesis, signal transduction, neuronal communication, membrane compartmentalization, and modulation of gene expression. Due to their structural and physiological roles, alterations in their metabolism lead to abnormal lipid raft organization and consequent deregulation of lipid raft-dependent signalling in several neurodegenerative diseases. The amyloidogenic processing of proteins involved in the pathogenesis of major nervous system diseases, including Alzheimer's disease and Parkinson's disease, requires lipid raft-dependent compartmentalization at the membrane level.

Lipid rafts also play multifaceted roles in myelin organization and stability and in myelin–axon interactions, thus their dysregulation affects both demyelination and remyelination. Recombinant human IgM22(rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis (MS). In vitro analysis revealed that rIHgM22 binds to sulfatide, phosphatidylinositol and phosphatidylserine. Moreover, the composition of the lipid microenvironment of its antigen can modulate the affinity of the antibody, suggesting that reorganization of lipid membrane might be relevant in its biological activity. Analysis of the effect of rHIgM22 on glycosphingolipid metabolism in mixed glial cell cultures and in astrocytes revealed no significant effects on the lipid pattern, while in OPCs and OLs the levels of gangliosides GM3 and GD3, known for their ability to interact with and modulate the activity of different growth factor receptors, are increased. Considering all this, we propose that rHIgM22 protective effects might be mediated by alterations of lipid-dependent membrane organization and/or signalling in different cell types present in the MS lesions' nice and that a complex cross talk between different cell types is underlying the ultimate repair effect elicited by this antibody.

P1

Alterations of cortical connectivity in a mouse model of premature brain injury

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Cortical circuits are built at perinatal times and gradually refined in an activity-dependent manner during the postnatal period of critical plasticity. Although lesions of the CNS occurring during this period recover better than those occurring later in life, they are often associated with long-term cognitive deficits, which suggests that neuronal circuits rewiring, in particular within the cortex, may either be incomplete or inappropriate. Here we used chronic hypoxia, a mouse model of very premature birth, to study the long-term impact of premature brain injuries on glutamatergic neuron's maturation and cortical circuit's formation. Our results reveal gradual and profound alterations of glutamatergic neurons dendritic arborizations following chronic hypoxia, that differentially affect their apical and basal dendritic compartments. Using retrograde tracing, we show that these dendritic alterations are paralleled by a global cortical hyperconnectivity as well as a redistribution of long-distance cortical connections. Finally, testing of sociability reveals an impairment for social novelty in young adult hypoxic mice, which amplifies in adulthood. Altogether, our results highlight how premature brain injuries, such as those resulting from very premature births, impact cortical neuron maturation and connectivity, as well as associated behaviors.

P2

Reverse engineering of the synaptic tagging and capture mechanisms

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Memory and learning require fine-tuning of the connections between neurons in our brains. To strengthen a connection, a neuron needs to deliver hundreds of proteins from a range of different signaling pathways, but only to synapses that have been specifically stimulated. These proteins, known as plasticity-related proteins (PRP) can be locally recruited and produced in response to synaptic stimulation. Despite decades of research, the molecules and signaling pathways that participate in this mechanism, known as "synaptic tagging" have not been categorically identified, possibly because there are many different tags. Instead of asking “What is the synaptic tag?”, we have focused on “What does it take to be a synaptic tag?” aiming to identify the biophysical characteristics PRPs should have. To address these questions, we have engineered artificial proteins, Synthetic PRPs (SynPRPs), to test mechanisms of activity-dependent targeting and determine how they collaborate to strengthen connections. We have demonstrated that these synthetic PRPs are phosphorylated by CaMKII and consequently bind postsynaptic density protein 95 (PSD95) in a phospho-dependent manner. Additionally, we have tested if synPRPs are captured in activated synapses by expressing synPRPs in mouse hippocampal neuronal cultures. Subsequently we have analyzed synaptic distribution and colocalization with PSD95 after an in vitro chemical long-term potentiation (cLTP) stimulation. We have observed that synaptic clusters of SynPRPs + PSD95 increase 2-fold their synaptic localization after cLTP in cultured hippocampal neurons. Our results could indicate that SynPRPs fulfill the criteria to be captured by a synaptic tag in activated synapses. Moreover, biophysically understanding the synaptic tag and capture mechanisms could lead to prevent alterations of synaptic tagging in cognitive dysfunctions.

P3

Designing a Protocol Adopting an Artificial Intelligence (AI)–Driven Approach for Early Diagnosis of Late-Onset Alzheimer's Disease

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First, we aim to develop an AI-driven causal model to efficiently and accurately identify upstream definitive genetic markers (DGMs) of late-onset Alzheimer's disease (LOAD), based on the Alzheimer's disease neuroimaging initiative (ADNI) dataset (ADNI n.d.-a), which covers comprehensive genetic and biological data of cognitively normal (CN), suffering from mild cognitive impairment (MCI), and AD subjects. Second, we verify if the MCI group of the Han Chinese population in Hong Kong carrying the DGMs will have a higher probability of subsequently developing LOAD, as compared to the CN group of the same population, via a 5-year longitudinal observational study. Third, we identify the cognitive, linguistic, psychological, and behavioral markers concurrent to the 50 MCI subjects that carry DGMs, using 50 CN subjects as the reference, via the same observational study. Study progress will be discussed.

Keywords: Depression, Diabetic Rats, Neurophysiological changes, Herbal drugs

P4

Somatic Mutations and Alzheimer's Dementia

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Alzheimer's disease (AD) represents a global health challenge, with an estimated 55 million people suffering from the non-curable disease across the world. While Amyloid-β plaques and Tau neurofibrillary tangles in the brain are classically associated with AD progression, it has become evident that diverse coding and non-coding regions of the genome may significantly contribute to disease pathology. The diversity of factors associated with AD pathogenesis, along with the wide range of ages within which the disease can be manifested suggests that a series of triggering events may be required to initiate disease. Since a greater number of somatic mutations can be found in the brains of AD patients compared to others, there is a great potential for mutational events to drive disease. In this review we lay out the current perspectives on the principal genetic factors associated with AD and the potential causes and contribution of somatic mutations to the pathogenesis of Late Onset Alzheimer's Disease (LOAD). This research is supported in part by the 2021 US National Academy of Medicine Catalyst Award on Healthy Longevity, awarded to a team led by Principal Investigator VOK Li, and Co-Investigators JCK Lam, J Downey, and I Gozes. We thank Ms Shanshan Wang of the University of Hong Kong for assisting with the literature review and bibliography compilation.

[a] Equal Contributions as Senior Authors
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P5

Alteration of mouse cognition and neural circuits formation resulting from mutations on the autism-linked gene *Nuak1*

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The development of functional neural circuits relies on tightly regulated cellular processes controlled by complex cascades of signaling pathways, culminating in the proper development of axon terminals and synaptic connections. A disruption of these molecular mechanisms can lead to life-altering neurodevelopmental disorders such as autism spectrum disorders (ASD), mental retardation, schizophrenia or cognitive defects. Our team identified previously that the autism-associated protein kinase NUAK1 plays a central role in controlling axonal development in the mouse cortex (Courchet et al. Cell 2013). In addition, we recently described that Nuak1 is haplo-insufficient for mouse cortical development (Courchet et al. 2018). Knockout of Nuak1 leads to alterations of cortical connectivity and a wide array of behavioral alterations including sociability defects, deficits in learning and memory and abnormal sensory gating. Building on this work, we created a mouse line with a mutation of the Nuak1 gene mimicking a de novo mutation identified in autistic patients. The targeted knock-in mutation (Q434*) was achieved using the Crispr-Cas9 strategy. We will present the first data of characterization of this novel, humanized mouse line. Furthermore, we turned to a candidate-approach to identify which signaling pathway and transcription factor could explains the roles of NUAK1 in cued- and contextual fear memory. Overall, the comparison of this model with conditional knockout models will allow to better characterize the cognitive defects associated to Nuak1 and to identify the alterations in the neural circuits underlying these behavioral alterations.

Keywords: autism spectrum disorders, NUAK1, mouse model, memory, CREB.

P6

A cell-based model to validate the pathogenic nature of epilepsy-associated variants of the RORB gene

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Epilepsy is a complex disease with a high contribution of genetic factors, including several hundreds of Mendelian disorders. In those case, pathogenic variants disrupt the expression or function of proteins essential for neuronal development, synaptic transmission, or ion currents. Yet the identification of variants of unknown clinical relevance in patients raises the need to validate that the mutations affect some of the functions of the gene. We focused on RORB, which encodes the retinoid-related orphan receptor beta (RORb), and was recently associated to moderate intellectual disability and epilepsy. We characterized that knockdown of RORb expression altered axon morphogenesis in mouse cortical neurons. Conversely, overexpression of RORb led to a tractable axonal phenotype (ie. increased axonal complexity). Taking advantage of this phenotype, we generated plasmids expressing mutant RORb proteins mimicking variants identified in epileptic patients, and validated that these mutations impair the functions of the RORb protein. Overall, our study demonstrates the pathogenic nature of the variants tested in our cell model.

P7

Differential Regulation of CREB and AKT by cyclic AMP in the developing retina: The role of A1 adenosine receptors

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Aims: Neurotransmitters activate distinct signaling pathways and regulate specific functions during development such as survival and differentiation of neurons. We are currently studying the development of important transcription factors and signaling pathways in the developing chick retina, where previous work showed that cyclic AMP levels are regulated by dopamine and adenosine. Here we studied the effects of activation or inhibition of adenylyl cyclase on CREB and AKT phosphorylation in different stages of retina development.

Methods: Retinas from 10-day-old chick embryos (E10), an early stage of development, and E16, a more developed stage, were dissected and stimulated for 30 minutes in Hanks saline with forskolin (10 μM) or the adenosine A1 receptor agonist cyclohexyladenosine (CHA, 100 nM) to respectively activate or inhibit adenylyl cyclase. After the incubation period, retinas were lysed and analysed by western blotting using antibodies against phosho-CREB or phospho AKT, or the respective total proteins.

Results: Forskolin strongly stimulates CREB phosphorylation in E10 retinas, but a much smaller stimulation was observed in E16 retinas. However, in an interesting way, forskolin promotes a decrease of AKT phosphorylation in E10 or even with a higher degree in E16 retinas. Moreover, although A1 adenosine receptors are negatively coupled to adenylyl cyclase, CHA stimulates CREB and to a smaller extent Akt in E10, but not in E16 retinas, in a Src kinase, PKC and Erk-dependent way.

Conclusions: Our results indicate that cyclic AMP and its regulation by A1 adenosine receptors differentially controls different signaling pathways during retinal development.

P8

Timing and Region-Specific Reference Gene Selection within the Rat Brain in the Lithium Pilocarpine Epilepsy Model

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qRT-PCR is a powerful commonly used tool for gene expression analysis requiring the right choice of stably expressed reference genes (RGs) for accurate normalization. In this work, we aimed to select the optimal reference genes for qRT-PCR normalization within the rat brain at different stages of the Li-pilocarpine model of acquired epilepsy. We have tested the expression stability of nine housekeeping genes: Actb, Gapdh, B2m, Rpl13a, Sdha, Ppia, Hprt1, Pgk1,and Ywhaz. We have developed set of 3 original multiplex qPCR assays for quick analysis of aforementioned gene expression. Based on geometric averaging of ranks obtained by four algorithms (geNorm, NormFinder, BestKeeper, Comparative Delta-Ct), we found that the stability of tested RGs varied significantly between different brain regions after pilocarpine induced status epilepticus and depends on timing (3 days, 7 days in latent phase of the model, or 2 months, i.e. chronic phase). Pgk1 and Ywhaz were the most stable, while B2m demonstrate the lowest stability in the analyzed brain areas. High RG stability were detected in the medial prefrontal cortex, amygdala, striatum, and dorsal hippocampus, whereas in the ventral hippocampus and temporal cortex 4-5 of 9 analyzed genes were inappropriate for expression normalization. The expression stabilities of tested RGs were lower 3 days (early latent phase of the model), and especially 2 months after pilocarpine induced seizures compared to 7 day post-seizure rats. Interestingly, we found region-specific changes in the expression of B2m, Actb, Rpl13a, Hprt1 in the brain in a lithium-pilocarpine model of temporal lobe epilepsy, most pronounced in the latent phase. Thus, our data highlight the importance of careful selection of RGs for gene expression studies and demonstrate novel targets for investigation of epilepsy mechanisms among the housekeeping genes commonly used for normalization.

Supported by Russian Science Foundation, grant № 20-75-00127.

P9

Hippocampal differential expression underlying the neuroprotective effect of Δ9-tetrahydrocannabinol (THC) microdose on old mice

Shapira Guy[1,2], Israel-Elgali Ifat[1,3], Avnat Eden[1], Rachmany Lital[1], Sarne Yosef[1], Shomron Noam[1,2,3]

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Delta-9 tetrahydrocannabinol (THC) is the primary psychoactive compound of the Cannabis plant and an exogenous ligand of the endocannabinoid system (ECS). In past studies, we demonstrated that a microdose of THC (0.002 mg/kg, 3–4 orders of magnitude lower than the standard dose for rodents) exerts a long-term neuroprotective effect in model mice, alleviating damage from acute neurological insults and late-onset neurodegenerative pathology. Assays measuring the impact of the THC treatment on gene expression, morphology and biochemistry of the brain identified significant neurogenesis, increased abundance of anti-inflammatory proteins and reversal of gene expression alterations linked to neurodegeneration and aging. In our follow-up research, we use high-throughput transcriptome profiling of various brain region to investigate the potential benefits of the treatment for Parkinson's Disease, including promotion of cell-survival in vulnerable dopaminergic neurons, inhibition of chronic microglial inflammation and resistance to oxidative stress. We highlight the modulation of the Transforming growth factor beta (TGF-β) pathway and known Parkinson's Disease therapeutic targets, such as Sgk1. We propose the THC microdose as a novel treatment for Parkinson's disease and discuss its potential disease-modifying properties when used at the pre-clinical stage.