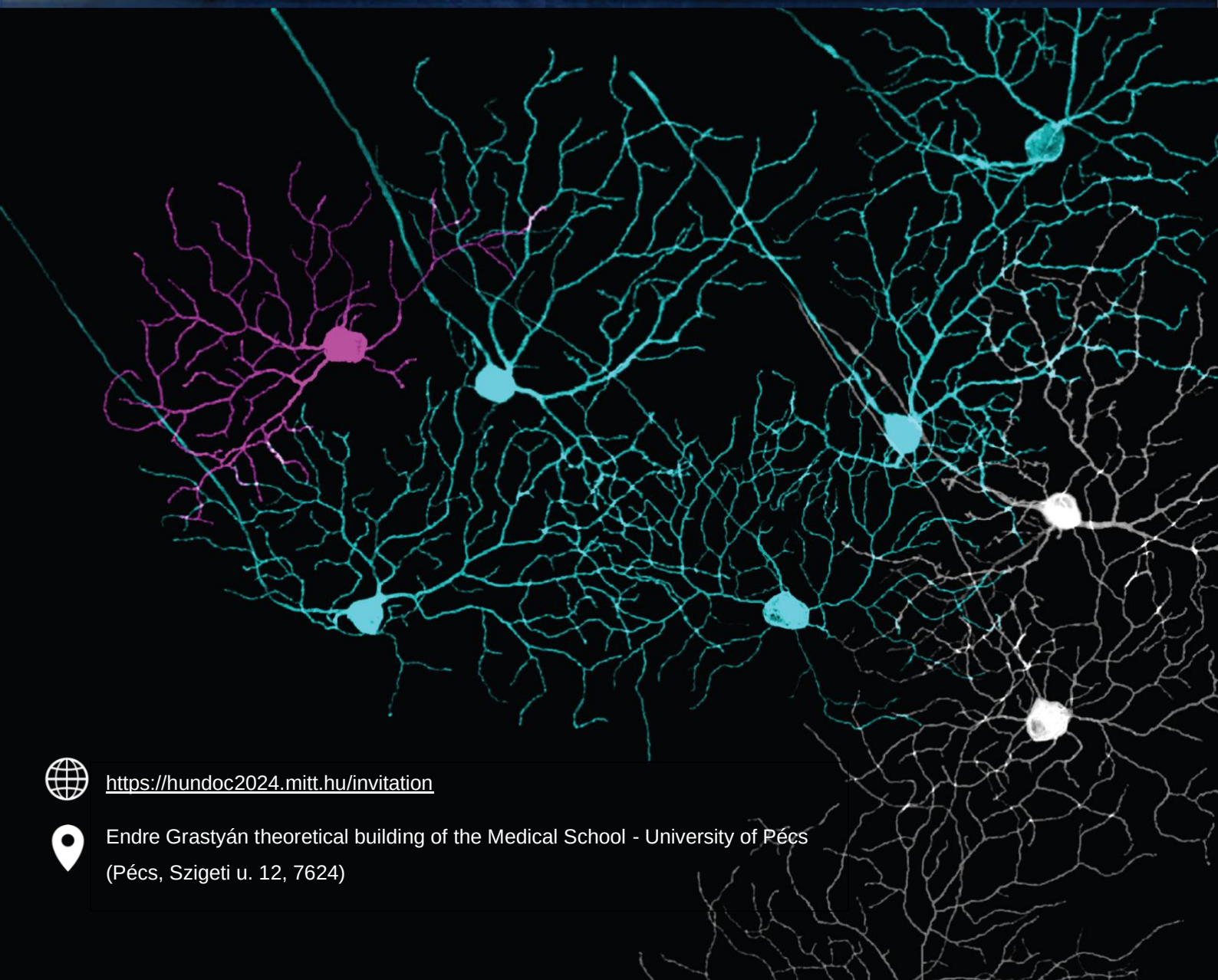
7th Hungarian Neuroscience Meeting for Undergraduate Students, Graduate Students and Young Postdocs

24th January, 2024, Pécs



https://hundoc2024.mitt.hu/invitation

Endre Grastyán theoretical building of the Medical School - University of Pécs
(Pécs, Szigeti u. 12, 7624)

Invitation

Dear Colleagues,

The 7th Hungarian Neuroscience Doctoral Conference for Undergraduate Students, Graduate Students and Junior Post-Docs (HUNDOC) will be held on the 24th of January. Similarly to previous years, it will be held the day before the International Neuroscience Workshop. Registration is separate and it is free of charge for MITT members who paid their annual fees (2000 HUF for students and 4000 HUF for postdocs) by November 15th. Non-members are asked to pay a registration fee (15 000 HUF).

We would like to welcome undergraduates, PhD students and junior post-docs to share their research at an open, stimulating and motivational forum. It'll be a fun day filled with good company, elevating speeches, and workshops and most of all fascinating science in the lively and lovely city of Pécs. Just like in previous HuNDoC conferences, we expect high number of young researchers. The HuNDoC conference will provide the catering and amazing programs, such as an open discussion with one of the greatest Hungarian neuroscientists, Prof. György Buzsáki. In the evening there will be a social event to further discuss and reflect on the happenings of the day (for further details, please see the program)

Every participant is required to submit an abstract, however ongoing projects are also welcome. Your results are expected to be presented in the form of an A4 Mini poster, with the possibility of being chosen to present your work in a short talk (10 min talk with 5 min discussion time) if you're interested.

We hope to see you there!

Any further questions, please do not hesitate to contact us at hundoc2024@gmail.com.

The organizing committee:

Barbara Fülöp, Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs

Szilárd Szőcs, Department of Physiology, Medical School, University of Pécs

Gergely Szarka, Department of Neurobiology, Faculty of Natural Sciences, University of Pécs

Special thanks for the support!

In the name of the organizers and all participants we would like to thank:

The Hungarian Neuroscience Society (https://mitt.hu/en) for sponsoring the event in both the spirit and funding.

Prof. György Buzsáki, Christina Miskolczi, Dr. Zsuzsanna Varga, Dr. Andrea Horváth-Sarródi for agreeing to elevate our conferences with their presentations.

Ábrahám István Nano-Bio-Imaging Core Facility (https://aok.pte.hu/en/egyseg/2110) for supporting our image contest.

The Faculty of Medicine of the University of Pecs for providing the location.

Bősz Emilia for her support in organization of the conference.

Social Event

Pub quiz and socializing at Paulus

The event and serving of the food starts at 19:00

7624 Pécs, Ifjúság útja 4.

http://www.pauluscafe.hu

Program Overview

Wednesday, 24, January 2024

08:00 – 09:00 **Registration** (registration site will close at 12 o'clock)

09:00 – 09:15 **Welcome speech**

09:15 – 09:45 **Invited speaker**

How early-life experiences can shape behaviour in later life: Investigating the links between early-life social isolation, social abnormalities in adulthood and prefrontal network function

Christina Miskolczi (Institute of Experimental Medicine and Janos Szentagothai Doctoral School of Neurosciences)

09:45 – 11:00 Oral presentations I.

Presenters:

Abbas Anna Anoir: CARiNg-HD: Studying the effect of cariprazine in induced neurons directly reprogrammed from Huntington's disease patients' fibroblasts

Bosnyák Inez: Optimization of an ischemic retionpathy mouse model and evaluation of the consequenses of oxygen deprivation

Bod Réka: Bridging in vivo and in vitro recordings in the human epileptic neocortex: patient-wise comparative analysis of single-unit activities

Ignácz Máté: Neuroectodermal stem cells contribute to the functional and morphological improvement of chronic spinal cord injuries via multiple mechanisms

Milica Milicic: TRPA1 ion channel does not contribute to the chronic stress-induced activation of locus ceruleus noradrenergic neurons

11:00 – 12:30 Mini Poster Section

12:30 – 13:30 **Lunch**

13:30 – 14:15 **Workshop I.** Zsuzsanna Varga PhD Mastering Essential Time Management Skills for Academic and Professional Success

14:15 – 15:30 Oral presentations II.

Presenters:

Horváth Ágoston Csaba: Towards layer-by-layer infrared neuromodulation: presentation and functional characterisation of an intracortical optrode needle featured with a micromirror tip ending

Padányi Anna: Modulation and investigation of cortical excitability with non-invasive transcranial magnetic stimulation and electro-encephalography in awake non-human primates

Ruppert Zsófia: Fructose supplementation exacerbates systemic inflammation induced by high-fat diet and causes brain shrinkage in mice

Szendi Vivien: Calbindin neurons of the lateral septum control maternal behaviour via a thalamicseptal-hypothalamic circuit

Zichó Krisztián: Novel pontine inhibitory nucleus regulates reward experience

15:30 – 16:00 **Coffee break**

16:00 – 17: 00 **Workshop II.** Meeting with the professor (Prof. György Buzsáki, Brain Prize in 2011)

17:00 – 17:45 **Workshop III.** Andrea Horváth-Sarródi M.D. What Doesn't Kill You Makes You Stronger? - Life beyond failures

17:45 – 18:00 **Closing remarks – awards**

19:00 – 24:00 **Social Event** - Pubquiz and socializing at Paulus - Pécs, Ifjúság útja 4, 7624

Miskolczi, Christina

How early-life experiences can shape behaviour in later life: Investigating the links between early-life social isolation, social abnormalities in adulthood and prefrontal network function

1 Institute of Experimental Medicine
2 Janos Szentágothai Doctoral School of Neurosciences

During early life, brain regions regulating social behavior undergo dynamic developmental changes. The prefrontal cortex (PFC) is a key regulator of social behavior, where experience-dependent network development is tied to the maturation of parvalbumin-positive (PV+) interneurons. Social adversities (e.g. abuse or neglect) experienced during this time could alter maturation and lead to abnormal social behavior in later life. However, our knowledge regarding the impact of early-life social stress on PFC operation and PV+ cell function is still scarce. Here, we used post-weaning social isolation (PWSI) to model early-life social neglect in mice and to study the associated changes in the PFC, with additional focus on PV+ interneurons. Our results show that PWSI induced disturbances in social behavior in adulthood, including abnormal aggression and fragmented behavioral organization. PWSI mice also displayed altered PFC neuronal activity during resting state and in response to a social challenge (resident-intruder test), as revealed by c-Fos co-activity patterns between PFC subregions. Additionally, we found that PWSI enhanced the extra- and intracortical excitatory drive and decreased the inhibitory drive arriving onto PV+ interneurons. In conclusion, PWSI impacts PV+ input properties and leads to altered excitatory/inhibitory balance in the PFC, which potentially contributes to the abnormal PFC network activity and social behavioral disruptions seen in PWSI mice. Our data advances our understanding on how early-life social stress can impact the maturing PFC and lead to the development of social abnormalities in adulthood.

1. Abbas, Anna

CARiNg-HD: Studying the effect of cariprazine in induced neurons directly reprogrammed from Huntington's disease patients' fibroblasts

Abbas Anna[1,2], Idris J. Jimoh[2], Lea Danics[1,2], Balázs Kis[1,2], Anikó Göblös[3], Roger A. Barker[4], Zoltán L. Veréb[3], Johan Jakobsson[5], Lajos Kemény[1,3], Judit Mária Molnár[2], Karolina Pircs[1,2,5]
1 HCEMM, Hungary
2 Semmelweis University, Hungary
3 University of Szeged, Hungary
4 University of Cambridge, United Kingdom
5 Lund University; Sweden

Huntington's disease (HD) is an uncurable autosomal dominant progressive neurodegenerative disorder. The role of the dopaminergic system in the development of HD symptoms is crucial, as the central dopaminergic pathways are overactivated in HD. The dopaminergic overactivity can be reduced by several drugs. However, their effectivity on psychiatric symptoms is limited. Moreover, the treatment of apathy and cognitive symptoms still remains challenging in HD. Cariprazine, a third-generation antipsychotic, is acting as a dopamine D3 and D2 receptor agonist. Previous results have shown positive effects in HD patients after cariprazine treatment. Clinical studies indicated positive effects in early-stage HD patients after cariprazine treatment in some psychiatric symptoms such as depressed mood, apathy and cognitive function in patients. Moreover, cariprazine also improved dopamine imbalance in the prefrontal cortex.

Aims: In this project, we aim to study the effect of cariprazine in a novel in vitro model system of HD using donor-derived aged-induced neurons. Our goal is to understand the putative beneficial effects of cariprazine in HD patients and to better understand its mechanism of action by focusing on autophagy. Using reverse translational strategy, we use cariprazine treatment in induced neurons directly reprogrammed from ctrl, HD drug-naive and cariprazine-treated HD patients' fibroblasts. For detection, we use immunocytochemistry (ICC) followed by high-content automated microscopy (HCS). We suppose that the described abnormal neurite morphology and the neurite-specific impairment of subcellular autophagy are positively altered following cariprazine treatment.

2. *Adegbite, Janet*

Investigation of the effect of SAHA on a human cell culture model of ischemic stroke

Janet Adegbite[1], Anikó Szecskó[1,2], Koppány Párdi[1], Gergő Porkoláb[1,2,] Mária A. Deli[1], Szilvia Veszelka[1]
1 Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
2 University of Szeged, Szeged, Hungary

Cardiovascular diseases such as myocardial infarction and ischemic stroke are the most common causes of disability and mortality worldwide due to atherosclerosis, thrombotic or embolic events that reduce blood flow and cause severe neuronal damage and blood-brain barrier (BBB) disruption. Our research focuses on

the protection of brain endothelial cells and BBB function after stroke as a critical tool to prevent severe post-stroke consequences. The major transcriptional regulator of blood flow responses of microvascular endothelial cells is Krüppel-like factor 2 (KLF2). Although its protective effect against cerebral ischemia is still unclear, laminar shear stress has been shown to promote endothelial survival and to have antithrombotic, antiadhesive and anti-inflammatory qualities in cardiovascular diseases. The aim of the study was to investigate the effect of SAHA (suberoyl anilide hydroxamic acid, vorinostat), a clinically used drug that is able to enhance KLF2 expression and has good BBB permeability. Human cell cultured BBB model was used under three different conditions: normoxia, oxygen-glucose deprivation (OGD), and reperfusion after OGD (OGD/R) and then tested the effect of SAHA against BBB dysfunctions. To examine the impact of SAHA on cell viability after OGD we used impedance-based cell analysis. The effect of SAHA on BBB integrity both in normoxia and OGD/R was monitored by measuring transendothelial electrical resistance (TEER), and fluorescence spectrophotometry was used to test the penetration of marker molecules (albumin and fluorescein) through a culture model of BBB. Moreover, immunocytochemistry was utilized to investigate the localization of KLF2. SAHA was able to protect against the TEER reduction caused by OGD and OGD/R. Penetration of both the paracellular BBB marker, fluorescein, and the transcellular marker, albumin elevated after OGD/R suggesting increased permeability of the BBB model, but treatment with SAHA was able to protect this adverse effect. Based on immunocytochemistry results, KLF2 expression was increased after OGD/R compared to normoxia but SAHA could not elevate it more. In conclusion, SAHA was able to prevent the BBB damage after ischemic stroke but the cellular mechanism underlying the protective effect does not seem to be related to KLF2.

This work was funded by OTKA Young Researcher Excellence Program (OTKA-FK 143233) by National Research, Development and Innovation Office of Hungary.

3. Balkó, Eszter

The effect of impaired AgRP neuronal function on body weight, lifespan and behaviour in calorie-restricted mice

Eszter Balkó[1], Mátyás Kapiller[1], Boglárka Barsy[1,2], Lilla Dénes[1], Ferenc Mátyás[1,2], Péter Sótonyi[1], Bence Rácz[1], Tamás L. Horváth[1,3]

1 University of Veterinary Medicine, Budapest, Hungary

2 ELKH Inst. of Exptl. Med., Budapest, Hungary

3 Department of Comparative Medicine, School of Medicine, Yale Uinversity, New haven, CT, USA

Neurons expressing hypothalamic agouti-related peptide (AgRP) and neuropeptide Y (NPY) play a crucial role in the development of hunger. AgRP neurons are located exclusively in the arcuate nucleus of the hypothalamus and together with POMC neurons form the basis of the melanocortin system: a set of CNS circuits regulating food intake and energy balance. However, the impact of AgRP neurons on complex, non-feeding related behaviour is less understood and could potentially represent an important link to various mental disorders, such as anorexia nervosa. Using the AgRP^{DTR} mouse model we performed various behavioural tests to study the effects of neonatal AgRP neuronal ablation on exploratory behaviour and anxiety. As AgRP neurons are primarily active during negative energy balance and starvation-like states, we also examined the ability of animals with impaired AgRP neuronal function to adapt to a CR regimen. Several studies have shown that CR can be successfully implemented to slow down ageing processes and increase lifespan. Considering the role of AgRP neurons in energy balance regulation, we also test the hypothesis that AgRP neurons are essential for the beneficial effects of CR. Our results indicate that perturbation of AgRP neuronal function can lead to sex-dependent changes in metabolic and behavioural adaptation to CR.

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4. Balog, Bolidzsár Zsolt

Positive valence regulated by pontine inhibitory cells: fiber photometry evidence

Boldizsár Zsolt Balog[1,3], Krisztián Zichó[1,2], Réka Z. Sebestény[1], Hunor Sebok[1], Áron Orosz[1,2], Gábor Nyiri[1]
1 Laboratory of Cerebral Cortex Research, Department of Cellular and Network Neurobiology, HUN-REN Institute of Experimental Medicine, Budapest, Hungary
2 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary
3 Tamás Roska Doctoral School of Sciences and Technology, Pázmány Péter University, Budapest, Hungary

The lateral habenula (LHb) is an evolutionarily well-preserved structure responsible for motivational and cognitive functions. Using viral gene delivery methods in transgenic mice, we found a novel, inhibitory, pontine cell population projecting to the LHb. We analyzed the activity of this GABAergic (gamma-aminobutyric acid) pontine nucleus using head-fixed fiber photometry measurements. In water-deprived mice, we found that the consumption of water droplets led to an increase in its activity, whereas aversive airpuffs also activated this nucleus. We also found that the nucleus was significantly activated by an otherwise neutral tone if it was previously associated with a positive or a negative experience. Furthermore, when we presented multiple airpuffs with a relatively short interval between airpuffs, we observed a short-term accommodation represented by a decrease in its activation to subsequent airpuffs. Our results suggest that this novel, inhibitory nucleus plays a role in the processing of aversive and rewarding experiences and may prevent overactivation of the lateral habenular negative processing circuitry. Therefore, these cells may play a role in mood-related pathologies.

Funding: This project was supported by the ÚNKP-23-3-II-SE-24 New National Excellence Program of the Ministry of Innovation, ÚNKP-23-2-II-SE-25 New National Excellence Program of the Ministry of Innovation, by the EFOP-3.6.3-VEKOP-16-2017-00009 Semmelweis 250+

5. Balogh, Boglárka

Developing and validating a new model for mouse subretinal haemorrhage

Boglárka Balogh[1,2], Dr. Tamás Kovács-Öller[1,2]
1 University of Pécs, Faculty of Sciences, Institute of Biology
2 University of Pécs, Szentágothai Research Centre, Retinal Neurobiology Research Group

Subretinal haemorrhage (SRH) is due to accumulation of blood between the retina and the retinal pigment epithelium (RPE), or between the RPE and the choroid. It can occur spontaneously as a result of changes in the elasticity of blood vessels but is often caused by hypertension or trauma. Subretinal blood is toxic to the retina and untreated SRH can lead to rapid tissue damage accompanied by photoreceptor and RPE degradation, resulting in visual impairment, and possible blindness.

In our research, we aim to create a new mouse model for SRH to better delineate the exact area of the bleeding itself and its effects regarding cellular survival and immune cell activation. Also, in our model system we show a possible treatment option that can alleviate the effects of secondary injuries due to the spreading of death signals and inflammation next to the bleeding site.

We created 4 injection groups of C57BL/6 mice with 24-hour survival times. For the SHAM group, filtered PBS was injected subretinally, in group I native, autologous blood from the tail vein of the animals were injected, in group II Cholera toxin B-AlexaFluor594 conjugate was co-injected with the autologous blood, while in group III fluorescently labelled GADPH siRNA was co-injected with blood to test it's effectivity in gene regulation. We used Iba1, GFAP and Casp3 markers for posthoc IHC staining and to show the level of immune activation in relation to SRH As far as we know it, our method is the first to induce and treat SRH with native blood and siRNA to not only reach a deeper insight to the pathology but to alleviate the effects as well.

Acknowledgement: Supported by the ÚNKP-23-3-I-PTE-1950 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund

6. Balogh-Lantos, Zsófia

Cell type- and layer-specific intracortical effects of continuous infrared neural stimulation revealed by high-density laminar recordings in the rat neocortex

Zsófia Lantos[1,2], Richárd Fiáth[1,3], Ágoston Csaba Horváth[1], Zoltán Fekete[1]
1 Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary
2 Roska Tamás Doctoral School of Sciences and Technology, Pázmány Péter Catholic University, Budapest, Hungary
3 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary

Infrared (IR) neuromodulation research has consistently shown temperature to be an

important neuronal state variable over the last decade. Multiple studies have described the ability to stimulate or block peripheral nerve activity with IR radiation. The promise of IR inhibition in treating neurodegenerative diseases, such as epilepsy, underscores the significance of further elucidating its biophysical mechanism. The effects of IR neuromodulation on cortical neurons in vivo were examined in this study via high-density laminar recordings. The neocortex of anesthetized rats was exposed to pulsed and continuous infrared light (1550nm) using a photonic microtool. Over 7500 single

units were recorded from 8 rats using a Neuropixels probe. Putative principal neurons and inhibitory interneurons with suppressed or increased activity were identified, highlighting cell- and layer-specific responses. We analyzed the temporal dynamics during stimulation trials and their correlation with temperature changes. Pulsed

light preferentially excited units over suppression, while continuous light tended to suppress. The temperature increases varied with frequency and were correlated with the number of responsive units. Examining alterations in the baseline firing rate across trials indicated a long-lasting effect of stimulation, maintaining the excitability of the affected neurons at an elevated level. Furthermore, analysis of individual neuron responses at varying frequencies revealed diverse patterns. This study offers new insights into the mechanisms of infrared neuromodulation by accurately characterizing layer- and cell-type-specific responses. By analyzing thousands of neurons, our findings on neuronal sensitivity to infrared irradiation parameters may assist in

optimizing future applications.

The authors are grateful for the funding of the National Development and Innovation Office (NKFIH FK134403 and TKP2021-EGA-42 to Z.F.) and the support of the Hungarian Brain Research Program (NAP2022- I-8/2022). R.F. was supported by the Bolyai János S cholarship of the Hungarian Academy of Sciences.

7. Bíró, László

Persistently increased post-stress activity of paraventricular thalamic neurons is essential for the emergence of stress-induced alterations in behavior.

Anna Jász[1,2]†, László Biró[1]†, Zsolt Buday[1,2], Bálint Király[3,4], Orsolya Szalárdy[5], Krisztina Horváth[6], Gergely Komlósi[1], Róbert Bódizs[5], Krisztina J. Kovács[6], Marco A. Diana[7], Balázs Hangya[3], László Acsády[1]

1 Laboratory of Thalamus Research, Institute of Experimental Medicine; Budapest, Hungary
2 Neurosciences PhD School, Semmelweis University; Budapest, Hungary
3 Lendület Laboratory of Systems Neuroscience, Institute of Experimental Medicine; Budapest, Hungary
4 Department of Biological Physics, Institute of Physics, Eötvös Loránd University, Budapest, Hungary
5 Psychophysiology and Chronobiology Research Group, Institute of Behavioral Sciences, Semmelweis University; Budapest, Hungary
6 Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine; Budapest, Hungary
7 Université Paris Cité, CNRS, Saint-Pères Paris Institute for the Neurosciences, F-75006 Paris, France

A single exposure to a stressful event can lead to long-term alterations in behavior. While long-term, stress-induced modifications in neuronal networks are well-studied, the initial steps leading to these changes are incompletely understood. Here we show that acute stress exposure induced an increase in the firing activity of mouse calretinin-positive neurons in the paraventricular thalamus (PVT/CR+) lasting for multiple days, in parallel with altered spontaneous behavior. Reducing PVT/CR+ neuronal activity for only one hour after the stress event was sufficient to rescue both the multiday increase in PVT/CR+ firing rate and the stress-induced behavioral change. Inhibition applied 5 days later was still able to ameliorate stress-induced behaviors. These data demonstrate that persistent alteration of firing activity in PVT/CR+ neurons after the stress event is critical to establish the stressed behavioral phenotype and offer a window of opportunity for therapeutic intervention in acute stress related diseases.

8. Bod, Réka

Bridging in vivo and in vitro recordings in the human epileptic neocortex: patient-wise comparative analysis of single-unit activities

Réka Bod[1,2], Berta Börcsök[1,3], Kinga Tóth[1], Loránd Erőss[4], Dániel Fabó[4], István Ulbert[1,3,4] and Lucia Wittner[1,4]

1 Research Centre for Natural Sciences, 1117 Budapest
2 Semmelweis University Doctoral School, János Szentágothai Neurosciences Program, 1085 Budapest
3 Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, 1083 Budapest
4 National Institute of Mental Health, Neurology and Neurosurgery, 1145 Budapest

Introduction: Epilepsy affords an excellent opportunity to explore cellular electrophysiology in humans, both in vivo and in vitro conditions. While long-term, single-site in vivo recordings might offer advantages in action potential pattern reconstruction and in the preservation of neuronal connections, in vitro electrophysiology provides a broader tissue exploration, but within a restricted space comprising neurons with cut connections. Both paradigms yield valuable insights into cellular electrophysiology, yet coherent investigation of neuronal firing patterns in these distinct conditions is lacking. In this study we compared the firing properties of single neurons recorded in the same patients, both in vivo and in vitro

conditions.

Methods: An identical recording system was used to conduct in vivo and in vitro intracortical recordings comprising a 24-channel laminar microelectrode with 150 µm distance between contacts, in epileptic patients (n=4) as well as in their postoperative tissue slices. We employed spike sorting algorithms docked by spikeinterface (tridesclous, spykingcircus2, and pykilosort) for single-unit analysis. We performed analysis of unit waveforms, correlograms, firing rates and burstiness index, as well as

determined the excitatory and inhibitory feature of the single neurons.

Results: Identifying 330 single neurons in vitro and 22 in vivo, we observed a significantly higher overall firing rate for in vitro (2.738 Hz) than in vivo cells (1.321 Hz, $p<0.0001$). The firing rate of principal cells was not different (2.71 Hz in vitro vs. 2.73 Hz in vivo), while interneurons discharged with a higher rate in vitro, than in vivo (2.93 vs. 0.74 Hz). The burstiness of all cells was significantly higher in vivo (6.986%)

than in vitro (3.506%, $p=0.0006$). Patient-wise in vivo and in vitro unit numbers correlated strongly ($r=0.703$). Layer-specific analysis revealed high correlations (0.926 for firing rates, 0.762 for burstiness indices).

Discussion: Disparities in firing rates and burstiness highlight the differences between in vivo and in vitro conditions. In vitro neurons, especially inhibitory cells with limited synaptic connections show higher activity than those in the intact neocortex. The lower burstiness in vitro might also be linked to the partial loss of synaptic inputs, but the different environment provided by the bathing solution might also account for the observed differences.

Funding:

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Bodo, Angelika

A pilot study using DREADD technology to develop a novel model of cognitive impairment

Bodo A. [1,2,3], Bali Zs.K. [1,3], Bruszt N. [1,2,3,4], Reisinger Cs. [1,3,4], Hernadi I. [1,2,3,4]
1 Translational Neuroscience Research Group, Grastyán Translational Research Centre,
2 Medical School
3 Szentágothai Research Centre
4 Faculty of Sciences, University of Pécs

Introduction: Designer Receptors Exclusively Activated by Designer Drugs (DREADD) is a novel chemogenetic technology where genes of modified human receptors without any endogenous ligands are expressed. These receptors can be reversibly activated by specific actuators, small molecules selectively binding to their DREADDs and not to any naturally occurring receptors.Thus, DREADD is a powerful tool that can be widely applied in basic research and preclinical drug development.

Aims: The primary aim of our pilot experiments were to investigate the changes in performance of rats during multiple behavioral experiments as a result of silencing the targeted brain areas using DREADDs. Our long-term goal is to develop a novel translational model representing the pathophysiology of cognitive decline in humans.

Experimental models and methods: We stereotaxically injected 500 nl of adeno-associated virus vector serotype 5 (AAV5) carrying the gene of the modified human M4D(Gi) cholinergic receptor into either the hippocampus (HC), the anterior cingulate cortex (ACC) or the infralimbic cortex (IL) in rats. After recovery, the training of rats started for the psychomotor vigilance task (PVT) in the Coulbourn

Habitest operant conditioning system. In PVT, parameters informing about general alertness (arousal) and sustained attention were measured 30 min after the administration of 3 different doses of DCZ or VEH s.c. based on a Latin square design along with 6 non-operated control animals. For the assessment of explicit memory, we performed novel object recognition (NOR) tests 30 min after DCZ administration and calculated discrimination index (DI) based on exploration time of the novel and the old object. Spatial learning skills were tested using the Morris water maze (MWM) task 30 min after injecting high doses (1.0 mg/bwkg) of DCZ in a between-subject design, measuring escape latency on 3 training days and the time spent in the goal quadrant (Q) during the probe trial.

Results: In the IL group, increased reaction time was detected in the PVT, whereas HC operated animals showed significantly prolonged motor response, and a decreased number of missed trials. In the NOR tests, the HC targeted animals could recognize the novel object only if they did not receive DCZ treatment. The MWM test showed significantly increased escape latency in the ACC group compared to the three other groups, indicating impaired spatial learning.

Acknowledgement: This project was funded by the Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-16 project of the University of Pécs. This research work was conducted with the support of the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture and Innovation. Funding: PTE-KITEP-2023-248

9. Bogdány, Tamás

Role of vestibular input in lucid dreaming

Bogdány Tamás[1]
1 Institute of Experimental Medicine, Budapest, Hungary

My current research focus is the interindividual differences concerning lucid dream experiences, vestibular related dream contents, wakeful imagination, and balance ability. It is hypothesized that those who experience more lucid dreams and/or are more proficient in lucid dreaming, have better balancing ability due to their integrated vestibular system at the multimodal level. The idea that vestibular processing is involved in lucid dream experiences - and that this relationship can be measured e.g. through balance ability - dates back to the beginning of lucid dreaming research.

It is based on the relationship between the vestibular system and REM sleep, the prevalence of lucid dreaming during REM sleep, and some observation regarding the internally (vestibular input) weighted reference frame of lucid dreamers in some perceptual tasks, such as Subjective Visual Vertical estimation. Accessing lucid dreaming directly in a laboratory environment is challenging, therefore methods that can capture the phenomenon outside the dream environment, such as performance measures obtained during wakefulness, have been preferred. In my study participants were interviewed about their lucid dreaming frequency (LDF), lucid dreaming skills (LUSK), dream contents (such as flying, falling, rolling, or floating), measured in their balance ability (modified Clinical Test of Sensory Interaction in Balance, mCTSIB), and the vividness of wake-induced mental imagery (Vividness of Mental Imagery Questionnaire - VMIQ- 2). Results show that frequent lucid dreamers do differ in their performance related to vestibular processing and give the courage to probe the system with other methods.

10. *Bosnyák, Inez*

Optimization of an ischemic retinopathy mouse model and evaluation of the consequences of oxygen deprivation

Inez Bosnyak[1], Alexandra Vaczy[1], Nelli Farkas[2], Dorottya Molitor[1], Balazs Meresz[1], Evelin Patko[1], Lina Li[1], Eniko Horvath[1], Reka Radics[1], Tamas Atlasz[1,3], Dora Reglodi[1]

1 Department of Anatomy, ELKH-PTE PACAP Research Team, Medical School, University of Pecs, Pecs, Hungary

2 Institute of Bioanalysis, Medical School, University of Pecs, Pecs, Hungary

3 Department of Sportbiology, Faculty of Sciences, University of Pecs, Pecs, Hungary

The retina has one of the highest metabolic activities and oxygen consumption, so insufficient blood supply leads to visual impairment. Retinal hypoxia has a key role in the development of age-related macular degeneration, glaucoma, diabetic retinopathy, and retinopathy of prematurity, among others. The incidence of these diseases is increasing, however, no effective treatment without side- effects is available. Furthermore, the pathomechanism of the above-mentioned conditions is not fully understood. Based on these facts, our aim was to develop an optimal ischemic retinopathy mouse model to investigate the retinal damage in a time-dependent manner and to analyze the sensitivity of different cell types.

Retinal ischemia was induced by bilateral common carotid artery occlusion (BCCAO) for 10 (n=12), 13 (n=14), 15 (n=14), or 20 (n=18) minutes, or by right permanent unilateral (n=17) common carotid artery occlusion (UCCAO) in 4-month-old CD1-IGS mice. The data obtained were compared with a sham-operated group (n=18). Optical coherence tomography was used for following the changes in retinal thickness 3, 7, 14, 21 and 28 days after the surgeries. After standardizing the results, the confidence intervals were compared for statistical analysis. Different immunohistochemical analyses were also performed. We evaluated the number and distribution of ganglion cells in the central and peripheral regions on whole-mount retina preparations. Vascular density was studied by lectin staining. Rods and cones were labelled too. For immunolabeled samples, non-parametric tests were used to analyze the results.

We observed significant changes in almost all retinal layers during the experimental period, but the absolute values of these changes are not very remarkable. The number of ganglion cells significantly decreased in the peripheral region in the 20-minute BCCAO group, and in the central and peripheral regions in the UCCAO group 4 weeks after the intervention. The percentage of vascular density was higher in the 20-minute BCCAO and in the UCCAO groups, suggesting neovascularization. In addition, cone loss was also observed in these groups.

Our results suggest that the 20-minute BCCAO is a good model for investigating the consequences of ischemia and reperfusion in the retina in a time-dependent manner, while the UCCAO causes more severe damage in a short time, so can be used for testing new drugs.

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11. Bruszt, Nóra

Long-term effects of memantine and two alpha7 nicotinic acetycholine receptor compounds on novel object recognition memory of aged rats

Nóra Bruszt[1,2], Zsolt Kristóf Bali [1,2], Lili Veronika Nagy[1,2,3], Szuhád Leila Khalil[1,2], Áron Olivér Kolozsvári [1,2], István Hernádi [1,2,3]

1 Grastyán E. Translational Research Centre, University of Pécs, Pécs, Hungary

2 Translational Neuroscience Research Group, Centre for Neuroscience, Szentágothai Research Centre, University of Pécs, Pécs, Hungary

3 Department of Neurobiology, Faculty of Sciences, University of Pécs, Pécs, Hungary

Aging has been demonstrated to be the main risk factor for the emergence of neurocognitive disorders (NCDs) that are characterised with progressive neuronal dysfunction and deterioration of cognitive abilities. Currently, there is no proper cure for NCDs yet, thus the discovery of new avenues for the treatment of NCDs would be crucial in the field. As we previously demonstrated, naturally aged rats show marked age-related cognitive impairment and certain pathological aspects of human NCDs, thus they are relevant model for testing pharmacological effects of new treatment strategies.

Our aim was to evaluate the pro-cognitive effect of the non-competitive NMDA receptor antagonist memantine and two different α7 nicotinic acetylcholine receptor (α7-nAChR) agents (the orthosteric agonist PHA-543613 and a novel positive allosteric modulator, called CompoundX) using a sub-chronic (two-week long) treatment regimen in aged rats. The long-term declarative memory of the animals was measured with the novel object recognition paradigm (NOR). Memantine was applied in the following doses: 0.03 mg/kg, 0,3 mg/kg, 3.0 mg/kg. PHA-543613 was administered in the doses of 0.03 mg/kg, 0.1 mg/kg and 2.0 mg/kg and CompoundX was administered in the doses of 0.1 mg/kg, 0.3 mg/kg and 3.0 mg/kg.

Results showed that memantine at 0.03 mg/kg and 3.0 mg/kg doses improved discrimination performance of aged rats during the treatment period, and their effects were maintained even after the end of treatments. However aged animals who received memantine at 0.3 mg/kg dose showed poor memory performance. PHA-543613 at 0.1 and 2.0 mg/kg dose improved discrimination performance during but not after the treatment period, while PHA-543613 at the lowest 0.03 mg/kg dose had no effects neither during nor after the treatment period. CompoundX at 0.3 and 3.0 mg/kg dose decreased age-related cognitive decline during the treatment period, however at 0.1 mg/kg dose no effects were observed.

To sum up, permanent improvement can be achieved with the applied sub-chronic pharmacological treatments against age-related cognitive impairment. Our results can contribute to the development of new and effective therapeutic strategies for human NCDs.

12. Buday, Zsolt

Characterisation of inhibitory and excitatory afferents of the paraventricular thalamic nucleus

Zsolt Buday[1, 2], Laszlo Biro[1], Kata Kota[1], Akos Babiczky[3], Ferenc Matyas[3], László Acsady[1]
1 Laboratory of Thalamus Research, Institute of Experimental Medicine; Budapest, Hungary
2 Neurosciences PhD School, Semmelweis University; Budapest, Hungary
3 Neuronal Network and Behavior Research Group, Institute of Experimental Medicine; Budapest, Hungary

The paraventricular thalamic nucleus (PVT) plays a crucial role in regulating emotional and motivational functions by innervating various forebrain areas through its excitatory axon collaterals. Modulating PVT activity, either through activation or inhibition, leads to significant changes in the processing of fear, arousal, and stress-related signals, influencing homeostatic behaviors. Maintaining the optimal balance between excitation and inhibition is essential for PVT function. However, the sources of glutamatergic and GABAergic afferents to the PVT remain unclear.

A substantial cell population within the PVT expresses calretinin (CR). These CR+ cells constitute a major source of thalamic inputs to the prelimbic cortex, amygdala, and nucleus accumbens, exhibiting selective c-Fos expression in response to stress. Whether PVT/CR+ neurons display selectivity in their afferent connections remains unexplored. Thus, our study aimed to address three key questions: i) the origins of excitatory and inhibitory inputs to PVT, ii) the selectivity of these inputs for PVT/CR+ cells, and iii) the extent to which these different inputs converge or segregate within the PVT.

Employing retrograde and anterograde viral labeling in vGLUT2-Cre, vGAT-Cre, and vGLUT2-Cre/vGAT-Flp double transgenic mouse strains, we analyzed subcortical inputs. Additionally, Rbp4-Cre, NTSR1-Cre, and FoxP2-Cre strains were utilized to label cortical inputs. Our findings revealed that subcortical afferents to PVT are widely distributed, yet the origin of excitatory and inhibitory inputs predominantly segregates. Co-innervation by both GABAergic and glutamatergic afferents was observed uniquely from the periaqueductal gray. Furthermore, axons from different subcortical areas significantly overlapped and exhibited high selectivity for the CR+ zone in the core region of PVT.

In contrast, the majority of cortical inputs originating from layer 5 pyramidal cells selectively targeted the lateral, transient zone of PVT, which contains fewer CR+ cells. Significant cortical inputs to the core region were exclusively found in FoxP2 animals, labeling deep layer 6 cells. These results underscore the integration of excitatory and inhibitory information by PVT from distinct subcortical centers, with a predominant targeting of CR+ neurons. In summary, our data elucidate the processing of cortical and subcortical information by distinct cell populations within the PVT.

13. <u>*Buzás-Kaizler, András*</u>

The role of neuroinflammatory mechanisms in the perinatal asphyxia-induced long-term neuropsychiatric disorders

András Buzás-Kaizler[1,2,3], Hanga Kelemen[1,2], Kornél Demeter[1], Zsolt Borhegyi[1], Éva Mikics[1]
1 Institute of Experimental Medicine,
2 Semmelweis University, Budapest, Hungary,
3 National Academy of Scientist Education

Background: Perinatal asphyxia (PA) is one of the most prevalent causes of neonatal death, and its mild-moderate form also contributes to the development of many neuropsychiatric disorders (e.g. ADHD, autism). Despite the major health risk posed by PA, its pathomechanism has remained elusive to date, due to the lack of reliable preclinical models. The literature suggests that neuroinflammatory mechanisms may play a crucial role in the long-term outcome and a prior inflammation may worsen the effects of PA. Our laboratory has developed a novel mouse model, which, unlike the already-used models, allows us to investigate the pathomechanism of PA without the disturbing effects of surgical interventions.

Aims: The aim of our research was the examination of the long-term effects of PA on behavior and the role of prior neuroinflammation in the increased vulnerability to hypoxia in the CNS, furthermore, the investigation of the underlying neuroinflammatory mechanisms.

Methods: To provoke the preliminary inflammation, IL-1β was administered subcutaneously on postnatal days 2-6 to both male and female mice. To induce PA, the mice inhaled an asphyxic gas mixture containing decreased O2 and increased CO2 on postnatal day 7. The comprehensive behavioral study was initiated in young adult age, focusing on the behavioral domains that alter in PA-associated neuropsychiatric disorders. Emotional and social behavior, motor functions, and stress-coping were assessed in classical behavior tests, cognitive abilities were examined in the IntelliCage and Automated Training System devices.

Results: male animals that underwent both the IL-1β administration and the asphyxic insult showed significant changes in various domains related to attention, impulsivity, and cognitive flexibility. Additionally, PA itself increased the anxiety levels. In female animals, PA caused significant differences mostly in emotional behavior and stress-coping.

Conclusions: A prior systemic inflammation exacerbated the effects of PA, presumably by increasing vulnerability to a hypoxic-ischemic insult in brain areas connected to higher-order cognitive and emotional functions. The aforementioned behavioral changes, as well as the sex- dependency, correspond with the findings of human studies. Therefore, the model provides usan outstanding opportunity to investigate cellular alterations of the brain, which may help us to reveal the pathomechanism of PA and the development of efficient treatments.

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14. Cser, Nárcisz

Investigation of targeted and untargeted magnetic nanoparticles on a human blood-brain barrier model

Nárcisz Cser[1], Lilla Barna[1], Anikó Szecskó[1], Mária Mészáros[1], Szilvia Veszelka[1], Sándor Valkai[1], András Dér[1], Mária A. Deli[1]

1 Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary

The blood-brain barrier (BBB) plays a crucial role in maintaining the homeostasis of the brain environment by limiting the passage of various substances, including drugs, from the bloodstream to the brain. While BBB is essential for protecting the brain, it poses a significant challenge for the delivery of therapeutic agents to treat neurological disorders. Magnetic nanoparticles (MNPs) with different surface coatings and BBB targeting ligands can be promising candidates to enhance brain penetration of pharmaceutics. By using external magnetic fields, MNPs can be guided precisely to the target site allowing for drug delivery to the brain. Our aim was to investigate the ability of untargeted and targeted MNPs to cross a human BBB culture model with the use of external magnet.

Red fluorescent, starch-coated MNPs were targeted with glutathion through biotin-neutravidin binding. Cell viability was assessed by impedance measurement, MTT assay, and double cell nuclei staining. BBB functions were assessed by measuring transendothelial electrical resistance and the permeability of both MNPs and marker molecules was evaluated. Confocal microscopy was used to visualize fluorescently labeled MNPs and immunostained tight junction proteins. Magnetic field was not-toxic to endothelial cells up to 100 mT and MNPs did not damage cells up to a concentration of 300 µg/ml. Both untargeted and targeted MNPs crossed the culture inserts without cells, and the total amount of MNPs could be recovered from basal compartments within 60 minutes. In contrast, only 2% of MNPs were successfully passed across the BBB while the integrity of the barrier was maintained. Both non-targeted and targeted MNPs penetrated into endothelial cells and showed different localization as observed by confocal microscopy. The non-targeted MNPs consistently resided in almost all cells, whereas the targeted MNPs only entered a subpopulation of cells. The presence of magnetic field did not affect the localization of MNPs. We showed that neither the magnetic field nor the MNPs affected the viability of endothelial cells. The penetration of MNPs through the BBB was significantly limited. The uptake and distribution of non-targeted and glutathione-targeted MNPs in endothelial cells showed different patterns.

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15. Csikós, Klaudia

Are cortical columns ubiquitous? High-resolution identification of functional domains in cat visual cortex using 3D functional ultrasound imaging

Klaudia Csikós[1,2], Ábel Petik[1], Domonkos Horváth[1], Fanni Somogyi[1,2], Attila Dobos[1], Botond Roska[4], Alan Urban[3], Daniel Hillier[1,2,4,5]

1 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary

2 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary

3 Neuro-Electronics Research Flanders, Leuven, Belgium

4 Institute for Molecular and Clinical Ophthalmology Basel, Basel, Switzerland

5 Visual Circuits and Repair Group, German Primate Center, Gottingen, Germany

In the visual cortex of carnivore and primate model species, elementary visual features like stimulus orientation or motion direction are organized into functional maps. It remains unclear how the layout and interrelations of cortical maps observed from surface layer activity extend into deeper layers of the cortex. Electrophysiology allows for precise temporal resolution, but it falls short in providing comprehensive spatial coverage, impeding a holistic comprehension of functional organization. On the other hand, optical imaging techniques have contributed valuable insights into cortical organization, but they are limited to < 1 mm penetration depth, which translates to the coverage of ~layer 2/3 in large brains. Here we ask whether the existence of canonical columnar functional architecture can be confirmed using high-resolution 3D activity imaging. Results: We performed longitudinal 3D functional ultrasound imaging from a large part of the cat visual cortex. We analyze stability and spatial clustering of 3D architecture of functional domains across the visual cortex and identify rules governing the layouts of distinct functional maps. Conclusion: Comprehensive identification of the functional architecture in the visual cortex at unprecedented coverage and resolution provides an alternative perspective on the classical columnar notion of functional architecture in the cat visual cortex.

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16. Darai, Luca

Medial prefrontal cortical neurons projecting to the preoptic area and the thalamus differently affect social behaviours of rats

Luca Darai[1], Dávid Keller[2,3], Árpád Dobolyi[1]
1 Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
2 Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
3 Institute for System Physiology, Faculty of Medicine, University of Cologne, Cologne, Germany

The medial prefrontal cortex (mPFC) plays a crucial role in the control of social behaviour.Dysfunction in this area may contribute to neuropsychiatric disorders, such as autism spectrum disorder or schizophrenia. mPFC neurons project to different subcortical areas, exerting influence from the mPFC. We determined the projection pattern of two types of projection neurons and examined their role in social behaviour using chemogenetics. Stimulatory and inhibitory designer receptors were expressed in the mPFC neurons projecting to the medial preoptic area (MPOA), and also in mPFC neurons expressing the calcium/calmodulin-dependent protein kinase II (CaMKII) using viral gene transfer. The mPFC neurons projecting to the MPOA gave rise to collaterals to several subcortical areas, such as the accumbens nucleus, ventral pallidum, lateral septum, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus, and medial amygdala but not to the thalamus. In contrast,

the CaMKII neurons of the mPFC projected to the paratenial, mediodorsal, submedius, and reticular thalamic nuclei but not to the MPOA and other targets of MPOA projecting neurons. When stimulating the mPFC neurons projecting to the MPOA, reduced sociability was measured in the three-chamber test. In turn, the direct social interactions between freely moving animals remained unchanged. Stimulation of mPFC CaMKII-containing cells resulted in reduced time spent with conspecifics in the three-chamber test and a decrease in the duration of several elements of social interactions between freely moving rats. The chemogenetic manipulation of the examined mPFC projection neurons exerted an effect on the sociability of the animals indicating their role in regulating social behaviours. The differences in the effects of manipulation of the two types of mPFC projection neurons suggest their involvement in the regulation of social behaviours in different ways.

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17. Dénes, Diána

Examination of retinoprotective compound in type 2 diabetes mellitus

Diana Denes[1], Alexandra Vaczy[1], Lina Li[1], Dorottya Molitor[1], Andras Varga[1], Reka Radics[1], Evelin Patko[1] , Edina Szabo[1], Dora Reglodi[1], Tamas Atlasz[1,2]
1 MTA-PTE PACAP Res. Group, Dept. of Anatomy, Medical School, University of Pecs, Pecs, H-7624, Hungary
2 Dept. of Sportbiology, Faculty of Sciences, University of Pecs, Pecs, H-7624, Hungary

Introduction: Nowadays, type 2 diabetes mellitus (T2DM) is considered as a global pandemic,nearly 250 million people are affected by this disease. A common complication of T2DM is diabetic retinopathy (DMR), which is a leading cause of vision loss. Based on numerous studies it is known that pituitary adenylate cyclase-activating polypeptide (PACAP) also has a protective role in various ophthalmological diseases, including DMR in case of type 1 diabetes mellitus. The protective effect of the polypeptide in the eye is mainly mediated by PAC1 receptor. Our study aimed to demonstrate the protective role of the PAC1 receptor in a rodent

model of DMR in type 2 diabetes.

Materials and Methods: Two-month-old male Wistar rats were used in this experiment. Type 2 diabetes was induced with the combination of low-dose streptozotocin (STZ) (30mg/ttkg) and high-fat diet. Four experimental groups were created: control+systane, control+PACAP fragment, diabetes+systane, and diabetes+PACAP fragment. A group of rats was treated topically two times per day for 16 weeks with a selective PAC1 receptor agonist PACAP fragment (1µg/drop). Eight weeks after STZ injection the model was validated by a fasting oral

glucose tolerance test (OGTT) and C-peptide ELISA test. Animals have been monitored during the whole experiment to track the progression of the disease. Body weight, skinfold thickness, intraocular pressure, electroretinography (ERG), and optical coherence tomography (OCT) measurements were carried out.

Results: With fasting oral glucose tolerance test (120 mins), the development of diabetes was justified. Using ELISA test, we found that the level of C-peptide in the diabetic group was almost the same compared to the control once. Increased ketone level with the progression of

the disease was observed in the diabetic group. Body weight, skinfold thickness and intraocular pressure measurements showed no significant differences between the control and the diabetic

group. Significant differences could be detected in visual function between the two groups at 16 weeks (in the case of a-wave, b-wave and OP amplitudes), where the diabetes PACAP fragment treated group was similar to the control groups. OCT measurements were correlated with the data of the ERG test, thus a greater reduction was detectable in the total retinal thickness in the diabetic+systane group compared to the diabetic+PACAP fragment group.

Conclusions: In summary, the specific PACAP fragment had a retinoprotective role in T2DM which suggest that PAC1 receptor could be a promising therapeutic approach for the treatment of DMR.

18. Dziewiczová, Lila

Involvement of CRHR2 in behavioral and molecular-biological outcomes in animal model of PTSD

Lila Dziewiczová[1,2], Ľubica Horváthová[1], Jana Osacká[1], Andrej Tillinger[1]
1 Biomedical Research Center of Slovak Academy of Sciences, Institute of Experimental Endocrinology, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic
2 Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Ilkovičova 3278/6, 841 04 Bratislava, Slovak Republic

Post-traumatic stress disorder (PTSD) is a mental disorder that can be triggered by experienced traumatic event. It is characterized by dysregulated fear and stress responses in which corticotropin releasing hormone (CRH) system plays an important role. The CRH acts via two receptors, CRHR1 that plays role in stress responding and anxious arousal and CRHR2 that is responsible for dampening the stress response. In PTSD altered CRH and glucocorticoid (GC) levels were detected. Involvement of GC has been underlined by changed levels in FK506 binding protein 51 (FKBP5), a key regulatory factor in GC signaling. Our goal was to assess if intranasal application of CRHR2 agonists (urocortin 2 (Ucn2) and urocortin 3 (Ucn3)) suppresses negative behavioral and molecular-biological outcomes related to PTSD in animals. We used the single prolonged stress (SPS) paradigm to induce PTSD related symptoms in animals. Vehiculum, Ucn2 or Ucn3 were applicated once immediately after SPS exposure. After one week of sensitization period animals were exposed to elevated plus maze, open field (OF) and 24 h afterwards decapitated. In the plasma we measured corticosterone (CORT) levels and in the hypothalamic paraventricular nucleus (PVN) we measured gene expression of selected markers connected to stress response. In the OF PTSD animals spent less time in the central zone and were more immobile but this effect was suppressed by Ucn3. PTSD animals had decreased levels of plasma CORT what Ucn3 reversed again. In the PVN animals exposed to SPS exhibited increased level of CRH mRNA and decreased levels of GR, CRHR1 and FKBP5 mRNA. Ucn3 reversed effect of SPS only in case of FKBP5. Obtained results indicate that intranasal administration of Ucn3 affected changes induced by PTSD in animals. On behavioral level Ucn3 suppressed PTSD-induced anxiety-like behavior, however, in the PVN results were ambiguous. Therefore, further studies will be needed to elucidate the mechanisms through which Ucn3 affects anxiety-like behavior in animals.

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19. Eckert, Zsófia

Emotional and cognitive predictors of comorbid anxiety and depression

Zsofia Eckert[1, 4]; Diana Pejtsik[1, 3]; Zoltan K. Varga[1]; Olga Wronikowska [2]; MateToth [1]; Mano Aliczki[1]; Zsolt Borhegyi[1]; Kornel Demeter[1]; Eva Mikics[1]
1 Insitute of Experimental Medicine
2 Medical University of Lublin
3 Semmelweis University.
4 University of Veterinary Medicine Budapest

Depression and anxiety are highly prevalent disorders. They often co-occur with each other resulting in more severe symptoms and less effective pharmacotherapy. The neurobiological background of this co-occurrence is not yet fully understood, and currently there are no established animal models to examine it. Therefore, we strived to better comprehend the underlying neurobiological, emotional and cognitive factors of comorbid anxiety and depression (CAD), by establishing a mouse model with high clinical translational validity. For this reason, we designed a behavioural sampling protocol to identify stable behavioural traits. We used multi-sampling of 3 anxiety and 3 depression tests repeated 3 times with each animal, followed by the Learned Helplessness (LH) test, a depression model. To detect stable emotional traits, we averaged the results from the repeated tests. With the help of machine learning predictions, we were able to reduce the testing protocol to the Light-Dark test (LD), the Forced Swim test (FST) and the LH. Using only these three tests repeated multiple times, a distinct subpopulation of animals could be reproducibly characterised which show low trait anxiety and active coping (resilient subpopulation), and another showing high trait anxiety, passive coping, and learned helplessness (comorbid population) that can be used to model the main symptoms of CAD. Furthermore, to examine the underlying cognitive factors behind the distinct subpopulations, a new group of mice were exposed to cognitive tasks in an automated home-cage system (IntelliCage). We found that the comorbid group demonstrated cognitive inflexibility and worse spatial learning abilities, as well as heightened punishment sensitivity compared to the resilient subpopulation. To summarise, we were able to develop a translational model of comorbid anxiety and depression which is reliable and enables us to conduct further research into the neurobiological background of these disorders. This model provides us with the opportunity to develop more effective pharmacological therapy for patients suffering from CAD.

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20. Egyed, Máté

Oxytocin receptor-expressing neurons in the medial preoptic area affect social behavior in rats

Máté Egyed[1], Vivien Szendi[1], Gina Puska[1,2], Árpád Dobolyi[1]
1 Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
2 Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary

Oxytocin is released in the brain in response to social interactions. Oxytocin receptors (OxtR) are involved in regulating social behavior in rodents. The hypothalamus is a regulatory center of the social behaviors whose anterior region, the medial preoptic area (MPOA), which contains a high density of neurons expressing oxytocin receptors. While the role of MPOA in maternal behavior has been extensively studied, there is limited evidence of its effect on social interactions among adults. Thus, we aimed to investigate the functional role of its OxtR-expressing neurons in interfemale social behavior through chemogenetic manipulation. We electively stimulated OxtR neurons in transgenic female Sprague Dawley rats expressing Cre recombinase in their OxtR neurons. We injected an adeno-associated virus expressing excitatory or inhibitory designer receptors (DREADDs) in a Cre-dependent manner into the MPOA. Upon stimulation of OxtR neurons through the injection of DREADD receptor

ligand, the rats spent more time body sniffing, grooming, mounting, and chasing conspecifics compared to the previous and subsequent control days when they only received vehicle injections. Additionally, there was a significant decrease in time spent in non-social contexts after stimulation. A three-chambers test was used to investigate the impact of MPOA OxtR neurons on sociability. The results showed that the rats spent more time in the chamber with the familiar mate than in the empty or unfamiliar conspecific associated chambers. The inhibition of the MPOA OxtR neurons did not affect these behaviors. Furthermore, neither the stimulation nor the inhibition had an effect on depression-like behavior based on the forced swim test. The target areas of MPOA OxtR expressing neurons were revealed through tract tracing. These neurons project to several brain regions, including the periaqueductal grey matter and the lateral septum. Both regions have been shown to participate in the control of social behavior. These preliminary data suggest that MPOA OxtR neurons have a wide and profound effect on social behavior in adult female conspecifics.

21. ElZafarany, Abdurrahman

Validation of a trainable pixel classifier for the detection of neurons in flat-mounted retinas

Abdurrahman ElZafarany[1], Katalin Fusz[1], Tibor Zoltán Jánosi[1], Paula Lily Hirsch[1], Péter Kóbor[1], Kristóf László[1], Ildikó Telkes[1], Péter Buzás[1]

1 Institute of Physiology, Medical School and Centre for Neuroscience, University of Pécs, Hungary

Background: A model for Autism Spectrum Disorder (ASD) has been widely used throughprenatally exposing rats to valproic acid (VPA). VPA is commonly given at around day 12.5 of gestation, which coincides with intense early retinal neurogenesis. We are interested in whether the pattern of retinal cell mosaics is altered in these animals. Pattern analysis requires recording the 2D coordinates of hundreds of neurons from flat-mounted retinas, a time-consuming, usually manual procedure. The current work aims to validate the Labkit machine learning tool of the Fiji image analysis application for semi-automatic identification and localization of specific retinal cell types in microscopic images to cut down the time needed to study retinal differences.

Methods: Retinal whole-mounts of valproate-treated and control animals were immunostained for parvalbumin, Prox1 and S-opsin to differentiate AII amacrine cells (AIIACs), parvalbumin-immunopositive wide field amacrine cells (PV+ wfACs), horizontal cells (HCs) and S-cones (SCs) and DAPI was added to stain cell nuclei. Three-dimensional microscopic image stacks were taken with a confocal microscope. Labkit was trained by an experienced observer to classify image pixels as belonging to each cell type of interest, and the images were segmented accordingly. The 3D Object Counter tool was used to delineate solid objects composed of pixels classified to each cell type, and their centroid coordinates were taken as estimates of cell position. To test the validity of the classification by Labkit, the resulting cell counts and positions were compared to ones taken by an independent observer.

Results. Our preliminary analysis of 1615 cells in 5 regions of interest showed that the cell counts for AIIACs determined with Labkit correlated strongly with those obtained manually ($r=0.91$, $p=0.035$) and the two cell counts were statistically not different ($p=0.81$, paired t-test). The average nearest neighbor distance between cells counted manually and by using Labkit was 3.7 pixels. About 5.2% of the manually counted cells had no nearest neighbor within an average cell diameter of 8 pixels, suggesting that they were not detected by the Labkit classifier.

Conclusion: In the case of AIIACs, the results indicate that cell counts determined by Labkit and those obtained manually are statistically not different. Training and post-processing may be improved to reduce the number of false negative and false positive detections.

22. Fadel, Ward

Classification of Surface Laplacian Based Motor Imagery EEG Tensors with Deep Neural Networks

Ward Fadel[1, 2], Istvan Ulbert[1, 2]
1 Faculty of Information Technology and Bionics, Pazmany Peter Catholic University, Budapest, Hungary
2 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary

Brain-Computer Interfaces (BCI) have the potential to enable people to control their environments using brain signals, and the feature extraction stage is a cornerstone in building BCI systems. We propose a novel approach in which raw EEG data is transformed into spherical spline Surface Laplacian-based multichannel 2-D Tensors for classification using a deep neural model. Within the challenging Physionet motor imagery dataset, encompassing 107 subjects, we applied power spectral density estimates of the Surface Laplacian-filtered Mu [8-13 Hz], Beta [13-30 Hz], and low Gamma [30-45 Hz] bands to generate three-channel images using azimuthal projection and Clough- Tocher interpolation. These images serve as inputs for the Deep Neural Network models (ResNet, CNN).

The study demonstrates a significant improvement in the classification accuracy of four classes using the proposed signal-to-image transformation within the deep neural network framework (62% for ResNet and 59.42% for CNN) compared to the baseline method, Support Vector Machine (50.49%). Additionally, the inclusion of the low Gamma band notably enhanced motor imagery classification accuracy. This work opens the door to a new domain where the advantages of deep neural networks in image classification can be leveraged to gain deeper insights into EEG-related issues.

23. Farkas, Szidónia

Manipulating the cholinergic neurons in Alzheimer's disease: validation of a mouse model

Szidónia Farkas[1,2,3], Adrienn Szabó[2,3], Viktor Jasper[1,3], Kinga Nyers-Marosi[1,3], Bence Petrovai[1,3], István M Ábrahám[1,3], Tamás Kovács[1,3] and Dóra Zelena[1,2,3]

1 Molecular Neuroendocrinology Research Group, Institute of Physiology, University of Pécs, Hungary

2 Laboratory of Behavioral and Stress Studies, Institute of Physiology University of Pécs, Pécs, Hungary

3 Centre for Neuroscience, Szentágothai Research Centre, Pécs, Hungary

Introduction: Alzheimer's disease (AD) is an increasing health and social problem ranking 7th among the most common causes of mortality worldwide. The cholinergic system is its most affected neurocircuit, therefore, it is a common therapeutical target. However, revealing its exact role requires further studies.

Aim: A genetical mouse model was created, that represented the progression of AD and, additionally, expressed Cre recombinase enzyme in cholinergic neurons allowing their targeted manipulation. Here we aimed to validate the model.

Methods: Two strains were cross-bread: B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) and B6;129S6-Chattm2(cre)Lowl/J. After serial genotyping, a colony, homozygote for four genes (PSEN1, APPSwe, tauP301L and Cre; 3xTg-ChAT-Cre) was established. To test the functionality of the Cre enzyme a stimulating DREADD virus (AAV8-hSyn-DIO-hM3Dq-mCherry) was injected unilaterally into the nucleus basalis magnocellularis (NBM) and clozapine-N-oxide-induced c-Fos activation was compared between the two hemispheres. For behavioural characterization different tests were performed: Y-maze, single pellet skilled reaching (SPSR), fox odor test (FOT), splash test (ST) and social discrimination test (SDT). The progressive appearance of Aβ plaques and pTau aggregates were confirmed by immunohistochemistry.

Results: Immunostainings confirmed the expression of Cre-dependent fluorophore in ChAT positive cells as well as the appearance of the pathological hallmarks (Aβ and pTau). The c-Fos activity was significantly increased at the virus injected hemisphere. In the behavioral tests 3xTg-ChAT-Cre mice showed decreased locomotor activity (Y-maze, SDT, FOT), increased anxiety (FOT, ST) and weaker fine motoric skills (SPSR) compared to control animals.

Conclusions: The newly created animals have a functional Cre recombinase enzyme in cholinergic cells. Additionally, the animals showed the pathophysiological hallmark of AD in specific brain areas and kept the typical behavioral alteration found in 3xTg-AD mice before. Thus, this strain seems to be appropriate for further studies.

24. *Fazekas, Fruzsina*

Automated analysis of changes in intracellular Ca2+ concentration in highly polarised cells

Fruzsina Fazekas[1], Tibor Zelles[2, 3, 4], Eszter Berekméri[2, 3]
1 University of Veterinary, Budapest, Department of Zoology
2 Semmelweis University, Department of Pharmacology And Pharmacotherapy
3 Semmelweis University, Department of Oral Biology
4 Institute of Experimental Medicine, Laboratory of Molecular Pharmacology

Calcium is the most researcherd ion in cell biology. We can monitor calcium ions by calcium imaging since the 60s. From that more program developed to research the calcium levels of the cell. In most programs the region of interest (ROI) selection is part of the process, which means selecting the areas to be investigated during the experiments. ROIs are traditionally determined manually, and no standardised proceeding exists, therefore causing a subjective element of the measurment. In addition most of these programs are not freely available, and their development is slower than that of science. Thereby the individual labors need to write their own code, which requires programming skills, and it is hard for other laboratories to implement these programs. However automated analysing process would be more time saving, and less human source demanding, which is a problem of the now available softwares. Our aim was to create a free, autmated, open-source analysing program with objective ROI selection, especially for highly polarised cells. The open-source R language was used for the development to be able to use the same platform for the statistical analysis as well. We minimised the number of R packages to be independent from the package development. Downloading and importing the packages can be slow, hence increasing the runtime. The required time was optimalised during the writing process. The code is able to measure different type of cells, especially the highly polarised ones, for instance retinal cone and cochlear Deiters cells. Further goal is to implement the code into Python to provide wider availability.

25. Földi, Péter

Noxious stimulus-responsive neurons in the ventral PAG and dorsal raphe nucleus

Péter Földi[1,2], Kinga Müller[1,2], Norbert Hájos[1]
1 HUN-REN - Institute of Experimental Medicine, 'Lendület' Laboratory of Network Neurophysiology, Budapest, Hungary,
2 Semmelweis University, János Szentágothai Doctoral School of Neurosciences, Budapest, Hungary

The ventral periaqueductal grey (vPAG) including the ventrolateral PAG and dorsal

raphe nucleus plays a critical role in controlling anxiety, fear memory formation, autonomic processes and most particularly, it is involved in descending modulation of pain processing. It has been shown that different neuron types, such as dopaminergic and serotonergic cells are part of this circuitry – besides the glutamatergic and GABAergic neurons – however their exact functions remained unclear. Additionally, malfunctioning of the circuit operation in the vPAG contributes to several neuropsychiatric disorders, the treatment of which is still a great challenge. Therefore, understanding the functional properties of neurons in this region can be critical in proposing new therapeutic approaches. Here, we explored the single-unit activity in the vPAG in urethane-anesthetized mice in response to different types of noxious and neutral stimuli by applying two different recording approaches. First, we performed acute silicone probe recordings to determine how neurons respond to different type of stimulations. Additionally, using the juxtacellular recording technique with the same set of external stimuli and post hoc immunocytochemistry to identify the recorded cell types, we distinguished functionally different neuron types in the vPAG. Analysing the firing features and neurochemical content of the recorded neurons, we found that dopaminergic neurons can be separated into two groups based on their response latency and vasoactive intestinal polypeptide content, suggesting their different involvement in noxious stimulation processing. Further, we revealed that serotonergic neurons are heterogeneous and can be clustered into five groups based on their responses upon noxious stimulation. Based on our observation serotonergic and dopaminergic neurons were exclusively responsive to non-neutral stimuli.Our current results show that the firing of the monoaminergic neurons in the vPAG circuitries is distinctly modified by noxious stimuli, implicating their different contribution to

pain processing in this clinically important brain region.

26. Fülöp, Barbara

Potential analgesic effect of fractalkine receptor (CX3CR1) antagonist in mouse model of chronic stress-induced pain

Barbrara, Fülöp[1]; Viktória, Kormos[1]; Katalin, Rozmer[1]; Ádám Dénes[2]; Nikolett, Lénárt[2]; Éva, Borbély[1]; Zsuzsanna, Helyes[1,3,4]

1 Department of Pharmacology and Pharmacotherapy, Medical School & Centre of Neuroscience, University of Pécs, Pécs, Hungary
2 Eotvos Lorand Research Network, Chronic Pain Research Group, University of Pécs, Pécs, Hungary
3 National Laboratory for Drug Research and Development, Budapest, Hungary
4 PharmInVivo Ltd., Pécs, Hungary
5 "Momentum" Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary

Chronic psychosocial distress plays a role in the development/exacerbation of several painful diseases (e.g. fibromyalgia, neuropathy). Drug treatment for these diseases has not been resolved, so it is important to examine the pathomechanism more precisely to identify new therapeutic targets. The role of neuroinflammation and the fractalkine receptor (CX3CR1), which is primarily expressed on microglial cells in the brain, has already been proven in stress and inflammatory pain. Based on our unpublished results, we were able to demonstrate the role of the receptor in CX3CR1 knockout mice in the development of pain caused by chronic immobilization stress (chronic restraint stress: CRS). In this research, we investigated the potential analgesic effect of the fractalkine receptor antagonist AZD8797 in a mouse model of stress-induced pain. Male C57Bl/6J wild-type (WT) mice were exposed to CRS for 2 weeks. From the beginning of the stress protocol, AZD8797 (1 mg/kg) or vehicle was administered intraperitoneally twice daily. The mechanical pain threshold was measured with a dynamic plantar aesthesiometer, and the cold tolerance of the hind

paw was measured weekly with the withdrawal latency from icy water test. At the end of the second week, behaviour tests were performed. Significant mechanical hyperalgesia developed for the second, cold hyperalgesia for the first week. However, mechanical sensitization of the hind paw did not develop in the presence of the antagonist. Cold hyperalgesia was developed to the same level in both vehicle and AZD8797-treated animals in response to stress. In the forced swim test, time spent immobile was decreased due to stress, but only in vehicle-treated animals. In the open field test, animals treated with AZD8797 spent significantly more time in the center compared to the vehicle-treated group regardless of stress application. Based on our results, the fractalkine receptor may play an important mediating role in the development of chronic stress-induced pain. Acting on the CX3CR1 receptor, AZD8797 successfully attenuated the mechanical sensitization caused by CRS, further strengthening the potential use of CX3CR1 as a drug target.

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27. Furuglyás, Kristóf

Determining the phase of oscillations at epileptic deep sources based on surface EEG measurements

Kristóf Furuglyás[1,6], Márton Kis[1,2,3*], Bálint Horváth[1], Andrea Pejin[1,2,3], Nóra Forgó[1,2,3], István Langó[1,2], Zoltán Chadaide[1,2,3], Tamás Laszlovszky[1], Zoltán Somogyvári[1,4+], Antal Berényi[1,2,3,5+]*

1 Neunos ZRt, Szeged 6720, Hungary.
2 MTA-SZTE 'Momentum' Oscillatory Neuronal Networks Research Group, Department of Physiology, University of Szeged, Szeged 6720, Hungary.
3 HCEMM-SZTE Magnetotherapeutics Research Group, University of Szeged, Szeged 6720, Hungary.
4 HUN-REN Wigner Research Centre for Physics, Institute for Particle and Nuclear Physics, Department of Computational Sciences, Theoretical Neuroscience and Complex Systems Research Group, Budapest 1121, Hungary.
5 Neuroscience Institute, New York University, New York, NY 10016, USA.
6 Eötvös Loránd University, Doctoral School of Physics, Pázmány Péter sétány 1/A, 1117 Budapest, Hungary

Our objective is to determine the accurate phases of oscillations at deep epileptic sources based on surface EEG measurements. To achieve this goal, we conducted experiments by replaying recorded seizures on deep brain electrodes inserted into human cadavers at various positions. Simultaneously, EEG signals were recorded on the skull using a 32-channel subcutaneous electrode system, enabling the measurement of phase relations between deep and surface electric potential recordings.

Two distinct methods, the lead-field projection method and the Gabor-Nelson method, were employed to infer deep activity from surface measurements. Both approaches operate under the assumption that the measured signals were generated by a localized deep current source dipole. The lead-field projection method assumes knowledge of the deep source's location, necessitating the solution of the forward model by calculating the lead-fields of unit amplitude dipoles at the known position. This forward solution requires MRI of the head and segmentation of different tissues based on the image.

In contrast, the Gabor-Nelson method does not assume prior knowledge of the deep source's position and only requires information about the electrode positions on the skull, eliminating the need for an MRI image and tissue segmentation.

Comparison of the signals replayed at different intracranial sources to the corresponding reconstructed dipole activity revealed that while the lead-field projection method reconstructed deep sources with slightly greater precision, the Gabor-Nelson method also yielded appropriate results. Our findings provide valuable insights into the comparative efficacy of these methods for accurately determining the phases of deep epileptic sources based on surface EEG measurements.

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28. Futácsi, Anett

Neurodegeneration in a transgenetic rat model of Alzheimer's disease

Anett Futácsi[1,2], Kitti Rusznák[1,2], Gergely Szarka[3,4], Ove Wiborg[5] and Boldizsár Czéh[1,2]
1 Structural Neurobiology Research Group, Szentágothai Research Centre, University of Pécs, 7624 Pécs, Hungary
2 Department of Laboratory Medicine, Medical School, University of Pécs, 7624 Pécs, Hungary
3 Histology and Light Microscopy Core Facility, Szentágothai Research Centre, University of Pécs, Pécs, Hungary
4 Retinal Neurobiology Research Group, Szentágothai Research Centre, University of Pécs, 7624 Pécs, Hungary
5 Department of Health Science and Technology, Aalborg University, 9220 Aalborg, Denmark

Alzheimer's disease is the most common form of dementia. The incidence of the disease increases with age, affecting 1% of 60-year-olds, and 30% of 85-year-olds, altogether approximately 55 million people worldwide (WHO data). Several studies provide evidence that abnormally folded amyloid beta and tau proteins that accumulate in amyloid plaques are responsible for the neurodegeneration, causing progressive deterioration of the nervous tissue and subsequent behavioral disturbances and memory-loss. It is well documented that the aggregation of the β-amyloid leads to neuro-inflammation and neuronal cell death, however the progression and exact mechanism still remains to be clarified.

Here, we investigated neuronal and glial changes in the brains of a transgenic Alzheimer's rat model, the TgF344-AD rats. This model shows the overexpression of human amyloid precursor protein (APPsw) and presenilin 1 (PSEN1E9), which play an important role in the progression of the disease. TgF344-AD rats express 2.6 times more human APP and 6.2 times more human PSEN1 in the brain. With post-mortem immunohistochemistry, we labelled amyloid plaques, microglial cells and astrocytes, as well as GABAergic interneurons in the hippocampus. Systematic, unbiased cell counting was carried out to assess putative cellular changes.

Our preliminary data indicate a pronounced neuro-inflammatory response in the hippocampus of the TgF344-AD model, involving mainly microglial cells, but among the GABAergic neurons only in the number of cholecystokinin-positive cells were altered.

Contrary to expectations, we could did not detect a correlation between amyloid plaque load and the neuronal and glial changes.

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29. Gálber, Mónika

The long-term impact of early life stress on resting state functional connectivity in depressed patients

Mónika Gálber [1,2], Szilvia Anett Nagy [1,3,4,5], Gergely Orsi [3,4,5,6], Gábor Perlaki [3,4,5,6], Mária Simon [1,7] Boldizsár Czéh [1,2]

1 Neurobiology of Stress Research Group, Szentágothai Research Centre, University of Pécs, Pécs, Hungary
2 Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary
3 HUN-REN-PTE, Clinical Neuroscience MR Research Group, Pécs, Hungary
4 Department of Neurosurgery, Medical School, University of Pécs, Pécs, Hungary
5 Pécs Diagnostic Centre, Pécs, Hungary
6 Department of Neurology, Medical School, University of Pécs
7 Department of Psychiatry and Psychotherapy, Medical School, University of Pécs, Hungary

Early life stress, such as childhood maltreatment (CM), is a major risk factor for developing major depressive disorder (MDD). However, it is not clear exactly why maltreated individuals are more susceptible to develop psychopathology and how adverse childhood experiences influence the development of brain architecture. To gain a better insight into how early life stress influence brain functions, we performed resting state functional magnetic resonance imaging (fMRI) in maltreated and non-maltreated depressed patients. Afterwards, we analyzed the fMRI data to detect putative between-network functional connectivity (FC) differences as a consequence of early life stress.

We recruited 37 depressed patients, n=18 maltreated and n=19 non-maltreated, together with 20 matched healthy controls. History of maltreatment was assessed using the Hungarian version of the 28-item Childhood Trauma Questionnaire. Functional connectivity differences between the groups were investigated using CONN Connectivity Toolbox.

We found several between-network functional connectivity alterations in the connections of the default mode network with the executive control, salience and cerebellar networks. The strongest differences (Fals discovery rate corrected $p < 0.00001$) were the increased resting state functional connectivity strengths between the basal ganglia network and salience, executive control and sensorimotor networks, and between sensorimotor and cerebellar, visual and default mode networks in maltreated patients compared to the non-maltreated depressed group.

Our results confirm that depressed patients with a history of early life stress have numerous alterations of between-network FC strengths not only in their fronto-limbic circuits, but also in sensorimotor, visual, auditory and cerebellar networks compared to non-maltreated depressed patients.

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30. Gazdik, Melinda

Synaptic communication and network activity are enhanced in human neuronal cultures differentiated from iPSCs associated with Kleefstra syndrome

Melinda Gazdik[1], Eszter Geiszelhardt[1], Ben Mahmoud Maissa[1], Attila Szűcs[1], Katalin Schlett[1], Krisztián Tárnok[1]

1 Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Autism spectrum disorder (ASD) is commonly considered as a neurodevelopmental disorder. Studies using whole-brain imaging have demonstrated abnormal connectivity in brain networks of individuals with ASD, and genetic studies have identified ASD-related mutations in genes that regulate synaptic development and function.

In this study we compared neurotypical human neurons (NT) with those affected by ASD-inducing Kleefstra syndrome (KS-ASD), using induced pluripotent stem cell technology to model human neuronal maturation and network formation, in vitro. Kleefstra syndrome is characterized by mutations in the EHMT1 gene which plays a role in heterochromatin formation and regulation of gene expression. The mutation can result in dysfunction of synaptic plasticity, synaptic scaling, learning, and memory formation, which may be related to abnormal neural network formation.

We performed weekly patch clamp measurements in whole-cell configuration to monitor the maturation and synaptic development of the induced NT and KS-ASD neuronal cultures for nine weeks. In voltage clamp experiments, KS-ASD neurons displayed robust spontaneous excitatory postsynaptic currents (sEPSC) even from the 1st week of cultivation. These populations of sEPSCs contained both fast and slow events and they were effectively blocked by the AMPA receptor antagonist, CNQX, and by the GABAA receptor antagonist, bicuculline, respectively. From the 1st week of differentiation, the AMPA inputs were dominant, but from the 2nd week, the GABA / AMPA input ratio was stable (40/60%).

To compare the network activity of KS-ASD and NT cells, weekly MEA (multielectrode array) measurements were performed during the first 7 weeks of maturation. In KS-ASD cultures, increased network activity was observed even from the first week of differentiation, however, he activity of the KS-ASD cultures decreased for the fifth week. In the frequency no difference was observable between KS-ASD and NT cells, but the amount of burst-like firing electrodes was higher in KS-ASD cultures. We also found evidence for synchronized and remarkably regular burst oscillations characterized by sharp peaks in the Fourier-spectra of the recorded spike trains.

Based on our observations, Kleefstra syndrome influences the neuronal network forming properties. In KS-ASD cultures, the network activity is more rapid even on the first 3 weeks, but the activity drops from the fourth week, so the premature or excessive neuronal maturation may hinder further network integrative function.

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31. Geiszelhard, Eszter

Functional maturation is accelerated in iPSC-derived human neurons obtained from patients with Kleefstra syndrome

Eszter Geiszelhardt[1], Melinda Gazdik[1], Ben Mahmoud Maissa[1], Attila Szűcs[1], Katalin Schlett[1], Krisztián Tárnok[1]

1 Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Kleefstra Syndrome (KS) is a rare neurodevelopmental disorder associated with intellectual disability, dysmorphic features and autism spectrum disorder (ASD). KS is caused by specific mutations in the EHMT1 gene, which plays a crucial role in the formation of heterochromatin. Synaptic plasticity, synaptic scaling, learning and memory formation is severely affected in KS, however, the impact of the functional loss of EHMT1 on the neuronal development remains unclear. In this study, we compared the *in vitro* differentiation and functional maturation of neurotypic human neurons to those affected by Kleefstra syndrome using neural progenitor cells (NPCs) derived from reprogrammed blood cells of a young neurotypical female donor (NT) or from a young female Kleefstra syndrome patient (KS-ASD). By weekly patch clamp measurements in whole-cell configuration we monitored the maturation of the induced NT and KS-ASD neuronal cultures for nine weeks. In KS-ASD cultures, the cultures exhibited a higher percentage of firing cells even from the first week, compared to the NT cells, which showed the firing phenotype only from the third week. In case of firing type neurons, analysis of the action potentials revealed a characteristic shoulder or *kink* in their phase portrait. Kink is associated with temporal mismatch in the activation of Na-currents in the soma and the axon. In KS-ASD cultures, kink was detectable even from the first week, compared to NT cultures, where it appeared only from the fourth week. The passive membrane properties, such as membrane resistance and time constant did not show difference between the two types of cells. However, the active properties, like spike amplitude, spike halfwidth, differed in KS-ASD vs. NT cultures, but these values decreased by the maturation in both culture types. Based on literature data, the excitability of cells can be related to the AIS (axon initial segment), which contains abundant voltage gated Na channels. We also followed the development of the AIS by ankyrin G immunostaining. KS-ASD neurons exhibited a more proximal AIS localisation from the first week on while in NT neurons, AIS shifted closer to the soma gradually during the first 3 weeks. Based on our morphological and electrophysiological studies, we find clear evidence for accelerated neural maturation in KS. Accelerated neural development may also influence the development of synaptic connectivity and network activity by affecting their integrative functions.

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32. Gőgös, Rebeka

Effects of mental fatigue on stability and flexibility of visually guided movements

Rebeka Gőgös1, Gergő Jákóczi, András Matuz[2], András N. Zsidó[3], Árpád Csathó[2]
1 Institute of Biology, Faculty of Sciences, University of Pécs, Pécs Hungary
2 Department of Behavioural Sciences, Medical School, University of Pécs, Pécs Hungary
3 Institute of Psychology, Faculty of Humanities and Social Sciences, University of Pécs, Pécs Hungary

Cognitive functions mediated by prefrontal activity and limited in capacity seem to be particularly sensitive to fatigue caused by prolonged task performance. Such cognitive functions include movement planning and movement execution. Results from a previous study suggested that the preparation (i.e. initiation) phase of visually guided pointing movements tends to be impaired with increasing fatigue induced by Time-on-Task. However, no research has been addressed to fatigue sensitivity of movements in terms of movement stability and flexibility. Therefore, in two experiments (N_1 = 26, N_2 = 27), we investigated the stability and flexibility of movements in visually guided movement tasks. Flexibility was considered the ability to adapt to unexpected changes during a movement trial and to modify the initiated track of movements. Stability was considered the ability to maintain the performance level at the presence of distractors. In the first experiment, we examined the stability of movement with a mouse-tracking version of the Eriksen flanker task. An arrowhead centered on the screen and flanked by distractors (congruent or incongruent) indicated the direction of the target stimulus participants needed to point with the cursor. The stability of movement under fatigue was assessed based on participants' ability to inhibit incongruent distractor information with increasing Time-on-Task. In the second experiment we examined the flexibility of movement along with trial conditions where the spatial position of the target stimulus changed unexpectedly after its appearance (change-trials). The difference between change-trials and non-change trials were examined with increasing Time-on-Task. Variables of movement preparation, movement execution, and subjective fatigue were recorded. Gaze position recording was also assessed to control fixation. In both experiments, the results indicated a clear detrimental effect of Time-on-Task on movement initialization suggesting that enhanced level of fatigue is manifested in slow movement preparation. Nevertheless, both stability and flexibility of movements remained unchanged with increasing Time-on-Task induced fatigue.

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33. Harcsa-Pintér, Noémi

Analyzing the audiovisual associative learning performances of adult migraine patients

Noémi Harcsa-Pintér[1], Kálmán Tót[1], Gabriella Eördegh[2], Gábor Braunitzer[3], Anett Csáti[4], János Tajti[4], Attila Nagy[1]

1 Albert Szent-Györgyi Medical School, University of Szeged, Department of Physiology, Sensomotory Research Lab, Szeged, Hungary

2 Department of Health Sciences and Health Management, Faculty of Health Sciences and Social Studies, University of Szeged, Szeged, Hungary

3 Laboratory for Perception & Cognition and Cognitive Neuroscience, Nyírő Gyula Hospital, Budapest, Hungary

4 Department of Neurology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Acquired equivalence learning involves recognizing stimuli as equivalent based on shared consequences. Previously, we assessed migraine patients and a healthy control group in a visual learning task, revealing poorer acquisition but stronger generalization in patients. This study investigates if similar patterns emerge in an audiovisual equivalence learning paradigm mirroring the original visual test structure.

Participants were tasked with linking auditory cues, including human voice, engine starting, meowing cat, and guitar strumming, to corresponding visual stimuli represented by cartoon faces of a man, woman, boy, and girl.

Examining data from 44 adult migraine patients and 44 age-, gender-, and education-matched healthy controls, we observed that, unlike the visual study, migraine patients exhibited significantly enhanced performance during the acquisition phase of the audiovisual test. However, no significant differences emerged in performances during the retrieval and generalization phases.

These findings hint at a potential overcompensating effect in migraine patients during multisensory information processing. This phenomenon might contribute to improved performance in equivalence learning, potentially offsetting the significant functional alterations observed in the visually guided generalization functions of migraine patients.

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34. Hernádi, Zsófia

Beneficial effects of large home-cage with environmental enrichment for schizophrenia-like rat

Zsofia Hernadi[1], Leatitia G. Adlan[1], Gabriella Kekesi[1], Alexandra Büki[1], Gábor Tuboly[2], Gyöngyi Horvath[1]

1 Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

2 Department of Neurology, Hódmezővásárhely - Makó Healthcare Services Center, Hódmezővásárhely, Hungary

As interindividual differences are acknowledged as a key determinant in diagnosis and treatment, understanding the biological mechanisms underlying individuality becomes increasingly important. Wide-range of behavioral disturbances were observed in different acute behavioral tests of a multiple-hit schizophrenia rat model (named Wisket), but the results might have been influenced by acute stress effects. The goal of this study was to characterize the behavior of control Wistar (C) and Wisket (W) rats in large home-cages with environmental enrichment (named Home Manner; HM) to reveal the potential beneficial effects this circumstance.

Rats (n=9/group) were housed in large home-cages (57×60×55.5 cm) with three floors equipped with dispensers to deliver pellets into two trays and environmental enrichment for 13 days. As the animal touched a tray, it provided 1 (small dose; SD) or 3 (large dose; LD) pellets, depending on the side, and if an animal learnt to prefer the tray providing the LD, delay discount design was provided to evaluate the impulsivity of the animals. Detailed behavioral analysis (including exploratory activity, eating time: time until the feeder runs out, and delay time) was applied to reveal the differences between groups and between the individual subjects.

Based on exploratory pattern of the animals, the rats could be categorized into four subgroups (SGs) with four active sides (named subsets), accordingly:

SG1: Activity at both trays (C:5; W: 4; SG1_LD, SG1_SD);

SG2: Activity only at the LD tray (C:1; W:2; SG2_LD);

SG3: Activity exclusively at SD tray (C:2; W:1; SG3_SD);

SG4: No activity at either trays (C:1; W:2), no detailed analyses were available.

Altogether data obtained from four subsets could be analyzed. Significant differences were obtained between the subsets in the HM, but only a few exploratory parameters showed alterations in the Wisket animals compared to controls within the different subgroups. Thus, the W rats showed high level of exploration activity in SG3. The cognitive functions seemed to be impaired in the W group within the SG1, accompanied with enhanced impulsivity compared to C animals. The personalized analyses revealed high behavioral variability in both groups.

This study highlights the importance of categorized and personalized analyses of behaviors in rodents, to improve the translational value of preclinical schizophrenia models.

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35. Hormay, Edina

Different aspects of the taste reactivity test to interpret gustatory responses elicited organismic changes

Edina Hormay[1,2,3], Kitti Mintál[1,2,3], Réka Bettina László[1,2], István Szabó[1], Attila Tóth[1,3], Kristóf László[1], Nina Győrfi[1,3], László Lénárd[1], Zoltán Karádi[1, 2, 3]

1 Institute of Physiology, University of Pécs, Medical School, Pécs, Hungary

2 Endre Grastyán Behavioral Physiology Research Division, Homeostatic Control Research Group, University of Pécs, Medical School, Pécs, Hungary

3 Endre Grastyán Behavioral Physiology Research Division, Cellular Bioimpedance Research Group, Szentágothai Research Centre, University of Pécs.

Background: The taste reactivity test is a long-known and broadly accepted method in animals for assessing organismic responses to gustatory stimuli [1]. In this test, small quantities of various taste stimulus solutions are delivered directly into the oral cavity of a freely moving specimen (in our case, adult male Wistar laboratory rats), which are capable to initiate and maintain the appetitive and consummatory phases of the drinking process. The manifest mimetic and movement responses are video recorded, the elicited acceptive and/or rejective response patters are evaluated, and the resulting data pool is used to estimate characteristic factors that determine the execution and regulation of fluid intake behavior.Objectives: In our laboratory the modified protocol of Grill and Norgren [1] was used for semi-quantitative [2] and quantitative [3] data analysis in order to examine the differences and similarities of various protocols.

Methods: With the aid of chronically implanted intraoral canulae, the taste reactivity responses of 22 male Wistar rats were evaluated to low and high concentrations of the 5 basic tastes. The semi-quantitative analysis included 3 well experienced, independent judges who evaluated any given recordings by their ingestive vs aversive scores from level 0 to level 3 (levels were defined by presence ratio of all characteristic symptoms /mouth, tongue, paw, head and overall movement responses /, as well as by duration and intensity of them/). During quantitative analysis (performed in details later), the video recordings were evaluated in 25 ms frame sequences, determined by the dominant movement patterns.

Results and Conclusion: The summed data of various protocols did only minimally differ from each other, tendencies and significances appeared to be very similar regardlessof the protocol version. By contrast, the different pattern analyses proved to complement each other, and this provided us a refined analysis outcome.

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36. Horváth, Ágoston Csaba

Towards layer-by-layer infrared neuromodulation: presentation and functional characterisation of an intracortical optrode needle featured with a micromirror tip ending

Ágoston Csaba Horváth[1], Borbála Csomai[1], Ákos Mórocz[1], Ágnes Szabó[1], Péter Fürjes[2], Estilla Zsófia Tóth[3,4], Richárd Fiáth[1,3], Zoltán Fekete2

1 Pázmány Péter Catholic University, Faculty of InformationTechnology and Bionics, Budapest, Hungary

2 Centre for Energy Research, Institute of Technical Physics and Materials Science, HUN-REN, Budapest, Hungary

3 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, HUN-REN, Budapest, Hungary

4 Semmelweis University Doctoral School, Budapest, Hungary

The application of infrared (IR) light irradiation as a neuromodulation technique has been proven as safe and applicable in numerous studies. Either the pulsed or the continuous wave mode of IR stimulation (INS) showed promising neural responses under various conditions in vitro and in vivo as well. One of the advantages of INS compared to the classical electrical stimulation is that INS does not induce photoelectric artefacts in electrophysiological recordings. Another advantageous property of INS is that the propagation of light can be shaped easier than in case of electrical signals. Therefore, the stimulus can be more directional, the neuromodulation impact can be localized.

In this work we present an intracortical IR optrode needle, that can be implanted into the brain tissue and performs optical stimulation and multi-site electrophysiological recording simultaneously. This silicon (Si) based microimplant has two modalities integrated into a single device: extracellular electrophysiological sensing and IR waveguiding. The needle-like Si shaft (0.19×0.17 mm) of the optrode holds 16 platinum (Pt) recording sites (900 µm2) with 100 µm inter-site distance. This multimodal probe can even be implanted to layers deeper than 4 mm in the tissue. IR waveguiding property is embedded into the Si substrate material of the same shaft. The optrode's shaft ends in a parabolic micromirror. This tip shape aims to direct the outcoupled IR light laterally towards neighbouring tissue, therefore more photons get absorbed closer to the recording sites causing the positioning of the maximum heating effect in the vicinity of Pt recording sites.

The proposed work shows the outcomes of numerous different characterisation methods to demonstrate the in vivo applicability of this optrode. Various optical investigations and thermal tests were made to calibrate the optically induced heating preceding the in vivo use. The first in vivo tests were made in the somatosensory cortex of anaesthetised rats. Our findings owing to the IR illumination in the recorded extracellular electrophysiological data are presented from various aspects.

Acknowledgement:

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37. *Ignácz, Máté*

Neuroectodermal stem cells contribute to the functional and morphological improvement of chronic spinal cord injuries via multiple mechanisms

Tamás Bellák[1], Krisztián Pajer[1], Máté Ignácz[1], László Gál[1], Zoltán Fekécs[1], Dénes Török[1], Annamária Marton[2], Csaba Vizler[2], Sudheer Babu Sangeetham[1], Antal Nógrádi[1]
1 University of Szeged, Albert Szent-Györgyi Medical School, Department of Anatomy, Histology and Embryology, Szeged, Hungary
2 Instititute of Biochemistry, Biological Research Centre, Szeged, Hungary

Spinal cord contusion injury leads to severe tissue loss and subsequent deficit of motor, sensory and vegetative functions below the lesion site. In this study we investigated whether transplantation of neuroectodermal stem cells into the injured rat spinal cord is able to induce significant morphological and functional improvement in a chronic spinal cord injury model. Mouse embryonic clonal neuroectodermal stem cells (NE-TR-4C) were grafted intraspinally five weeks after a thoracic spinal cord contusion injury performed in SD rats. Control animals underwent contusion injury without stem cell transplantation. Functional tests (BBB test, video-based locomotor pattern analysis) and detailed morphological analysis were performed to evaluate the effects of grafted cells in different time points. Grafted animals showed significantly better functional recovery compared with control animals. Morphologically, the contusion cavity was significantly smaller, and the amount of spared tissue was significantly higher in grafted animals than in controls. Retrograde tracing studies showed a statistically significant increase in the number of FB-labelled neurons rostral (spinal cord segments, raphe nuclei, somatomotor cortex) to the injury. The extent of functional improvement was related to the amount of inhibitory factors (GFAP, CS-56) around the cavity and microglial reactions in the injured segment. Five days after transplantation the majority of grafted cells appeared to survive, formed clusters and a small proportion of the cells differentiated into neurons and astrocytes. Ten days after grafting the majority of the grafted cells appeared as nonviable fragments in microglia/macrophage cells. These data suggest that grafted neuroectodermal stem cells are able to induce morphological and functional recovery after chronic spinal cord contusion injury despite the limited survival of transplanted cells.

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38. Kelemen, Hanga

Investigating the Impact of Neuroinflammation on Long-Term Neuropsychiatric Consequences of Perinatal Asphyxia

Hanga Kelemen[1], Kornél Demeter[1], Eszter Sipos1, Barbara Orsolits1, Gyula Balla1, András Buzás-Kaizler1, Áron Kerényi1, Manó Aliczki1, László Biró1, Zoltán Balogh1, Miklós Szabó2, Andrea Fekete2, Kai Kaila3, Ádám Dénes1, Éva Mikics1
1 Institute of Experimental Medicine, Budapest, Hungary
2 1st Department of Paediatrics, Semmelweis University, Budapest, Hungary
3 Neuroscience Center, University of Helsinki, Finland

Introduction: Perinatal asphyxia (PA) poses a significant threat to neonatal well-being and is linked to enduring cognitive impairments and neurodevelopmental disorders. Despite its prevalence, the underlying mechanisms of persistent brain injury resulting from asphyxia remain elusive, hindering targeted therapeutic interventions during the critical early period of neuroplasticity. In this study, we employed a translational rodent model of PA to explore neuropsychiatric outcomes and the associated neurobiological cascades, with a specific focus on microglia as key neuroinflammatory mediators and potential intervention targets.

Methods: Male Wistar rat pups were exposed to an asphyxia-inducing gas mixture (4% O2, 20% CO2) for 15 minutes under normothermic conditions at the age of 7 days. Long-term behavioural assessments encompassing motor, emotional, and cognitive domains were conducted from infancy to adulthood. Immunohistochemical analyses targeting brain regions implicated in observed deficits was performed to unravel the role of PA-induced neuroinflammation in the neuropsychiatric alterations. Additionally, we explored the impact of the anti-inflammatory agent interleukin-1 receptor antagonist (IL-1RA) administered early in the post-PA period on acute microglial morphology, long-term behavior, and histology.

Results: PA led to heightened anxiety, notable motor impulsivity and cognitive deficits, particularly in operant learning attention and spatial memory indicating predominantly prefrontal cortex-dependent phenotypical deficits. These behavioural changes were accompanied by a sustained modification of the excitatory/inhibitory balance in the affected brain region. Analysis of microglial morphological subtypes in the acute post-asphyxia period revealed alterations in the prefrontal cortex. Administration of IL-1RA significantly mitigated cognitive deficits in adulthood and influenced short- and long-term histological changes.

Conclusion: Our findings underscore the significant prefrontal cortex-dependent behavioral consequences of PA, characterized by enduring excitatory/inhibitory dysregulation following acute neuroinflammatory changes. The study suggests that a systemically administered anti-inflammatory approach using IL-1RA may offer a promising treatment avenue for the long-lasting cognitive deficits manifesting due to asphyxia.

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39. *Kelemen, Viktor*

Investigation of autism spectrum disorder using principal component analysis in a rat model of autism

Viktor Kelemen[1], Tímea Májer[1], Zsuzsanna Faragó[1], Sándor Borbély[1], Attila Szűcs[1], Petra Varró[1], Ildikó Világi[1]

1 Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest

Autism is a neurodevelopmental disorder characterized by impaired social communication, limited interest, and repetitive behavior patterns. On the neuronal networks level, autism is associated with loss of excitation-inhibition balance and increased risk of developing epilepsy.

The valproic acid (VPA) rat model is commonly used to study autism-like behaviors. Prenatal VPA exposure increases the risk of developing autism in offspring.

In our study, pregnant Wistar rats received an i.p. injection of VPA on gestation day 12.5 (dose 500 mg/bwkg). Pups were tested for various motor functions and behaviors from day P3 to week 6. Then, electrophysiological studies were performed in two age groups: 6 weeks and 3 months. Brain slices were prepared from the offspring for field potential measurements and parallel detection of intrinsic optical signals. Excitability changes were tested with spontaneous bursts evoked by Mg-free solution (MFR) and afterdischarges (ADs) evoked by brief bursts of high frequency electrical stimulation.

Autism is a spectrum disorder, meaning that it can manifest very differently in different individuals. The rodent VPA model seems to reproduce this aspect of the disorder, as alterations manifest to a different degree in each treated animal. Taking into account the results of behavioral tests and the severity of the malformations, we divided the treated animals into two groups (strongly vs weakly autistic) using principal component analysis.

Behavioral tests indicate that strongly autistic animals were able to perform surface righting reflex, negative geotaxis, auditory startle and visual placing reflex significantly later than the other VPA-treated and control rats. The results of social interaction tests indicate a stronger social defect in treated male rats.

VPA treatment showed an increase in seizure activity, suggesting an increased tendency to epilepsy. AD threshold was lower in strongly autistic group than in weakly autistic group and control slices. The length of ADs and burst length were higher in VPA-treated groups. In MFR, burst length and frequency were higher in treated groups than in control.

Tail and limb deformities in VPA-treated rats correlated with autism severity: the malformations were predictive of developmental delay, and electrophysiological data suggest increased neuronal excitability in strongly autistic rats compared with the other treated rats. Before drawing final conclusions, further analysis of the data is needed.

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40. Király, Ágnes

Complex examination of the pathophysiological mechanisms of pain in fibromyalgia-a human clinical study and rodent experiment

Ágnes Király[1,2,3], Zsuzsanna Helyes[1,2,3], Éva Szőke[1,2,3]
1 Department of Pharmacology and Pharmacotherapy, Medical School & Centre for Neuroscience, University of Pécs, 12 Szigeti Street, H-7624, Pécs, Hungary
2 National Laboratory for Drug Research and Development, Budapest, Hungary
3 Hungarian Research Network, Chronic Pain Research Group, Pécs, Hungary

Backgrounds and aims. Fibromyalgia (FM) is a common primary chronic pain condition associated with widespread musculoskeletal pain, predominantly in women. The mechanisms are unclear, but psychosocial distress and anxiety are likely to be the main etiological factors. The therapy is unsatisfactory; therefore, it is an "unmet medical need". Studies in translationally relevant animal models are important to identify key mediators. Although an optimal rodent FM model does not exist, chronic restraint stress (CRS)-induced pain in mice characterized earlier by our team seems to be appropriate to investigate the pathophysiological pathways and mechanisms. In the present study we perform unbiased transcriptomic investigation on PBMC and metabolomic analysis on plasma samples of CRS-exposed mice compared to non-stressed controls.

Methods. 12-week-old female and male C57BL/6 mice are exposed to CRS in ventilated centrifuge tubes for 6 h/day for 2 weeks, age and sex-matched animals without stress kept under standard circumstances serve as controls (n=12-15). During the CRS protocol their general health parameters (appearance, fur condition, stool and urine) are monitored daily and their weight is measured every other day. Pain threshold tests (dynamic plantar aesthesiometry, cold sensitivity in icy water) are performed before the protocol and repeated after the exposure to CRS. Blood samples are collected under deep anaesthesia via cardiac puncture, PBMC is isolated for next generation sequencing, and plasma for mass spectrometry. The data are analysed by complex bioinformatic tools, differentially expressed genes and metabolites are identified, potential pathways, signalling processes and networks are determined. The experiment was approved by the Animal Welfare Committee at the University of Pécs.

Results and conclusions. CRS induces approximately 20-25% mechanical and cold hyperalgesia on the paw after 2 weeks without any anxiety, depression-like behaviour or locomotor disturbances demonstrating the development of stress-induced pain. The analysis of the samples is still ongoing, the results are planned to be completed by March of 2024. We parallelly perform a study on FM patients with similar unbiased methodological approaches and the final aim is to compare the animal experimental data with the clinical results. We would like to determine the translational value of the mouse model for the investigation of potential pharmacological interventions.

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41. Kis, Balázs

Activation of autophagy by targeting the AMPK pathway as a treatment strategy for Huntington's disease

Balázs Kis1,2, Anna Abbas1,2, Lea Danics1,2, Anikó Göblös3, Roger A. Barker4, Johan Jakobsson5, Lajos Kemény3,6, Karolina Pircs1,2,5

1 HCEMM-SU, Neurobiology and Neurodegenerative Diseases Research Group, Budapest, Hungary.
2 Institute of Translational Medicine, Semmelweis University, Budapest, Hungary.
3 Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary
4 Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK.
5 Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, Lund University, Lund, Sweden.
6 HCEMM-USZ Skin Research Group, Szeged, Hungary

Huntington's disease (HD) is an autosomal dominant age-related neurodegenerative disorder caused by a mutated Huntingtin (HTT) gene containing an abnormal number of CAG repeats. Several studies documented altered autophagy in neurodegenerative disorders. Autophagy is an evolutionarily conserved lysosomal degradation pathway that ensures the cytoplasmic homeostasis. Our previous results using an induced neuronal (iN) model showed a subcellular cell type specific alteration of autophagy in HD-derived iNs. Moreover, mass spectrometry identified protein dysregulation in the AMPK pathway, which directly regulates the autophagy pathway.

In this project we will use an iN model to target the AMPK pathway in HD-iNs. We will use an all-in-one self-inactivating lentivirus to directly reprogram donor fibroblasts into neurons. The advantage of this methodology is that iNs maintain age and disease-related signatures of the donor cells as direct reprogramming bypasses the intermediary cell rejuvenation step inevitable in other approaches such as iPSC.

We will perform a mini drug screening with various drugs affecting the AMPK pathway using 5 Ctrl and 5 HD patient derived iNs. We will use high content automated screening microscopy to monitor the neuronal morphology and the expression of different autophagy markers after different drug treatments. We will continue to preclinically validate the best candidates that could rescue some key aspects of the disease phenotype presented in HD-iNs.

After careful pre-clinical validation of the best autophagy targeting hits, this proposal holds great potential to develop novel and better drugs specifically targeting components of the autophagic pathway firstly for HD, and later for other neurodegenerative proteinopathies (like Alzheimer's diseases, Parkinson's diseases and Frontotemporal dementia).

42. Kis Noémi

Cholinergic regulation of dendritic Ca2+ spikes controls firing mode of hippocampal CA3 pyramidal neurons

Noémi Kis[1,2], Balázs Lükő[1], Bela Erlinghagen[1], Mátyás Irás[1], Judit K. Makara[1]
1 Laboratory of Neuronal Signaling, HUN-REN Institute of Experimental Medicine, 1083 Budapest, Hungary
2 János Szentágothai School of Neurosciences, Semmelweis University, 1085 Budapest, Hungary

The hippocampus plays an important role in spatial navigation and contextual memory. Hippocampal pyramidal cells (PCs) frequently exhibit complex spike bursts (CSB), a firing pattern driven by regenerative dendritic Ca2+ plateau potentials. These events can induce rapid synaptic plasticity and initiate formation of new place fields in CA1PCs in spatially navigating mice. CSBs are also present in PCs of the CA3 area, and recent results suggest that place field-inducing plateau potentials and CSBs in CA3PCs are especially prolonged1. However, the dendritic mechanisms enabling such long-lasting events in CA3PCs are not well elucidated.

In our previous studies, conducted in acute rat brain slices, we observed large heterogeneity among CA3PCs both in CSB prevalence2 and in the kinetics of dendritic Ca2+ spikes3-4. While many CA3PCs express long duration (~50 ms) compound Ca2+ spikes, a group of cells exhibits unusually short (few ms long) Ca2+ spikes, which do not support sustained CSB firing. This raises the question whether specific conditions are required to gate the ability of these CA3PCs to fire prolonged plateaus.

Dendritic integrative functions are influenced by neuromodulation, including the cholinergic system that orchestrates memory processes. Acetylcholine (ACh) affects various ion channels including those mediating and shaping Ca2+ spikes in CA3PCs. This led us to investigate how dendritic Ca2+ spikes are regulated by cholinergic activity in CA3PCs.

The cholinergic agonist carbachol (2 µM) robustly prolonged pharmacologically isolated Ca2+ spikes, transforming short Ca2+ spikes into long-lasting forms. Carbachol also facilitated long-duration CSB firing in response to current injection or synaptic stimulation. On the other hand, nicotinic and muscarinic ACh receptor blockade did not affect Ca2+ spikes, indicating the heterogeneity of spikes is not due to variable cholinergic tone in the slice. Optogenetic stimulation of cholinergic axons in ChAT-Cre/Ai32transgenic mice increased CSB rate and duration, indicating that endogenous cholinergic activity can control Ca2+ spikes in CA3PCs.

We propose that cholinergic neuromodulation can gate the ability of CA3PCs with short-duration Ca2+ spikes to generate sustained plateau potentials, providing a state-dependent dendritic mechanism potentially contributing to memory encoding and retrieval.

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43. Kispál, Réka

Investigating the role of neuromodulators in mice during associative learning with a 50% reward schedule

Réka Kispál[1], Írisz Szabó[1], Bálint Király[1], Anna Velencei[1], Balázs Hangya[1]
1 Lendület Laboratory of Systems Neuroscience, Institute of Experimental Medicine

Reward prediction error (RPE) is the difference between actual and expected reward. According to reinforcement learning theory, value is updated based on the RPE. The release of dopamine (DA) in the ventral striatum (VS) was shown to represent RPE during classical conditioning. However, DA release in the prefrontal cortex (PFC), an area that is crucial to value-based decision making, was much less studied. Cholinergic neurons have been shown to play an important role in associative learning, suggesting that they are involved in the processing of stimuli that predict future outcomes. It has been previously demonstrated that the release of norepinephrine (NE) follows the threat prediction error, but its relationship with RPE has not been studied yet. We aimed to investigate the role of DA, acetylcholine (ACh) and NE in associative learning and the correlation of their release with the RPE.

To address this, we trained mice (n=20) on a sound detection Pavlovian conditioning task with a 50% reward schedule that allowed examining clean representations of +RPE (rewarded trials) and -RPE (reward omission trials), while we measured DA, ACh and NE release by fiber photometry. Behavioral updating of value representations based on the outcome of the previous trial was indexed by the licking activity of mice in the anticipation of reward.

As expected, anticipatory licking during the stimulus decreased after omitted rewards but increased after rewarded trials. DA release followed a similar pattern not only in the VS but also in the PFC. Moreover, we found significant positive correlations between DA release and anticipatory lick rate (ALR) difference both in VS and PFC. In the case of ACh, we did not observe anything similar in any of the examined brain areas. No clear correlation was observed between the change in ACh release in any of the brain areas and the change in the ALR. NE release in the BLA followed a similar pattern to ACh, but it decreased after rewarded trials and increased after omitted rewards in the PFC. Furthermore, we observed a negative correlation in the PFC between NE release and ALR changes.

These results indicate that the dopaminergic system broadcasts similar RPE signals to both striatal and frontal cortical targets. In contrast to DA, NE release decreased after rewarded trials and increased following omitted rewards and it was also negatively correlated with the intensity of the animals' anticipatory behavior.

44. Kiss, Ádám

Blink rate extraction from EEG recording

Ádám Kiss[1], András Kelemen[2], Attila Nagy[1]
1 Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged;
2 Department of Technical Informatics, Faculty of Science and Informatics, University of Szeged;

EEG recordings include many components, such as EMG or blink components. In classical processing these are excluded, however, they may contain additional incomes. A possible side-channel information is the blink rate. A possible extraction of it is presented in this paper.

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45. *Kolozsvári, Áron*

Electrophysiological and immunohistochemical comparison of the dentate gyrus projecting principal cells in the lateral and medial entorhinal cortex

Áron Kolozsvári[1,2], Szilárd Szőcs[1,2], Nóra Henn-Mike[1,2], Ágnes Agócs-Laboda[1,2], Klaudia Barabás[1,2], Csaba Varga[1,2]

1 Institute of Physiology, Medical School, University of Pécs

2 Centre for Neuroscience, Szentágothai Research Centre, University of Pécs

The entorhinal cortex (EC) is the primary input and output structure of the hippocampus forming a network hub in the cortico-hippocampal circuits. The medial (MEC) and lateral entorhinal cortex (LEC) convey spatial (grid cell) and contextual (odor, social) information to the hippocampus, respectively. This difference is largely attributed to the diverge inputs from other brain areas converging to these two subregions of the entorhinal cortex. However, the entorhinal cortex not only transmits information but also processes it. In order to understand the computational power in the two areas, we systematically compared the intrinsic electrophysiological properties of the dentate gyrus projecting stellate (MEC) and fan (LEC) cells in layer II.

We used in vitro whole-cell patch-clamp electrophysiology to compare their basic firing properties, action potentials and passive membrane characteristics. After electrophysiological recordings, we verified the biocytin-filled cells with specific immunohistochemical markers, reelin and WFS1.

We found that fan cells differed from stellate cells in many aspects. Stellate cells showed faster time constant and larger h-currents. During depolarization a spike-doublet appeared in stellate but not in fan cells at the beginning of the firing, which was consistently present during every sweep even at higher amplitude depolarization currents. When we used CsCl-containing intracellular solution, the doublets were absent in both stellate and fan cells. Cs+ acts as a voltage-gated potassium channels blocker, therefore, we assume potassium currents are involved in spike-doublet generation in stellate cells.

In order to shed light on the potential differences in voltage-sensitive ion channel expressions of these neurons, we will perform patch-seq single cell transcriptomic experiments and immunohistochemically verify the differences in expression levels.

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46. Koltai, Zsófia

Gene expression changes of heat shock proteins and myokines induced by physical activity in the brain and skeletal muscle

Zsófia Koltai[1], Ákos Menyhárt[2], Zsófia Ruppert[1], Bettina Rákóci[1], László Vígh[1], Zsolt Török[1], Melinda E.Tóth[1]

1 Laboratory of Molecular Stress biology, HUN-REN Biological Research Centre, Szeged

2 Department of Cell Biology and Molecular Medicine Albert-Szent Györgyi Medical School, Faculty of Science and Informatics University of Szeged

Several pieces of evidence suggest, that sedentary lifestyle is an important risk factor forneurodegenerative diseases. Our previous experiments have confirmed, that regular exercise has a positive effect on the cognitive function of mice, particularly in females. The aim of our current research is to identify the factors that may mediate the effects of physical activity and explore the causes of sex differences. Therefore, we analyzed the gene expression levels of myokines and heat shock proteins produced by the brain and skeletal muscle after exercise. C57BL/6 mice were running on a treadmill for one hour per day for a week. After the last exercise session, we collected blood samples and isolated RNA from the m. quadriceps femoris and the hippocampus samples. Changes in gene expression levels of various myokines and heat shock proteins were examined using real-time PCR. To confirm the qPCR results, immunofluorescent staining were performed on frozen muscle sections. After one week of training, the gene expression levels of small heat-shock proteins (Hsp25 and αB-crystallin) and the CD68 macrophage marker doubled in the muscle tissue of male animals. However, there was no significant difference in female mice. The level of Hsp70 mRNA increased by 2.5 times in females, while in male mice, it increased by 10 times compared to the control group. Additionally, immunofluorescent staining revealed distinct staining patterns of Hsp25 and Hsp70 in the muscle tissue of trained males. In the hippocampus, we observed the increase of Hsp25 and BDNF gene expression in response to exercise, without any significant sex differences. Our results show, that exercise training can increase the expression level of Hsp25 and BDNF in the brains of both sexes, which may contribute to its beneficial effects. However, the excessive expression of Hsp and CD68 genes in male mice suggests, that the same level of training intensity was more stressful for males than females. This could explain the observed sex difference in the improvement of cognitive functions and highlights the importance of personalised training.

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47. *Kovács, Beatrix*

High-sensitivity quantification of anti-AAV neutralization from preclinical model and human sera

Beatrix Kovacs [1, 2], Aron Szepesi [3, 4], Viktoria Szabo [5], Mirella Barboni [5], Zoltán Zsolt Nagy [5], Balazs Rozsa [3, 4, 6], Daniel Hillier [1, 6, 7]

1 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary
2 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary
3 Laboratory of 3D Functional Network and Dendritic Imaging, Institute of Experimental Medicine, Budapest, Hungary
4 BrainVisionCenter Ltd., Budapest, Hungary
5 Department of Ophthalmology, Semmelweis University, Budapest, Hungary
6 Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary
7 Institute for Molecular and Clinical Ophthalmology Basel, Basel, Switzerland

Adeno-associated virus (AAV) vectors are the principal vehicle for gene therapy. In a large share of humans, immunity against AAVs can reduce or completely inhibit transgene expression, and can lead to adverse outcomes. The level of immunity can be estimated by assaying AAV neutralization level of the blood serum. Surprisingly, there is no widely adopted sensitive assay for the quantification of AAV neutralization. We develop an in vitro generic neutralization assay and quantify its reproducibility and sensitivity via longitudinal evaluation of neutralization in preclinical models and humans. Our results may offer a precise, validated readout modality that can serve as solid ground for safety assessment of new AAV constructs.

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48. Kucsápszky, Nóra

Development of a novel organ-on-a-chip model combining a human cell-based cell culture model of the blood-brain barrier and human midbrain organoids

Nóra Kucsápszky[1], Judit P. Vigh[1,2] , Anna Kocsis[1], Ana R. Santa-Maria[1,3], Silvia Bolognin[2], Jens C. Schwamborn[2], Sándor Valkai[1], András Kincses[1], András Dér[1], Mária A. Deli[1], Fruzsina R. Walter[1]
1 Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
2 Doctoral School of Biology, University of Szeged, Szeged, Hungary
3 Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA
4 Luxembourg Centre for Systems Biomedicine, Developmental and Cellular Biology, University of Luxembourg, Belvaux, Luxembourg

Blood-brain barrier (BBB) is formed by brain endothelial cells and surrounding cell types,such as astroglia, pericytes, neurons and microglia creating the neurovascular unit. Cell culture models of the BBB are needed to describe brain microvasculature related phenomena. Dynamic BBB-on-a-chip models are important devices in the research of BBB pathology and pharmacology. Lab-on-a-chip models created earlier by our team enable visual observation, transendothelial electrical resistance (TEER) and permeability measurements across the brain endothelial monolayer, and also the introduction of fluid flow to mimic blood circulation. The aim of the present study was to develop a novel, more complex human cell culture-based BBB-on-a-chip model with the co-culture of midbrain organoids. Our device is composed of a porous cell culture membrane in between two polydimethylsiloxane (PDMS, a transparent polymer) layers. The layers are stuck together with the help of oxygen plasma treatment. Two plastic slides are sputter coated using a mask with gold and then platinum to make the electrodes for the measurement of the TEER. To hold the lab-on-a-chip together screws are used on the sides around the plastic slides. In the bottom slide three holes are drilled to place the organoid holders, which are made from the lid of PCR tubes. In the top slide, there are four holes to which the inlets and outlets are glued to enable culture media change and flow. In the model we co- culture human stem cell derived endothelial cells, brain pericytes and human midbrain organoids. The device is also used for the modelling of dynamic conditions with medium flow in the top compartment to mimic shear stress. Based on our experiments the improved device works reliably. The results show that the BBB maturation and the integrity of the barrier are appropriate in the presence of the midbrain organoids. To verify the goodness of the model we used TEER and permeability measurements. Immunostaining of the tight junction proteins was used to examine the morphology of the cells. We found that the dynamic modelling of the BBB is possible using these human cell types co-cultured with the midbrain organoids. This setup can be used in further experiments to study pathologies and drug permeation across the BBB.

Funding/Grants:

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49. Kumar, Prabhat

Glucose transporter 2 positive cells in the medial prefrontal cortex: unravelling their impact on posttraumatic stress disorder.

Prabhat Kumar[1], Csilla Lea Fazekas[1,2,3], Pedro Correia[1,2,3], Dora Zelena[1,2]
1 Institute of Physiology, Medical School, University of Pécs, Centre for Neuroscience, Szentágothai Research Centre, Pécs, Hungary
2 Institute of Experimental Medicine, Eötvös Loránd Research Network, Budapest, Hungary
3 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary

Posttraumatic stress disorder (PTSD) is a psychopathological condition induced by a traumatic event, such as physical or sexual assault or a natural disaster. It is known that neurons rely heavily on glucose as a primary energy source and energy demand of the brain increases in life situations. The median prefrontal cortex (m-PFC) is an important brain area regulating fear response, Moreover, it contains glucose sensing glucose transporter 2 (GLUT2) containing neurones. We hypothesized that manipulating these mPFC-GLUT2 cells by a chemo genetic technique will influence the behavioural outcome. GLUT2-Cre transgene mice was used, and designer receptor exclusively activated by designer drug (DREADD) sequence was injected into the m-PFC by the help of an adeno associated viral vector. Two weeks after vector injection foot shock trauma was applied. As previously post-trauma sucrose consumption for 24h was showed to be protective we combined manipulation of the glucose sensitive cells (i.e. injection of the DREADD ligand clozapine-N-oxide right after trauma) with 16% sucrose drinking. The acute stress disorder (ASD) was studied 24h, while PTSD-like freezing 14-day after the trauma. Immunohistochemistry against red fluorescent protein confirmed the correct hits in the PFC region. The mice drunk more from sucrose than from water measured for 24h after trauma. However, the body weight increase was the same in all groups throughout the experiment. In general, females seemed to be more affected than males. In contrast to our expectation the effect of stimulation of the GLUT2 positive cells of the PFC and post-trauma sucrose drinking did not show synergistic effect on freezing behaviour studied both in a context as well as in cue dependent way. Our research draw attention to the importance of energy supply in the development of psychiatric disorder, especially in the decision making m-PFC area.

Keywords: Posttraumatic stress disorder, Median prefrontal cortex, Glucose transporter 2, Sucrose, Fear memory, and Freezing.

50. Kvak, Erika

Application of dehydroepiandrosterone as a neuroprotective agent for the therapy of Alzheimer's disease in a mouse model

Erika Kvak [1, 2], Szidónia Farkas[1], Dóra Zelena[1]
1 Laboratory of behavioural and stress studies, Institute of Physiology, University of Pécs, Pécs, Hungary
2 Faculty of Health Sciences, University of Pécs, Pécs, Hungary

Introduction: Alzheimer's disease (AD) is currently one of the most significant neurodegenerative diseases, and its effective treatment remains a challenge. The dehydroepiandrosterone (DHEA) is an androgen molecule, which protects in vitro against amyloid-ß (Aß) toxicity, and so might potentially improve cognitive functions. As steroid might influence wide range of processes both short (via membrane receptors) and long term (via intracellular receptors) we can expect beneficial effect already after one injection.

Methods: Six months old male 3xTg-AD mice (B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) were treated intraperitoneally with DHEAS (a water-soluble sulphate salt of DHEA, 10 mg/10ml/kg) and compared to vehicle treatment. Behavioural tests (Y-maze and social discrimination) were performed 30 minutes after the injection, and after 24/48 hours we transcardially perfused the animals. We performed immunohistochemistry on 30 µm thick sections for acetylcholinesterase (cholinergic fibre density) and amyloid-β accumulation.

Results: In previous studies we observed that the 3xTg-AD animals exhibited increased anxiety and cognitive disorders. While the Y-maze test did not reveal any significant effects of DHEAS on specific motor and cognitive deviations, changes in social behaviour were evident in the social discrimination test. As often seen in AD, amyloid plaques and neurofibrillary tangles appeared in the brains of these mice, as well as cholinergic fibre destruction in sensory cortex. The treatment was able to influence these morphological changes.

Conclusion: In conclusion, our findings support the potential of DHEAS as a protective agent for nerve cells, suggesting its usefulness as a novel therapeutic option for neurodegenerative diseases, including AD.

51. *Lam Tri, Duc*

Endothelial Antibody Factory" at the Blood Brain Barrier: Novel Approach to Therapy of Neurodegenerative Diseases

Lam Tri Duc[1,2], Claudine Kieda[3,4], Claude Nicolau[5,6], István Krizbal[1], Attila E. Farkas[1]
1 Institute of Biophysics, HUN REN Biological Research Centre Szeged, Szeged, Hungary
2 Doctoral School of Biology, University of Szeged, 6726 Szeged, Hungary
3 Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine, Warsaw, Poland
4 Centre for Molecular Biophysics, UPR 4301 CNRS, Orleans, France
5 ALSaTECH, Inc., Boston, MA, USA
6 Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA

Nowadays, aging is an increasing problem for the societies and economies of developed countries. Many researchers employ various types of stem cells such as iPSCs for cell-based treatment and regenerative medicine because of the benefits of noninvasive isolation techniques, reduced immunogenicity, and greater proliferation potency. Endothelial precursor cells (EPCs) are considered the best candidates for immunotherapy of neurodegenerative and many other diseases. In this study, EPC lines were established from aorta– gonad–mesonephros (AGM) of murine 10.5 dpc embryo, namely MAgECs (mouse aorta– gonad–mesonephros endothelial cells) 10.5. We wanted to test the hypothesis that EPCs can be used as vectors for treating neurological disorders by integrating them into the vasculature and secreting therapeutic molecules. Therefore, MAgEC 10.5 RT cells were further modified to also express the anti-β-amyloid and anti-TDP43 Fabs (cells named MAgEC 10.5 RT anti-TDP-43). My study aimed to report preclinical data as a preliminary proof of concept for the development of a novel approach using ex-vivo transfected EPCs as cellular producers of anti-TDP-43 and anti-β-amyloid antibody fragments (Fabs). We used these tools to study the delivery of antibody fragments into the brain tissue. After injection, their brains were sectioned at various times. We observed EPCs localizing in the brain up to 7 days after injection. The relationship between EPCs and NVU components was studied using immunofluorescent microscopy. We observed that most EPCs completely fill the capillary lumen in brain sections 4 hours after injection. In contrast, EPCs were seen flattening against and sticking to vessel walls after 28 hours post-injection. Seven days after injection, EPC cells were found to form tight junctions with preexisting endothelial cells and between themselves, revealed by Claudin 5 staining. EPCs further modified to express the anti-TDP43 Fab, were also observed in the vasculature for up to 7 days and these cells continued to secret of Fabs. Our results confirm that production and secretion of Fabs leads to accumulation of anti-TDP43 Fab at the blood brain barrier and the Fab also appears past the barrier inside brain parenchyma.

52. Láng, Tamás

A thalamo-preoptic pathway inhibits intermale aggression in rats

Tamás Láng[1], Botond Drahos[1], Dávid Keller[1,2], Árpád Dobolyi[1,3]
1 Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest 1094, Hungary
2 Institute for Systems Physiology, Faculty of Medicine, University of Cologne, Cologne 50931, Germany
3 Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest 1117, Hungary

We have established that the posterior intralaminar thalamic nucleus (PIL) receives ascending input from the somatosensory system and that projection from the PIL to the preoptic area of the hypothalamus increases social grooming between adult female rats. It remained open if PIL neurons promote any other type of social touch between conspecifics. Therefore, in the present study, we focused on the role of PIL neurons, and the PIL-preoptic pathway in intermale aggressive behavior.

For chemogenetic manipulation of PIL neurons, we injected adeno-associated virus into the nucleus, which expressed excitatory and inhibitory DREADDs fused with mCherry. In a separate experiment, we selectively tagged socially c-Fos-activated neurons in the PIL with DREADDs. To induce aggression, the animals were separated for 2 months. On the first day of the experiments, a vehicle was injected followed by aggressive behavioral test 1.5 hours later. An unfamiliar intruder was placed in the subject animal's cage resulting in an aggressive response. On the second day, the same test was repeated starting 1.5 hours after clozapine- N-oxide (CNO) injection to activate the DREADDs. Chemogenetic stimulation decreased aggression and increased duration of positive valance contacts while inhibition of the PIL neurons resulted in an increase in aggression and a decreased duration of positive valance contacts.

To establish the activity of PIL neurons and their neuronal targets during aggressive behavior, we measured the number of c-Fos-ir cells. While the PIL and its target brain regions showed elevated c-Fos activation following aggression, the inhibition of PIL neurons during aggressive behavior decreased c-Fos expression in the PIL and in one of its target brain areas, the medial preoptic area (MPOA). In turn, CNO injection into animals previously injected with an AAV encoding the stimulatory DREADD resulted in an elevated c-Fos expression in the PIL and the MPOA in the absence of social interactions. Therefore, we also investigated the PIL-MPOA pathway by injecting a stimulatory DREADD-expressing virus into the PIL and local CNO injection into the MPOA via intracerebral cannulas in order to activate the fiber terminals in the MPOA originating from the PIL. The activation of the pathway decreased aggression and increased positive valance contacts.

Based on these results we suggest that PIL neurons reduce intermale aggressive behaviorpossibly by their projections to the medial preoptic area.

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53. Layous, Róbert

Optical recording of unitary synaptic connections between CA3 pyramidal cells using Voltron imaging

Róbert Layous[1], Árpád Mike[1], Eszter Sipos[1], Bálint Tamás[1], Ádám Szatai[1], Antónia Arszovszki[1], János Brunner[1], János Szabadics[1]
1 HUN-REN Institute of Experimental Medicine, Budapest

Understanding the impact of synaptic connections and the rate of connectivity between individual neurons is crucial for drawing the blueprint of neuronal networks. We employ a novel voltage imaging method to map unitary synaptic connections with high efficacy between large number of neurons. Voltron is a genetically encoded voltage indicator that is capable of detecting both action potentials and small subthreshold events, such as unitary EPSPs. We used Voltron imaging in acute slices to test the connectivity between CA3 pyramidal cells. These connections are thought to be crucial for major hippocampal memory functions; however, there are controversies in the literature about how densely CA3 pyramidal cells excite each other. Voltron was expressed sparsely in CA3 neurons using a mixture of two rAAVs expressing the Voltron protein Cre-dependently and the Cre enzyme. 4-8 weeks after the rAAV injections, we prepared acute slices that were incubated with a fluorescent dye (Janelia Fluor 549) that covalently binds to the perisomatically expressed Voltron protein. We measured the membrane voltage changes in up to hundreds of neurons using epifluorescent illumination with a CMOS camera at high speed (1 kHz) in a large field of view (375x235 micrometers). We detected spontaneous spiking activity of CA3 pyramidal at room temperature. Typically, we identified 10-40 active cells during one imaging session (2.5-4 minutes long imaging) within one field of view. If an individual cell elicited a sufficient number of spikes (>10 APs), we were able to observe subthreshold responses with sufficient signal-to-noise ratio in other cells that were also identified based on their spontaneous spiking. Thus, we correlated both the sub- and suprathreshold responses of potentially connected pairs (n = 1895 pairs). Only those pairs were accepted as connected where both response types showed clear excitatory effects (n = 85 connections). As the Voltron signal persisted after fixation and labelled the perisomatic dendrites and spines of the imaged cells, the spiking cells were anatomically verified as pyramidal cells or different types of GABAergic cells. In some of the experiments we tested the involvement of AMPA receptors in the detected pyramidal cell connections by using a specific inhibitor, NBQX and a positive modulator, cyclothiazide. Altogether we showed that Voltron imaging can reveal the synaptic connectivity of neuronal networks with high efficacy.

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54. Li, Lina

The Protective Effect of PACAP38 in Type 2 Diabetic Retinal Disease

Li Lina[1], Evelin Patko[1], Andras Varga[1], Diana Denes[1], Dorottya Molitor[1], Edina Szabo[1], Balazs Meresz[1], Dora Reglodi[1], Alexandra Vaczy[1], Tamas Atlasz[1,2]

1 Department of Anatomy, HUN-REN-PTE PACAP Research Team, Centre for Neuroscience, Medical School, University of Pecs

2 Department of Sportbiology, Faculty of Sciences, University of Pecs

Introduction: The continuously growing diabetes population has caused a major concern with type 2 diabetic retinal disease (T2DRD), which is a leading cause of permanent blindness, however the underlying pathophysiological mechanism of T2DRD has not been fully understood yet. Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated from ovine hypothalamus based on its stimulating effect on the adenylate cyclase enzyme in anterior pituitary cells. PACAP38 (PACAP with 38 amino acids) activates anti-apoptotic pathways, inhibits pro-apoptotic signaling pathways, and creates an anti-inflammatory environment in the retina. The aim of the present study was to test the possible retinoprotective effect of topical administration of PACAP38 in type 2 diabetic animal model induced by high fat diet and the intraperitoneally injected low-dose streptozotocin (STZ).

Methods: Wistar rats were divided into 4 groups: control, control+PACAP38, diabetes and diabetes+PACAP38 groups randomly. Type 2 diabetes was induced with the combination of STZ (30mg/kg) and high-fat diet. All rats were treated topically two times a day for 4 months: control+PACAP38 and diabetes+PACAP38 groups were applied with PACAP38 eye drops (1ug/ drop), while the control group and diabetes group were administered with vehicles (artificial tears). Diabetes model was validated by a fasting oral glucose tolerance test (OGTT) and C-peptide ELISA test. Animals have been checked during the whole experiment to monitor the progression of the disease. Electroretinography (ERG), optical coherence tomography (OCT), post-mortem immunohistochemistry staining, and vessel analysis were measured in the retina samples.

Results: OGTT, C-peptide ELISA test, and the investigation of blood parameters proved the development of type 2 diabetes. Significant differences could be detected in visual function between the two diabetic groups at 16 weeks (in the case of a-wave, b-wave and OP amplitudes), where the diabetes PACAP38-treated group was similar to the control ones. OCT measurements correlated with ERG data where the total retinal thickness was preserved in the diabetes+PACAP38 group. The retinal microvascular structure and the ganglion cell number were also protected by PACAP38 in the retina.

Conclusions: Topically administered PACAP38 has displayed its potent neuroprotective effect against type 2 diabetic retinal disease, therefore it could be a promising therapeutic approach for the treatment of T2DRD.

55. *Lükő, Balázs*

Analyzing dendritic signals of hippocampal CA3 cells using in vivo calcium imaging.

Balázs Lükő[1], Snezana Raus-Balind[1], Balázs Ujfalussy[3], Judit Makara[4]
1Laboratory of Neuronal Signaling, HUN-REN Institute of Experimental Medicine, 1083 Budapest, Hungary;

In vivo calcium imaging is a popular tool enabling us to extract signals from thousands of cells of a behaving animal. In this project however our aim is not to look at a population of cells but rather to record from as many different dendritic segments of the same cell as possible and compare the properties of the calcium events that we can find in them. My responsibility in the project is to prepare an analysis pipeline that can be applied to the 2-photon calcium recordings made by Snezana Raus-Balind with the help of Balázs Ujfalussy all being supervised by Judit Makara

We are recording from mice running on a treadmill while they are presented two pseudo-randomly alternating linear corridors with the help of three monitors creating a VR-like experience. The animals can acquire a water reward by licking a port at a specific zone in each corridor so they are actively engaged in a spatial task. To label the cells we use the fluorescent indicator GCamp8s which is sparsely expressed in the field of view.

Our cells of interest are the hippocampal CA3 pyramidal cells because of multiple reasons. From the technical side these cells are optimal for studying dendrites as they can be measured in a horizontal orientation and as a result distal and proximal dendrites of the same cell can be measured from in the same imaging plane. From the scientific side we have studied the dendritic Ca2+ spikes extensively (see Magó et al) in the last couple of years but our data always came from in vitro slices so it is an important question for us what kind if Ca2+-events we can measure in more natural conditions.

Our basic questions are whether there are localized events and if there are how they compartmentalize, where they originate and how they propagate. To be able to address these questions we first need to extract the signals by performing motion correction, ROI definition and signal extraction. The traces are then first smoothed and flattened to make it possible to reliably detect events. As a result we have a structured data that can be used to answer our scientific questions like how a specific event looks like in the different dendritic compartments, does a specific cell have a significant spatial tuning or we can even take a look whether a particular event influences the spatial tuning of the cell in subsequent laps. These steps are all performed using custom written python scripts written by 3 and I all put together in a way that anyone can easily navigate through the analysis using Jupyter Notebooks.

Distinct dendritic Ca2+ spike forms produce opposing input-output transformations in rat CA3 pyramidal cells. Magó Á, Kis N, Lükő B, Makara JK.

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56. Lyakhova, Victoria

The Lateral Septum and its multifaceted role in anxiety

Victoria Lyakhova[1,2], Dániel Schlingloff[1], Ágnes Simon[1], Balázs Hangya[1]
1. Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience, Budapest, Hungary
2. János Szentágothai Doctoral School of Neurosciences, Semmelweis University

The Lateral Septum (LS) plays an important role in controlling emotional states such as anxiety, aggression, and controls social behaviours, presumably in a sex-dependent manner. Despite the relatively sizeable amount of experimental data supporting these LS functions, the mechanistic understanding of how LS regulates these processes is hindered by a series of contradictory results. Although the LS is thought to contain exclusively GABAergic cells, we found that a fraction of LS cells expresses cholinergic neuronal markers. In this study, we try to understand the function of this specific neuronal population named LS cholinergic cells (LSCNs), using LSCN-specific viral expression of optogenetic actuators combined with behavioural testing by using paradigms such as the Open Field Test, the Elevated Plus Maze and the Light-dark Box to assess anxiety levels, and the fox odour test combined with c-Fos staining to explore neuronal activation following exposure to an inherently aversive stimulus.

Our preliminary results show that stimulation of the LSCNs has an anxiogenic effect on observed behaviour independent of sex. The fox odour test revealed activation in LS, however, with no overlap with LSCNs. Since traditional fiber optogenetics obstruct the usability of other paradigms, such as the Elevated Plus Maze or sociability tests, due to physical constraints on the animals exerted by optic cables, we started to adopt wireless optogenetics to overcome this limitation. In the future, we aim to expand our investigations with an array of techniques, including optogenetic inhibition, fiber photometry, electrophysiology, and anatomy in order to better understand this critical yet enigmatic structure.

57. Mérész, Balázs

Potential new treatment target in bacterial keratitis

Balazs Meresz[1], Inez Bosnyak[1], Dorottya Molitor[1] , Bela Kocsis[3], Kornelia Farkas[4], Dora Reglodi[1], Tamas Atlasz[1,2], Alexandra Vaczy[1]

1 MTA-PTE PACAP Res. Group, Dept. of Anatomy, University of Pecs Medical School, Pecs, H-7624, Hungary

2 Faculty of Sciences, Dept. of Sportbiology, University of Pecs, Pecs H-7624, Hungary

3 Dept. of Medical Microbiology and Immunology, University of Pecs Medical School, Pecs, H-7624, Hungary

4 Institute of Bioanalysis, University of Pecs Medical School, Pecs, H-7624, Hungary

Introduction: Bacterial keratitis is an infection of the corneal tissue caused by various bacterial species. Etiology and pathogenesis are influenced by diverse factors. One of the most common factors is contact lens wear. Other predisposing factors include different eye injuries, previous ocular surgery, corneal edema, and drug induced effects with corticosteroids. Despite the progress made in diagnosis and treatment keratitis is still responsible for the most sight-threatening lesion. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide found in the central nervous system and peripheral organs, known for its neuroprotective and anti-inflammatory effects, which are mainly mediated through PAC1 receptor. The aim of our research is to investigate the protective role of the PAC1 receptor in endotoxin-induced keratitis model in mice.

Methods: In our study, we induced bacterial keratitis in a CD1-IGS mouse strain by intraperitoneal injection of lipopolysacharide (LPS). To investigate the role of PAC1 receptor, half of the animals were treated intravitreally with PAC1 receptor antagonist, maxadilan. 24 hours after injection, optical coherence tomography (OCT) was used to assess inflammation and treatment-induced changes in the anterior segment of the eye. Routine histological examinations were performed to confirm OCT results. To map inflammatory pathways, we determined the expression of 40 cytokines in the different groups. Five weeks after the LPS administration, a four-step scoring system known from the literature was used to determine the degree of keratitis.

Results: Based on OCT and post-mortem histology results, we found a significant increase in central corneal thickness 24 hours after LPS indejction, which was attenuated by maxadilan treatment. Cytokine assays showed that maxadilan inhibited the activation of inflammatory pathways. Five weeks after maxadilan injection grading score showed less severe keratitis in the group receving LPS and treated with maxadilan compared to the group receving endotoxin alone.

Conclusion: Our results suggest that targeting the PAC1 receptor could be a promising therapeutic approach for the treatment of bacterial keratitis.

58. *Mihalj, Denisa*

GABAergic synaptic abnormalities in olfactory brain regions of mice with autism-likesymptoms

Denisa Mihalj[1], Zdeno Pirnik[1,3], Veronika Borbelyova[2], Zuzana Bacova[1], Jan Bakos[1,3]
1 Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
2 Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
3 Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Dysfunctional sensory systems, including altered olfactory function, have recently been reported in individuals with autism spectrum disorder (ASD). Disturbances in processing odors may be linked to γ-aminobutyric acid (GABA)ergic synaptic abnormalities in the olfactory brain regions. At GABAergic synapses, gephyrin is a key scaffolding molecule that contributes to the maintenance of appropriate excitatory and inhibitory balance by regulating GABA receptor trafficking. However, the precise molecular mechanism by which GABAergic transmission affects the olfactory system in ASD is not fully understood. Therefore, the present study aimed to evaluate selected components of the GABAergic system in olfactory brain regions and primary olfactory neurons isolated from Shank3-deficient (-/-) mice, which exhibit autism-like behavioral phenotypes. Shank3 deficiency led to a significant reduction in GEPHYRIN/GABAAR colocalization in the piriform cortex and primary neurons isolated from the olfactory bulb. Gene expression analysis revealed significantly lower mRNA expression of presynaptic GABA transporter 1 in the olfactory bulb and Collybistin in the frontal cortex of Shank3-/- mice than in WT mice. A similar trend of reduction was observed in Somatostatin expression in the frontal cortex of Shank3-/- mice. Overall, it appears that Shank3 deficiency is associated with changes in GABAergic synapses in brain regions that are important for olfactory information processing, which may represent a basis for understanding the functional impairments in ASD. Supported by APVV-21-0189, VEGA 2/0057/23, and HAS-SAS-2022-02.

59. Milicic, Milica

TRPA1 ion channel does not contribute to the chronic stress-induced activation of locus ceruleus noradrenergic neurons

Milica Milicic[1], Balázs Gaszner[2], Gergely Berta[3], Erika Pintér[1], Viktória Kormos[1]
1 Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, H-7624, Pécs, Hungary.
2 Department of Anatomy, Medical School and Research Group for Mood Disorders, University of Pécs, H-7624, Pécs, Hungary.
3 Department of Medical Biology, Medical School, University of Pécs, H-7624 Pécs, Hungary.

Background: We have previously proven the involvement of transient receptor potential ankyrin 1 (TRPA1) in stress adaptation. The lack of TRPA1 affects both urocortin 1 (member of corticotropin-releasing horomone (CRH) family) content of the Edinger-Westphal nucleus, and serotonin immunoreactivity in dorsal raphe nucleus. The noradrenergic locus ceruleus (LC) is also an important player in mood control. Here we aimed at investigating whether the TRPA1 is expressed in the LC. We also put forward to test if the response to chronic variable mild stress (CVMS) is affected by the lack of TRPA1.

Methods: The *Trpa1* mRNA expression was examined by RNAscope *in situ* hybridization in the LC of intact C57BL/6J mice. We investigated TRPA1 knockout and wildtype mice in the three-weeks CVMS model of depression. Tyrosine-hydroxylase (TH) and FOSB double immunofluorescence was used to test the functional-neuromorphological changes in the LC.

Results: No TRPA1 expression was detected in LC. The TH content was not affected by CVMS exposure. The FOSB immunosignal was induced by CVMS in the LC, however, it did not co-localize with the TH neurons. Unexpectedly, a strong CVMS-associated FOSB activation was detected in the pontine micturition center, Barrington's nucleus (BN), located next to the LC.

Conclusion: The TRPA1 is not expressed in the LC. Lack of functional TRPA1 receptor neither directly nor indirectly affects the TH content of LC neurons in CVMS. Current studies are in progress to determine how the BN activation contributes to the development of overactive bladder and nocturnal enuresis that are both chronic stress-induced conditions.

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60. Mintál, Kitti

Autism spectrum disorder associated behavioral symptoms and their relationship with the gastrointestinal microbiome

Kitti Mintál[1,2], Attila Tóth[1,2], Edina Hormay[1,2], Béla Kocsis[3], Kristóf László[1], Anita Bufa[4], Tamás Marosvölgyi[4], Renáta Cserjesi[5], Zoltán Vizvári[2,6], László Lénárd[1,2], Zoltán Karádi[1,2]
1 Institute of Physiology, Medical School, University of Pécs
2 Medical and Engineering Multidisciplinary Cellular Bioimpedance Research Group, Szentágothai Research Centre, University of Pécs
3 Department of Medical Microbiology and Immunology, Medical School, University of Pécs
4 Institute of Bioanalysis, Medical School, University of Pécs
5 Institute of Psychology, ELTE Eötvös Loránd University
6 Department of Environmental Engineering, Faculty of Engineering and Information Technology, University of Pécs

Introduction: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder where difficulties of social interactions have been considered to be the most severe symptoms. The escalating use of antibiotics and the consequent disruption of the gastrointestinalmicrobiome has been implicated in the development of various neurological and psychological symptoms through the microbiome-gut-brain axis. Therefore, changes in the microbiome community are supposed to influence the central regulatory processes and affect brain functions which ultimately lead to behavioral alterations.

Aim: The first aim of the present study was to assess whether depletion of the gastrointestinal microbiome could induce ASD-like behavioral symptoms. The second goal was to demonstrate the benefits of a probiotic mixture of ours on ASD-like behavioral symptoms.

Method: The impact of the alterations on the social behavior was examined in adult male Wistar rats. Animals have been divided into six groups - 1. antibiotics treated; 2. antibiotics and probiotic treated; 3. probiotic treated; 4 valproic acid treated; 5. valproic acid and probiotic treated; 6. control groups. As antibiotics treatment, rats were given broad spectrum antibiotics mixture dissolved in their drinking water for 4 weeks. Probiotic treated groups daily received our probiotic mixture (containing beneficial bacterial species) with their food for 14 days. Valproic acid treated groups were created as pregnant rats received a single dose of valproic acid on the 12.5th day of gestation and then their male pupils were used in the experiments. Social behavioral test was conducted following the respective modifications of the microbiota.

Results: Our findings demonstrate significant group-differences in the social behavioral test. Antibiotics-induced microbiome alterations during adulthood triggered severe deficits in social behavior similar to those seen in the valproic acid treated rats. However, the probiotic treatment was able to alleviate the antisocial behavior both in the antibiotics- and the valproic acid treated rats.

Conclusions: The present findings well demonstrate that the gastrointestinal microbiome plays important role in the organization of social behavioral processes, and also substantiate that our specific probiotic mixture can alleviate both the antibiotics and the valproic acid generated antisocial behavioral symptoms.

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61. Molitor, Dorottya

Negative Effects of Maternal Smoking on Retinopathy of Prematurity

Dorottya Molitor[1], Alexandra Vaczy[1], Edina Szabo[1], Evelin Patko[1], Balazs Meresz[1], Dora Reglodi[1], Tamas Atlasz[1,2,3]

1 Department of Anatomy, MTA-PTE PACAP Research Team, University of Pecs Medical School, 7624 Pecs, Hungary

2 Szentagothai Research Center, University of Pecs, 7624 Pecs, Hungary

3 Department of Sportbiology, Faculty of Sciences, University of Pecs, 7624 Pecs, Hungary

Introduction: Premature birth can be associated with a series of disorders affecting the quality of future life. One of these conditions is a neurovascular disease of the retina, which called retinopathy of prematurity (ROP). The oxygen-induced retinopathy (OIR) is a well-established model of ROP characterized by vessel abnormalities such as vasoobliteration and neovascularization. It is known that there are several factors which can result premature birth, such as smoking during pregnancy. Our aim of this study was to examine the vulnerabilities of maternal smoking on OIR with immunohistochemical and molecular biological methods.

Materials and Methods: Pigmented strain of laboratory mice (C57BL/6) were used in this experiment. During the pregnancy mice had to smoke two times a day for 30 minutes in a special chamber. To induce retinopathy pups were exposed to 75% oxygen +/- 2% from postnatal day (PD) 7-12 then returned to room air. On PD17, after anaesthesia, animals were decapitated, and the retinas were removed for histological and biochemical analysis. Isolectin GS-IB4 was used to label the endothelial cells of the retinas, then a computational tool was used for further quantitative analysis of the retinal vascular networks. VEGF, HIF1-α, iNOS, Erk, and pErk antibodies were detected and quantified by western blotting of pooled retinas distributed by treatment groups.

Results: Our computational analysis of retinal vasculature showed quantitative changes in several parameters (such as vessel density, branching index, total number of junctions) as well as in case of the protein expressions examined by western blotting. Two primary angiogenic factor, HIF-1α and VEGF showed elevated levels in the retinas exposed to nicotine during the smoking process compared to the samples affected by ROP alone.

Conclusion: Based on our results we showed that maternal smoking caused a greater degree of retinal damage in ROP, thus prevention and screening of this disease can be considered essential in preterm infant care.

Keywords: retinopathy, prematurity, smoking

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62. Mundrucz Laura

Anticonvulsant effect of the nonsteroidal anti-inflammatory drug meclofenamate *via* TRPM4 inhibition

Erzsébet Kövesdi[1], Laura Mundrucz[1], Attila Gyéresi[1], Máté Deák[1], Balázs Gaszner[2], Andy Pironet[3], Cecília Szekeres-Paraczky[4,5], Zsófia Maglóczky[4], Péter Gombás[6], Rudi Vennekens[3] Viktória Kormos[7] and Miklós Kecskés[1]

1 Institute of Physiology, Medical School, University of Pécs, H-7624, Pécs, Hungary
2 Department of Anatomy, Medical School and Research Group for Mood Disorders, Centre for Neuroscience, Szentágothai Research Centre, University of Pécs, H-7624, Pécs, Hungary.
3 Laboratory of Ion Channel Research, Biomedical Sciences Group, Department of Cellular and Molecular Medicine, VIB-KU Leuven Center for Brain & Disease Research, KU Leuven, 3000, Leuven, Belgium
4 Human Brain Research Laboratory, HUN-REN Institute of Experimental Medicine, H-1083 Budapest, Hungary
5 Szentágothai János Doctoral School of Neuroscience, Semmelweis University, 1085 Budapest, Hungary
6 Department of Pathology, St. Borbála Hospital, H-2800 Tatabánya, Hungary
7 Department of Pharmacology and Pharmacotherapy, Centre for Neuroscience, Medical School, University of Pécs, H-7624, Pécs, Hungary

Keywords: TRPM4, meclofenamate, epilepsy, mossy cell, hippocampus

Abstract

TRPM4 is a Ca2+-activated non-selective cation channel regulating diverse physiological functions of excitable cells. It has been shown previously that TRPM4 is present and functionally active in hilar mossy cells and modulates seizure susceptibility in the mouse model of temporal lobe epilepsy. Here we demonstrate that in vivo application of meclofenamate a novel antagonist of TRPM4 before the induction of status epilepticus reduces the frequency and duration of seizures in mice. Furthermore, we showed that mossy cell loss is reduced selectively in the ventral hippocampus upon meclofenamate treatment following status epilepticus. Interestingly, we have found higher expression of TRPM4 in ventral compared to dorsal mossy cells pointing to a dorso-ventral inhomogeneity of mossy cells. Moreover, using patch clamp recordings, we proved that meclofenamate modulates spontaneous activity and AP dynamics of MCs. Finally, we detected TRPM4 expression in human mossy cells as well. This data indicates that pharmacological blocking of TRPM4 may serve as an effective antiepileptic strategy with possible human translational relevance.

63. Nehr-Majoros, Andrea Kinga

Analgesic effects of cyclodextrin derivatives via modulation of Transient Receptor Potential Ankyrin 1 ion channel function

Andrea Kinga Nehr-Majoros[1], Noémi Bencze[1], Maja Payrits[1], Ágnes Kemény[2], György Sétáló Jr.[2], Zsuzsanna Helyes[1], Éva Szőke[1]
1 University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, Pécs
2 University of Pécs, Medical School, Department of Medical Biology and Central Electron Microscopic Laboratory, Pécs

The non-selective cation channel Transient Receptor Potential Ankyrin 1 (TRPA1) is highly expressed on nociceptive sensory nerve terminals and primary sensory neurons, where it is involved in pain integration and inflammation. TRPA1 activation is facilitated by cholesterol- and sphingolipid-rich lipid microdomains (lipid rafts) located in the membrane. Previous experiments have demonstrated that cyclodextrin (CD) derivatives forming a complex with cholesterol thus depleting it from membrane raft regions can reduce receptor activation, thereby exerting analgesic effect. Our aim is to further investigate the effect of these lipid-protein hydrophobic interactions on TRPA1 activation and to identify CD derivatives as analgesic and anti-inflammatory agents with novel mechanisms of action.

In our experiments, we compared three different CD derivatives selected on the basis of our previous results: random methylated β-cyclodextrin (RAMEB), (2-hydroxypropyl)-β-cyclodextrin (HPBCD) and sulfobutylether-β-cyclodextrin (SBECD). In vitro cholesterol depletion of CD derivatives was detected by fluorescence microscopy after Filipin III staining in Chinese hamster ovary cell line. We investigated the analgesic effect of CD pretreatment in the mouse model of formalin-induced acute inflammatory pain. Nocifensive behavior was measured in two phases: in the first phase (0-5 min) direct activation of free sensory nerve endings was observed, while in the second phase (20-45 min) pain evoked by the release of inflammatory mediators was observed. The cholesterol depleting effect of intraplantar CD treatment was measured by colorimetry of mouse plantar skin using Abcam Cholesterol Assay kit.

Filipin III fluorescence staining showed that 3 mM RAMEB, 10 mM HPBCD or 10 mM SBECD treatment significantly reduced the cholesterol content of the CHO cell membrane. CD pretreatment (3 mM RAMEB, 10 mM HPBCD and 10 mM SBECD) reduced the duration of nocifensive behavior during the second phase of formalin-induced acute inflammatory pain. Intraplantar CD derivative treatment reduced the total cholesterol content in the plantar skin of mice compared to the cholesterol content measured in control animals treated with physiological saline revealed by Abcam Cholesterol Assay kit.

According to our results we conclude that RAMEB, HPBCD and SBECD are able to deplete cholesterol from CHO cells applied in vitro and from the plantar skin of mice after intraplantar injection in vivo, also exerting analgesic effect in case of TRPA1-mediated acute pain. These results suggest, that the applied CD derivatives may be potential new compounds for peripheral analgesia with a novel mechanism of action.

64. *Nguyen, Eszter*

Effect of electrical microstimulation parameters on in vivo neuronal calcium responses in the visual cortex of anesthetized mice

Eszter Nguyen[1,2], Csaba Horváth[1,3], Melinda Rácz[1,3,4], Frederik Ceyssens[5,6], Maarten Schelles[5], Michael Kraft[5,6], István Ulbert[1,2], Lucia Wittner[1,2], Richárd Fiáth[1,2]

1 Integrative Neuroscience Group, Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary

2 Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary

3 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary

4 Selye János Doctoral College for Advanced Studies, Semmelweis University, Budapest, Hungary

5 Micro- and NanoSystems (MNS), Department of Electrical Engineering (ESAT), KU Leuven, Leuven, Belgium

6 Leuven Institute for Micro- and Nanoscale Integration (LIMNI), Leuven, Belgium

Sensory neuroprostheses aim to restore sight or hearing through electrical microstimulation via implanted neural interfaces. Although this field has made significant progress in recent years, there are still gaps in our understanding of how to precisely target and activate specific neurons or neuron populations by intracortical microstimulation. One possible solution to achieve more precise control over neuronal activity without increasing the number of electrodes could be the application of advanced stimulation patterns such as current steering and dynamic stimulation. In this study, we developed flexible three-shank probes containing twenty-one small iridium-oxide electrodes to assess the effects of advanced electrical microstimulation strategies on cortical activity obtained using in vivo two-photon calcium imaging. The fabricated probes were implanted into the visual cortex (V1) of Thy1-GCaMP6 transgenic mice anesthetized with ketamine/xylazine, and a custom-made high-channel-count neural stimulator device was used to generate electrical stimulation patterns. Here, we present preliminary results from the first experiments, where we used a two-photon laser scanning microscope (laser wavelength between 820-920 nm) to image the calcium activity in layer 2/3 of V1, adjacent to the implanted probes. Calcium imaging (raster scanning at 31 Hz)was performed through a 20x water immersion objective with a numerical aperture of 1, providing a field of view of 550 µm × 550 µm. In initial in vivo electrical stimulation experiments, we explored different stimulation parameters and observed diverse spatial and temporal activation patterns of neurons. The calcium imaging datasets we captured are utilized by multiple open source calcium analysis tools that have been recently developed. Presently, we are designing a processing and analysis pipeline that integrates suite2p and CalmAn. Following data processing steps, there is an opportunity to analyze the spatial and temporal activation patterns of neurons triggered by advanced electrical microstimulation. Our plans for the future involve exploring the influence of diverse advanced stimulation patterns on the activity of the visual cortex and identifying promising stimulation strategies that can improve the resolution of state-of-the-art visual cortical prostheses.

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65. Okore, Lavender Awino

The Role of Strategy Co-creation and Localization Practices in Sustainable Healthcare Investment Decisions

Lavender Awino Okore[1], Prof. Dr. Edina Molnár[2]
1 Institute of Management and Organization Sciences, Faculty of Economics and Business, University of Debrecen, 4032 Debrecen, Hungary.
2 Institute of Social Sciences, Faculty of Health Sciences, University of Debrecen, 4032 Debrecen, Hungary.

Sustainable healthcare investments is an imperative pursuit in developing country contexts as social problems become part of the evolving global concerns. Proactively deploying applied neuroscience at the nexus of contemporary localization techniques is a frontier that goes beyond the common conventional practices in the realm of Multiple Criteria Investment Decisions (MCID). The study investigates the moderating role of neuroleadership in facilitating a participatory planning process in African Healthcare Systems. We introduce the Multicriteria Decision Aid Approach (MCDAA) to support subjective investment decisions. Premised on the foregoing, the study builds on neuroeconomics theories and cognitive functions, to project critical data points at the grassroot level and triangulate it into an investment model. We leverage social decision making perspectives, cognitive biases and heuristics to build a co-created healthcare investments advocacy model that embeds localisation. Optimally, there exists an integrated approach across investments that will enable the attainment of development goals. While developed countries have systems that drive efficient investment practices and pursue health development objectives, developing countries are lagging. The insights show that there is little to no use of maximum personalization which calls for country landscaping, engagement and planning in service level strategy at the last mile. Notably, across CBOs, CSOs, NGOs and FBOs there is little to no structurally anchored strategic approach, despite the perceived and evidenced dependency. We observe that increasing local ownership of the healthcare investment advocacy agenda in developing could enhance the effectiveness and impact of the investments.

Keywords: Sustainable Healthcare Investments, Multiple Criteria Investment Decisions (MCID), Multicriteria Decision Aid Approach (MCDAA), Community Based Organizations (CBOs), Civil Society Organizations(CSOs), Non-governmental Organizations(NGOs), Faith Based Organizations(FBOs).

66. Orosz, Áron

The projection pattern of a vGlut3 neurons in the hippocampus

Áron Orosz[1,2], Boldizsár Balog[1,2], Albert Barth[1,2], Krisztián Zichó[1,2], Tamás Zsoldos[1,2], Gábor Nyiri[1,2]
1 Laboratory of Cerebral Cortex Research, Department of Cellular and Network Neurobiology, HUN-REN
2 Institute of Experimental Medicine, Budapest, Hungary

Learning and memory mechanisms are fundamental processes in the brain and represent the highest level of cortical functioning, but they require strong subcortical inputs, not all of which are known. In particular, from an evolutionary perspective, processing memories of negative experiences is one of the most important functions of the brain, yet there is no complete model of how fear memories are formed, refreshed and recalled. Several brain areas are involved in the processing of negative experiences, such as the lateral habenula, amygdala and hippocampus (HIPP). Previously, our group has shown that median raphe region (MRR) vGluT3 neurons specifically innervate HIPP stratum lacunosum-moleculare (SLM) interneurons (SLIN) via fast excitatory synapses while also targeting the gyrus dentate gyrus. In turn, MRR vGluT3 cells also receive input from MRR vGluT2 cells and from the medial prefrontal cortex, which is able to represent position and context and is involved in decision making and working memory. Thus, we hypothesized that MRR vGluT3 neurons process convergent inputs from higher order cortex to regulate the formation and recall of HIPP memory and temporal memory associations, including, for example, the formation of fear memory traces. For that we investigated the MRR vGluT3 neurons anatomical projection furthermore.

67. Padányi, Anna

Modulation and investigation of cortical excitability with non-invasive transcranial magnetic stimulation and electro-encephalography in awake non-human primates

Anna Padányi[1,2], Balázs Knakker[1], Evelin Kiefer[1], Szuhád Khalil[1], Antonietta Vitális-Kovács[1], István Hernádi[1,2,3]

1 Grastyán E. Translational Research Centre, University of Pécs,
2 Medical School,University of Pécs,
3 Faculty of Sciences, Institute of Biology, University of Pécs

Age-related neurocognitive disorders (NCD), beyond functional cognitive decline, are well-characterised by the disruption in the balance of excitatory and inhibitory (E/I) cortical networks. Thus, utilising non-invasive transcranial magnetic stimulation (TMS) and electroencephalography (EEG) methods which are similarly applicable in both patients and in translationally relevant preclinical animal models, we established baseline cortical excitability in rhesus macaques, followed by measurements under diazepam to intentionally modulate the E/I balance.

First, we trained the animals to perform a simple eye-fixation task, during which we recorded scalp-EEG from 27 electrodes by a telemetric amplifier system (min 20 sessions). In parallel, we measured individual motor thresholds (MT) by stimulating over the hand area of the primary motor cortex (assisted by neuronavigation) and quantifying the evoked motor responses using electromyography. MT – indicative of excitability threshold – was measured with 2.12% within-subject SD and 0.865 Intraclass Correlation Coefficient, suggesting a good reliability of MT measurements. To further characterise baseline cortical excitability, we recorded two consecutive input-output (I/O) curves with multiple stimulation intensities ranging from 50-150% of MT, semi-randomly ordered with 8 single-pulses at each intensity.

Then, we systemically introduced diazepam ($GABA_A$ PAM) – that is known to shift E/I balance – in 3 doses: 0.1, 0.3 and 1mg/kg. In scalp-EEG, a marked increase in low-frequency (alpha-beta) oscillatory power and a decrease in high-frequency (gamma) power with a strong frontal focus was observed indicating a shift of the E/I balance towards inhibition. In the TMS protocol, the first I/O curve (10 min post-administration) shifted to the right, showing a similarly pronounced decrease in excitability. Both the main effects of the stimulation levels ($F_{1,382}=84.12$, $p<0.001$) and the treatment ($F_{3,382}=28.00$, $p<0.001$) were statistically significant, with no interaction between the two factors ($F_{3,382}=1.11$, $p=0.344$).

In summary, combining scalp-EEG and single-pulse TMS offers a complementary and reliable preclinical research method for investigating cortical baseline excitability, as well as for detecting changes in E/I balance. Thus, the research provides the potential for deeper understanding of cortical excitability in a translationally relevant manner with the future goal of development of better treatment options in NCDs.

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68. Páli, Emese Kincső

Testing dual targeted, dopamine coupled nanoparticles on cell culture models of the blood-brain barrier

Emese Kincső Páli[1], Mária Mészáros[1], Gergő Porkoláb[1,2], Anikó Szecskó[1,2], Mária A. Deli[1], Szilvia Veszelka[1]

1 Biological Barriers Research Group, Institute of Biophysics, Biological Research Centre, Szeged, Hungary

2 Doctoral School of Biology, University of Szeged, Szeged, Hungary

The protective mechanisms of the BBB prevent most potential neuroprotective drugs from entering the brain, thus complicating the pharmacological treatment of central nervous system diseases. One of the promising strategies to solve this problem is the use of brain-targeted nanoparticles (NPs) as delivery into the CNS.

Previous results from the literature and our group suggest that ligands targeting transporters of endothelial cells coupled to NPs can enhance the delivery of NPs into endothelial cells. Last year we successfully targeted three-armed polyglutamic acid nanocarriers with the combination of alanine and glutathione and tested them on cell cultures, but these NPs were not coupled with any active compound. This year we coupled the alanine-glutathione targeted, fluorescently labeled, three-armed polypeptide nanocarriers with dopamine (3-PLG-A-GSH-R6G-DOPA) and tested on the cell cultured model of the BBB.

Our aim was to investigate the physicochemical properties, toxicity, cellular uptake and permeability of NPs on the model of the BBB and to test the delivery of these NPs into organoids derived from healthy and Parkinson's disease patient-specific stem cells.

The physicochemical properties of the particles were investigated by dynamic light scattering measurements. Real-time impedance measurements were performed to test the effects of NPs on the viability of endothelial cells. We investigated the cellular uptake of NPs into human brain endothelial cells (hECs) and the uptake mechanism by spectrofluorimetric measurement and by confocal laser microscopy. Furthermore, the penetration of the NPs across the BBB model and penetration into organoids was investigated.

The size of the NPs was ~500nm and particles have a negative surface charge. The NPs had no toxicity effect on the hEC during 24h. The targeted NPs showed time-dependent significantly higher cellular uptake compared to the control group. The treatment of free ligands inhibited the internalization of targeted NPs in cells. The dualtargeted NPs showed significantly higher permeability across the BBB compared to the non-targeted group. The targeting ligands also increased the penetration of NPs into PD organoids.

The alanine and glutathione proved to be an appropriate brain targeting combination and significantly increased the BBB crossing of dopamine coupled to polypeptide nanocarriers. Our results may contribute to the development of more efficient drug delivery systems to the CNS in the future.

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69. Párdi, Koppány

Improvement of blood-brain barrier properties by a combination of small molecules has a protective effect in a cell culture model of ischemic stroke

Koppány Párdi[1], Anikó Szecskó[1,2], Janet Adegbite[1], Gergő Porkoláb[1,2], Mária A. Deli[1], Szilvia Veszelka[1]

1 Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary

2 University of Szeged, Szeged, Hungary

During an ischemic event, the blood flow interruption and the subsequent oxygen-glucose deprivation (OGD) cause blood-brain barrier (BBB) disruption and neuronal death, which may have life-threatening consequences. Protection of the BBB as a therapeutic target is a novel concept in medicinal strategies. Our aim was to target three different signalling pathways of BBB by small molecule combinations, improving barrier functions and increasing the expression of Krüppel-like factor 2 (KLF2), the main transcriptional regulator of the blood flow response. We cultured human endothelial cells under three different conditions: normoxia, OGD, and reperfusion after OGD (OGD/R) and then tested the effect of our small molecule combination against BBB dysfunctions. After OGD, the viability of cells was significantly decreased measured by impedance-based manner, but the treatment of small molecule combinations increased the cell index even higher than that of in normoxia. Both OGD and OGD/R condition resulted in reduced transendothelial electrical resistance of the cell layers compared to normoxia, suggesting that paracellular barrier is less tight, but this was significantly elevated by small molecule treatment. Penetration of both the paracellular BBB marker, fluorescein, and the transcellular marker, albumin elevated after OGD/R suggesting increased permeability of the BBB model, but treatment with a combination of small molecules was able to protect this adverse effect. Finally, we investigated whether the protective effect of small molecule inducers against OGD/R is mediated through an increase in KLF2 expression. Against our expectations, immunocytochemical staining showed that OGD/R increased the KLF2 levels compared to normoxia, followed by a decrease after treatment with small molecule combination. Since the cellular mechanism underlying the protective effect was not linked to KLF2, the most important BBB tight junction protein, claudin-5 was investigated. Immunocytochemical staining of claudin-5 was dramatically altered after OGD/R but staining was similar to normoxia after treatment with small molecule BBB inducers. Our results suggested that tightening the BBB by increasing claudin-5 levels through a combination of our small molecule BBB inducers may be protective in ischemic stroke.

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70. Pejtsik, Diana

A new approach for revealing trait anxiety and its molecular predictors in rodent models

Diana Pejtsik[1,3], Zoltan K Varga[1], Mate Toth[1], Zoltan Balogh[1], Mano Aliczki[1], Laszlo Szente[1,3], Gyula Y Balla[1], Levente Kontra[2], Zsofia Eckert[1], Eva Mikics[1]

1 Institute of Experimental Medicine, Laboratory of Translational Behavioural Neuroscience

2 Institute of Experimental Medicine, Bioinformatics Unit

3 Semmelweis University, Janos Szentagothai Doctoral School of Neuroscience

The reliability and validity of preclinical anxiety testing is essential for translating animal research into clinical use. However, the most commonly used anxiety tests lack inter-test correlations and have repeatability issues that need to be clarified. Translational animal research should be able to capture stable individual traits to aid the development of personalised medicine. However, with the current approaches that typically involve using one type of test one time, it is only possible to measure transient states of animals that are heavily influenced by the experimental conditions. Here, we propose a validated, optimised test battery which can reliably capture trait anxiety in rats and mice of both sexes. Instead of developing novel tests, we combined widely-used tests (elevated plus-maze, open field and light-dark test) to understand their repeatability issues and low inter-test correlations, and provide instantly applicable adjustments for better predictive validity. We repeated these tests three times to capture multiple anxious states, which we combined together to generate summary measures (SuMs). Using correlations and machine learning, we found that our approach resolves between-test correlation issues of anxiety tests and provides better predictions for subsequent outcomes under anxiogenic conditions or fear conditioning. Moreover, SuMs were more sensitive to detect anxiety differences in an etiological model of social isolation. Finally, we tested our sampling method's efficiency in discovering anxiety-related molecular pathways through RNA sequencing of the medial prefrontal cortex. We identified four times more molecular correlates of anxiety using SuMs, which pointed out functional gene clusters that had not emerged in association with single testing. Furthermore, we also found that 50% of the most robust molecular findings were also found to be correlated with anxiety in the amygdala. Overall, temporally stable SuMs are necessary to capture trait anxiety in rodents, providing better predictions for potential therapeutic targets.

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71. *Peña Torres, Giancarlo*

Effects of Mambalgin-1 Acid-Sensing Ion Channel 1a Blocker on the Retinal Ganglion Cell Activity

Giancarlo Peña Torres[1,1,2], Gergely Szarka[1,2,3,4], Béla Völgyi[1,2,3,4]
1 Szentágothai Research Centre, University of Pécs, Pécs, Hungary
2 Department of Neurobiology, University of Pécs, Pécs, Hungary
3 Center for Neuroscience, University of Pécs, Pécs, Hungary
4 NEURON-066 Rethealthsi Research Group, Pécs, Hungary

Acid-sensing ion channels (ASICs) were identified to function as molecular detectors for acidity since their activity was first measured in 1980 by Krishtal & Pidoplichko in trigeminal ganglia and dorsal root ganglion cells. These cation channels were then found to appear in neurons across the entire body (Waldmann et al., 1997; Xu & Wu, 2021). ASICs play a significant role in various physiological processes, including retinal function (Ettaiche et al., 2006; Tan et al., 2011). While previous studies have showed their effect regarding the detection and signaling of pH change in ischemia and hypoxia scenarios, utilizing tarantula venom peptide (PcTx1) (Sherwood et al., 2009), the actual role they might play in image formation as a circuitry element is yet to be determined. We study the role of ASIC1a in mouse retinal ganglion cells (RGCs) by using a specific peptide inhibitor, mambalgin-1, isolated from black mamba snake venom, to further understand how ASIC1a channels are involved in signal transduction and cellular activity. We monitored the spontaneous and light-evoked spike activity of the RGCs. We performed Ca^{2+}- imaging experiments and extracellular multi electrode array (MEA) recordings, measuring spontaneous activity and stimulating with a complex light stimulus paradigm to see cell subtype specific effects. After the administration of the toxin, light response kinetics were altered in a subtype specific manner, with decreased spontaneous activity.

72. Péntek, Loretta

Physiological changes in the mouse retina induced by traumatic brain injuries

Loretta Péntek[1,2], Bálint Fazekas[5], Tamás Kovács-Öller[2,3,4]
1 Szentágothai Research Centre, University of Pécs, Pécs, Hungary
2 Department of Neurobiology, University of Pécs, Pécs, Hungary
3 Center for Neuroscience, University of Pécs, Pécs, Hungary
4 NEURON-066 Rethealthsi Research Group, Pécs, Hungary
5 Department of Neurosurgery, University of Pecs, H-7623 Pecs, Hungary

Traumatic brain injuries (TBI) are known to initiate complex neuroinflammatory processes, but their effects on the retina are not always fully explored. In my experiment, I examine retinal ganglion cells and microglia, which are key components of the retina's function and immune response. The methods I use are Ca-imaging and immunohistochemical staining (IHC). After inducing TBI, using the Ca-imaging technique, we can find out what functional changes take place in the retinal ganglion cells over time, and we can examine the structure and number of microglia with IHC. My goal is to find out if TBI affects ganglion cell firing 24 and 48 hours after injury and if the frequency of prolonged calcium events increases. Also, whether there is a change in the morphology of the microglia, are they activated. My results suggest that TBI changes the Ca signal transmission of ganglion cells and, in parallel, microglial activation patterns also showed a dynamic response, which indicates inflammatory processes in the retina after TBI. Thus, the effects of TBI extend beyond the brain domain, affecting retinal ganglion cells and microglial responses. Future studies may delve deeper into the long-term consequences of TBI on retinal function and explore potential therapeutic interventions.

73. Pham, Daniel

PAC1 receptor colocalization with Ca^{2+}-binding proteins and cochlea-efferent markers in the auditory pathway of PACAP knockout and wild-type mice

Daniel Pham[1], Balazs Daniel Fulop[1], Gergo Gyurok[1], Balazs Gaszner[1], Dora Reglodi[1], Andrea Tamas[1]

1 University of Pécs, Medical School, Department of Anatomy, ELKH-PTE PACAP Research Team

Introduction: The neuroprotective and cytoprotective effects of PACAP are demonstrated in several previous examinations. Recently, in PACAP KO mice, we showed elevated hearing tresholds along with higher apoptosis rate and increased synthesis of Ca2+-binding proteins (parvalbumin, calretinin) of hair cells in the organ of Corti.

Methods: In this present study, we examined the role of PACAP in the auditory pathway of 1.5, 4, and 8-month-old mice. The synthesis of Ca2+-binding proteins and of PAC1 receptor were visualized with calretinin-parvalbumin-PAC1 receptor immunostaining in the cochlear nuclei of PACAP KO and WT mice. Choline acetyltransferase (ChAT)-tyrosine hydroxylase (TH)-PAC1 receptor triple immunostaining was performed in the nuclei of the superior olivary complex participating in cochlear efferentation.

Results: PAC1 receptor showed colocalization with parvalbumin and calretinin positive cells in the ventral cochlear nucleus. The number of parvalbumin positive cells significantly increased with the age in both genotype, however, the number of PAC1 receptor containing parvalbumin positive cell had a less pronounced increase. In the dorsal cochlear nucleus we also found a similar, but less pronounced elevation in the KO animals. In young animals, PAC1 receptor was colocalized more with parvalbumin positive cells than with calretinin positive cells in the dorsal cochlear nucleus in both genotypes. In the superior olivary complex, PAC1 receptor was detected in the third of ChAT and TH positive cells. We did not find significant differences between the age groups and the genotypes.

Conclusions: The age-related increase of parvalbumin in the auditory pathway is known as a compensatory mechanism. Based on our experiment, this elevation is less marked in the cells of the ventral cochlear nucleus which also synthesize PAC1 receptor. Higher PAC1 receptor association with parvalbumin cells in the dorsal cochlear nucleus could show that PACAP does not affect all cells similarly in this nucleus. PAC1 receptor colocalize with ChAT and TH positive neurons - which take part in the efferent innervation of cochlea - in both genotype. Our experiments prove that PACAP plays a role in the auditory system not only in the cochlea, but also in other parts of the auditory pathway.

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74. Plesz, Szonja Bianka

Exploring the gut-brain connection: How does microbiome composition relate to cognitive behavior in Wisket model rats?

Szonja Bianka Plesz[1], Leatitia Gabriella Adlan[1], Alexandra Büki[1], Gyöngyi Horváth[1], Balázs Ligeti[2], Bence Ágg[3,4], Zoltán S. Zádori[5], Gabriella Kékesi[1]
1 Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
2 Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary
3 Cardiometabolic and HUN-REN-SU System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
4 Pharmahungary Group, Szeged, Hungary
5 Department of Pharmacology and Pharmacotherapy,
Semmelweis University, Budapest, Hungary

Increasing evidence suggests a bidirectional relationship between the gut microbiome and the central nervous system, thus dysbiosis may also play a key role in the etiology of neurodevelopmental disorders, including schizophrenia. Studies revealed that dysbiosis may also affect cognitive performance and memory, impaired in schizophrenia. The rats of the triple-hit Wisket model (repeated ketamine treatment; post-weaning social isolation; selective breeding) show various schizophrenia-like behavioral phenotypes, such as decreased locomotor activity and cognitive deficit. Thus, we aimed to determine the correlation between gut microbiome composition and cognitive behavior in Wisket model rats.

Three-month-old male and female, control (n=8) and Wisket (n=10) rats were involved in the study. The food-rewarded Ambitus test was used to assess the animal's behavior. The Ambitus apparatus is a rectangular corridor system that includes side boxes with food rewards. Rats performed two sessions per day three hours apart. In session 1, all inside and outside boxes were baited (16 rewards), whereas in session 2, only the inside boxes were baited (8 rewards). Based on the number of collected rewards and completion time the motivation index was calculated, which predicts the cognitive performance. After behavioral testing, fecal samples were collected to verify the microbiome composition by using deep sequencing of bacterial 16S rRNA. Behavioral changes and fecal microbiome composition were correlated by multiple regression analysis.

A motivational deficit was indicated in the Wisket group by the significantly lower motivation index bygroup ($F(1,16)=7.95$; $p<0.05$), by session ($F(1,16)=52.60$; $p<0.001$), and group and session interaction ($F(1,16)=5.86$; $p<0.05$). The relative abundance of the families *Bifidobacteriaceae, Clostridiaceae*, *Erysipelotrichaceae*, *Lachnospiraceae*, *Lactobacillaceae, Oscillospiraceae,* and *Sutterellaceae* positively; while that of the families *Acidaminococcaceae*, *Eubacteriaceae,* and *Prevotellaceae* negatively correlated with the motivation index.

The present results suggest that targeting the microbiome may provide a promising opportunity for the development of new therapies to alleviate or prevent schizophrenia-related cognitive symptoms.

75. Puskás, Júlia

Exploring excitability changes in ex vivo and in vitro prefrontal cortical networks in a rodent model of autism

Júlia Puskás[1], Viktor Kelemen[1], Zsuzsanna Faragó[1], Ildikó Világi[1], Schlett Katalin[1], Attila Szűcs[1], Krisztián Tárnok[1], Petra Varró[1]

1 Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Individuals with ASD experience social interaction and communication skill deficits, as well as repetitive behaviours. These impairments are related to modifications in neural network connectivity and excitability in various brain areas, including the prefrontal cortex. which is as a "social hub," responsible for interpreting others' emotions and evaluating social situations. Neuronal alterations behind idiopathic ASD can be investigated in rodents using the valproate (VPA) model. In this model, a single dose of VPA is administered during pregnancy to induce ASD-related alterations in offspring. In this study, we focused to investigate established alterations in prefrontal cortical networks in brain slices from VPA-treated adult rats.

During the experiments, Valproic acid was administered to rats dams on the 12.5th day of pregnancy. The treatment resulted in substantial postnatal development delays of pups and impaired their social behaviour, suggesting that VPA has led to cellular and/or network-related alterations.

Field potential studies were conducted on acute prefrontal slices to characterise the network activity of both young and adult rodents of both sexes. Our findings indicate a significant increase in excitability among the male subjects in the treatment group. While underlying factors remain unclear, this finding suggests a potential alteration in network-level activity. Further exploration of cellular activity and network development is warranted.

To investigate alterations in prefrontal cortical cells and networks during early development, which result in a modified state in adulthood, we aim to develop an organotypic slice culture model. Organotypic prefrontal slices from 6-day-old mice were prepared for experimentation. We can analyse active and passive membrane properties of neurons, as well as spontaneous network activity, using whole-cell patch-clamping. Recordings of slices at different ages in vitro demonstrate parallel development with the behaviour of acute slices from young animals.

Thus, the slice culture exhibits expected changes related to maturation. These preliminary results indicate that the model is suitable for characterising the VPA-induced ASD-related changes during early development stages.

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76. Rahmoun, Hanaa

Contribution of the Cx36-type neuronal gap junctions and the HCN4 channel to induce spontaneous Ca2+-waves in the retina

Hanaa Rahmoun[1], Gergely Szarka[1,2], Béla Völgyi[1,2], Tamás Kovács-Öller[1,2]
1 University of Pécs, Faculty of Sciences, Pécs, Hungary
2 University of pecs, Szentágothai Research Centre, Pécs, Hungary

Patients taking Ivabradine, a heart medication, often experience sensations of enhanced brightness in their visual field (PMC2189731). This side effect of the treatment was prevalent contrary to Ivabradine's impermeability through the blood-brain barrier. Whether this is due to collateral effects on neurons of the retina is still debatable. Active components of Ivabradine block ionic currents mediated by HCN ion channels (Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel), also called as pacemaker channels due to their essential role in generating rhythmic activity within groups of heart or brain cells. Since all four HCN channel isoforms (HCN1-4) are expressed in the mouse retina, here we investigated this issue in the in vitro retina preparation of the mouse. Retinal bipolar cells have a type-specific inventory of various HCN channels that are highly concentrated at their synaptic terminals. By using Ca++-imaging methods we monitored the spontaneous activity of retinal ganglion cells (RGCs), the postsynaptic partners of HCN-expressing bipolar cells. We found that spontaneous RGC activity significantly decreased for most recorded cells when Ivabradine (in an equal concentration of 10 mM) was administered. This Ivabradine effect was reversible and following washing the spontaneous RGC activity was reverted to near control levels. This Ivabradine effect was also confirmed in a specific retinal microcircuit where the spontaneous activity of the transient OFF alpha RGCs (t-OFF alpha RGC), the postsynaptic partner of the HCN4 expressing type 3 OFF-cone bipolar cell, was also diminished by the same pharmacological treatment. The above data, in theory, may underlie the clinically observed effects of Ivabradine and thus argue against its impermeability through the blood-retina barrier.

77. *Rákóczi, Bettina*

Investigating the role of HSPB1 in chronic neuroinflammation in a mouse model of Alzheimer's disease

Bettina Rákóczi[1,2], Zsófia Ruppert[1,2], László Vígh[1] Zsolt Török[1], Melinda E. Tóth[1]
1 Laboratory of Molecular Stress biology, HUN-REN Biological Research Centre, Szeged;
2 Doctoral School in Biology, University of Szeged, Szeged, Hungary

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, characterized by the accumulation of amyloid-beta (Aβ) plaques in the brain. According to the literature microglia and astrocyte cells may help to remove these aggregated proteins through phagocytosis. However, over activation of the glial cells together with chronic inflammation can cause further tissue damage. In addition to activated glial cells, heat shock proteins (HSPs) are also observed around the Aß plaques. The primary role of these chaperons is to maintain the protein homeostasis, however, several evidence suggest that they have additional functions, such as regulating inflammation. Previously our group observed that overexpression of a small HSP, the HSPB1 improved the symptoms of AD in mice, though the mechanism behind these processes is not clear. Therefore, the aim of this study was to investigate the role of HSPB1 in chronic neuroinflammation and its effect on glia activation in a mouse model of AD.

As a model of AD, we used the APP/PS1 mouse strain, which was crossed with an HSPB1 overexpressing line to study the effects of the chaperone. AD model mice show a higher susceptibility to premature mortality due to a high incidence of seizures. However, overexpression of HSPB1 in APP/PS1 females remarkably reduced the mortality rate. This is in line with our previous results suggesting that HSPB1 can ameliorate the increased neuronal excitability in APP/PS1 mice. Moreover, immunofluorescent staining of frozen brain sections confirmed the accumulation of transgenic HSPB1 and the presence of activated glia cells around the Aß plaques. Neuroinflammation and glia activation in the hippocampus were indicated by qPCR as well. In the APP/PS1 group the expression levels of IL-1ß, IL-6, TNFα, astrocyte and microglia marker genes were significantly higher than those in the healthy wild-types. However, HSPB1 overexpression led to an increase in the level of M2 anti-inflammatory microglia marker genes in the hippocampi of both male and female APP/PS1 mice.

Our results confirm that HSPB1 may have protective effects against the symptoms of Alzheimer's disease, which was demonstrated by the lower mortality rate observed in the APP/PS1/HSPB1 mice. Moreover, overexpression of HSPB1 may influence the activation of the microglia cells promoting tissue repair.

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78. Riszt, Rafaella Mínea

Application of the self-ordered spatial search paradigm for the investigation of primate working memory

Rafaella Mínea Riszt[1,2], Balázs Knakker[1], Antonietta Vitális-Kovács[1], Judit Inkeller[1], Anna Padányi[1,2], Evelin Kiefer[1], István Hernádi[1,2]
1 Grastyán E. Translational Research Centre, University of Pécs
2 Medical School, University of Pécs

The self-ordered spatial search (SOSS) task is a non-verbal touchscreen paradigm probing visual spatial working memory (SWM) that can be applied in non-human primate research in a preclinical translational experimental setting. We examined the performance of the animals in the task on control baseline days and after a transient cognitive impairment induced by the muscarinic acetylcholine-receptor antagonist scopolamine.

Fourteen adult male rhesus monkeys performed the SOSS task in 1-hour sessions with 300-500 trials each. In every trial the animals were shown 4-8 identical cyan colored squares on a touchscreen, and they had to touch each square in an arbitrary order once, and only once. Each touch was followed by a 0.5-2s long delay period after which all squares reappeared at the same location. Besides accuracy, we analysed continuous (a stimulus is touched twice in a row) and recurrent (a stimulus is touched again, but not directly after the previous touch) perseverative error rates (CPE and RPE, respectively). This distinction is important because while CPEs can be caused by simple motor perseveration, RPEs are more likely to involve failures of memory processes. The distribution and ratio of the errors throughout the trials can also give us information about the animals' task solving strategies and limitations of memory capacity.

Increasing the set size resulted in selectively increased proportion of the RPEs that presumes higher levels of SWM involvement. Even though scopolamine treatment dose-dependently deteriorated performance, it was not selective to any of the errors. The close-to-zero amount of CPEs in all choices and the higher RPE rate in the later phase of the choice sequence suggest an "n-back" capacity of working memory in this task.

Our present version of the SOSS task design involving different set sizes appears to be a sensitive assessment of behavioural correlates of SWM processes. The observed transient amnestic effects of muscarinic antagonist agent scopolamine proved the applicability of the SOSS task for preclinical cognitive research. Associating the observed effects to specific working memory processes and further optimizing the SOSS task will make it suitable for efficacy assessment of novel cognitive enhancer pharmaceutical drug candidates.

79. Ruppert, Zsófia

Fructose supplementation exacerbates systemic inflammation induced by high-fat diet and causes brain shrinkage in mice

Zsófia Ruppert[1,2], Patrícia Neuperger[2,3], Bettina Rákóczi[1,2], Nikolett Gémes[2,3], Brigitta Dukay[1], Petra Hajdu[1], Péter Mária[1], Gábor Balogh[1], László Tiszlavicz[4], László Vígh[1], Zsolt Török[1], László Puskás[3], Gábor J. Szebeni[3,5,6], Melinda E. Tóth[1]

1 Laboratory of Molecular Stress Biology, Institute of Biochemistry, HUN-REN Biological Research Centre, Temesvári krt. 62., Szeged H-6726, Hungary

2 PhD School in Biology, University of Szeged, Közép fasor 52., Szeged H-6726, Hungary

3 Laboratory of Functional Genomics, HUN-REN Biological Research Centre, Temesvári krt. 62., Szeged H-6726, Hungary

4 Department of Pathology, Albert Szent-Györgyi Medical School, University of Szeged, Állomás utca 1., Szeged H-6720, Hungary

5 Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Közép fasor 52., Szeged H-6726, Hungary

6 CS-Smartlab Devices Ltd., Ady E. u. 14., Kozármisleny H-7761, Hungary

Obesity is associated with a systemic low-grade chronic inflammation, caused by the increasing amount of adipokines and cytokines secreted by the adipose tissue. These molecules can disrupt and cross the blood-brain barrier, leading to neuroinflammation in different brain regions, including the hippocampus. As these processes are not yet fully understood, we aimed to study obesity-related systemic inflammation and local changes in the brain, using two diet-induced obesity models of different severity.

3-month-old, male C57BL/6 mice were divided into 3 groups (n=15/group). The control group got a normal diet (ND). Obesity model groups were fed with high-fat diet (HFD) or HFD supplemented with 30% fructose solution (HFD+F) for 5 months. Glia activation was determined by Iba1 and GFAP immunostaining, while DCX+ neurons were counted to detect the hippocampal neurogenesis. Relative gene expression changes of visceral white adipose tissue (vWAT) and whole brain were analyzed by RT-PCR, while serum inflammatory cytokines were measured using Multiplex Immunoassay.

Body weight was increased by the HFD alone, although F supplementation caused further weight gain. In parallel, RT-PCR analysis revealed that the higher bodyweight was associated with significantly elevated expression levels of pro- (TNFa) and anti-inflammatory (Il10; TGFb) cytokines in the HFD+F group compared to ND and HFD animals. This was also confirmed by immunoassay, as the serum TNFα concentration showed an elevated level only in the HFD+F group. Moreover, gene expression levels of microglial activation markers and heat-shock genes showed a slight increase in the brains of HFD+F animals. However, no glial activation was detected in any brain regions of the obesity models. Interestingly we found, that the HFD+F-consuming group had a lower brain weight than the ND, which was accompanied by an increased number of DCX+ cells in the hippocampus.

Overall, F supplementation exacerbated the HFD-induced systemic inflammation as indicated by the higher body weight, the elevated cytokine expression in the WAT, and the increasedserum TNFα level. On the other hand, neuroinflammation was not pronounced in any of the obesity models. However, the reduced brain weight may indicate brain shrinkage related to obesity and possible compensatory neurogenesis in the HFD+F-fed animals.

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80. *Sandle, Joanna*

Group I metabotropic glutamate receptor-mediated modulation of mono- and disynaptic transmission in the human neocortex

Joanna Sandle[1], Gábor Molnár[1], Martin Tóth[1], Katalin Ágnes Kocsis[1], Pál Barzó[2], Karri Lamsa[1,3], Gábor Tamás[1]

1 University of Szeged, Department of Physiology, Anatomy and Neuroscience
2 University of Szeged, Department of Neurosurgery
3 Hungarian Centre of Excellence for Molecular Medicine Research Group for Human neuron physiology and therapy

Activation of group I metabotropic glutamate receptors (mGluR) in the brain mediates changes in neuronal excitability, synaptic transmission, and network activity of cortical circuits and has been linked to learning-related plasticity, brain state-modulation as well as various human neurological disorders but studies investigating their effect in human brain are scarce and mostly focused on excitatory neurons.

We studied the effects of group I mGluR activation on monosynaptic glutamatergic synapses arriving onto interneurons and disynaptic GABAergic synapses using dual whole-cell patch clamp recordings in layer 2/3 of human neocortex. We found that activation of group I mGluRs by the agonist DHPG modulated interneurons in a subtype-dependent manner. We observed depression of excitatory synaptic transmission strength primarily in non fast-spiking, adaptive firing interneurons whereas most fast-spiking basket cells and axo-axonic cells exhibited potentiation of their synaptic excitatory input by the agonist. Disynaptic inhibitory connections showed an increase in diIPSP/C amplitudes, while diIPSP/C failure rates and latencies remained unaltered. Parallel experiments in Wistar rats showed DHPG-mediated strengthening of glutamatergic input to fast-spiking basket cells.

Our results demonstrate the diverse synaptic strength modulation of Group I mGluRs both in glutamatergic synapses arriving onto interneurons and GABArgic synapses originating from them suggesting a cell type-specific mechanisms of action in the human neocortex.

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81. *Sebők, Hunor*

An alternative cholinergic innervation of the hippocampus

Hunor Sebők[1], Boldizsár Balog[1], Virág Takács[1], Márton I. Mayer[1], Dr. Gábor Nyiri[1]
1 Institute of Experimental Medicine, Budapest, Laboratory of Cerebral Cortex Research

Cortical areas, such as the memory encoding hippocampus, are highly regulated by subcortical pathways, many of those originating from the basal forebrain. Cholinergic cells of the medial septum (MS) and the horizontal diagonal band (HDB) play a vital role in attention and memory formation. While the cholinergic innervation of the hippocampus by the MS is well established, we discovered an additional cholinergic pathway from the HDB. Using tracing techniques combined with immunohistochemistry and electron microscopy, we found that HDB cholinergic cells target distinct hippocampal layers than the MS and target primarily the hilar mossy cells of the dentate gyrus. Additionally, preliminary results of our chemogenetic behavioral experiments suggest that HDB cholinergic cells can drive hippocampal novelty detection and memory formation. Our results can provide new insights into the cholinergic involvement in hippocampal processes and neurodegenerative diseases.

82. Sere, Péter

Is thalamus involved in seizure generalization?

Péter Sere[1], Blanka Kozma[1], Anna Horváth-Zalatnai[1], Ágnes Schnell[1], Péter Barthó[1]
1 Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Magyar Tudósok körútja 2, Budapest 1117, Hungary

Seizures often originate in an epileptic focus, from where they spread to large areas of the brain. The potential pathways of generalization are of high importance as potential targets of therapeutic intervention. Here, we are testing the hypothesis that seizures generalize from a cortical epileptic focus via thalamic pathways, rather than via direct cortico-cortical connections. Higher-order thalamic nuclei are especially likely to be involved, as they project to large areas of the cortex.

We employed an optogenetic acute epilepsy mouse model, where brief (10-15s) stimulation of the layer 6 corticothalamic pathway evoked grand-mal seizure with high probability and repeatability. Animals were traced through several months, with seizures induced daily.

Thalamic nuclei (primary and higher-order somatosensory vental posterior and posterior) were inhibited by microinjections of tetrodotoxin, or kainate lesion. So far, neither tetrodotoxin inhibition or kainate lesion of either somatosensory nuclei prevented reliably the generalization of seizures.

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83. Simon, Dávid Vince

Unravelling the role of hemokinin-1 in age-related deterioration of motor coordination and muscle strength

Dávid Vince Simon[1,2,3], Eszter Kepe[1,2,3], Zsuzsanna Helyes[1,2,3], Éva Borbély[1,2,3]
1 Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs
2PharmInVivo Ltd.
3 National Laboratory for Drug Research and Development, Magyar tudósok krt. 2. H-1117 Budapest, Hungary

Musculoskeletal problems and consequent fractures significantly reduce the quality of life in old age. A group of neuropeptides, tachykinins, have regulatory functions in the central and peripheral nervous system, and this group includes haemokinin-1 (HK-1). It is present in high concentrations in the cerebellum and reproductive organs and can be detected in bone and muscle. We investigated its role in locomotor coordination and muscle function and we were also looking for possible sex differences in 3-4, 12 and 18 month old C57BL/6 wild type and HK-1 deficient (Tac4KO) male and female mice.

In the static rod test, which is used to investigate locomotor coordination, mice are placed on the ends of rods of different thicknesses and the time it takes them to turn and reach the end of the rod is measured. In the grid test, the mice have to cling upside down on a metal grid. In the horizontal bar test, the animals have to grip the bars with their forelegs and climb out to the edge of the bar. These last two tests measure muscle strength.

No difference was found between the wild and gene-deficient groups in the young animals. In the ageing animals (12 and 18 months), both males and females, a significant deterioration in locomotor coordination was observed in the static bar test, which was significantly more severe in 12-month-old male Tac4 gene-deficient animals compared to respective wild types, but the opposite effect of gene deficiency was observed in 18-month-old females. A significant decline in muscle strength was also detected in older wild-type animals in both tests. In the grid test, the loss of muscle strength was significantly smaller in females compared to males, a phenomenon also observed in the horizontal bar test.

Our results suggest that HK-1 may play a complex regulatory role in locomotor coordination in old age, where important sex differences can be observed. However, HK-1 do not affect muscle strength. Therefore, elucidating the mechanism of action of HK-1 and its interactions with sex hormones may be important for drug developmental purposes.

84. Somogyi, Fanni

Screening AAV delivery routes, capsids and promoters for cortex-wide functional and long-term stable access to brain function in large animal species

Fanni Somogyi[1,3], Beatrix Kovács[1,3], Klaudia Csikós[1,3], Ábel Petik[1,3], Domonkos Horváth[1,2], Eszter Juhász[1], Réka Bod[1], Attila B. Dobos[1], Lucia Wittner[1,5], Dániel Hillier[1,2,4]

1 Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary

2 Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary

3 Szentágothai János Neurosciences Division, Semmelweis University Doctoral School, Budapest, Hungary

4 Institute for Molecular and Clinical Ophthalmology Basel, Basel, Switzerland

5 Neurology and Neurosurgery, Institute of Mental Health, Budapest, Hungary

In neuroscience, a central question revolves around understanding how signals relevant to behavior and cognition are processed within neural circuits, engaging thousands of neurons across multiple brain regions. Despite over a century of research, our current comprehension of brain-wide neural circuits enabling various brain functions in large-animal species (e.g., cats, primates) remains limited. In stark contrast, significant progress has been made in dissecting mouse brain function, enabled by the availability of transgenic driver and reporter lines.

We set out to emulate the quality and reproducibility of transgenic mouse reporter lines in large-animal models by establishing a long-term stable gene delivery method that achieves functional protein levels. Results: We quantified transduction efficiency upon screening a set of constructs in the cat brain. We identified one construct that yields brain-wide labeling upon a single injection.

Conclusion: We provide a precise, highly reproducible brain-wide transduction method that has not been available up to now, one that approximates the qualities attributed to transgenic reporter mouse lines. Using this method, genetically targeted dissection of both local and brain-wide functional circuits may gain broad application in large-animal models.

85. *Sparks, Jason*

PACAP contributes to the maintenance of endotoxin fever through the regulation of pyrogenic cytokines and cyclooxygenase-2

Jason Sparks[1], Nora Furedi[1], Kata Fekete[2], Margit Solymar[2], Katalin Ordog[3], Laszlo Deres[3,4], Krisztina Pohoczky[5], Agnes Kemeny[5,6], Dora Reglodi[1], Eszter Pakai[2], Andras Garami[2]

1 ELKH-PTE PACAP Research group, Department of Anatomy, Medical School, University of Pecs, Pecs, Hungary

2 Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pecs, Pecs, Hungary

3 1st Department of Medicine, Medical School, and Szentagothai Research Centre, University of Pecs, Pecs, Hungary

4 HAS-UP Nuclear-Mitochondrial Interactions Research Group, Budapest, Hungary

5 Department of Pharmacology and Pharmacotherapy, Medical School, University of Pecs, Pecs, Hungary

6 Department of Medical Biology, Medical School, University of Pecs, Hungary

Introduction and Aim: Pituitary adenylate cyclase-activating polipeptide (PACAP) signaling is involved in various inflammatory processes. A common manifestation of systemic inflammation is fever, which is usually induced in animal models with the administration of bacterial lipopolysaccharide (LPS). A role for PACAP signaling was suggested in LPS-induced fever, but the underlying mechanisms of how PACAP contributes to febrile response have remained unclarified.

Methods: We administered LPS (120 µg/kg, intraperitoneally) to mice with the Pacap gene, i.e., the gene encoding the PACAP protein, either present (Pacap+/+) (n=15) or absent (Pacap−/−) (n=14) and measured their thermoregulatory responses, serum cytokine levels, and tissue cyclooxygenase-2 (COX-2) expression.

Results: We found that the LPS-induced febrile response was attenuated in Pacap−/− mice compared to their Pacap+/+ littermates starting from ~120 min postinfusion. Administration of LPS resulted in amplification of COX-2 mRNA expression in the lungs, liver, and brain of the mice in both genotypes at 210 min postinfusion. In the LPS-treated groups, the upregulation of the COX-2 mRNA in Pacap−/− mice was significantly attenuated in the liver, whereas it was augmented in the lungs and the brain compared to Pacap+/+ mice. Serum concentration of the pyrogenic cytokines interleukin (IL)-1α and β were significantly increased in Pacap+/+ mice in response to LPS compared with saline, whereas the change was not significant between the treatment groups in Pacap−/− mice. In case of IL-1α and β, the intergenotype difference between the LPS-treated groups was also significant. The serum concentrations of IL-6, IL-10, and TNFα were higher in LPS-treated than in saline-treated mice of both genotypes, however, the rise in IL-10 was significantly attenuated in Pacap−/− mice compared to Pacap+/+ mice.

Conclusion: PACAP signaling is necessary for normal fever maintenance. Our results suggest that PACAP contributes to the later phases of LPS-induced fever by modulation of COX-2 protein expression in the periphery and the brain, as well as by augmentation of pyrogenic cytokine levels in the circulation. These findings advance the understanding of the crosstalk between PACAP signaling and the “cytokine-COX-2” axis in systemic inflammation, thereby open up the possibilities for new therapeutic approaches.

86. Suhaili, Iffah Syafiqah binti

Uncovering Neural Saccadic Responses using EEG during Natural Viewing of Paintings

Iffah Syafiqah binti Suhaili[1], Zoltan Juhasz[1]
1 Bioelectric Brain Imaging Laboratory Department of Electrical Engineering and Information Systems University of Pannonia, Veszprem, Hungary

Electroencephalography (EEG) is widely used in cognitive research due to its low cost, non-invasive nature, and high temporal resolution. The traditional EEG method, Event-Related Potential (ERP), averages multiple trials together to measure brain activity. However, ERP is limited to strictly controlled experiments resulting in fast, phase- and time-locked stimulus responses, and with experiment settings that minimise head and eye movements. Consequently, it is not suitable for everyday activities with natural viewing conditions, diverse stimuli and longer durations. These conditions may greatly increase the probability of the occurrence of unwanted artefacts, and reduce the phase-locking properties of the responses. To overcome these limitations, eye-tracking devices are frequently used to capture eye movements, however they do not provide information about brain activity.

In this study, participants viewed a series of paintings (40 abstract and 40 representational), and their brain activity was measured using EEG. Each painting was displayed on the screen for 8 seconds, followed by 4 seconds for the participants to provide a key-press response indicating their preference ("like" or "dislike"), and a 1-second number cue. Brain activity was registered on the scalp of the participants using a 128-channel Biosemi ActiveTwo EEG measurement instrument with a sampling rate of 2048 Hz. Pre-processing steps, including filtering and independent component analysis (ICA), were then applied to separate neural and non-neural activity sources.

Our results revealed an interesting independent component with a unique spike train pattern, identified through ICA, that we identified as a series of lambda waves. Previous research has shown that microsaccades, along with saccade-induced lambda waves, can be observed as artefacts in EEG, and eye-tracking combined with ERP analysis has proven effective in monitoring visual attention during free-viewing tasks. To our knowledge, no research has utilised ICA to identify these spikes. Our analysis suggests a potential connection between these spikes, lambda waves, saccades, and microsaccades. The ICA approach enables us to detect subtle eye movements and either implicitly assess the level of attention or remove unwanted occipital activation from natural viewing experiment EEG data that could otherwise overshadow the underlying neural activity.

87. *Szabó, Adrienn*

Social Behavior in the Absence of Uncoupling Protein 2 (UCP2)

Adrienn Szabó[1], Erika Kvak[1], Dóra Zelena[1]
1 Institute of Physiology, Medical School, University of Pécs, Centre for Neuroscience, Szentágothai Research Centre, Pécs, Hungary

Uncoupling proteins (UCP) act as transporters in the mitochondrial inner membrane, regulating the discharge of the proton gradient generated by the respiratory chain for functions such as thermogenesis, redox balance maintenance, and reduction of reactive oxygen species. Within this family, UCP2 emerges as a multifaceted player in the central nervous system, influencing processes like cellular stress, cell proliferation, and neuroprotection. As UCP2 is co-expressed with oxytocin and vasopressin, the well-known social hormones, UCP2 could have a role in these processes as well.

Our aim was to explore the role of UCP2 in social behaviour comparing UCP2 knockout (KO) and wild-type (WT) rats using social discrimination test. As possible background mechanism, we examined the differences in the vasopressinergic and oxytocinergic system in the brain using immunohistochemical and PCR methods.

Our discoveries illuminate the potential impact of UCP2 on molding social behavior. Additionally, we scrutinized the viability of the UCP2 knockout rats as a possible model for therapeutic interventions aimed at this protein in neuropsychiatric disorders marked by diverse social disorders. This research could provide valuable insights for animal modelling of such diseases and the development of potential therapeutic strategies.

88. Szabó, István

Enhancing Pediatric Amblyopia Screening: Unraveling Optimal Stereovision Test Combinations with a Perceptron Model

István Szabó[1], Zsófia Csizek[1], Eszter Mikó-Baráth[1,2], Anna Budai[1], András Frigyik[3], Ágota Pusztai[4], Vanda Á. Nemes[1,2], László Závori[1], Diána Fülöp[1], András Czigler[1], Kitti Szabó-Guth[1,2], David Piñero[5], Péter Buzás[1,2], Gábor Jandó[1,2]

1 Institute of Physiology, Medical School, University of Pécs, Hungary,
2 Center for Neuroscience, University of Pécs, Hungary,
3 Faculty of Sciences, University of Pécs, Hungary,
4 Department of Ophthalmology, Medical School, University of Pécs, Hungary,
5 Department of Optics, Pharmacology and Anatomy, University of Alicante, Spain

Objective: This study aims to determine the most effective stereovision test combination for screening amblyopia and amblyogenic risk factors in children, using various density static and dynamic random dot stereograms with or without noise.

Methods: A Perceptron model was trained with four-dimensional input data, representing scores from four tests: static random dot stereogram (SRDS) with 8% dot density, dynamic random dot stereogram (DRDS) with 1% dot density, DRDS with 0.7% dot density, and DRDS with 1% density including 0.5% uncorrelated noise. The Neural Network Toolbox was employed for optimization and cross-validation to ensure model generalizability. The study tested various combinations of input variables to evaluate their impact on the model's performance, focusing on convergence rates and Area Under the Receiver Operator Characteristic Curve (AUC) metrics. One hundred repetitions were used to test the convergence of the network and assess variability.

Results: The study revealed that modifications to the output function and the use of the Levenberg-Marquardt training algorithm significantly accelerated the Perceptron's convergence. The Perceptron's generalization ability, assessed through AUC metrics, remained stable across both training and testing sets, indicating robustness against overfitting. Pairwise comparisons of different configurations, with Bonferroni's correction applied, showed significant differences in performance variables. The findings suggest that single test performance is inferior to combinations involving two or more tests. The best results were observed when combining static and at least one dynamic test. The noisy stereogram contributed the least to overall performance; excluding it from the four-dimensional version did not significantly deteriorate performance. However, including all four tests may enhance stability.

Conclusion: The study successfully identified effective combinations of stereovision tests for screening amblyogenic risk factors in children. The Perceptron model, with its effective convergence and reliable generalization ability, proved a valuable tool in determining the optimal mix of tests, emphasizing the importance of combining static and dynamic elements in stereovision assessments.

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89. Szabó-Guth, Kitti

Ensuring Visual Acuity Measurement Reliability in Cases of Reduced Visual Acuity: A Preliminary Study Using Bangerter Foils

Kitti Szabó-Guth[1], János Radó[1], Péter Hegyi[1], Eszter Mikó-Baráth[1], Gábor Jandó[1]
1 Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary

Our research group has developed an Android-based software (EuvisionTab®), designed for comprehensive vision testing. Currently, the software is undergoing testing and fine-tuning. In this phase of testing, we are focusing on the visual acuity (VA) module to determine whether the system performs reliably, even when dealing with reduced visual acuity. In addition to EuvisionTab, we are conducting measurements using two other VA methods (ETDRS and FrACT) to gather additional valuable insights by comparing their results. To simulate different levels of reduced visual acuity, we are using eight Bangerter occlusion foils of varying strengths. These occluders are translucent plastic filters that are applied to the front of spectacle lenses, reducing light transmission and visual acuity in a graded manner. Each measurement is repeated ten times to ensure reliability. The measurements are conducted monocularly, with subjects wearing their vision correction. The VA examination is carried out using a 10,4" Samsung Galaxy tablet, presenting Landolt C optotypes at a distance of 5 meters in a darkened room. While the data collection is ongoing, preliminary measurements suggest that the selected vision testing systems perform reliably even in cases of reduced visual acuity. The variance of the results falls within the statistical limits that define the systems as reliable.

90. Szántó, Milán

Investigation of dexamethasone loaded polymer micelles on the culture model of nasal epithelium and the blood-brain barrier

Milán Szántó[1], Ilona Grósf[1], Bence Sipos[2], Mária Deli[1]
1 Institute of Biophysics, Biological Research Centre, ELKH, Szeged, Hungary
2 Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, Hungary

Administration of drugs to the central nervous system is a challenging area due to the blood-brain barrier (BBB). It is a structure that prevents the passage of biomolecules from the blood to the brain. The anatomical basis of the BBB formed by endothelial cells, which control the permeability of drugs by interendothelial tight junctions and efflux transport systems. Consequently, the effective treatment of neurodegenerative diseases has been a challenge. Intranasal administration can occur as an alternative drug delivery route to the brain mainly through the sensory neuronal pathway or indirectly by the passage across the BBB from the systemic circulation. This allows a rapid solution to bypass the BBB where the intact drug can penetrate through the nerval pathways directly into the brain. Another problem is that potential drug candidates cannot access the brain in a therapeutically relevant concentration. Hence, novel drug delivery systems are needed. Polymeric micelles can offer increased solubility, enhanced drug release and permeability through biological barriers. In our study, we investigated the effect and penetration of dexamethasone, a steroidal anti-inflammatory drug with poor water solubility, encapsuled into polymeric micelle on the human culture model of the nasal epithelium and the blood-brain barrier. Real-time impedance measurement showed a slight decrease in cell index of the nasal epithelial and the brain endothelial cells after 30- and 60 minutes of the treatment. We observed increased penetration of the dexamethasone loaded polymeric micelle across the nasal epithelial and the brain endothelial cell layer compared to the dexamethasone suspension treated group at 30- and 60-minute timepoints. After the permeability experiment, we tested the cell layer integrity by measuring the transendothelial- and epithelial electrical resistance (TEER) and the penetration of two different marker molecules. The dexamethasone polymer micelles did not damage the cell layers as reflected by the unaltered TEER values and the low permeability values of the marker molecules. Based on our results, polymer micelles can be effectively used to enhance the permeability of dexamethasone through the nose into the brain.

91. *Szendi, Vivien*

Calbindin neurons of the lateral septum control maternal behaviour via a thalamic-septal-hypothalamic circuit

Vivien Szendi[1], Gina Puska[1,2], Szilvia Oláh[1], Máté Egyed[1], Ágnes Fanni Seres[1], Miklós Márton Takács[1], Melinda Cservenák[1], Árpád Dobolyi[1]
1 Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
2 Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary

The lateral septum (LS) is a forebrain area that has a role in forming prosocial behavioural elements such as maternal care. LS is supposed to have a role in maternal behaviour based on lesion studies, however, the responsible cellular networks are not known yet. Therefore, we aimed to characterize maternally activated septal neurons and their function in maternal regulation. First, we established that all of the pup-induced c-Fos+ septal neurons are inhibitory GABAergic neurons. A population of these GABAerg neurons in the ventral subdivision of the LS (LSv) contain calbindin (Cb+). The number of c-Fos-activated Cb+ neurons was markedly higher in mothers following pup exposure than pup deprivation. We demonstrated by viral based, cell type-specific anterograde tracing that Cb+ LSv neurons send massive projection to the medial preoptic area (MPOA) a centre for regulating maternal behaviour and confirmed this projection by retrograde tracing. To establish how maternal input arrives at the Cb+ LSv neurons, we first established that these neurons contain receptors of the maternally induced neuropeptide, parathyroid hormone 2 (PTH2) using Cb immunolabelling in reporter mice expressing ZsGreen in PTH2 receptor-expressing neurons. These receptors are likely activated by PTH2 released from the PTH2 terminals we also identified around Cb+ neurons in the LSv. Moreover, a synaptic connection was established between PTH2+ fibres and maternally activated inhibitory neurons in the LSv using electron microscopy. We also demonstrated that PTH2+ fibers in the LSv originate in the posterior intralaminar thalamic nucleus (PIL) from PTH2+ neurons, which all show c-Fos-activation in mothers following pup exposure compared to pup-deprived controls. The MPOA also receives PTH2+ input from PIL, thus we investigated and showed that the same neurons of the PIL project to both LSv and MPOA neurons. Functional investigation of LS Cb+ neurons in Cb-Cre mice demonstrated that the inhibition of these neurons reduced the time spent with the licking of the pups but caused no other behavioural effects. Therefore, we conclude that the activation of the inhibitory Cb+ neurons is required for the pup licking behaviour, which may be executed by their projection to the MPOA neurons. These data indicate that the LSv and MPOA form an interconnected subcircuit driven by somatosensory input from the pups via the PIL PTH2+ neurons in maternal brain.

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92. Szeredi-Faragó, Zsuzsanna

Hippocampal local field potentials evoked by 4-aminopyridine in valproate rat model of autism spectrum disorder

Zsuzsanna Szeredi-Faragó[1], Viktor Kelemen[1], Attila Szűcs[1], Petra Varró[1]
1 Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest

Autism is a neurodevelopmental disorder described by several major symptoms for example impairment of sociability, impaired communicational skills, stereotypic and repetitive behaviors. According to a leading hypothesis about the background of autism, the disequilibrium of the excitation/inhibition processes within neuronal networks plays a key role. This imbalance may be caused by the hyper- or hypoactivation of the glutamatergic system or the GABAergic system. During my experiments, I compared local field potentials (LFPs) in hippocampal areas ex vivo using rat brain slices of control and in utero valproate (VPA) exposed animals.

The rodent valproate autism model was applied: pregnant Wistar rat dams were treated with VPA on the 12.5 gestation day, autistic traits were confirmed with behavioral testing and later 6-week-old and 3-month-old male and female offspring was used for electrophysiological experiments. I used multi-channel microelectrode array (MEA) system to detect synchronous activity in the hippocampus which was evoked by 50 µM 4-aminopyridine (4-AP) perfusion, a potassium channel blocker used as a convulsant in several epilepsy models. Local field potentials were recorded in 400 µm thick horizontal rat brain slices.

Preliminary analysis of local field potentials revealed alterations in hippocampal excitability of VPA males, namely both 6-week-old and 3-month-old male treated subjects showed deviation from their control counterparts. The hippocampal area of 6-week-old treated males seems to be more excitable than the control 6-week-old males, as the frequency of LFPs was increased, although the amplitude of local field potentials was lower. On the contrary, the hippocampus of 3-month-old treated males was less excitable while having similar amplitude of LFPs as the control 3-month-old males. On the other hand, females do not exhibit similar differences. Additionally, a spatial propagation of field potentials were observed from the CA3 region of the hippocampus towards the CA1 region.

Interestingly, even control females and males show differences. Recordings from 6-week-old control female hippocampus revealed higher frequency of LFPs compared to the 6-week-old control males, suggesting that the control, untreated animals also have gender-related differences, which could be altered by the VPA treatment.

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93. *Szöts, Ildikó*

Age-related differences in the morphophysiological properties of human layer 2/3 pyramidal cells

Ildikó Szöts[1], Martin Tóth[1], Gábor Molnár[1], Gábor Tamás[1], Pál Barzó[2]
1 HUN-REN-SZTE Research Group for Cortical Microcircuits, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged
2 Department of Neurosurgery, University of Szeged, Szeged

The basic excitatory neurons of the cerebral cortex, the pyramidal cells, are the most important signal integrator for the local circuit. They have quite characteristic morphological and electrophysiological properties that are known to be largely constant with age in the young and adult cortex. However, the brain undergoes several dynamic changes throughout life, such as in the phases of early development and cognitive decline in the aging brain. We set out to search for intrinsic cellular changes in supragranular pyramidal cells across a broad age range: from birth to 85 years of age and we found differences in several biophysical properties between defined age groups. During the first year of life, subthreshold and suprathreshold electrophysiological properties changed in a way that shows that pyramidal cells become less excitable with maturation, but also become temporarily more precise. According to our findings, the morphological features of the three-dimensional reconstructions from different life stages showed consistent morphological properties and systematic dendritic spine analysis of an infantile and an old pyramidal cell showed clear significant differences in the distribution of spine shapes. Overall, the changes that occur during development and aging may have lasting effects on the properties of pyramidal cells in the cerebral cortex. Understanding these changes is important to unravel the complex mechanisms underlying brain development, cognition and age-related neurodegenerative diseases.

94. Takács-Lovász, Krisztina

Altered purine, fatty acid and ester, aminoacid and hormone profiles in migraineurs during the ictal and interictal periods

Krisztina Takács-Lovász [1], Tímea Aczél[1], Kata Bölcskei[1], József Kun[1,6], Mihal Ciborowski[5], Witold Bauer[5], Barna Vásárhelyi[7], Gellért B. Karvaly[7] and Zsuzsanna Helyes[1,2,3,4]

1 Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs

2PharmInVivo Ltd.

3 National Laboratory for Drug Research and Development, Magyar tudósok krt. 2. H-1117 Budapest, Hungary

4 Hungarian Research Network, Chronic Pain Research Group, University of Pécs

5 Univeristy of Byalistok, Byalistok

6 Bioinformatics Research Group; Bioinformatics and Genomics Core Facility, Szentágothai Research Center, University of Pécs

7 Department of Laboratory Medicine, Semmelweis University, Hungary

Migraine is a primary headache affecting 10% of people worldwide. The pathophysiological mechanisms are still not clearly understood, and the therapy is often unsatisfactory. Therefore, disease- and headache-specific mediators and potential novel therapeutic targets need to be identified by hypothesis-free unbiased approaches using patients' plasma samples.

Here we analyzed the metabolomic changes in the plasma of patients during ictal and interictal periods in comparison with healthy controls.

Thirty six female and 1 male subjects were enrolled in this study: 24 episodic migraine patients with or without aura and 13 healthy controls. The studied groups were similar, matched in age, and anthropometric measurements such as body mass index (BMI). Samples were run using 4 different instrumental setups, with the liquid chromatographic separation of 106 metabolites, and the flow injection analysis of 524 metabolites using MxP® Quant 500 kit. For data analysis, samples with >20 % CV were filtered out, MetaboAnalyst 5.0 online available tool was used for this step. Log transformation was performed to normalize the data.

Enrichment Analysis with Metaboanalyst 5.0 revealed pantothenate and CoA phosphatidylcholine and phosphatidylethanolamine biosynthesis, citric Acid, alpha linolenic acid and linoleic acid metabolismsphingolipid metabolism, fatty Acid elongation in mitochondria, bile acid biosynthesis, carnitine synthesis between healthy and patients in migraine phase. Glycolysis and pyruvate metabolism were hit in Pathway analysis.
Among ictal and interictal, Enrichment analysis showed altered inositol, starch and sucros metabolism, bile acid metabolism, steroid Biosynthesis, pyrimidine metabolism, arachidonic acid Metabolism, propanoate Metabolism, trypthophan Metabolism. Pathyway analysis for this comparison collected ascorbate and aldarate metabolism, pentose and glucoronate interconversions.

Mitochondrial mechanisms such as fatty acid elongation, citric acid metabolism might be disease-dependent and primary bile acid and arachidonic acid biosynthesis headache-dependent. These results suggest that metabolomic analysis gives valuable insight into the mechanisms of migraine.

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95. Toller, Kata Anna

Examination of pituitary adenylate cyclase-activating polypeptide (PACAP) in patients with atrial fibrillation undergoing pulmonary vein isolation

Kata Anna Toller[1,2], Peter Kupo[1], Beata Polgar[3], Kristof Janosi[1], Tunde Toth[2], Dorottya Debreceni[1], Dora Reglodi[2], Andrea Tamás[2]

1 Heart Institute, Clinical Centre, University of Pecs Medical School

2 Department of Anatomy, ELKH-PTE PACAP Research Team, Centre for Neuroscience, University of Pecs Medical School

3 Department of Medical Microbiology and Immunology, Clinical Centre, University of Pecs Medical School

Pituitary adenylate cyclase-activating polypeptide (PACAP), which has cardioprotective effects, has previously been shown to increase in plasma levels in myocardial infarction and decompensated heart failure, whereas it decreases in chronic heart failure.

In our study, we collected blood samples from patients (n=46) undergoing pulmonary vein isolation for atrial fibrillation from the femoral vein at the beginning of the procedure, from the right atrium before septal puncture, from the left atrium before and after the beginning of ablation, from the femoral vein at the end of the procedure and from the cubital vein the next day after the procedure. PACAP levels were determined by ELISA. Patients were divided into intact (n=29) and scarred (n=17) left atrial groups by electroanatomical map of the patients' left atrium. PACAP levels were compared in the total population and in the two groups and correlated with left atrial size and other comorbidity data.

Significantly higher levels of PACAP were detected in atrial blood samples and in post-operative femoral vein samples compared to peripheral blood samples collected at the beginning of the procedure and 1 day after the procedure. We measured higher PACAP levels in the left atrium before ablation and in the femoral vein after the procedure compared to our left atrial samples at the end of the ablation. In patients with scarred left atrium, we found lower PACAP levels in the left atrium after ablation compared to the intact group.

Our study was the first to show a significant difference between PACAP levels in atrial and peripheral blood samples. The elevated PACAP levels measured in atrial samples may be due to myocytes and neurons, whose PACAP production decreases depending on the degree of scarring following ablation-induced injury in the left atrium, but transient systemic elevation of PACAP levels is demonstrated in the periphery, suggesting a potential biomarker role for PACAP in these pathologies.

Supervisors: Dr. Andrea Tamás Associate Professor, Dr. Péter Kupó Assistant Professor

Support: PTE-KITEP-2023-335, NAP 3.0 Project, Kriszbacher Scholarship

96. Török, Izabella

Possible role of the TRPA1 in a mouse model of chronic alcohol consumption

Izabella Török[1], Ammar Al-Omari[1], Viktória Kormos[1]
1 Department of Pharmacology and Pharmacotherapy, Pécs, Hungary

Aims: The centrally projecting Edinger-Westphal nucleus (EWcp) contributes to control of alcohol consumption by its urocortin1 (UCN1) and cocaine- and amphetamine-regulated transcript (CART) co-expressing peptidergic neurons. UCN1/CART/EWcp is the primary seat of central transient receptor potential ankyrin1 (TRPA1) cation channel. We hypothesized that ethanol and its metabolites, may directly activate TRPA1 ion channels in the EWcp, thus may play a role in alcohol consumption.

Methods: Free-choice dark-phase mouse model of chronic alcohol (10% ethanol) consumption was performed involving male *Trpa1* knock-out (KO) and wild type (WT) mice for 3 months. Alcohol preference was measured. *Trpa1*, *Cart* and *Ucn1* mRNA expression was quantified by RNAscope *in situ* hybridization.

Results: Decreased *Ucn1* and *Cart* mRNA density was found in the control KO group with lower initial alcohol preference, which is consistent with the literature data that low *Cart* and *Ucn1* expressing mouse strains have lower alcohol preference.

The mRNA expression of all three genes tested was significantly reduced upon chronic alcohol exposure which may explain the lower alcohol preference in WT mice at the end of the experiment. In contrast, *Ucn1* mRNA levels were unchanged and *Cart* mRNA expression was reduced to a lesser extent in KO mice, which may explain the increased alcohol preference in the KO animals at the end of the experiment.

Conclusions: Decreased *Trpa1*, *Ucn1* and *Cart* mRNA expression upon chronic alcohol treatment, associated with reduced alcohol preference strongly suggests the regulatory role of this ion channel in alcohol consumption.

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97. *Tót, Kálmán*

The effect of semantic meanings on audiovisual equivalence learning

Kálmán Tót[1], Gabriella Eördegh[2], Noémi Harcsa-Pintér[1], Ádám Kiss[1], Bátor Brassó[1], András Kelemen[3], Attila Nagy[1]

1 Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged

2 Department of Health Sciences and Health Management, Faculty of Health Sciences and Social Studies, University of Szeged

3 Department of Technical Informatics, University of Szeged

Equivalence learning is a type of associative learning, where the person learns that two stimuli are linked and they are equivalent with each other, if they share the same outcome, and this equivalence can be generalized to new instances. The Rutgers Acquired Equivalence Test (RAET) is a visual learning task to investigate this kind of learning. Based on the test we developed three audiovisual equivalence learning tests with the same structure. In all three tests the antecedents are the same four sound stimuli, but the consequents are visual stimuli with different complexity and semantic meanings. Our question is whether the semantic meanings affect the performances in the learning tests. The results of our research with healthy volunteers showed that if more semantic label corresponds to the applied visual stimuli, it leads to better performances in the learning test.

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98. Tóth, Boglárka

Thalamocortical circuits in motor learning

Boglárka Tóth[1], Eriko Kuramoto[1,2], Hajnalka Boko [1], László Acsády[1]
1 Institute of Experimental Medicine, Budapest Hungary
2 Kagoshima University, Kagoshima Japan

The thalamus has been classically seen as the final relay station of sensory information towards the cortex. Recent knowledge however suggests that it is actively involved in cortical functions. All cortical areas receive thalamic input, which carries not only sensory information but is essential for maintaining cortical function. The role of thalamocortical circuits has been demonstrated in many cortical computations. Thalamic inputs are divided into driver and modulator types. The basis for this division is their effects on relay cells. These inputs differ in many properties, such as size, site of origin, electrophysiological properties and electron microscopic structure. Many thalamic nuclei receive driver inputs from layer 5 pyramidal cells of the cortex (L5) and modulator inputs from L6. The properties and effects, of drivers with cortical origin have only been investigated in sensory areas. The influence of frontal cortical areas on the thalamus, which plays a central role in the preparation and learning of goal directed movements, is still poorly understood. In our study, we investigated the morphology and behavioral impact of L5 driver inputs from the secondary motor cortex (M2) in the ventromedial nucleus (VM). To investigate the anatomy of the pathway, we injected GFP-containing virus into RBP4-cre mice (L5-specific strain), in the M2 and primary sensory cortical area (S1) and measured the maximum cross-sectional area of the boutons in the VM and posterior nucleus area on confocal images. Boutons originating from the M2 were significantly smaller, compared to those of S1 origin.

To investigate the effect of the pathway on behavior, we injected ArchT-containing virus into the M2 region of RBP4-cre mice and axon terminals were inhibited in the VM region. The effect of L5 inhibition in VM was investigated in open field, in place aversion test, and during locomotion training on a horizontal wheel. Inhibition of the pathway did not affect the animals' movement in open field, didn't provoke place aversion or influenced the average speed on the wheel.

These data show that M2 L5-VM corticothalamic pathway is morphologically distinct from those in sensory areas. Precise function of the unique L5 corticothalamic pathway remains to be determined.

99. Varga, Bálint

Fronto-temporal interactions in associative recall explored by multielectrode recordings

Bálint Varga[1,2], Marcell Stippinger[1], Fülöp Bazsó[1], Attila Bencze[1,3], Zoltán Somogyvári[1], László Négyessy[1], Hisashi Tanigawa[4,5], Tamás Kiss[1]
1 Wigner Research Centre for Physics, Dept. of Computational Sciences, Budapest, Hungary
2 Semmelweis University, Semmelweis Doctoral School, Budapest, Hungary
3 Óbuda University, Electrophysics Institute, Budapest, Hungary
4 Zhejiang University, Interdisciplinary Institute of Neuroscience and Technology, Hangzhou, China
5 Niigata University, Department of Physiology, Niigata, Japan

Object-based visual working memory relies critically on the fronto-temporal cortical network. Investigating interactions within this network is crucial for comprehending the neural mechanisms of goal-directed recall in memory. A delayed color recall task, wherein colors were associated with achromatic images of scenes and geometric drawings, was employed to examine interactions between the temporal and prefrontal cortex through high-density electrocorticography (ECoG) in two macaque monkeys. Regions with similar functionality were identified by clustering the time-varying signal of electrodes in the ECoG arrays over the prefrontal and temporal cortices. The clustering results aligned with the dorso-ventral and rostro-caudal functional segregation of cortical areas. Analysis of task-specific sub-regional activities revealed a dynamically changing pattern throughout the task in both the temporal and prefrontal cortices. Granger causality analysis in various frequency bands explored the relationship of sub-regional activities within and between the two cortical regions. Mnemonic activities showed higher interregional causal relationships between regions than within them, specifically in the theta and alpha bands, while these differences largely diminished at higher frequencies. Similar observations were noted during the cue period. Phase-Amplitude Coupling (PAC) analysis is underway to gain a deeper understanding of the oscillatory dynamics of fronto-temporal interactions during goal-directed recall in memory.

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100. *Várkonyi, Dorottya*

Unravelling the Dynamics of Thermoregulation in the Triple Transgenic Mouse Model of Alzheimer's Disease

Dorottya Várkonyi [1], Csilla Lea Fazekas [1], Choi Muyong[2], Dóra Zelena [1]
1 Pécsi Tudományegyetem, Általános Orvostudományi Kar, Élettani Intézet
2 Pécsi Tudományegyetem, Általános Orvostudományi Kar

According to the literature low body temperature is considered a risk factor for Alzheimer's disorder (AD) and may contribute to a significant worsening of its histological pathology. Less is known about the alteration of the thermoregulation in AD subjects. Therefore our aim was to identify it in the popular triple transgenic mouse model (3xTg-AD) of AD. In addition to continuous telemetric monitoring of circadian temperature changes, we used cold and warm stimulation as well as an NK3 agonist (senktide)-induced drop in core body temperature modelling post-menopausal heat waves. Six-month-old female animals are compared to wild-type (WT). As the important thermoregulatory centrum, the medial preoptic area is rich in oestrogen-sensitive cells, the stimuli were used also after ovariectomy (OVX). No difference was found after cold stimulation (neither in the forced swim test in cold water), while there was a marginal difference between genotypes after warm stimulation. The senktide was effective (drop in temperature) in WT animals but not in 3xTg-AD animals. So far, OVX did not influenced the outcome. Our results confirm that the 3xTg-AD animals have disturbed thermoregulation, which was more sensitive to external heat (both warm stimulus and senktide-induced increase in tail temperature). The one week OVX does not seem to be long enough to substantially influence the thermoregulation. The ineffectiveness of senktide in 3xTg-AD animals might have a potential as new biomarker for AD, however, further studies needed in this aspect.

101. *Vida, Sára*

Role of microglia in SORL1-dependent neurodegeneration and Alzheimer's disease

Sára Vida[1], Balázs Pósfai[1], Krisztina Tóth[1], Nikolett Lénárt[1], Csaba Cserép[1], Zoltán Máté[2], Ferenc Erdélyi[2], Zsolt Lele[3], István Katona[3], Henne Holstege[4], Olav M. Andersen[5], Ádám Dénes[1]
1 Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary
2 Medical Gene Technology Unit, Institute of Experimental Medicine, Budapest, Hungary
3 Molecular Neurobiology Research Group, Institute of Experimental Medicine, Budapest, Hungary
4 Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands
5 Department of Biomedicine, Aarhus University, Aarhus, Denmark

Understanding the mechanisms of neurological diseases is one of the most urgent challenges of medicine. In recent years, the contribution of microglia, the main regulator of immune processes in the brain to homeostatic and neuroprotective processes has gained increasing interest. Alzheimer's disease (AD) is a progressive neurodegenerative disease and the leading cause of dementia worldwide. In this study, we explore the involvement of microglia in the pathogenesis of AD, focusing on microglia-neuron somatic junctions through which microglia monitor and shape neuronal function. Specifically, we study the role of microglia in the context of the sortilin-related receptor SORL1, a key risk gene in hereditary AD.

In this translational study, we have collected fixed tissue, native brains and cerebrospinal fluid of triple-transgenic AD-model (PSEN1//App_swe//tauP301L) and control mice, from three different age groups (60-80 days, 220-240 days, 490-520 days) and from both sexes. We have also developed a new CRISPR/CAS9 targeting strategy combined with in utero electroporation to achieve genetic deletion of Sorl1 in a subpopulation of cortical neurons in transgenic mice. Measurement of inflammatory biomarkers and high-resolution immunofluorescent imaging have been performed to study the effects of SORL1 deletion on cellular- and inflammatory responses. We also obtained 3 groups of human CSF samples: SORL1-mutation carrying AD patients, age-matched non-SORL1-associated AD and CTRL patients, as well as fresh frozen human tissue from these groups.

Our results show significant alterations of microglial morphology and function during the aging of mice with chronic neurodegeneration, as well as marked, related changes in the expression of SORL1. Proteomic analysis of human samples revealed a strong neuroinflammatory background to SORL1-associated AD, suggesting the involvement of microglial actions. Our ongoing investigations may shed light to some novel inflammatory mechanisms underlying the pathogenesis of AD.

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102. Vitális-Kovács, Antonietta

Training of a simple touch-screen-based continuous performance task in cynomolgus macaques

Antonietta Vitális-Kovács[1], Balázs Knakker[1], Judit Inkeller[1], Rafaella Mínea Riszt[1,3], Anna Padányi[1,3], Evelin Kiefer[1], István Hernádi[1,2,3]
1 Grastyán E. Translational Research Centre, University of Pécs
2 Faculty of Sciences, Institute of Biology, University of Pécs
3 Medical School, University of Pécs

Continuous performance test (CPT) is a neuropsychological test used to assess

motivation and sustained attention. The basic notion of the task is to reinforce the agent when it responds to a visual stimulus on touchscreen presented at various positions.

In this study, in a preclinical translational research environment, we used a simple touch-screen-based CPT paradigm to train naïve young adult macaques to use the touchscreen and build knowledge that is generalizable towards understanding more complex cognitive paradigms in the future. Our primary aim was to train the monkeys to be able to routinely utilize the touchscreen and understand the underlying basic task contingencies.

Eight male and four female cynomolgus macaques had been trained for 6 months for one session per weekday with CANTAB (Cambridge Neuropsychological Test Automated Battery), a touch screen cognitive testing device specially designed for non-human primates. All subjects started with the same settings, and then, we gradually adjusted task parameters (session length, stimulus size, stimulus duration) towards target values while monitoring individual motivation, performance and distribution of effective working time during the sessions. We observed how many training days it took the animals to reach the final task complexity and tested different settings to find which can help to develop the necessary skills and performance at a certain difficulty level. We also constantly monitored the animals' body weight and adjusted their additional food intake in the home cage to maintain a desirable motivational level in the task.

By the 14th training day, all animals reached the 30-min session length, from this time they continued with individual differences. On the 76th training day, 8 (75%) animals reached the 55-min session length. By the 70th training day, 7 (56%) animals had reached the smallest stimulus size, 3 of them worked with adequate motivation and performance, so from the 86th training day we continued to improve their precision and generalization by changing the stimulus shape.

Based on our experience macaques may need at least 80 training days to learn the CPT task. Daily monitoring and individualized adjustments are necessary for the continuous development. As CPT is an essential first step to master more complex cognitive tests, the presently described training process will be useful for optimizing the initial touchscreen training regimes of naïve animals in the future.

103. *Vörös, Dávid*

Sulpirid blocks the anxiolytic effect of oxytocin in elevated plus maze test

Dávid Vörös[1,2] , Orsolya Kiss[1,2] , Tamás Ollmann[1,2,3,4] , Kitti Mintál[1,2,5] , László Péczely[1,3,4] , OlgaZagoracz[1,2,3,4] , Erika Kertes [1,2,3,4] , Veronika Kállai[1,2,3,4] , Bettina Réka László [1,3] , Beáta Berta[1,2,3,4] , AttilaToth[1,2,3,5] , László Lénárd[1,2,3,6] and Kristóf László1,2,3

1 Medical School, Institute of Physiology, University of Pécs, Szigeti Str. 12, 7602 Pécs, Hungary

2 Neuropeptides, Cognition, Animal Models of Neuropsychiatric Disorders Research Group, Medical School, Institute of Physiology, University of Pécs, 7602 Pécs, Hungary

3 Neuroscience Center, University of Pécs, 7602 Pécs, Hungary

4 Learning in Biological and Artificial Systems Research Group, Medical School, Institute of Physiology, University of Pécs, 7602 Pécs, Hungary

5 Cellular Bioimpedance Research Group, Szentágothai Research Center, University of Pécs, 7602 Pécs, Hungary

6 Molecular Endocrinology and Neurophysiology Research Group, Szentágothai Center, University of Pécs, 7602 Pécs, Hungary

The neuropeptide oxytocin is known for its involvement in regulating both social and non-social behaviors. Within the limbic system, the central nucleus of the amygdala holds a crucial role in functions such as learning, memory, anxiety, and reinforcement mechanisms. In rodents, the central nucleus of the amygdala has been found to be rich in oxytocin receptors. Previous research from our team has demonstrated that oxytocin in the central nucleus of the amygdala exhibits an anxiolytic effect in a dose-dependent manner.

The current study aimed to investigate the potential interaction between oxytocin and the D2 dopamine receptor antagonist sulpiride concerning anxiety, particularly in the elevated plus maze test. Four group of Wistar rats were subjected to bilateral microinjections namely 10 ng oxytocin pretreated by vehicle solution, vehicle solution pretreated by 4 µg of D2 dopamine receptor antagonist, 10 ng oxyitocin pretreated by 4 µg of D2 dopamine receptor antagonist or vehicle solution respectively. The groups received the D2 dopamine receptor antagonist 15 minutes before either the 10 ng oxytocin treatment or the vehicle solution into the central nucleus of the amygdala.

Rats that received 10 ng oxytocin spent a significantly longer time on the open arms of the elevated plus maze. Pre-treatment with the D2 dopamine receptor antagonist did not result in a significant time difference compared to the control group, and administering the dopamine D2 receptor antagonist alone did not impact the time spent on open arms. These results suggest that the anxiolytic effects of oxytocin in the central nucleus of the amygdala involve the dopamine system, as the D2 dopamine receptor antagonist can effectively block the anxiety-reducing effects induced by oxytocin.

104. *Vörös, Kinga*

Felodipine efficiency analysis on induced neurons derived from Huntington's disease FELL-HD clinical trial patients

Kinga Vörös[1,2], Dimitris Apostolopoulos [3], Lea Danics [1,2,4], Shaline Fazal [3], Roger A Barker [3], Karolina Pircs [1,2,5].

1 Semmelweis University, Budapest, Hungary
2 HCEMM-SU, Budapest, Hungary
3 University of Cambridge, Cambridge, UK
4 ELKH-SU, Budapest, Hungary
5 Lund University, Lund, Sweden

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, caused by CAG expansions in the huntingtin gene (*HTT*), which results in the aggregation of the mutated huntingtin protein (mHtt). HD is uncurable, and after disease onset around 30-40 years of age patients die within the next 10-20 years. Autophagy, a lysosomal degradation pathway ensuring cytoplasmic homeostasis is dysfunctional in HD, contributing to insufficient mHTT protein removal. The Fell-HD clinical trial is based on repurposing Felodipine, an already licensed L-type calcium channel blocker and antihypertensive drug with a low chance of side effects. Felodipine significantly increases autophagy in animal models of HD and subsequently reduces the level of toxic mHTT, neurodegeneration, and disease symptoms, like tremors and loss of motor coordination.

In this current project, in parallel with the FELL-HD trial, we are testing felodipine drug efficacy in induced neurons (iN) directly reprogrammed from the FELL-HD cohort's skin fibroblasts. Transdifferentiated iNs keep the genetic and aging signatures of the donor bypassing any stem cell or neuroprogenitor phase during conversion. We converted 8 control and 18 HD patients' fibroblasts with mild symptoms to iNs with the same conversion efficiency and purity. Our previous results indicated an accelerated aging in HD-iNs defined by DNA methylation. Therefore, we are investigating in the current cohort the presence of any accelerated aging using the Horvath epigenetic clock. Felodipine cell survival measurements showed no toxicity. We used 0.1μM and 1 μM felodipine treatment for 24h and 72h. After 28 days of conversion in 96-well plates iNs will be counterstained with neuronal and autophagy markers to determine neuronal morphology and subcellular autophagy changes using high-content automated screening microscopy.

These preclinical results will be directly compared and correlated with FELL- HD trial outcome and the patients cognitive and motor scores. This project using an in vitro preclinical iN model can potentially provide predictive information about drug effectiveness, opening a new dimension in clinical trial optimization and personalized medicine.

105. *Wang, Zeyu*

Extracting Neural Oscillations in Multi-channel EEG: Multivariate Empirical Mode Decomposition vs Digital Filtering

Zeyu Wang[1] and Zoltan Juhasz[1]
1 University of Pannonia, Veszprem, Hungary

Neural oscillations are the most prominent feature of EEG data and have emerged as a paradigm of reference for EEG research. Countless studies in the past decades have demonstrated that perceptual, cognitive, motor, and emotional processes are tightly linked with specific patterns of oscillations. Neural oscillations are observed and studied at multiple spatiotemporal scales of the brain, and their signal features can be extracted by the time- frequency analysis of measured EEG data, which is widely implicated in signal processing and neural computations.

Digital filters are fundamental operations in various stages of EEG signals processing pipeline (noise removal, baseline correction, feature extraction, etc.), and are also the most widely used oscillation extraction method. However, due to the non-stationary nature of EEG signals, methods based on convolution and pre-defined functions, such as digital filters are sometimes unable to accurately extract intrinsic neural oscillations in EEG. An obvious concern is that digital filters may distort the temporal features of the target: peaks or transitions may be smoothed, steps may turn into pulses, and artificial features may appear. Another concern is distorting causality between features within the signal, or between the signal and external events such as stimulation.

Empirical Mode Decomposition (EMD) is an effective tool for the analysis of non-linear and non-stationary signals, which can decompose signals according to the time scale features of the data itself, without any pre-set basis functions. In a non-stationary signal, there are multiple oscillation modes at any time instance, which means that the signal at each time point contains multiple instantaneous frequencies, EMD can decompose the non-stationary signal into narrowband components (Intrinsic Mode Functions, IMFs) to separate the mixed instantaneous frequencies, and each component contains one oscillation mode. Due to the adaptive and data-driven nature, EMD is able to extract more accurate information about neural oscillations, phase coherence, and brain connectivity.

In the poster, we compare the performance of extracting neural oscillations with EMD and digital filters using multi-channel synthetic signals and EEG signals measured in the eyes- closed resting state. Our preliminary results suggest that although digital filters are widely used, EMD seems to have more advantages in neural oscillation extraction.

106. *Zagorácz, Olga*

Neuropeptide QRFP enhances memory in passive avoidance paradigm

Olga Zagorácz[1], Tamás Ollmann[1], László Péczely[1], Kristóf László[1], Anita Kovács[1], Beáta Berta[1], Veronika Kállai[1], Erika Kertes[1], Dávid Vörös[1], Daniella Dusa[1], Ádám Szabó[1], László Lénárd[1, 2]*
1 Institute of Physiology, University of Pécs Medical School, Pécs, Hungary
2 Molecular Neurophysiology Research Group, University of Pécs, Szentágothai Research Center, Pécs, Hungary

The incidence of dementia is on the rise worldwide with nearly ten million new cases occuring every year. To address this problem, active research of numerous neuropeptides, including the RFamide peptides and their properties on cognition, was initiated. Previously we have demonstrated that pyroglutamylated RFamide peptide (QRFP) enhances consolidation of spatial memory in rats. This effect was related (at least in part) to neuropeptide Y (NPY) mechanism. The aim of the present study was to examine the effects of QRFP on aversive memory.

Administration of two doses of QRFP (200 ng and 400 ng) was investigated in a step-through passive avoidance paradigm. NPY Y1 receptor antagonist BIBP3226 was applied to elucidate whether it can prevent effects of QRFP. All the drugs were applied directly into the lateral hypothalamic area (LHA), the primary location of QRFP synthesis within the CNS.

QRFP administered in 200 ng dose significantly increased the step-through latency in passive avoidance response, while a higher dose was revealed to be ineffective. The antagonist itself and combined Antagonist + QRFP treatments led to elongated step-through latency as well. The results remained significant one week after the first testing. These data suggest that neuropeptide QRFP facilitates passive avoidance learning, but this phenomenon cannot be linked to the NPY system.

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107. *Zichó, Krisztián*

Novel pontine inhibitory nucleus regulates reward experience

Krisztián Zichó[1,2], Réka Z. Sebestény[1], Boldizsár Balog[1,3], Virág Takács[1], János Brunner[4], Charlotte Seng[5], Áron Orosz[1,2], Hunor Sebők[1], Csaba Földy[5], János Szabadics[4], Gábor Nyiri[1]

1 Laboratory of Cerebral Cortex Research, Department of Cellular and Network Neurobiology, HUN-REN Institute of Experimental Medicine, Budapest, Hungary

2 János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary

3 Tamás Roska Doctoral School of Sciences and Technology, Pázmány Péter University, Budapest, Hungary

4 Laboratory of Cellular Neuropharmacology, HUN-REN Institute of Experimental Medicine, Budapest, Hungary

5 Laboratory of Neural Connectivity, Brain Research Institute, Faculties of Medicine and Science, University of Zürich, Zürich, Switzerland

The lateral habenula (LHb) regulates behavioral flexibility that is essential for effective decision-making. LHb activity can facilitate aversion or reward-seeking behavior as a function of its excitatory/inhibitory inputs. However, despite LHb abundant connections with hindbrain areas the role of brainstem inputs are still unclear. Using viral tract tracing, immunohistochemistry, fluorescent- and electron microscopic imaging, in vitro electrophysiological recordings, fiber photometry calcium-imaging and optogenetic behavioral experiments in transgenic mice, we investigated a previously unrecognized gamma-aminobutyric acid (GABA)-ergic cell population in the pons. These cells localized beneath the 4th ventricle and establish inhibitory synapses in LHb. They strongly activated by both rewarding and aversive experience, but not by neutral environmental stimuli, suggesting their role in valence encoding. Their stimulation induced both acute and conditioned place preference in behavioral experiments, indicating its potential role in reward behavior. Conversely, inhibition of these pontine neurons caused acute and conditioned place avoidance, and their inhibition during aversive experience increased conditioned fear behavior even further, suggesting their fundamental role in fear regulation. Our results suggest that a novel GABAergic pontine nucleus has a major role in processing positive valence and reward-seeking behavior.

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