ELTE
EÖTVÖS LORÁND
UNIVERSITY

HUNGARIAN
NEUROSCIENCE
SOCIETY

International Neuroscience Conference

29-30 January 2026 - Budapest, Hungary

Poster abstracts book

Intravitreous Application of Neuroectodermal Stem Cells Following Optic Nerve Injury

Tamas Bellak[1]; Peter Balazs Kocsis[2]; Levente Laszlofy[1]; Rebeka Kristof[1]; Adam Meszaros[1]; Krisztian Pajer[1]; Annamaria Marton[3]; Csaba Vizler[3]; Antal Nogradi[1]
[1]University of Szeged, Department of Anatomy, Histology and Embryology, Laboratory of Neural Regeneration, Szeged
[2]University of Szeged, Department of Ophthalmology, Szeged
[3]Biological Research Center, Institute of Genetics, National Biotechnology Laboratory, Szeged

Optic nerve injury has severe consequences, including the degeneration of retinal ganglion cells, narrowing of the visual field, and ultimately vision loss. The aim of our experiments was to determine the extent of retinal ganglion cell loss following optic nerve injury and to investigate the neuroprotective effect of intravitreally transplanted neuroectodermal stem cells.
In this study, we used 10–12-week-old female Sprague-Dawley rats (weight: 220–250 g). Contusion of the left optic nerve was induced using forceps. Three days after the injury, rats were divided into three groups: the treated group, which received intravitreal transplantation of neuroectodermal stem cells (NE-TR-4C); the control group, which received an injection of cell culture medium (HDMEM); and the injured-only group. After the injury, rats were allowed to survive for 1, 2, or 12 weeks. Subsequently, we assessed the number of retinal ganglion cells, the extent of astrocyte and microglia reactions, the fate of the transplanted stem cells, and changes in the optic nerve and optic disc. Visual performance was also evaluated.
In the injured only and control (medium-injected) groups, the number of retinal ganglion cells continuously decreased, with no significant differences between these two groups. In contrast, stem cell treatment mitigated cell loss. This improvement may be associated with modulation of the astrocytic response and microglial activation. Four and eleven days after transplantation, a significant portion of the transplanted cells survived and formed clusters near the retina. Immunohistochemical analyses showed that these cells differentiated toward a neuronal lineage, although they did not integrate into the retinal layers. No significant differences were observed in the optic disc between groups; however, the fiber density of the optic nerve was higher in stem cell-treated animals. Visual behavior testing revealed a significant change in the time spent in the dark chamber between the treated and the other two groups.
Our results suggest that intravitreally applied NE-TR-4C stem cells can rescue retinal ganglion cells destined for degeneration, promote neuroprotection and regeneration.

Automated and highly reproducible generation of neural spheroids

Maissa Ben Mahmoud[1]; Eszter Geiszelhardt[1]; András Czirók[2]; Júlia Tárnoki-Zách[2]; Katalin Schlett[1]; Krisztián Tárnok[1]
[1]Eötvös Loránd University, Institute of Biology, Department of Physiology and Neurobiology, Budapest
[2]Eötvös Loránd University, Institute of Physics and Astronomy, Department of Biological Physics, Budapest

In vitro modelling of human neurodegenerative diseases in cell cultures is a technically favorable approach for the pharmaceutical industry. However, the need to develop healthy versus pathological tissue-like environment formed by the interaction of multiple cell types possesses significant challenges. Two-dimensional (2D) cell culture systems often lack crucial hormonal factors and physical and chemical signals that change spatially and temporarily in the developing nervous system. Brain spheroids, however, represent a sophisticated three-dimensional (3D) structure derived from stem cells to emulate the cell-type composition and tissue organization of the embryonic brain.
The primary objective of the present study was to enhance the in vitro modelling prospects of neural tissue through the partial automation of the 3D tissue cultivation. We used a robotic system (SpheroMaker, BioPhys-Concepts Kft., Hungary) to disperse iPSC-derived neural progenitor cells (NPCs) from neurotypic and Kleefstra syndrome origin into ultra-low adhesive U bottomed wells of tissue culturing plates. Cell aggregates were monitored for 4 weeks by optical microscopy and image analysis.
Cell dispersal and culture medium exchange using the robotic system produced highly reproducible aggregates with less than 5% variability in diameter without loss of spheroids. All spheroids contained NeuN-expressing differentiated neurons with IIIbeta-tubulin positive processes, indicating that regardless of the culture systems, neuronal differentiation was successfully initiated. However, spheroid growth differed between conditions: spheroid formation was faster in Kleefstra-derived cells, and the growth rate of neurotypic spheroids was higher from the third week of culture. We also demonstrated that a clearing method is necessary to visualize neurons up to 100 µm beneath the surface of the spheroids. In order to circumvent the technical difficulties of clearing small (300 to 500 µm diameter) spheroids, an automated millifluidic fluid exchange system is also under development.
In conclusion, the highly reproducible and automated development and treatment of 3D neuronal spheroids is a promising tool for the pre-clinical testing of pharmaceutical compounds in nervous tissue-like environments.

Intraspinal delivery of chondroitinase-mRNA-LNP promotes morphological and functional recovery after chronic spinal cord contusion injury

Eszter Gál[1]; Tamás Bellák[1]; Zoltán Fekécs[1]; Krisztián Pajer[1]; Dénes Török[1]; Norbert Pardi[2]; Antal Nógrádi[1]
[1]*University of Szeged, Department of Anatomy, Histology and Embryology, Laboratory of Neural Regeneration, Szeged*
[2]*University of Pennsylvania, Department of Microbiology, Philadelphia*

Spinal cord contusion injury leads to severe tissue loss and subsequent deficits of motor, sensory and vegetative functions below the lesion. Many of the human lesions remain untreated and a chronic injury develops. In this study we investigated whether the injection of chondroitinase-mRNA-LNP into the injured rat spinal cord is able to induce morphological and functional improvement in a chronic spinal cord injury model. Chondroitinase-mRNA-LNP was injected into the lesion cavity 10 weeks after thoracic (T10) contusion injury performed in Sprague-Dawley rats. The GFP-mRNA-LNP animals received an intraspinal injection of GFP encoding mRNA. The injured group underwent a contusion injury without mRNA-LNP administration. During the survival period functional tests (BBB, video-based locomotor pattern analysis system) were performed, followed by detailed morphological analysis. Six weeks after the mRNA-LNP treatment the retrograde tracer Fast Blue was applied distal to the injury to determine the extent of axonal sparing/regeneration.
The chondroitinase-mRNA-LNP treated animals showed significant improvement in functional recovery compared with GFP-mRNA-LNP and injured animals. Morphological results confirmed a significantly smaller lesion area with a greater amount of remaining tissue in the chondroitinase-mRNA-LNP group. Retrograde tracing studies showed a statistically significant increase in the number of FB-labelled neurons rostral to the injury. These data confirmed better preservation/regeneration of the proprio- and supraspinal tracts. Immunohistocemical findings also supported that the treatment promoted axonal preservation/regeneration of the corticospinal tract. The extent of functional improvement was related to the decreasing amount of inhibitory chondroitin-sulphate proteoglycans around the lesion area. Five days after the treatment, chondroitinase enzyme was detected in the microglia/macrophage cells around the lesion site. This finding confirmed the uptake and active expression of chondroitinase-mRNA-LNP by immune cells.
These results suggest that the intraspinal use of chondroitinase-mRNA-LNP is able to induce morphological and functional recovery after chronic spinal cord contusion injury by modulating the lesion’s microenvironment.

What is the barrier to methane? Regional Investigation of the Effects of Methane Inhalation on Blood–Brain Barrier Permeability and Brain Mitochondrial Function in a Rat Model of Sepsis

Ataollah Azarkish[1]; Balázs Pifka[1]; Ákos Kovács[1]; Eszter Flanek[1]; Levente Gulácsi[1]; Krisztina Spisák[1]; László Juhász[1]; Andrea Szabó[1]; Marietta Zita Poles[1]
[1]Albert Szent-Györgyi Medical School, Institute of Surgical Research, Szeged

Introduction: Sepsis-associated encephalopathy (SAE) is closely linked to early disruption of the blood–brain barrier (BBB) and neuroinflammation, contributing to impaired cerebral function. Mitochondrial dysfunction is a key mechanism underlying sepsis-associated brain injury. We have demonstrated the anti-inflammatory properties of methane (CH4), including preservation of mitochondrial function. This study aimed to investigate the effects of CH_4 inhalation administered at different time windows during sepsis progression on BBB permeability and brain mitochondrial function in an intra-abdominal sepsis model.
Methods: Adult male Sprague–Dawley rats (n=68; 370 ± 30 g) were assigned to sham-operated (n=14), or septic (n=54) groups. Sepsis was induced via ip. injection of 5 mL/kg fecal inoculum. Septic rats were further divided into untreated (n=18) and CH_4-treated septic groups, receiving 2.2% methane at defined time intervals (3–6 h, 21–24 h; n=18 each) during sepsis progression. Animal well-being was assessed using Rat-Specific Sickness (RSS) score at 6, 16 and 24 h. At 24 h rats were anesthetized, Rat-specific Organ Failure Assessment (ROFA) scores, and mitochondrial respiration were measured in the cortex, cerebellum, hippocampus and striatum. In a second set of experiments, BBB permeability was examined regionally using Evans-blue dye.
Results: Sepsis markedly increased RSS and ROFA scores, significantly decreased mitochondrial respiration, and altered the BBB integrity. CH_4 inhalation applied at 3–6 h of sepsis progression improved RSS scores but had modest effects on ROFA scores and no impact on BBB permeability. Treatment at 21–24 h significantly reduced ROFA levels and decreased BBB leakage across all examined brain regions. Early CH4 treatment also had moderate effect on mitochondrial oxygen consumption, late phase CH4 treatment also significantly increased complex II- and IV-linked oxidative phosphorylation in all examined brain regions. Mitochondrial membrane integrity was also preserved by CH4 inhalation.
Conclusion: Methane inhalation demonstrates significant potential as an adjunctive treatment for sepsis and SAE. Our results highlight a time-dependent efficacy: early CH4 administration provides modest clinical benefits, whereas late-stage intervention more effectively restores organ function and preserves BBB integrity. This suggest that CH4 may be particularly efficacious in maintaining BBB function during the established phase of sepsis.

Anti-inflammatory and neuroprotective effects of inflammasome inhibitors following contusion spinal cord injury

Barnabás Pájer[1]; Krisztián Pajer[1]; Tamás Bellák[1]; Rebeka Kristóf[1]; Zoltán Fekécs[1]; Dénes Török[1]; Ádám Mészáros[1]; Antal Nógrádi[1]
[1]University of Szeged, Anatomy, Histology and Embriology, Laboratory of Neural Regeneration, Szeged

Introduction: Following contusion spinal cord injury, severe neuroinflammation develops in the affected area. Activated inflammasomes play a key role in initiating the pro-inflammatory cascade. The aim of our study was to examine what morphological and functional regeneration can be achieved after spinal cord injury by inhibiting the activation of the NLRP3 inflammasome alone, as well as the combined inhibition of NLRP3 and NLRC4 inflammasomes.
Methods: In our experiments, we induced contusion spinal cord injury at the level of the thoracic segment 10 in Sprague–Dawley rats. Some animals were treated for 4 days with a specific NLRP3 inflammasome inhibitor (MCC950), while others received a dual NLRP3 and NLRC4 inhibitor (C75), administered once daily by intraperitoneal injection at a dose of 10 mg/kg. Control groups received either no treatment or only the vehicle of the administered compounds. In our short-term experiments, we assessed the gene expression of inflammatory markers using qPCR, and mapped microglia/macrophage responses by immunohistochemistry. In long-survival groups, we identified intact/regenerated tracts using retrograde labeling, and evaluated gait using video-based motion analysis.
Results: MCC950 and C75 treatment significantly reduced the expression of the pro-inflammatory genes IL-1β, NLRP3, and caspase-1, while C75 additionally decreased NLRC4 expression in the injured region. The density of microglia/macrophage cells was significantly lower in the treated groups at three and seven days post-injury. The number of retrogradely labeled propriospinal and supraspinal neurons was significantly higher in the treated groups. Our morphological analyses revealed substantial neuroprotection, and our functional assessments showed significant motor improvement in both treatment groups.
Discussion: Based on our results, we conclude that inhibition of the NLRP3 inflammasome alone, as well as the combined inhibition of NLRP3 and NLRC4, significantly reduces neuroinflammation following contusion spinal cord injury. In the long term, this was reflected in the preservation of proprio- and supraspinal pathways and in overall neuroprotection.

On-demand activation of secretagogin expression regulates neuroblast migration and cortical regeneration

Csenge Dr. Tóth-Kőrösi[1,2]; János Dr. Hanics[1,2]; Tibor Dr. Harkány[3,4]; Alán Dr. Alpár[1,2]
[1]*Semmelweis University, Department of Anatomy, Budapest*
[2]*SE NAP B Research Group of Experimental Neuroanatomy and Developmental Biology, Budapest*
[3]*Medical University of Vienna, Department of Molecular Neurosciences, Center for Brain Research, Vienna*
[4]*Karolinska Institutet, Department of Neuroscience, Solna*

Ten thousands of newly born cells move in the rostral migratory stream to reach their target, the olfactory bulb. We previously identified a neuronal population in this pathway which externalize an abolishing enzyme to dismantle the extracellular matrix, thereby promoting neuroblasts in their migration. This mechanism is activity dependent and is regulated by the calcium-sensor protein secretagogin. Here, we show that neuroblasts can exit the stream and find an alternative route towards the prefrontal cortex. We labelled the newly born neuroblasts with BrdU and found that their path is lined by secretagogin-expressing neurons, similarly to the migratory stream. These secretagogin neurons are stationary but can migrate themselves, and their number increases upon system activation. Lesion of the prefrontal cortex increased the number of those neuroblasts which migrate towards the lesioned cortex, whereas the number of neuroblasts moving towards the olfactory bulb remained unchanged. Adult cortex typically lacks secretagogin-expressing neurons. Nevertheless, secretagogin-expression cells appeared in the penumbra as well as along the route leading to it. These secretagogin expressing cells were neither migrating neuroblasts, nor astrocytes, nor microglia. They were in close relation and proximity to BrdU-labelled neurons, likely paving their way towards their destination, the penumbra region. We suggest that secretagogin is expressed on-demand in brain injury and shapes regenerative processes.

Neuroimmune crosstalk and pharmacological modulation of segment regeneration

Gabriella Laura Tóth[1]; János Schmidt[2]; Edit Pollák[3]; Zsolt Pirger[1]; István Fodor[1]; László Molnár[1]
[1]*HUN-REN Balaton Limnological Research Institute, Tihany*
[2]*Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs,*
[3]*Institute of Biology, Faculty of Natural Sciences, University of Pécs, Pécs*

Scarless regeneration in annelids is critically mediated by the central nervous system and by free-floating coelomocytes, analogues of mammalian leukocytes, which migrate to and accumulate in large numbers at the amputation site. However, the exact cellular and molecular mechanisms underlying this process have remained largely unexplored. In vertebrates, immune cells play a key role in neuroimmune crosstalk by producing and responding to classical neurotransmitters. We investigated whether coelomocytes contain classical neurotransmitters and, if so, whether neurotransmitters signals integrated by coelomocytes contribute to segment regeneration.
Using mass spectrometry and targeted (immuno)histochemistry, we detected GABA, glutamate (Glut), dopamine (DA), and serotonin (5-HT) in coelomocytes of the earthworm model, *Eisenia andrei.* Transcriptomic analysis of coelomocytes showed both evolutionarily conserved and lineage-specific features: transcripts encoding Glut decarboxylase, GABAB receptor, 5-HT transporter, DA transporter, 5-HT receptors, and DA receptors were identified, whereas transcripts for tyrosine hydroxylase, tryptophan hydroxylase, aromatic L-amino acid decarboxylase, GABAA receptor, and GABA transporters were not detected. The presence of GABAergic components in coelomocytes suggests an ancient and fundamental role for inhibitory signaling in immune regulation, while the lack of monoamine-synthesizing enzymes indicates that certain aspects of neuroimmune communication evolved later in vertebrates.
Subsequent mass spectrometry analysis of coelomocytes isolated at 0, 3, 7, and 14 days post-amputation showed a significant increase in Glut, 5-HT, and DA levels and a decrease in GABA levels at days 3 and 7, indicating a potential role of neurotransmitters in early stages of regeneration. Pharmacological disruption of neurotransmitter signaling using different concentrations of haloperidol (D1/D2 antagonist), ondansetron (5-HT3 antagonist), GABA, and their mixture resulted in a significant reduction in regenerated segment number and impaired tissue organization.
Our results 1) reinforce the concept that neuroimmune communication is an ancient and evolutionarily conserved feature of animal physiology and 2) demonstrate the key role of coelomocyte-mediated neurotransmitter homeostasis in wound healing and tissue regeneration. Our findings may inform new strategies in regenerative medicine and immune modulation in higher organisms.

Less invasive intraperitoneal delivery of human IL-10 mRNA–LNP promotes morphological and functional recovery after spinal cord contusion injury

Zsombor Perdi[1]; Zoltán Fekécs[1]; Tamás Bellák[1]; Rebeka Kristóf[1]; Dénes Török[1]; Csaba Szigeti[1]; Norbert Pardi[2]; Krisztián Pajer[1]; Antal Nógrádi[1]
[1]University of Szeged, Szent-Györgyi Albert Medical School, Department of Anatomy, Histology and Embryology, Szeged
[2]University of Pennsylvania, Department of Medicine, Philadelphia

Introduction
Spinal cord injury causes extensive tissue damage and loss of motor, sensory, and autonomic functions. Our laboratory previously used intraspinal delivery of human IL-10 mRNA packaged in lipid nanoparticles (LNP) to successfully treat spinal cord contusion injury, achieving significant recovery. Here, we tested whether intraperitoneally administered hIL-10 mRNA–LNP could be taken up by local macrophages, transported to the injured spinal cord, and induce morphological and functional improvements.
Materials and Methods
Contusion injury was induced at the Th6 spinal segment in female Sprague-Dawley rats, followed by intraperitoneal injection of hIL-10 mRNA–LNP. Control animals received no treatment. One and four days post-treatment, immunohistochemical methods were used to map hIL-10-positive macrophages in the injured spinal cord. During the survival period, functional tests (BBB score, video-based motion analysis) were conducted, followed by detailed morphological analysis. Affected tract preservation and regeneration were assessed via retrograde labeling with Fast Blue (FB).
Results
hIL-10-positive macrophages appeared in smaller numbers in the injured spinal cord on day 1 post-treatment and in greater numbers by day 4. Treated animals showed significantly greater functional recovery than controls. Lesion area was significantly smaller in the treated group compared to controls. Retrograde labeling revealed a significant increase in FB-labeled neurons rostral to the injury in the spinal cord, brainstem, and somatomotor cortex, indicating preservation or regeneration of proprio- and supraspinal tracts. The improvements likely stem from modulation of the microglia/macrophage response and astrogliosis extent.
Conclusion
These findings suggest that intraperitoneal mRNA–LNP delivery offers a less invasive yet effective therapeutic strategy for spinal cord injuries.
Funding
Supported by the RNA Innovation Competence Center of the Cluster of Natural Sciences at the Interdisciplinary Centre for Research Development and Innovation Excellence, University of Szeged.

Novel, engineered fusogenic liposome-based anti-oxidant delivery system improves the blood-brain barrier integrity in aging

Boglárka Dr. Csík[1,2,3]; Santny Shanmugarama[3,4]; Roland Dr. Patai[3,4]; Ádám Dr. Nyúl-Tóth[3,4]; Zoltán Dr. Ungvári[1,3,4]
[1]*Faculty of Medicine, Semmelweis University, Institute of Preventive Medicine and Public Health, Budapest*
[2]*Semmelweis University, Health Sciences Program, Doctoral College, Budapest*
[3]*University Of Oklahoma Health Sciences Center, Department of Neurosurgery, Vascular Cognitive Impairment, Neurodegeneration and Healthy Brain Aging Program, Oklahoma City*
[4]*University Of Oklahoma Health Sciences Center, Oklahoma Center for Geroscience and Healthy Brain Aging, Oklahoma City*

Vascular defects contribute to age-related neurodegeneration and cognitive impairment. Blood-brain barrier (BBB) breakdown and neurovascular uncoupling lead to cerebral blood flow (CBF) deficits, neuroinflammation and decreased cognitive function. The naturally occurring polyphenol, resveratrol (RSV) effectively targets cerebromicrovascular endothelial cells *in vitro* and *in vivo*, attenuating age-related oxidative stress and improving vascular health. However, *in vivo* bioavailability of resveratrol is very low, thus our goal was to create delivery systems to increase the efficiency of anti-oxidant, anti-inflammatory polyphenols. We have introduced a novel fusogenic liposomal (FL) delivery system with an engineered protein corona (PC) with specific apolipoproteins (ApoE). Our aim was to harness the protein corona formation to mitigate the negative effects of spontaneous PC formation, and to directly target our fusogenic liposomes to cerebrovascular endothelial cells and to increase uptake, with the ultimate goal of effectively delivering RSV to protect the cerebral microvasculature in aging. Our *central hypothesis* was that endothelial barrier disruption and dysfunction can be effectively reduced by delivery of our novel ApoE-FLs loaded with the antioxidant resveratrol. We characterized the uptake of ApoE-FL by brain endothelial cells *in vitro*, its circulation time and biodistribution *in vivo.* We showed significantly increased APOE-FL-RSV accumulation in brain endothelial cells *in vivo* compared to control liposomes (FL) uptake. We used advanced *in vivo* multiphoton imaging to longitudinally assess the beneficial effects of ApoE-FL-RSV on BBB and CBF; which were significantly improved in aged APOE-FL-RSV treated mice. Thus, we concluded that ApoE-FL-RSV can be utilized as pharmaceutical intervention in age-related vascular impairment and cognitive decline.

Pharmacological inhibition of transient receptor potential ankyrin 1 (TRPA1) ion channel may modulate the electric foot shock-induced acute stress responses in mice

János Konkoly[1]; Viktória Kormos[1]; Erika Pintér[1]; Aya Alakrami[1]; Dóra Zelena[2]; Prabhat Kumar[2]; Balázs Gaszner[3]
[1]*University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, Pécs*
[2]*University of Pécs, Medical School, Institute of Physiology, Pécs*
[3]*University of Pécs, Medical School,, Institute of Anatomy, Pécs*

Introduction:
The centrally projecting Edinger-Westphal nucleus (EWcp) is involved in stress adaptation. Previously, we found that TRPA1, a non-selective cation-channel is expressed by the urocortin1 (UCN1) positive EWcp neurons, and its expression is significantly increased in mice upon acute stress. Thus, we hypothesize that EWcp/UCN1/TRPA1 neurons may regulate acute stress responses, and our aim was to investigate the effect of pharmacological TRPA1 inhibition on the behavioural and neuromorphological changes upon acute stress.
Methods:
Two sets of *Trpa1* wild-type mice were pretreated with intraperitoneally administered vehicle (saline) or TRPA1 antagonist (A-967079) solutions. After 30 minutes, animals were subjected to the electric foot shock model of acute stress, and we parallelly evaluated the freezing behaviour. RNAscope *in situ* hybridization combined with UCN1 immunostaining was performed to assess the expression of *Trpa1* mRNA and the UCN1 peptide content in the EWcp.
Results:
Significantly increased freezing behaviour was observed in both (control/non-shocked and stressed/shocked) antagonist treated groups compared to the vehicles. The shock-induced higher freezing was added to this antagonist effect leading to significant difference between the stressed groups. The *Trpa1* copy number was higher in the antagonist treated controls than in the vehicles; however, no changes were found upon foot shock. There was no basal difference in the UCN1 peptide density between the control groups, but the antagonist treatment decreased significantly the peptide content upon stress.
Conclusions:
The elevated freezing behaviour and the decreased UCN1 content in the antagonist treated mice upon foot shock suggest that TRPA1 may modulate acute stress responses.
Funding:
J.K. was supported by the Research grant of Medical School, University of Pécs (KA-2024-12)

Novel drug candidate binds to delta subunit containing GABAA receptors and improves spatial memory

Orsolya Huzián[1,2]; Lajos István Nagy[2,3]; László Hackler Jr.[2]; Levente Knapp[2,3]; Ágnes Katalin Kocsis[4]; Ildikó Szöts[4]; Gábor Tamás[4]; László Géza Puskás[2,3]; Gábor Molnár[4]
[1]*Astridbio Technologies, Szeged*
[2]*Aperus Pharma, Szeged*
[3]*Avidin Ltd., Szeged*
[4]*University of Szeged, Department of Physiology, Anatomy and Neuroscience, HUN-REN-SZTE Research Group for Cortical Microcircuits, Szeged*

The hydroxyquinoline derivative Q134R is a promising drug candidate for the treatment of Alzheimer's disease with cytoprotective and cognition-enhancing properties. Radioligand binding assays showed that Q134R interacts with the TBPS binding site of GABA receptors. Electrophysiological recordings in mouse brain slices revealed that Q134R significantly increased tonic inhibitory currents in cortical neurogliaform and dentate gyrus granule cells, both known to express delta subunit-containing GABAA receptors. This effect was abolished in mice deficient in the GABAA delta subunit confirming the delta subunit dependency of Q134R's action. Furthermore, in a scopolamine-induced amnesia model, Q134R treatment significantly improved spatial memory performance in wild-type mice, but not in mice lacking in the delta subunit. These results suggest that Q134R enhances tonic inhibition through delta subunit-containing GABAA receptors, which may also play a role in mediating memory, which could serve as a protective mechanism in early-stage neurodegenerations such as Alzheimer's disease. These effects likely contribute to its broader therapeutic efficacy, potentially complement its previously reported interactions with signaling pathways such as NFAT and HIF-1, which are also implicated in Alzheimer's disease pathology.

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

Erika Eliza Kvak[1]; Szidónia Farkas[1]; Adrienn Szabó[1]; Dorottya Várkonyi[1]; Evelin Szabó[1]; Réka Szabó[1]; Dóra Zelena[1]
[1]University of Pécs, Medical School, Institute of Physiology, Behavioural Physiology and Stress Research Group, Pécs

Introduction: Alzheimer's disease (AD) is a degenerative disorder, and the most typical cause of dementia. Dehydroepiandrosterone and its water-soluble form, dehydroepiandrosterone-sulphate (DHEAS) are endogenous steroid hormones, which in vitro have neuroprotective effect. However, little is known about their in vivo efficacy as well as their timing and dosage. Based upon their chemical structure a single injection might have long-lasting effects. Indeed, an acute DHEAS treatment diminished morphological alteration 48h later. However, we could not detect behavioural effect 30 min after administration, therefore our aim was to study a later (24h) timepoint.
Methods: Seven-months-old male and female 3xTg-AD (B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) and wild-type control mice were treated intraperitoneally with DHEAS and compared to vehicle treatment (10mg\10mL/kg). To test learning and memory Y-maze and conditioned fear tests (CFT) were applied. After termination females' hippocampus were analysed by qPCR to determine the differences in the levels of mAPP, hAPP, and Iba-1 expression.
Results: In Y-maze test 3xTg-AD animals moved less than wild type and females had worse working memory than males. In the CFT, trauma reduced exploratory behaviour and increased time spent freezing. The 3xTg-AD animals moved less and spent more time freezing than wild-types. By qPCR we confirmed the presence of human amyloid precursor protein in 3xTg-AD mice only, without any influence of either the trauma or DHEAS treatment. However, the 3xTg-AD mice not only had higher mouse APP mRNA level, but DHEAS treatment showed a tendency ($p=0.079$) to reduce this level. The mRNA level of the microglia marker Iba-1 were increased by shock and there was a tendency for DHEAS to reduce this level ($p=0.054$) independently from genotype.
Conclusion: At behavioural level the single DHEAS injection 24h later was not able to correct behavioural alterations. However, there was a tendency to diminish neuroinflammation and amyloid accumulation. Further studies with other timepoints are still needed to explore the full cognitive potential of DHEAS.

Treatment of beta-amyloid-induced increased tonic conductance and cognitive deficits by an alpha 5 GABA inverse agonist

Aoife O Connell[1]; Beatriz Calvo-Flores Guzman[2]; Cameron Keighron[3]; Ying Zhai[1]; Jordi Boix[2]; Katie Peppercorn[4]; Warren Tate[4]; Henry Waldvogel[2]; Richard Faull[2]; Johanna Montgomery[2]; Leo Quinlan[3]; Andrea Kwakowsky[1]
[1]University of Galway, Pharmacology and Therapeutics, Galway
[2]University of Auckland, Auckland
[3]University of Galway, Physiology, Galway
[4]University of Otago, Dunedin

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder characterized by cognitive impairment, which may arise from disruptions in the excitatory/inhibitory balance within the brain. Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system, plays a crucial role in maintaining the excitatory/inhibitory balance and regulating neuronal activity involved in memory. In AD, changes in alpha 5 GABA A receptor (a5GABAAR) expression and activity increase tonic inhibition, disturbing the neuronal excitatory/inhibitory balance and ultimately impairing cognitive processes. Therefore, targeting a5GABAAR offers a promising therapeutic strategy. This study examined the potential of an a5GABAAR-selective inverse agonist, a5IAs, in treating beta-amyloid-induced cognitive deficits and the mechanism underlying this using ex vivo microelectrode array and patch clamp electrophysiology. a5IA significantly reduced beta-amyloid-induced long-term spatial memory deficits and long-term potentiation. a5IA reversed beta-amyloid-induced increases in neuronal excitability, as indicated by input-output curves, and mitigated elevated tonic conductance. These findings highlight a5IA's ability to restore beta-amyloid-induced cognitive function and underscore that targeting a5-GABAARs with inverse agonists may be a promising and important direction for the development of novel therapies for the treatment of AD, as well as for further drug development to identify the ideal a5-GABAAR-targeting drug.

Cell type-specific rearrangement of perisomatic inhibition in the valproate model of autism

Judit M. Veres[1]; Anita Varga[2,3]; Reéb Zsófia[1,3]; Viktor Román[2,4]; Norbert Hájos[1,5,6]
[1]HUN-REN Institute of Experimental Medicine, Laboratory of Network Neurophysiology, Budapest
[2]Gedeon Richter Plc., Laboratory of in vivo Pharmacodynamics, Budapest
[3]ELTE Eötvös Loránd University, Institute of Biology, Doctoral School of Biology, Budapest
[4]Semmelweis University, Richter Department, Budapest
[5]Indiana University Bloomington, The Linda and Jack Gill Center for Neuroscience, Bloomington
[6]Indiana University Bloomington, Department of Psychological and Brain Sciences, Program in Neuroscience, Bloomington

Autism spectrum disorder (ASD) is characterized by disrupted excitatory/inhibitory balance, with evidence suggesting interneuron dysfunction across cortical networks. This study investigated the cell type-specific changes in perisomatic inhibition in the valproate (VPA) mouse model of autism, focusing on two major interneuron populations: parvalbumin-positive basket cells (PVBCs) and cholecystokinin-positive basket cells (CCKBCs). Pregnant mice received VPA at embryonic day 12.5, and male offspring demonstrated core ASD-like behavioral phenotypes, including decreased ultrasonic vocalizations, increased tactile sensitivity, and reduced sociability. Using immunohistochemistry and confocal microscopy, we observed an increased density of total neurons but a specific decrease in PV-expressing interneurons in the basolateral amygdala of VPA-treated mice. Analysis of perisomatic inhibitory terminals revealed a significant reduction in PV-positive bouton density around principal neurons, while CB1 receptor-positive boutons (representing CCK interneuron terminals) showed no change. Functional validation through optogenetic stimulation of PV cells and whole-cell recordings from BLA principal neurons confirmed decreased PV-mediated inhibitory input number with steeper input-output relationships in VPA-treated mice. These findings indicate a cell type-specific rearrangement of perisomatic inhibition in the BLA of VPA-treated mice, with specific weakening of PV-mediated inhibition that may contribute to amygdala hyperreactivity in autism. This interneuron-specific dysfunction may represent a potential target for therapeutic interventions in autism spectrum disorders.

Local Microglial Activation in a Subretinal Hemorrhage Mouse Model

Boglárka Balogh[1,2]; Marietta Zille[3]; Loretta Péntek[1,2]; Béla Völgyi[1,2,4]; Tamás Kovács-Öller[1,2,4]
[1]*Szentágothai Research Centre, University of Pécs, Pécs*
[2]*Institute of Biology, Faculty of Sciences, University of Pécs, Department of Neurobiology, Pécs*
[3]*University of Vienna, Division of Pharmacology and Toxicology, Department of Pharmaceutical Sciences, Vienna*
[4]*NEURON-066 Rethealthsi Research Group, Pécs*

Subretinal hemorrhage (SRH) is caused by the accumulation of blood between the retina and the pigment epithelium (RPE) or between the RPE and the choroid. It can occur spontaneously, but is frequently associated with age-related macular degeneration, hypertension, diabetes, or head trauma. Understanding the mechanisms underlying SRH and its effects on the retina is crucial for developing effective treatments.
We developed a novel technique for modelling SRH by co-injecting blood and a dye-coupled tracer, Cholera toxin subunit B-Alexa555 (CtB-A555) into the subretinal space of C57BL6 mice, to better localize and understand the disease and how it can cause microglial activation and inflammation. To visualize the microglial activation, we used Iba1 marker for post-hoc immunohistochemical staining and ex vivo imaging.
Our results show that microglia change morphology. They were clustered at the injection site and in the zones neighboring the blood injection. In contrast, the non-affected areas of the same eye did not exhibit these morphological changes, while SHAM-PBS injection had less effect on microglia. These findings provide evidence of microglial activation in response to SRH. Utilizing advanced imaging techniques, we were able to accurately localize the affected regions, which included not only the immediate retinal area over the blood clot but also the neighboring regions. Furthermore, in wild-type C57BL6 mice, we observed that microglia exhibited high motility in ex vivo retinal samples post-SRH induction. These microglia actively phagocytosed blood and tracer molecules while surveying the trauma site.

In conclusion, our novel SRH model using CtB-A555 co-injection provides a valuable tool for studying the pathophysiology of subretinal hemorrhage and its impact on retinal microglia. The ability to precisely localize and monitor microglial activation opens new avenues for therapeutic research and treatments in SRH and related retinal conditions.

Early Effects of Traumatic Brain Injury on Retinal Ganglion Cells and Microglia in a Mouse Model

Loretta Péntek[1]; Endre Czeiter[2,3]; Krisztina Amrein[2,3]; Boglárka Balogh[1]; Dorina Puskás[1]; Béla Völgyi[1,2]; Tamás Kovács-Öller[1,2]
[1]*Faculty of Sciences, University of Pécs, Pécs*
[2]*János Szentágothai Research Centre, University of Pécs, Pécs*
[3]*Medical School, University of Pécs, Pécs*

Background: Traumatic Brain Injuries (TBI) are serious, often fatal, neurological injuries that can affect not only the brain but also the retina, which is closely connected to it through the optic nerve. Retinal ganglion cells (RGCs) and the axons that emerge from them play a key role in vision, and their damage can lead to visual impairment. Microglia, as immune cells of the CNS, respond quickly to injury by initiating inflammatory processes, and therefore may be potential biomarkers. Since the retina is easily accessible and easy to study, it is an ideal model to map the changes in the nervous system caused by TBI.
Aims: The aim of our research was to explore cellular and functional changes in the retina following TBI after 24 and 48 hours, to examine neuroinflammation and neurodegeneration.
Methods: TBI was induced in laboratory mice with the Marmarou model, and retinal changes were examined after 24 and 48h. Spontaneous RGC activity was monitored by ex vivo calcium ion imaging, while microglia activation and detection of cell death were analyzed by immunohistochemical staining. Microglia movement was detected by time-lapse videos.
Results: Based on our results, TBI alters calcium signaling in RGCs, which is associated with increased spontaneous neuronal activity. In parallel, microglia are activated in both layers of the retina. 1 day after TBI, the level of active-Casp3 in the cells increased compared to the SHAM-control group. However, 24h after TBI, activity was no longer visible, presumably because the ganglion cells were undergoing apoptosis.
Conclusions: Our results suggest that signs of brain injury also appear in retinal cells. Spontaneous RGC activity increased 24h post-TBI, but decreased below control levels 48h later, indicating cell death. Significantly increased microglial activation, suggesting the presence of inflammatory processes in the retina.

Developing cell-based bioassays for clinical trials – the use of patient-derived induced neurons to study autophagy in the Fell-HD clinical trial

Kinga Vörös[1]; Dimitrios Apostolopoulos[2]; Souha Klibi[3,4]; Győző Szenci[5]; Anna A. Abbas[1]; Ágnes Varga[1,4]; Roland Zsoldos[1,4]; Balázs Kis[1]; Bendegúz Sramkó[1]; Lea Danics[1]; Shaline Fazal[2]; Szabolcs Takáts[5]; Csaba Kerepesi[3,4]; A. Roger Barker[2]; Karolina Pircs[1,4,6]
[1]HCEMM-SU, Institute of Translational Medicine Semmelweis University, HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Budapest
[2]University of Cambridge, Cambridge; [3]HUN-REN SZTAKI, Budapest
[4]HUN-REN-SZTAKI-SU Rejuvenation Research Group, Budapest
[5]Eötvös Loránd University, Budapest; [6]Lund University, Lund

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG expansions in the huntingtin gene (HTT). These expansions produce mutated huntingtin protein (mHtt). HD is incurable and typically presents in mid-life. It progresses to death over a 20-year period. Autophagy, a lysosomal degradation pathway that ensures cytoplasmic homeostasis, is dysfunctional in HD, thus contributing to mHTT protein accumulation.

Preclinically, it has been shown that felodipine can upregulate autophagy and clear protein aggregates in cells, including neural cells in HD. Thus a phase II clinical trial was undertaken (Fell-HD) to assess the tolerability and feasibility of testing this drug in patients with early-stage HD while also looking for any signal of efficacy. Given we cannot look at autophagy in the living human brain, we sought to do this using induced neurons (iN) directly reprogrammed from skin fibroblasts from the FELL-HD participants. Transdifferentiated iNs keep the genetic and aging signatures of the donor bypassing any stem cell or neuroprogenitor phase during conversion. We converted 7 control and 18 Fell-HD patient-derived fibroblasts to iNs with the same conversion efficiency and purity. DNA methylation array and analysis in iNs showed accelerated aging in some patients. Moreover, most HD-iNs showed a less elaborate neuronal morphology and increased HTT expression using qPCR. We used 0.1 µM and 1 µM felodipine treatment for 24h to assess its effects. After 28 days of conversion followed by Felodipine treatment iNs were fixed and counterstained using neuronal (TAU) and autophagy markers (p62, LC3, LAMP1) to determine neuronal morphology and subcellular autophagy changes using high-content automated screening microscopy. Additionally, HTT measurements were again performed using qPCR after treatment. Our results showed that Felodipine enhanced autophagy in only a subset of patients while having no obvious adverse effects on HD-iNs. Lastly, we compared and correlated our preclinical results with FELL-HD trial and found a correlation between autophagy impairments and patient-specific treatment response.

In summary, this project, utilizing an in vitro preclinical iN model, presents a novel approach to investigating pathways targeted by drugs that cannot be studied in the living human brain, thereby opening up a new dimension in testing agents in the clinic.

Chemogenetic inhibition of focal epilepsy in an optogenetic mouse model

Péter Sere[1]; Anna Zalatnai[1]; Tamás Kitka[2]; Péter Barthó[1]
[1]*HUN-REN TTK, KPI, Alvási Oszcillációk Kutatócsoport, Budapest*
[2]*CERATe Therapeutics, Budapest*

We used a recently developed acute mouse model to investigate the potential cortical and subcortical targets for preventing seizure generalization. The model is based on optogenetic stimulation of the layer 6. corticothalamic pathway in NTSR1-cre or NTSR1-ChR mice. After a brief kindling period, tonic-clinic seizures can be instantly and repeatedly evoked by a brief pulse train (15s, 8Hz) in the primary somatosensory cortex, mimicking an epileptic focus. Inhibiting the cortical induction site or the somatosensory thalamus by tetrodotoxin succefully arrested seizures, while primary motor cortex inhibition did not prevent the seizure generalization. Finally, we tested chemogenetic inhibition of the induction site, using both traditional DREADD, as well as a custom G-protein coupled receptor newly developed by CREATe Therapeutics. Administration of respective actuators for either receptor effectively prevented induced seizures.

Investigation of the role of gap junctions in the generation of synchronous physiological and epileptiform activity in human neocortical tissue, in vitro.

Amirmahdi Mojtahedzadeh[1,2]; Hanga Dormán[2]; Estilla Zsófia Tóth[2]; Réka Bod[2]; Gábor Nagy[3]; Attila Bagó[3]; László Halász[3]; Loránd Erőss[3]; Johanna Szabó[3]; Dániel Fabó[3]; István Ulbert[2,3,4]; Lucia Wittner Wittner[2,3,4]; Kinga Tóth[2]
[1]*Semmelweis University, Budapest*
[2]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Center for Natural Sciences, Budapest*
[3]*Centre of Neurosurgery and Neurointervention, Semmelweis University, Budapest*
[4]*Faculty of Information Technology and Bionics, Péter Pázmány Catholic University, Budapest*

Gap junctions (GJ) have a role in shaping both physiological and pathological synchronies. A relation between gap junctions (GJs) and epileptiform activity has been found in animal studies. GJ-blockers decrease epileptiform activity suggesting that GJ communication has a synchronizing effect during seizure-like activity.
Here we examine the effect of the GJ-blocker carbenoxolon on the synchronization mechanisms during physiologically occurring spontaneous population activity (SPA) and during bicuculline (BIC)-evoked epileptiform activities in *in vitro* neocortical slice preparations from postoperative brain tissues of epileptic and non-epileptic patients. We performed local field potential gradient recordings.
In case of non-epileptic samples, SPA disappeared in 50% of the slices and decreased in frequency (to 21%) and amplitude (to 28%) in 50% of cases. In epileptic samples a total blockade of events was not observed. The frequency and amplitude decreased in half of the slices (to 53- and 55%), in the rest the amplitude increased (to 128%). In non-epileptic tissues carbenoxolon could not block the evoked epileptiform activity, but in 40% of the slices decreased it. In 60% of the slices the amplitude of the events increased (to 134%).
Our results indicate that GJ-blockade alters both physiological and pathological network synchrony in human neocortical tissue. SPAs were most sensitive in non-epileptic samples, whereas epileptic tissue showed heterogeneous or compensatory responses.

Comparative study of the locus coeruleus in wild-type and PACAP gene knock-out mice with aging

Marcell Schmidt[1]; Dániel Pham[1]; Balázs Dániel Fülöp[1]; Balázs Gaszner[1]; Tünde Tóth[1]; Adél Jüngling[1]; Dóra Reglődi[1]; Andrea Tamás[1]
[1]University of Pecs Medical School, Department of Anatomy, HUN-REG-PTE PACAP Research Team, Centre for Neuroscience, Pécs

The neuroprotective effect of pituitary adenylate cyclase-activating polypeptide (PACAP) has been demonstrated in several models of Parkinson's disease, and its deficiency is known to lead to accelerated aging processes and increased vulnerability in PACAP knockout (KO) mice. In our previous studies, we described age-dependent neuronal loss and an increase in the number of microglia in the substantia nigra (SN) and ventral tegmental area (VTA), which are the nuclei of the dopaminergic system, in PACAP KO mice. The aim of our present study was to observe age-related morphological changes in the locus coeruleus (LC), which, as the nucleus of the noradrenergic system, is also involved in the pathogenesis of Parkinson's disease.
Our studies were performed on young (4 months old) and aged (1.5 years old) wild-type (WT) [n=5-4] and PACAP KO [n=8-6] mice. Noradrenergic neurons in the LC were labeled with the enzyme tyrosine hydroxylase (TH), and microglia were labeled with the Iba1 marker. Microglial activity was classified based on morphological criteria.
In 1.5-year-old PACAP KO mice, we detected a significant decrease in TH+ cell numbers with age and compared to wild-type animals of the same age. We observed a significant increase in the number of microglia with age in PACAP KO mice, which were morphologically inactive.
Our results suggest that in the absence of endogenous PACAP, age-related neurodegenerative processes are also accelerated in the locus coeruleus. The observed differences are consistent with the changes previously observed in the SN and VTA regions, supporting the broad neuroprotective and immunoregulatory role of PACAP. Furthermore, the involvement of the LC suggests that in the absence of endogenous PACAP, not only the dopaminergic but also the noradrenergic system becomes more vulnerable, which also supports the hypothesis that endogenous PACAP may play an important role in the mechanism of age-related neurodegeneration.

The effect of cholinergic cell manipulation on learning and memory consolidation in female triple transgenic Alzheimer model mice

Dorottya Csiszár[1]; Adrienn Szabó[1]; Dorottya Várkonyi[1]; Erika Eliza Kvak[1]; Szidónia Farkas[1]; Dóra Zelena[1]
[1]Medical School, University of Pécs, Institute of Physiology, Laboratory of Behavioural and Stress Research, Pécs

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, affecting learning and memory. The cholinergic system plays a central role in modulating these functions, however, the exact process is not fully understood. We were focusing on the medial septum (MS) of the basal forebrain, and our aim was to reveal the behavioural consequences of the manipulation of the MS cholinergic neurons. We utilized the 3xAD-ChAT-Cre strain, which was created by crossbreeding the triple transgenic 3xTg-AD line with ChAT-Cre animals, bearing a Cre recombinase enzyme in the cholinergic cells and showing progressive AD-related pathology. Targeting the MS cholinergic cells in 9-month-old females, chemogenetic technique was used with stimulatory and inhibitory DREADD (designer receptor exclusively activated by designer drug) sequences delivered by an adeno-associated viral vector (AAV). The animals were tested for short-term (Y-maze), spatial (Morris water maze, MWM) and working memory (Radial Arm Maze) as well as on an object recognition task (Novel Object Recognition). The model was working properly as we observed the accumulation of pathological hallmarks in the brain of the 3xAD-ChAT-Cre mice and the red fluorescent proteins, co-delivered with DREADDs, was present in the MS cholinergic cells. The effect of chemogenetic manipulation was not equivocal: the modulation either improved or impaired performance in certain tasks.
Our results indicate that MS cholinergic modulation influences memory processes in a task-specific manner and could potentially affect AD-related hippocampal pathology.

Survival of human grafted neuronal progenitor cells in human cortical organotypic slice cultures

Rebeka Stelcz[1,2]; Vera Deli[1]; Eszter Juhász[1]; Attila Bagó[3]; Loránd Erőss[3]; Boglárka Hajnal[3]; István Ulbert[1,3,4]; Ágota Apáti[1]; Lucia Wittner[1,2,3]
[1]*HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Semmelweis University Doctoral School, Budapest*
[3]*Semmelweis University Clinics of Neurosurgery and Neurointervention, Budapest*
[4]*Péter Pázmány Catholic University Faculty of Information Technology and Bionics, Budapest*

Organotypic slice cultures derived from postoperative human brain tissue offer a unique long-term model for studying neuronal development and integration under near-physiological conditions. This system supports tissue viability for several weeks, enabling investigations of optogenetic tools and stem cell integration.
In this study, GFP-expressing human induced pluripotent stem cell (iPSC) derived neural progenitor cells (NPCs) were injected into human cortical organotypic slices. Their survival, differentiation, and integration into the existing neuronal network were analysed using confocal microscopy. To enhance integration, we optimised the culturing medium composition, method, and timing of the injection.
In most cases, the NPCs initiated neuronal polarisation by growing neuronal protrusions; however, round cells with glial morphology were also observed. NPCs survived mainly at the margin of the tissue and at the injection site, while some migrated deeper into the tissue. These findings demonstrate that NPCs can survive and develop neuronal phenotypes in human cortical slice cultures for up to five weeks without the need for additional trophic factors.
Based on our preliminary findings, our system appears to be a promising platform for studying cell integration and circuit formation in stem cell-based therapies.

Investigating the pathological changes of neuromuscular junctions and perisynaptic immune processes in the TDP-43(A315T) transgenic mouse model of ALS

Boglárka Batka[1,2]; Orsolya Horváth[1,2]; Kinga Molnár[1,2]; Bernát Nógrádi[1,2]; Péter Klivényi[1,2]
[1]*Albert Szent-Györgyi Medical School, University of Szeged, Department of Neurology, Szeged*
[2]*Hungarian Research Network, HUN-REN-SZTE Neuroscience Research Group, Szeged*

Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disorder that results in the loss of muscle strength. Apart from the degeneration of upper and lower motoneurons, NMJ denervation plays a pivotal role in the disease. While systemic immune dysregulation has been implicated in ALS, it remains unclear whether immune-mediated processes contribute to NMJ denervation. Our aim was to characterize NMJ denervation and investigate the inflammatory mechanisms that might contribute to NMJ loss in ALS.
Methods: Experiments were performed on late-symptomatic (12–15-week-old) TDP-43A315T transgenic hemizygous male mice and wild-type (WT) littermates (n=5-5). Five skeletal muscles were investigated: three hindlimb muscles (*gastrocnemius* (GC), *tibialis anterior* (TA), *soleus* (SOL)), and two forelimb muscles (*biceps* (BIC), and *triceps* (TRC)). NMJ denervation and morphological changes were assessed via immunohistochemistry techniques and semi-automated NMJ-morph quantification. Immune cell infiltration was detected with immunostaining (CD45) and quantified by cell counting technique.
Results: The GC and TA muscles showed the highest ratio of denervated NMJs (21,7% and 13,8%) in transgenic TDP-43A315T mice, compared to WT controls. This result correlated with increased presence of polyinnervation and sprouting in the GC and TA muscle. Single-NMJ morphometry revealed that presynaptic alterations (decreased axon diameter, nerve terminal perimeter and area) were detectable in all the skeletal muscles we investigated. Postsynaptic parameters were also partially affected, suggesting secondary changes due to chronic denervation. In the innervation zone of the GC muscle, we observed robust immune cell infiltration.
Conclusions: Our results indicate that the TDP-43A315T mouse model displays hindlimb-dominant NMJ denervation pattern, however, presynaptic alterations were observed in all investigated muscles. The elevated immune cell count in the innervation zone of the GC muscle suggests that inflammatory processes might be key factor in ALS pathomechanism, warranting further investigation in search of potential therapeutic targets.

Investigating the effects of C1q on neuronal cells and its co-occurance with presynaptic activity markers

Márta Pőcze[1]; Vilmos Tóth[1]; Vanda Tukacs[2]; Stefánia Pap[2,3]; Dominik Mátyás[2,3]; Tamás Molnár[1]; Gábor Juhász[2]; Katalin A. Kékesi[2]; József Kardos[1]
[1]Eötvös Loránd University, Department of Biochemistry, ELTE NAP Neuroimmunology Research Group, Budapest
[2]InnoScience Hungary Ltd., Mátranovák
[3]Eötvös Loránd University, Department of Anatomy, Cell and Developmental Biology, Doctoral School of Biology, Budapest

C1q, the first element of the classical complement cascade selectively binds to synapses - as an „eat-me” signal for microglial phagocytic elimination - that show signs of local apoptotic changes, autophagy and mitochondrial stress. In pathological conditions, accompanied by excessive synapse loss, elevated concentrations of C1q can be observed in the CNS. However, it remains to be elucidated, to what extent C1q binds falsely to active, functional synapses in these diseased states.
Besides its opsonizing role, C1q also performs signalling pathway initiator functions. This includes its observed influence on autophagy and apoptosis in various cell types.
Here, we present our results from an ongoing study, on whether C1q leads to disease progression by incorrect opsonization or by non-opsonizing mediatory effects, using synaptosome cytometry to examine C1q's co-occurrence with presynaptic activity markers and via observing effects of C1q treatment of neuronal cells.

Trifluoperazine protects brain slices from hypoosmotic Injury

Melinda Szabó[1]; Rita Frank[1,2]; Ákos Menyhárt[1,2]; Eszter Farkas[1,2]
[1]Department of Cell Biology and Molecular Medicine, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Szeged
[2]Hungarian Centre of Excellence for Molecular Medicine, Cerebral Blood Flow and Metabolism Research Group, Szeged

Cerebral edema after acute brain injury is tightly linked to spreading depolarizations (SDs) and to changes in aquaporin-4 (AQP4) water channels in astrocytic endfeet. Trifluoperazine (TFP), a classical antipsychotic and calmodulin antagonist, has recently been shown to alter AQP4 expression and subcellular localization, suggesting potential anti-edema effects. We investigated whether TFP attenuates cytotoxic edema and SD, modulates AQP4 expression, and preserves tissue integrity in an osmotically stressed mouse brain slice model.
Acute coronal brain slices from adult C57BL/6 mice were perfused with hypoosmotic medium (199 mosm/L; HM60), which induced spontaneous SDs and tissue swelling, followed by an SD triggered by transient anoxia. SDs and tissue swelling were monitored by intrinsic optical signal imaging. Slices were incubated with vehicle or TFP (10 μM), subsequently subjected to TTC staining and NeuN, GFAP and AQP4 immunohistochemistry, and the stained sections were evaluated by quantitative image analysis.
HM60 induced marked tissue swelling, which was reduced by approximately half with TFP. Spontaneous SDs were less frequent and delayed by TFP compared with HM60 alone (953±463 s vs. 696±336 s; HM60+TFP vs. HM60), and latency of anoxia-induced SDs was also prolonged (71±38 s vs. 42±30 s; HM60+TFP vs. HM60). The cortical surface area invaded by SDs was reduced by TFP for both spontaneous (41.5±12.4 % vs. 56.7±13.8 %; HM60+TFP vs. HM60) and anoxia-induced SDs (42.0±11.3 % vs. 67.9±12.8 %; HM60+TFP vs. HM60). TTC staining and NeuN labeling showed better preserved tissue integrity under TFP (NeuN-positive area fraction 10.15±2.31 % vs. 7.83±2.36 %; HM60+TFP vs. HM60), whereas GFAP-positive astrocytic area remained similar (4.37±2.17 % vs. 5.18±4.85 %; HM60+TFP vs. HM60). In contrast, AQP4-immunopositive area was markedly reduced after TFP treatment (1.88±0.72 % vs. 3.66±0.96 %; HM60+TFP vs. HM60).
These findings indicate that TFP reduces SD occurrence and associated tissue injury while suppressing astrocytic AQP4 expression in cortical tissue under hypoosmotic challenge. This supports pharmacological modulation of astrocytic AQP4 as a promising therapeutic approach against SD-associated cytotoxic brain edema.

Optimized Cyclopean Steady-State VEP Pipeline for Objective Assessment of Human Stereopsis

János Radó[1]; Eszter Mikó-Baráth[1]; Péter Hegyi[1]; Gábor Jandó[1]; Péter Buzás[1]
[1]*University of Pécs Medical School, Institute of Physiology, Pécs*

Background: Objective electrophysiological assessment of stereopsis is essential in populations where behavioral testing is unreliable, such as young children or non-verbal patients. Cyclopean stimuli, including dynamic random dot correlograms (DRDC) and stereograms (DRDS), selectively engage binocular cortical mechanisms but optimal stimulation and data analysis parameters for steady-state visual evoked potentials (ssVEPs) remain insufficiently defined.

Methods: We recorded EEG responses from 22 healthy adults with normal stereo vision while presenting DRDC and DRDS stimuli using anaglyphic dichoptic separation. Stereo specific features of the stimuli alternated periodically at three temporal frequencies (0.94, 1.88, and 3.75 cycles/s). ssVEP responses were evaluated across occipital and parietal electrodes using circular T2 statistics at the fundamental and second harmonic frequencies. Monocular control conditions were applied to confirm stereo specificity.

Results: DRDC stimuli alternating at 1.88 cycles/s elicited statistically significant ssVEP responses in all participants when occipital and parietal electrodes were considered, and in over 90% using occipital electrodes alone. DRDS stimuli also produced robust responses but showed greater inter-individual variability and stronger reliance on parietal channels. Control measurements confirmed that the observed responses depended on intact binocular processing. Frequency composition differed by stimulus type, with DRDC responses dominated by the fundamental harmonic and DRDS responses by the second harmonic.

Conclusions: Full-field DRDC stimuli at approximately 2 cycles/s provide a highly reliable electrophysiological marker of stereopsis using minimal electrode configurations. These findings support the clinical and research application of optimized ssVEP paradigms for objective assessment of binocular visual function.

Transcriptomic alteration of the medial preoptic area in socially isolated rats

Tamara Hajdu[1]; Tamás Láng[1]; Fanni Dóra[1,2]; Botond Drahos[1]; Réka Kovács[1]; Árpád Dobolyi[1]
[1]*Semmelweis University, Anatomy, Histology and Embryology, Laboratory of Neuromorphology, Budapest*
[2]*Semmelweis University, Human Brain Tissue Bank, Budapest*

Social interactions are essential for adaptive behavior; their disruption induces long-lasting behavioral and neurobiological changes. Short-term deprivation may elevate social behaviours as a homeostatic regulation, while long-term isolation may have detrimental consequences. The medial preoptic area (MPOA), a key hypothalamic hub for social motivation, exhibits poorly understood molecular plasticity in response to social experience. Therefore, we combined behavioral phenotyping with transcriptomics to study the effects of 1-week social isolation in male rats.
Social behavior was assessed via direct social interaction, sociability, and social novelty tests, evaluated both manually and using an AI-based analysis. Isolated rats exhibited increased social investigatory behavior, characterized by elevated social sniffing and a stronger preference for familiar conspecifics, but a reduced time spent with novel animals and a lower social novelty index, indicating impaired social flexibility. AI metrics strongly correlated with manual evaluation, confirming their robustness.
RNA sequencing of punched MPOA samples from 12 controls and 12 isolated rats identified 263 downregulated and 27 upregulated genes (|log2 fold change|>0.5, adjusted $p<0.05$), indicating widespread transcriptional suppression. GO, Reactome and KEGG enrichment analyses highlighted purinergic and G protein–coupled receptor signaling, neuropeptide-mediated communication, inhibitory neurotransmission, and synaptic organization. Co-expression and STRING protein-protein interaction network analyses revealed coordinated transcriptional modules.
Subsequent qRT-PCR validation, correlating strongly with RNA-seq data (Pearson $r=0.8472$, $p<0.0001$), validated 11 downregulated and 1 upregulated gene. Downregulated genes included purinergic and prostaglandin receptors (*Gpr63, Gpr87, Gpr171, Ptger3*), as well as neuromodulatory, peptide receptor activity, and inhibitory components (*Gpha, Cck, Gabrd, F2rl2*), synaptic organization-related genes (*Ntng1, Cpne9*), and reduced cholinergic signaling capacity, including decreased *Chrna3* expression. The upregulated *Pcp4l* links to calcium signaling and excitability, suggesting a compensatory response.
These findings suggest that social isolation leads to a social phenotype characterized by increased social drive but reduced novelty preference, accompanied by coordinated transcriptional reorganization in the MPOA.

Novel prognostic biomarkers to detect disease progression for personalised ALS patient care

Zalán Kádár[1,2,3]; Rebeka Kristóf[1]; Ádám Mészáros[1]; Péter Klivényi[2,3]; Bernát Nógrádi[2,3]; Antal Nógrádi[1]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Department of Anatomy, Histology and Embryology, Laboratory of Neural Regeneration, Szeged
[2]Albert Szent-Györgyi Medical School, University of Szeged, Department of Neurology, Szeged
[3]Hungarian Research Network, University of Szeged, HUN-REN-SZTE Neuroscience Research Group, Szeged

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder and the most common motoneuron disease. It is primarily characterized by the loss of motor neurons, resulting in progressive muscle weakness. In later stages, the disease causes respiratory failure, which is the leading cause of death among patients. ALS exhibits high heterogeneity both in its clinical and pathomechanical features, which contributes to limited understanding of the underlying pathomechanistic events, and unsuccessful therapeutic trials. In addition to the limited efficiency of disease-modifying therapies, the lack of biomarkers remains a significant challenge in managing the disease. Systemic immune changes are considered to be major contributor to disease progression in ALS, thus we hypothesized that inflammatory cytokines might be promising prognostic biomarkers in ALS.
Blood samples from 42 individuals (31 ALS patients and 11 healthy controls) were used for RNA isolation. Gene expression changes of 19 major inflammatory genes were quantified with qPCR technique. Disease progression was quantified with the Revised ALS Functional Rating Scale and correlated with gene expression.
The gene expression of major components of multiple inflammasome pathways (AIM2, NLRC4, NLRP6, casp1, PYCARD, IL1b, IL18 and GSDMD genes) showed moderate-strong positive correlation with rate of ALSFRS-R decline. These results suggest that the elements of the inflammasome pathway might serve as potential prognostic biomarkers for disease progression in ALS, although further investigation on protein expression changes are needed.

Investigation of the effects of a short PACAP fragment in ischemic retinopathy

Inez Bosnyak[1]; Viktoria Lili Bauer[1]; Alexandra Vaczy[1]; Edina Szabo[1]; Dorottya Molitor[1]; Balazs Meresz[1]; Olivia Orosz[1]; Tamas Atlasz[2]; Gabor Toth[3]; Dora Reglodi[1]
[1]Department of Anatomy, HUN-REN-PTE PACAP Research Team, Medical School, University of Pecs, Pecs
[2]Department of Sports Biology and Kinesiology, Faculty of Sciences, University of Pecs, Pecs
[3]Institute of Medical Chemistry, University of Szeged, Szeged

Insufficient blood supply to the retina can quickly lead to vision loss or even blindness. The most common eye diseases today are caused by oxygen deficiency in the retina. The pathomechanism of these diseases is not fully understood, and there is still no highly effective, long-term, non-invasive therapeutic option. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide known for its cell-protective and anti-inflammatory effects, among other things. Of its three receptors, the PAC1 receptor is primarily responsible for its protective functions.
The aim of our study was to investigate a short, cyclic PACAP fragment that binds to the PAC1 receptor in an ischemic retinopathy mouse model.
Ischemic retinopathy was induced by permanent unilateral common carotid artery occlusion (UCCAO) in 4-5-month-old CD1-IGS mice. Half of the animals received PACAP1-5 eye drops twice a day for 28 days (Control-Systane, n=13; Control-PACAP, n=13; UCCAO-Systane, n=10; UCCAO-PACAP, n=10). The thickness of the retinal layers was examined by optical coherence tomography (OCT) on days 0 and 28. At the end of the experiment, the retinas were isolated, and immunohistochemical staining was performed on whole-retina preparations and cryostat sections. Furthermore, the level of 17 apoptotic factors was analyzed.
Based on our OCT measurements, the thickness of the nerve fiber layer decreased significantly as a result of oxygen deprivation ($p=0.015$), but this difference was not observed in the PACAP-treated group ($p=0.172$). The degeneration was not limited to the axons of the ganglion cells; as by day 28 the number of ganglion cells had also decreased significantly ($p=0.004$). The eye drops used did not prevent the significant decrease, but they did moderate the extent of the damage ($p=0.024$). Furthermore, calbindin expression in the horizontal cell area was significantly lower in the UCCAO group compared to the control group ($p<0.01$), but this change did not occur in the PACAP-treated group. These findings were also supported by the difference in the level of numerous apoptotic factors following PACAP1-5 treatment.
Based on our results, the short PACAP fragment examined may be a promising therapeutic molecule in the treatment of ischemic retinopathy.

Evaluation of the effect of the interleukin-1 receptor antagonist Anakinra© in a translational model of inflammation-sensitized hypoxic-ischemic encephalopathy

Renáta Fábián[1]; Ferenc Domoki[1]; Gyöngyi Kis[2]; Marietta Ábel[1]; Gábor Remzső[1]; Emma Balog[1]; Éva Rózsa[1]; Valéria Tóth-Szűki[1]; Viktória Kovács[1]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Department of Physiology, Experimental Neonatology Research Group, Szeged
[2]Faculty of Science and Informatics, University of Szeged, Department of Physiology, Anatomy and Neuroscience, Szeged

Introduction Hypoxic-ischemic encephalopathy (HIE) is a brain injury most commonly elicited by perinatal asphyxia (PA) that is often combined with maternal/fetal inflammatory states that can further worsen HIE mortality and the occurrence of adverse neurologic outcomes. The activation of the interleukin-1 receptor (IL-1r)/MyD88 pathway was found to be important in determining neuronal injury in rodent HIE models previously. In the present study, we first aimed to characterize a novel translational large-animal HIE model by eliciting brain injury with simulated PA combined with inflammation. Second, we tested whether Anakinra©, a human recombinant endogenous IL-1r antagonist exerts neuroprotection in this swine model.
Materials and methods Anesthetized, mechanically ventilated, normothermic newborn (postnatal day 1) pigs were divided into three experimental groups: PA, lipopolysaccharide (LPS)+PA, and LPS+PA+anakinra (n=8-7-6). PA was elicited with ventilation of a hypoxic-hypercapnic gas mixture for 20 minutes. LPS (1 µg/kgbw/h, iv) was given for 1.5h starting 1h before PA onset. Anakinra (10 mg/kgbw, sc) was given twice: at the 1st and the 24th hours after completion of PA. Animals were intensively monitored for 48 hours after PA. The animals were then euthanized and brains were harvested. Neuronal injury in various PA-vulnerable regions were determined by neuropathological examination of photomicrographs obtained from H&E stained sections using light microscopy. Furthermore, the mRNA abundance of IL-1beta and MyD88 were also determined in the same regions using RTqPCR.
Results LPS significantly increased PA-induced neuronal injury in most of the examined brain regions. However, Anakinra© in the applied dose did not exert a significant neuroprotective effect. For instance, the ratio of injured neurons in the hippocampal CA1 region was 26±3% vs. 56±11%* vs. 55±9%* (PA vs. LPS+PA vs. LPS+PA+anakinra, *p<0,05 vs. PA). We also found similarly enhanced expression of both IL-1beta and MyD88 in both LPS-treated groups compared to the PA group.
Discussion Our swine model replicates inflammation-induced sensitization of the newborn brain to PA thus it provides an appropriate translational model to study this aspect of HIE development. Further studies are required to establish whether pharmacological interventions targeting the IL-1/MyD88 pathway could confer significant neuroprotection.

APOE Genotype Profiling in Alzheimer's Disease Patients to Support iPSC-Based Investigations

Orsolya Pantl[1,2]; Edit Szabó[1]; Ágota Apáti[1]; Balázs Sarkadi[1]
[1]*Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Doctoral School, Semmelweis University, Budapest*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by neuronal loss, accumulation of amyloid-β plaques, tau pathology, and cognitive decline. Genetic factors strongly influence disease susceptibility, with the apolipoprotein E (APOE) gene representing the most prominent risk locus. The three major APOE alleles (ε2, ε3, ε4) are defined by two SNPs (rs429358 and rs7412), which introduce amino acid substitutions at positions 112 and 158 and underlie the protective effect of ε2, the neutral status of ε3, and the risk-enhancing role of ε4.
Using TaqMan-based genotyping, we analyzed APOE allele distribution in a cohort of AD patients and observed a significantly higher frequency of the ε4 allele compared with age-matched controls. The same genotyping approach was applied to DNA isolated from induced pluripotent stem cells (iPSCs), enabling clear identification of wild-type, heterozygous, and homozygous genotypes and facilitating direct investigation of patient-specific genetic backgrounds in vitro.
Building on these findings, we aim to develop iPSC-derived three-dimensional (3D) culture systems and blood–brain barrier (BBB) models to more faithfully recapitulate key aspects of AD pathophysiology. These platforms will support detailed analysis of disease-relevant cellular and molecular processes, allow precise evaluation of drug penetration across the BBB, and provide a testbed for potential therapeutic strategies. Integrating APOE genotyping with iPSC-based in vitro modeling establishes a translational framework for studying the mechanisms underlying Alzheimer's disease and for advancing preclinical research and personalized therapeutic development.

Behavioral consequences of the chemogenetic silencing of the ventral tegmental area in rats I.: Psychomotor vigilance and general motivation

Angelika Bodó[1,2]; Zsolt Kristóf Bali[1,2]; Nóra Bruszt[1]; Zsóka Mária Ábel[1,2,3]; Fruzsina Kovács[1,2,3]; István Hernádi[1,2,3]

[1]*University of Pécs, Szentágothai Research Centre, Translational Neuroscience Research Group, Pécs*

[2]*University of Pécs, Grastyán Translational Reserach Center, Pécs*

[3]*University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs*

The ventral tegmental area (VTA) plays an important role in psychiatric disorders via mesolimbic and mesocortical dopaminergic pathways, making it an ideal target for our experiments. We aimed to silence the VTA using the chemogenetic DREADD method and examined the induced symptoms from a behavioral pharmacological perspective. We stereotaxically injected 500 nl (n=10) and 300 nl (n=6) adeno-associated viral vectors into the VTA brain region of adult male LH rats that expressed hM4Di receptor and mCherry reporter gene in our target neurons under the control of hSyn promoter. 30 minutes prior to the experiments, the animals were treated subcutaneously with 3 doses of deschloroclozapine (DCZ; 0.03/0.1/0.3 mg/bwkg) actuator or vehicle (VEH). Attentional functions and general motivation were assessed using the psychomotor vigilance task (PVT). We also administered bupropion (5/15 mg/bwkg) in combination with DCZ for pharmacological validation . Then, we administered high DCZ doses for 5 consequential days as subacute treatment and for 10 days as subchronic treatment to investigate the longer-term effects of chemogenetic VTA suppression.

In the PVT task, the hM4Di group performed significantly more premature trials with a reduction in the number of missed trials following acute DCZ treatment. These effects were further enhanced by combination treatment with bupropion. Subchronically treated animals showed signs of developing DCZ tolerance as on the treatment days the differences between groups have diminished, while on the post-treatment days the number of missed trials significantly increased in the hM4Di group.

Taken together, acute VTA silencing resulted in a phenotype corresponding to hypomania-like behavior and sensitivity towards dopaminergic treatment. This contradiction may be explained by the more prominent hM4Di transfection in GABAergic compared to dopaminergic VTA neurons. In light of the above assumption, the sings of tolerance observed during subacute and subchronic treatment and the increasing post-treatment passivity may be interpreted as the result of increased compensatory mechanisms. These will confirmed by immunohistochemical detection of neuron-type specific hM4Di transfection and dopamine transporter overexpression, respectively. Our present results are consistent with the dopaminergic theory of bipolar disorder (BD), and thus, VTA silencing may become a relevant novel preclinical model for the investigation of BD.

Investigating the first and second hits in disease development: the vasopressin-deficient (AVP-Cre/Cre) genetic background and prenatal valproate-exposure affects early development of rat pups

Katalin Várallyai[1]; Evelin Szabó[1]; Kristóf László[1]; Dóra Zelena[1]
[1]*University of Pécs, Medical School, Center for Neuroscience, Institute of Physiology, Szentágothai Research Center, Behavioral Physiology and Stress research group, Pécs*

INTRODUCTION: Vasopressin (AVP) has been implicated in the patomechanism of several psychiatric conditions including autism spectrum disorder (ASD). AVP-deficiency in Brattleboro rats altered early development, suggesting the impact of the first, genetic hit. Surprisingly, the AVP-Cre rat line created with the aim to manipulate AVPergic cells, showed also sign of AVP-deficiency. However its effect is not equivocal on development. Moreover, it is not clear, how AVP-deficiency might influence the development of maternal valproate (VPA)-induced ASD-like symptoms, as second hit.
METHODS: Offsprings of maternally VPA-treated (500mg/kg ip., on postpartum day 13) AVPCre/Cre homozygous and normal Sprague-Dawley rats were observed between postnatal days 3-15. Somatic parameters (body weight and length), developmental milestones (eye opening, pinna detachement, ear canal opening, teeth abruption) and somatomotor reflexes (righting reflex, turning/seeking reflex, cliff avoidance, negative geotaxis) were followed. The animals were sacrificed on postnatal day 18 and their kidneys were examined for *V1a* and *V2* receptor mRNA by qPCR.
RESULTS: The AVP-deficiency in this rat line was previously confirmed by enhanced water consumption as well as lower *Avp mRNA* on several hypothalamic areas in a separate adult cohort. In pups we have found a slower somatic development in the AVP-Cre animals (body weight and length), while VPA-treatment lead to a shorter, broken tail in both genotypes. Among the developmental milestones there were no differences in pinna detachement, but VPA-treated animals accelerated the ear canal opening. We detected both genotype and sex-differences in eye opening, teeth abruption, and turning/seeking reflex. The *Avp* receptor mRNA levels were not different between the groups. This might be due to the mixed, unbalanced sexes, as in adults *V2* mRNA levels were lower only in males.
CONCLUSION: In accordance with the results from the Brattleboro rat line, AVP-deficiency in general seems to affect early development. The VPA-treatment also had impact without a clear interaction between the two factors.

The histone deacetylase inhibitor SAHA restores blood-brain barrier integrity in a human stem cell-based model of ischemic stroke

Koppány Párdi[1]; Anikó Szecskó[1,2]; Zuhao Cui[1,2]; Gergő Porkoláb[1]; Zsófia Hoyk[1]; Csilla Kovács[1]; Janet Folasade Adegbite[1]; Nárcisz M. Cser[1]; László Dér[3]; Krisztina Nagy[3,4]; Virág Fodor[1]; Mária A. Deli[1]; Szilvia Veszelka[1]
[1]HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Biological Barriers Research Group, Szeged
[2]University of Szeged, Doctoral School of Biology, Szeged
[3]HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Biophotonics and Biomicrofluidics Research Group, Szeged
[4]University of Szeged, Department of Experimental Physics, Institute of Physics, Szeged

Ischemic stroke is characterised by acute cerebrovascular occlusion, blood-brain barrier (BBB) breakdown and narrow therapeutic window. Its treatment is a clinical challenge due to the risk of reperfusion injury and limited efficacy of thrombolytic therapies, therefore, novel therapeutic approaches are needed. Histone deacetylase inhibitors (HDACi) have emerged as neuroprotective agents in stroke models, but their effect on preserving BBB integrity is still unexplored. Our aim was to investigate the effects of the HDACi suberoylanilide hydroxamic acid (SAHA) on BBB changes in a cell culture model of ischemic stroke.
In our experiments, the effects of SAHA were tested on a human BBB co-culture model under normoxia and during a 24-hour reoxygenation (OGD/R) following a 6-hour oxygen-glucose deprivation (OGD).
SAHA promoted BBB protection against OGD/R by increasing transendothelial electrical resistance and decreasing BBB permeability. SAHA also increased the level of tight junction protein claudin-5, and several ECM components associated with as glycocalyx and basement membrane. Moreover, SAHA downregulated proliferation, had a significant impact on endothelial cell morphology, and upregulated non-canonical Wnt signalling.
Our results suggest that SAHA could be a potential therapeutic drug for the treatment of ischemic stroke via BBB protection. Since SAHA has already been approved for human use as the anticancer drug vorinostat, its repurposing to restore BBB functions and prevent post-stroke damages may be greatly facilitated.

Peripherally induced acute neuroinflammation leads to electrophysiological and proteomic changes in the visual system of rats

Stefánia Krisztina Pap[1,2,3]; Dániel Mittli[1,2]; Dominik Mátyás[1,2,3]; Vanda Tukacs[3,1]; Éva Hunyadi-Gulyás[4]; Zsuzsanna Darula[4,5]; Katalin Adrienna Kékesi[3,1,2]; Gábor Juhász[3,1]
[1]ELTE Eötvös Loránd University, Institute of Biology, Laboratory of Proteomics, Budapest
[2]ELTE Eötvös Loránd University, Institute of Biology, Department of Physiology and Neurobiology, Budapest
[3]InnoScience Ltd., Mátranovák
[4]HUN-REN Biological Research Centre, Core Facility Proteomics Research Group, Szeged
[5]Hungarian Centre of Excellence for Molecular Medicine, Single Cell Omics Advanced Core Facility, Szeged

Peripheral immune activation can induce neuroinflammation, affecting not only higher cognitive functions, but also basic sensory functions such as vision. In this study, we investigated how acute systemic inflammation influences retinal and cortical visual processing in rats and explored underlying molecular changes using retinal proteomic analysis. Male Wistar rats were treated with intraperitoneal injections of lipopolysaccharide (LPS; 2 mg/kg), indomethacin (10 mg/kg) or a combination of the two. We performed self-controlled experiments, and baseline visual responses were recorded before treatment. We recorded electroretinograms (ERG) and visual evoked potentials (VEP) in freely moving rats during single and repetitive (10 and 20 Hz) light stimulation. LPS treatment caused a reduction in ERG amplitudes within 8 hours, whilst enhancing cortical VEP responses. Indomethacin alone had minimal effects; however, combined LPS + indomethacin treatment increased retinal responses, indicating anti-inflammatory modulation of visual alterations. Proteomic analysis of retinal samples revealed widespread changes in protein expression in the LPS and LPS + indomethacin groups, suggesting the underlying molecular mechanisms of inflammation-related functional alterations. Our findings demonstrate that peripheral inflammation significantly affects retinal and cortical visual activity and concomitant proteome level changes contributing to the visual dysfunction induced by peripheral immune challenge.

TRPA1 modulates urocortin 1 turnover in the centrally projecting Edinger-Westphal nucleus in a CGRP-induced migraine model

Ammar Al-Omari[1]; Péter Poszovácz[1]; Balázs Gaszner[2]; Viktória Kormos[1]
[1]*University of Pécs Medical School, Department of Pharmacology and Pharmacotherapy, Pécs*
[2]*University of Pécs Medical School, Department of Anatomy, Research Group for Mood Disorders, Pécs*

The urocortin 1 (UCN1)-expressing neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) regulate the function of migraine-related brain areas *via* direct urocortinergic connections. In the central nervous system, EWcp/UCN1 neurons uniquely co-expresses transient receptor potential ankyrin 1 (TRPA1) cation channel, which has also been linked to migraine. Here we aimed to investigate whether central TRPA1 receptors regulate the EWcp/UCN1 neurons' response to migraine.
The intraperitoneal calcitonin gene related peptide (CGRP) injection model of migraine was implemented and validated using light-dark box and von Frey assays in wild-type (WT) and TRPA1 knockout (KO) male mice. RNAscope *in situ* hybridization and immunofluorescence were used to examine the *Ucn1*, *Trpa1* mRNA expression and UCN1 peptide content in the EWcp. FOS immunohistochemistry was performed to assess acute neuronal activation in the EWcp and the antinociceptive lateral periaqueductal gray matter (lPAG).
CGRP administration induced light aversion, periorbital hyperalgesia and increased FOS immunoreactivity in the lPAG in both genotypes supporting the model validity. Additionally, *Trpa1* deficient mice exhibited reduced sensitivity to light, regardless of the treatment conditions. In the EWcp, CGRP treatment increased FOS immunosignal and *Ucn1* mRNA expression in both genotypes. Moreover, in WT mice, the treatment increased the EWcp UCN1 peptide and *Trpa1* mRNA levels, with no such changes observed in *Trpa1* KO animals. These findings suggest a possible role of central TRPA1 in migraine by regulating UCN1 dynamics in the EWcp. Targeting TRPA1 ion channels through pharmacological interventions may offer a new strategy for migraine treatment.

The response of peptidergic neurons in the Edinger-Westphal nucleus to chronic stress is modulated by pregnancy

Dániel Hegedüs[1]; Zsófia Havasi[1]; Zsófia Cezarina Németh[2]; Viktória Kormos[2]; Balázs Gaszner[1]
[1]*University of Pécs Medical School, Department of Anatomy, Research Group for Mood Disorders, Pécs*
[2]*University of Pécs Medical School, Department of Pharmacology and Pharmacotherapy, Pécs*

Obligatory peptidergic cocaine- and amphetamine-regulated transcript (CART) and urocortin 1 (UCN1) co-expressing neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) have been implicated in the stress adaptation, mood control, energy homeostasis and also in nest-building behavior of pregnant mice. Earlier studies have shown that these neurons express oxytocin receptors, and also estrogen receptors, but the existence of progesterone receptor (*Pgr*) has not been confirmed at mRNA level. To the best of our knowledge, no study has been published so far on the role of EWcp in response to chronic stress exposure during pregnancy. Therefore, we aimed at investigating the EWcp in the chronic variable mild stress (CVMS) model during pregnancy.
Hundred female C57BL6J mice were housed for one night with males. Half of the animals were subjected to the CVMS model for three weeks, while the other half served as controls. Pregnancy developed in 25% of control mice and 15% in CVMS-exposed animals. On the day before the expected delivery, mice were perfused and the brains were extracted. RNAscope in situ hybridization combined with immunofluorescence was performed on sections from the EWcp area. We examined the *Pgr, Ucn1, Cart* mRNA expression, moreover we semi-quantified the FOSB, CART, and UCN1 immunoreactivities.
We confirmed the co-existence of *Pgr* mRNA in EWcp CART/UCN1 neurons. Chronic stress increased the expression of *Pgr* and *Cart* mRNAs in not pregnant mice in contrast to pregnant animals where no change occurred. Interestingly, the *Cart* mRNA was also increased by pregnancy, but the CVMS exposure during pregnancy downregulated *Cart* in the EWcp. The FOSB neuronal activity and UCN1 peptide immunoreactivity increased in non-pregnant CVMS-exposed mice only. Pregnancy, but not CVMS exposure increased the CART peptide immunoreactivity in the female EWcp.
We conclude that the response of EWcp neurons to CVMS alters during pregnancy. The presence of progesterone receptors in the EWcp suggests that during pregnancy increased progesterone level may modulate the stress responsivity of the EWcp. UCN1 and CART peptides were differentially regulated. The CVMS-induced rise of *Cart* mRNA expression turned into a downregulation in pregnant mice, suggesting that CART may have a greater significance in the modulation of EWcp response to CVMS is pregnancy. Our data suggest that the deleterious effects of chronic stress during pregnancy may be mediated by the EWcp/CART.

Lost in Translation: A Single Developmental Assumption Led to a Decade of Timing Bias in Avian ASD Models

Paulina Hoppa[1]; Gergely Zachar[1]
[1]*Semmelweis Egyetem, Anatómiai, Szövet- és Fejlődéstani Intézet, Budapest*

The clinical translation of findings from animal models remains largely unsuccessful, particularly in the study of mental and neuropsychiatric disorders. This limitation is exacerbated by interspecies differences in neural organization and by insufficient consideration of evolutionary divergence. Comparative, multi-species approaches may improve translational validity by enabling the identification of conserved neurobiological mechanisms.

Valproic acid (VPA), an antiepileptic drug, has been shown to induce autism spectrum disorder (ASD)–like phenotypes, including impaired social behavior, in children exposed during embryonic development. Consequently, VPA-based ASD models have been developed in several species, including rodents, primates, zebrafish, and birds. However, optimal dosing and the identification of developmentally sensitive treatment windows in some species remain unresolved.

The present study aimed to compare VPA-induced ASD models across species by evaluating effect sizes associated with the strongest reported behavioral outcome, typically measures of sociability. A major methodological difference among models concerns the timing of VPA exposure: in rodents, treatment is usually administered at embryonic day (ED) 12.5–13.5. The VPA model was first adapted to domestic chickens by Nishigori et al. (2013), who identified ED14 as the effective treatment day, a protocol later extended to zebra finches using ED9 based on the established developmental equivalence between avian species.

We conducted a meta-analytic comparison of standardized effect sizes (Cohen's *d*) across species and found that VPA-induced impairments in social behavior are significantly smaller in birds than in mammals. Notably, most avian studies published since 2013 appear to employ a treatment window corresponding to a substantially more advanced developmental stage than that used in rodent models. While limited evidence suggests that such late exposure can induce mild ASD-like phenotypes in mammals, further experimental work is needed to establish developmentally appropriate treatment windows in birds. Based on cross-species comparisons of embryonic development, a more homologous window in avian models is likely to occur around ED 4–4.5 in both zebra finches and domestic chickens.

Impaired Thermoregulation in Female 3xTg-AD Mice: NK3 Receptor–Dependent Responses

Dorottya Várkonyi[1,2]; Erika Eliza Kvak Kvak[1,2]; Evelin Szabó[1,2]; Szidónia Farkas[1,2]; Choi Muyong[1]; Dóra Zelena[1,2]
[1]*Universiry of Pécs, Medical School, Institute of Physiology, Pécs*
[2]*University of Pécs, Szentágothai Research Centre, Centre for Neuroscience, Pécs*

Alzheimer's disease (AD) disproportionately affects women after menopause, suggesting a critical role for sex-specific factors in disease vulnerability. In addition to cognitive decline, AD is associated with systemic disturbances, including impaired thermoregulation. As its manipulation might have therapeutical consequences, it is import the reveal thermoregulatory alterations during AD progress.
To do so core body temperature (cBT) was continuously monitored using implantable telemetry in 3xTg-AD and wild-type (WT) females during cold and warm exposure tests and following acute administration of senktide, a selective NK3R agonist mimicking hot flushes. Ovariectomy (OVX) was used to accelerate disease progression.
Cold exposure induced comparable cBT responses across genotypes and hormonal states, with no significant differences observed. During warm exposure, intact 3xTg-AD females exhibited an exaggerated compensatory thermoregulatory response compared to WT controls. This genotype-related difference was no longer observed following OVX. Senktide treatment induced a significant reduction in cBT in WT females, consistent with NK3R-mediated vasodilatory thermoregulation, whereas this response was significantly smaller in 3xTg-AD mice. Ovariectomy significantly altered senktide-induced temperature dynamics and further accentuated genotype-dependent differences.
Quantitative PCR analysis of brown adipose tissue revealed no significant differences in the expression of uncoupling protein 1 (UCP1) or iodothyronine deiodinase 2 (Dio2); however, a marginally elevated expression of the β3-adrenergic receptor (Adrb3) was detected, suggesting subtle alterations in sympathetic regulation of peripheral thermogenesis.
Together, these findings demonstrate female-specific and hormone-dependent disruptions of thermoregulation in the 3xTg-AD model and highlight impaired NK3R-mediated pathways as a potential contributor to systemic physiological dysregulation in Alzheimer's disease.

SIRT3 deficiency unmasks EMPA-mediated modulation of Sirtuin expression following MPTP-induced neurotoxicity

Orsolya Horváth[1,2,3]; Kinga Molnár[1]; Péter Klivényi[1]
[1]University of Szeged, Albert Szent-Györgyi Medical School, Department of Neurology, Szeged
[2]University of Szeged, Doctoral School of Clinical Medicine, Szeged
[3]HUN-REN-SZTE Functional Clinical Genetics Research Group, Hungarian Research Network, Szeged

Introduction
Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, characterized by progressive dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc). Sirtuins (SIRT1-7) are NAD^+-dependent deacetylases that regulate key cellular processes, and have been implicated in neurodegeneration such as PD. Epidemiological studies suggest that individuals with type II diabetes (T2DM) have a higher risk of developing PD and may experience faster disease progression. Empagliflozin (EMPA) is a selective sodium glucose cotransporter-2 (SGLT2) inhibitor used to treat T2DM. EMPA exhibits emerging neuroprotective effects attenuating dopaminergic loss, and motor deficits in PD models. Therefore, the aim of this study was to assess the effects of EMPA in a MPTP-induced PD model while examining the mRNA expression of SIRTs, with particular emphasis on determining how the absence of SIRT3 influences EMPA efficacy.
Material and Methods
Experiments were carried out on 10 weeks old male wild-type (WT) and SIRT3 knockout (SIRT3KO) mice (WT control n=10, WT MPTP-treated n=8, WT MPTP+EMPA-treated n=9, SIRT3KO control n=5, SIRT3KO MPTP-treated n=9, SIRT3KO MPTP+EMPA-treated n=8). On the first day of experiment the animals received 15 mg/kg MPTP intraperitoneally in every two hours, in total 5 injections. From second day, animals were treated with 15 mg/kg EMPA per os for 21 days, once in a day. Twenty-four hours after the last EMPA treatment, brain samples were collected for SIRT1-7 mRNA expression analysis using real-time polymerase chain reaction (RT-PCR).
Results
In the striatum MPTP treatment elicited a modest downregulation of SIRT1 and SIRT6, while significantly suppressed SIRT2 ($p<0.05$), SIRT3 ($p<0.01$), SIRT4 ($p<0.01$), and SIRT5 ($p<0.05$) expression in WT animals. In SIRT3KO mice, MPTP reduced the expression of SIRT1, SIRT2, SIRT4, SIRT5, and SIRT6. EMPA treatment did not prevent MPTP-induced sirtuin decline in WT animals but restored, and in some cases enhanced, the expression of SIRT1 ($p<0.001$), SIRT2 ($p<0.05$), SIRT4 ($p<0.05$), SIRT5 ($p<0.001$), and SIRT6 ($p<0.01$) in SIRT3KO animals.
Conclusion
EMPA selectively enhanced the expression of SIRTs in the absence of SIRT3, indicating that its modulatory effects depend on existing sirtuin deficiencies. These findings suggest that EMPA may engage compensatory regulatory pathways to restore sirtuin network homeostasis under conditions of SIRT3 loss.
Funding: Thematic Excellence Programme TKP2021-EGA-32

Advancing Two-Photon Calcium Imaging in Glioma Research Through Open-Hardware and Open-Software Innovations

Gergely Katona[1]; Andrea Slézia[1]; András Dávid[2]; Ábel Petik[3]; Dániel Hillier[3]; Péter Nagy[4]; Kornélia Szebényi[5]; István Ulbert[6]; András Füredi[4]; Attila Kaszás[1]
[1]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Multimodal Neurotechnology Res.Group, Budapest
[2]Budapest University of Technology and Economics, Budapest
[3]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Visual Systems Neuroscience Res. Group, Budapest
[4]HUN-REN Research Centre for Natural Sciences, Institute of Molecular Life Sciences, Drug Resistance Research Group, Budapest
[5]HUN-REN Research Centre for Natural Sciences, Institute of Molecular Life Sciences, Metabolic Drug-interactions Research Group, Budapest
[6]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Integrative Neuroscience Research Group, Budapest

Glioblastoma multiforme (GBM) is the most aggressive form of brain cancer, with a median survival of approximately 15 months. Current standard treatments—including surgical resection, chemotherapy, electrotherapy, and anti-epileptic medication—have not produced substantial improvements in clinical outcomes over the past two decades. It has become increasingly evident that the tumor and the surrounding cortical tissue form a unique, self-reinforcing interaction that greatly worsens tumor progression. While several structural and molecular aspects of this interaction have been described, relatively few studies have investigated how the functional activity of peritumoral brain tissue is altered.
In our work, we set out to characterize functional changes in peritumoral cortical circuits using in vivo two-photon calcium imaging. We selected the mouse visual cortex as the experimental model because its local computations, intracortical connectivity, and cell-type–specific responses to diverse visual stimuli are well characterized in the healthy brain. Developing this paradigm requires us to overcome two major methodological challenges.
First, sensitive two-photon measurements must be performed during visual stimulation, which introduces significant stray-light contamination. To solve this, we developed the Photon Shield, an open-hardware light-shielding system consisting of a 3D-printed headplate and a rail-mounted cylindrical objective shield that can be assembled in under 90 seconds. The system provides robust and – importantly – highly reproducible protection against stimulation-induced stray light, enabling artefact-free imaging.
Second, accurate interpretation of neural activity requires tools for validating the output of widely used calcium-signal analysis algorithms, particularly CaImAn's CNMF and OnACID pipelines. For this purpose, we developed Pluvianus, a stand-alone graphical user interface for visual exploration and quality control of CaImAn results. Publicly available on GitHub, Pluvianus facilitates transparent curation, improves the reliability of extracted neural signals, and supports community-driven development.
Together, these methodological advances—light-artefact suppression with the Photon Shield and robust signal-quality assessment with Pluvianus—represent important steps toward accurately characterizing functional alterations in GL291-implanted Thy1-GCaMP6s mouse peritumoral cortical circuits.

CCL2-CCR2 axis as a driver of neuromuscular denervation in amyotrophic lateral sclerosis

Bernát Nógrádi[1,2,3]; Kinga Molnár[4,1]; Rebeka Kristóf[4]; Orsolya Horváth[1]; Yu-Ting Huang[2,3]; Zara Ridgway[2,3]; Amaia Elicegui[5]; Sandra Fuertes-Alvarez[5]; Sonia Alonso-Martin[5]; Gábor J. Szebeni[6]; Nikolett Gémes[6]; Abdullah Ramadan[2,3]; Hannah L. Smith[2,3]; István A. Krizbai[4]; Roland Patai[4]; László Siklós[4]; Péter Klivényi[1]; Helena Chaytow[2,3]; Thomas H. Gillingwater[2,3]
[1]University of Szeged, Department of Neurology, Szeged
[2]University of Edinburgh, Edinburgh Medical School: Biomedical Sciences, Edinburgh
[3]Euan MacDonald Centre for Motor Neuron Disease Research, Edinburgh
[4]HUN-REN Biological Research Centre, Institute of Biophysics, Szeged
[5]Biogipuzkoa Health Research Institute, Stem Cells and Aging Group, San Sebastian
[6]HUN-REN Biological Research Centre, Core Facility, Laboratory of Functional Genomics, Szeged

Systemic immune changes have been implicated in amyotrophic lateral sclerosis (ALS), but precise mechanisms and cellular targets remain unknown. Neuromuscular junction (NMJ) denervation is a major pathophysiological event in ALS, but it is unclear whether immune system dysregulation contributes to this process. Here, we report leukocyte and macrophage infiltration in ALS patient-derived skeletal muscle biopsies. We also found immune cell infiltration in the skeletal muscle of hTDP-43 and TDP-43M337V transgenic mice, occurring from pre-symptomatic stages and targeted to NMJ-enriched muscle regions. Proteomic analysis implicated the CCL2-CCR2 axis as a driving factor in this immune process. CCL2+ cells were enriched around NMJs in hTDP-43 mice, and in ALS patient skeletal muscle. Local treatment with CCL2-neutralising antibodies or normal IgG antibodies in hTDP-43 mice reduced leukocyte infiltration and ameliorated NMJ denervation. These results demonstrate that the CCL2-CCR2 axis drives immune cell infiltration targeting NMJs in ALS, identifying a potential avenue for therapeutic intervention to prevent NMJ denervation.

Thalamocortical network mechanisms underlying absence epilepsy in a humanized mouse model

Zsombor Juhász[1]; Aletta Magyar[1,2]; Péter Berki[1]; Péter Barthó[3]; Ferenc Mátyás[1,2]; Sándor Borbély[1,3]
[1]*HUN-REN Institute of Experimental Medicine, Neuronal Network and Behaviour Research Group, Budapest*
[2]*Semmelweis University, Semmelweis University Doctoral School, Budapest*
[3]*HUN-REN Research Centre for Natural Sciences, Sleep Oscillation Research Group, Budapest*

Absence epilepsy is common form of pediatric epilepsy that can persist in adulthood. It characterized by brief seizures arising from abnormal thalamocortical oscillations called spike-and-wave (SWD) discharges, which often linked to altered GABAergic inhibitory transmission. Still, underlying network mechanism of the generation and maintenance of SWDs are elusive.
The GABAA R43Q genetic mouse model of a human genetic epilepsy, harboring a single gene mutation in the GABAA g2 subunit, recapitulates absence-like phenotypes, thus, provides an ideal platform to investigate these issues. Utilizing in vivo extracellular recordings in R43Q mice and cross-correlation analysis of thalamic relay cell firing, we identified an abnormal intrathalamic connection: direct excitatory influence between thalamic cells. Furthermore, clustering coefficient analysis revealed high clusterization also within thalamic and cortical subnetworks, suggesting an increased small-world property that renders the network susceptible to seizure generation.
Since R43Q mutation reportedly reduces the cell surface expression of GABAA receptors containing g2 subunit, the phasic/tonic inhibition might be also imbalanced. Using in vitro patch clamp experiments we found that, tonic inhibition was also decreased in R43Q mice.
Finally, optogenetic manipulation of the layer 6 corticothalamic (L6 CT) pathway – possessing modulatory influence over network states – was tested on spontaneous cortical activity and seizures in freely moving and awake head-fixed mice. Sustained optogenetic stimulation of this pathway blocked ongoing cortical activities and SWDs. However, the offset effect was dependent on the actual cortical sleep/wake state. In light and deep sleep, the direct inhibition was followed by short sleep-spindle-like oscillations. In REM and awake states, SWDs could be emerged. Short stimulations of L6 CT cells resulted in biphasic activation in thalamic and cortical cells, while sustained stimulus elicited offset rhythmic firing in both cortical pyramidal and fast-spiking interneurons.
In summary, our data revealed that both thalamic and cortical networks undergo subtle modifications by forming abnormal connections and altered inhibitory transmissions within the thalamocortical network, potentially increasing the network's susceptibility to generating SWDs. Furthermore, we demonstrate that SWDs can be effectively blocked by activating the L6 corticothalamic pathway in a state-dependent manner.

Bursting Neuronal Activity In Vivo: Insights from Pre-Surgical Microelectrode Recordings in Patients with Drug-Resistant Epilepsy

Ágnes Kandrács[1,2]; Brieg Oude[1]; Anthony Pinto[1]; Bertrand Mathon[1,3]; Katia Lehongre[1]; Valerio Frazzini[1,4]; Vincent Navarro[1,4]
[1]Paris Brain Institute, Paris
[2]HUN-REN Research Centre for Natural Sciences, Budapest
[3]Pitié-Salpêtrière Hospital, Neurosurgery Department, Paris
[4]Pitié-Salpêtrière Hospital, Epilepsy Unit, Reference Center for Rare Epilepsies, Paris

The project aims to analyse neural activity in mesial temporal structures recorded with microelectrodes during intracranial monitoring in patients with drug-resistant epilepsy. Single-unit activity (SUA) can be isolated from long-term recordings, and neurons can be classified as interneurons (IN) or pyramidal cells (PC). Here, we investigated whether bursting neurons can be identified and characterised *in vivo* in patients with mesial temporal lobe epilepsy.
Intracranial macroelectrodes combined with research microelectrodes were implanted in mesial temporal structures in 25 patients. Local field potentials (LFP), SUA, and multi-unit activity (MUA) were recorded continuously for 2–3 weeks (24/7). Single units were isolated using standard spike-sorting methods during awake periods, and neuronal types were determined from action potential waveform features. Bursting behaviour was then analysed at both the SUA and MUA levels.
We isolated single-unit spikes from MUA, classified neurons as IN or PC, and applied a new burst definition to SUA and MUA. This revealed synchronous bursting across units and may help refine spike sorting. Neurons can be characterised by burstiness, as well as intraburst amplitude dynamics and interspike intervals (ISI). Burstiness was associated with firing rate and action potential half-width; fast bursting may reduce after-hyperpolarisation (AHP) and relate to higher burst propensity. When bursting was present, interneurons exhibited a greater number of bursts than pyramidal cells. Bursting properties also depended strongly on anatomical location and on whether recordings were obtained within the epileptogenic zone.
Overall, these findings suggest that bursting neurons can be identified and characterised *in vivo* in patients with mesial temporal lobe epilepsy.

Region-specific changes in NECAB2 and calbindin positive interneurons across brain regions involved in the epileptic circuitry in a kainic acid model of temporal lobe epilepsy

Ádám Szentes[1]; Anna Fehér[1]; Zsolt-András Nagy[1]; Melinda Toth[1]; Nándor-István Todor[1]; Károly Orbán-Kis[1]; Tibor Szilágyi[1]; Krisztina Kelemen[1]
[1]George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Physiology, Neurophysiology, Târgu Mureș

Calcium-binding protein expressing interneurons play a major role in fine-tuning brain circuits. Previous studies have demonstrated that temporal lobe epilepsy (TLE) is associated with selective remodeling of the neurochemical profiles of neurons across multiple brain regions, resulting in altered expression of calcium-binding proteins.
Our aim was to quantify changes in neuronal expression patterns of the calcium-binding proteins NECAB2 and calbindin (CB) in the hippocampal regions, the paraventricular thalamic nucleus (PVT), the amygdala (AMY), the endopiriform nucleus (EPN) and the perirhinal cortex (PRC) in the kainic acid-induced animal model of TLE.
In this study, 8-week-old male Wistar rats were randomly assigned to three experimental groups. Status epilepticus was induced by injecting kainic acid into the right lateral ventricle in the epileptic control (EPI), brivaracetam-treated (BRV), and levetiracetam-treated (LEV) groups, all subsequently developed spontaneous seizures in the chronic phase. Sham-operated control animals received saline solution. Starting from the third week post-surgery, animals in the treatment groups received for three weeks either daily oral brivaracetam or levetiracetam. The EPI and sham-operated groups received placebo treatment. Following this period, the animals were sacrificed, and fluorescent immunohistochemistry was performed on whole-brain sections to assess changes in NECAB2- and CB-expressing cell populations. Fluorescent images were acquired using a Slide Scanner microscope, and single-labeled and colocalizing cells were quantified manually.
A significant increase in NECAB2-positive cell density was observed in all examined regions of the left hippocampus as well as in the PVT, in the BRV group compared to the EPI group. In the AMY, the LEV group showed a significant increase. CB-positive cell density was increased significantly in the LEV group, in all regions, with the exception of PVT and the right dentate gyrus. The density of NECAB2/CB colocalizing cells was significantly increased in the AMY of all the epileptic groups (treated or untreated), and in the PRC of the LEV group.
In conclusion, kainic acid-induced TLE is associated with region-specific selective remodeling of NECAB2- and calbindin-expressing neuron populations across hippocampal and extra-hippocampal brain regions involved in epileptic circuitry, with distinct modulatory effects of brivaracetam and levetiracetam.

Modulation of neuronal firing by β-cyclodextrin-complexed rufinamide and phenytoin in an in vitro temporal lobe epilepsy model

Zsolt-András Nagy[1]; Előd Bomher[1]; Ágnes Csüdör[2]; Ádám Szentes[1]; Máté Sárosi[2]; Rita - Judit Kiss[1]; Károly Orbán-Kis[1]; Tibor Szilágyi[1]
[1]*George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mures, Department of Physiology, Târgu Mureș*
[2]*University of Debrecen, Debrecen*

Epilepsy affects approximately 50 million people worldwide. Many anti-seizure medications (ASMs) exhibit poor bioavailability, which contributes to the development of pharmacoresistance and remains a major challenge in epilepsy therapy. ß-cyclodextrin (BCD) is known to enhance the solubility of numerous active substances, thereby potentially improving ASMs efficacy and offering a novel therapeutic approach for epilepsy.
The objective of this study was to investigate the concentration-dependent effects of BCD-complexed rufinamide (RUF) and phenytoin (PHT) in an *in vitro* low-magnesium model of temporal lobe epilepsy.
Local field potential was recorded from the pyramidal layer of the CA3 region in hippocampal brain slices obtained from 7–14-day-old male Wistar rats. Seizure-like events (SLEs) were induced in the slices by perfusion with magnesium-free artificial cerebrospinal fluid (ACSF). After recording five baseline SLEs, slices were perfused with magnesium-free ACSF containing BCD-complexed RUF (50 and 100 µM) or PHT (25, 50 and 100 µM). Spikes occurring during SLEs were identified in the recordings, followed by the analysis of the frequency-based parameters of the events (area under the time–frequency curve - AUC, maximal and average spiking frequency, half peak interval and post peak decay slope).
The AUC captures both the duration and firing frequency of the ictal phase of SLEs, thereby providing an integrated measure of the neural load imposed on hippocampal networks. The AUC showed a significant decrease at all concentrations in both the RUF and PHT-treated groups compared to SLEs recorded in baseline conditions. The post-peak decay slope was calculated by linear regression of frequency data points between the peak and half-maximum values. This slope was significantly steeper in the presence of RUF (50 and 100 µM) and PHT (25 and 100 µM) compared with baseline conditions. The half peak interval, defined as the time from peak to half-maximum, also decreased significantly during ASM application.
Our results demonstrate that BCD-complexed RUF and PHT significantly modulates *in vitro* SLE activity. Both compounds reduced hippocampal network load and attenuated the intensity of ictal activity, as reflected by frequency-based parameters. Importantly, BCD complexation enhances the solubility and potential bioavailability of these ASMs while preserving their antiepileptic efficacy in hippocampal networks.

The power of zebrafish: cost-effective, quick, and reliable tool to trace the neurodegenerative effects of micro- and nanoplastics

Ferenc Budán[1]; Gábor Hancsicsák[1]; Anna Katinka Mukli[1]; Gejin De[1]; Alexandra Júlia Hencz[1]; Dávid Szép[1]; Renáta Gajdács-Dóró[1]; Haonan Li[1]; Ágnes Végvári[1]; József Pál[1]; Szilárd Pál[2]; Ádám Horváth[2]; Domokos Máthé[3,4]; Attila Sík[1]
[1]*University of Pécs, Medical School, Institute of Physiology, Pécs*
[2]*University of Pécs, Institute of Pharmaceutical Technology and Biopharmacy, Pécs*
[3]*Faculty of Medicine, Semmelweis University,, Department of Biophysics and Radiation Biology-HUN-REN TKI 1094, Budapest*
[4]*HCEMM-SU In Vivo Imaging Advanced Core Facility, Budapest*

Micro- and nanoplastics (MNP) are polymer-based particulates ranging in diameter from 500 µm to 1 nm. Their ubiquitous presence in the environment manifests a growing health hazard. In human brain (*frontal cortex*) samples from 2016 and 2024, autopsy has revealed a dramatic increase in MNP. In ex vivo brains of dementia patients even greater accumulation of MNP was observed. Hence, MNP can induce neuroinflammation. Thus, monitoring of the kinetics and the in vivo effects of MNP are warranted to assess the health hazards and to enable studies for testing potential elimination mechanisms.
The Zebrafish (*Danio rerio*) model can be suitable. It is widely used as model organisms in neuroscience research. The nervous system of zebrafish shares key structural and functional features with the mammalian nervous system. Their ability to produce hundreds of eggs and their rapid developmental rate enable researchers to obtain substantial amounts of data within a short time frame. Consequently, the zebrafish larval system further allows high-throughput behavioral analysis, as up to 96 larvae can be examined simultaneously. Additionally, by 5–7 days post-fertilization (dpf), zebrafish larvae already display a range of complex behaviors, including escape responses, hunting, and negative thigmotaxis.
At this developmental stage, researchers can perform real-time imaging of neuronal activity, apply genetic or chemical modifications, and rapidly screen candidate drugs for disorders like Alzheimer's and Parkinson's diseases or perform behavior tests. Experiments using 5 dpf zebrafish larvae often meet ethical standards for minimizing and refining animal use, as these early-stage larvae are not subject to certain regulatory restrictions, that enable high-throughput testing.
Furthermore, electroencephalography (EEG) recordings can be performed in 5 dpf larvae, enabling direct observation of epileptiform activity or its suppression. Thus, the potential epileptic effects of MNP may be monitored using this method, with respect to MNP type and exposure level.

Studying the Effect of Cariprazine in Induced Neurons Directly Reprogrammed from Huntington's Disease Patient Fibroblasts

Anna Anoir Abbas[1,2]; Idris Jimoh[2]; Balázs Kis[1,2]; Anikó Göblös[3]; Roger A. Barker[4]; Zoltán L. Veréb[3]; Johan Jakobsson[5]; Lajos Kemény[1,3]; Tibor Pankotai[1,3]; Judit Mária Molnár[2]; Karolina Pircs[2,1,5]
[1]HCEMM, Szeged
[2]Semmelweis University, Budapest
[3]University of Szeged, Szeged
[4]University of Cambridge, Cambridge
[5]Lund University, Lund

Huntington's disease (HD) is an uncurable autosomal dominant progressive neurodegenerative disorder. The role of the dopaminergic system in developing HD symptoms is crucial, as the central dopaminergic pathways are overactivated in HD. Several drugs can reduce dopaminergic overactivity. 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 showed a positive effect 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.
In this study, we investigate the effects of cariprazine using a novel in vitro model of HD, employing donor-derived aged induced neurons (iNs). Our objective is to elucidate the putative therapeutic effects of cariprazine and its mechanism of action, with a particular focus on autophagy. Using a reverse translational approach, we applied cariprazine treatment to iNs directly reprogrammed from fibroblasts of healthy controls, drug-naïve HD patients, and cariprazine-treated HD patients. High-content automated microscopy coupled with immunocytochemistry was employed for morphological and autophagy analyses. Our findings indicate that cariprazine treatment ameliorates the neurite abnormalities characteristic of HD-iNs. Furthermore, cariprazine modulated autophagic processes, suggesting a potential role in restoring subcellular autophagic dynamics. Notably, treatment with cariprazine led to a reduction in HTT gene expression. Comparative analyses with other D2/D3 antipsychotic agents suggest that these effects are attributed to the unique structural properties of cariprazine. This study provides compelling evidence supporting the therapeutic potential of cariprazine in HD.

Shared Cellular Hubs as Potential Therapeutic Targets in Osteoarthritis and Alzheimer's Disease

László Ducza[1]; Wang Zhangzheng[1]; Krisztián Juhász[1]; Csaba Matta[1]; Luca Paluska[1]; Al-Mnaseer Ahmed Mohammed Falih[1]; Mansour Hana[1]; Roland Takács[1]
[1]University of Debrecen, Faculty of Medicine, Anatomy, Histology and Embryology, Debrecen

Osteoarthritis (OA) and Alzheimer's disease (AD) are common age-related disorders increasingly recognized as comorbid. Yet, how peripheral joint pathology mechanistically influences central neurodegeneration has remained unclear. By integrating bulk and single-cell transcriptomic datasets from human OA cartilage and AD cortex, we provide the first directional cellular communication framework linking these diseases.
We identified 18 Comorbidity-Associated Upregulated Pathways shared between OA and AD, enriched in extracellular matrix (ECM) remodeling, inflammatory signaling, and cellular stress responses. Single-cell analysis uncovered distinct hierarchical communication hubs: in OA cartilage, a fibrochondrocyte subpopulation expanded markedly and emerged as a dominant ECM-driven sender, releasing collagen- and fibronectin-derived ligands toward inflammatory and proliferative chondrocytes. In AD cortex, we identified a disease-specific oligodendrocyte subtype as a major neuronal-glial receiver, integrating neurorexin (NRXN)-dependent neuronal input and astrocytic stress signals, accompanied by strong induction of heat-shock and proteotoxic stress markers.
These findings reveal a periphery-to-brain signaling architecture, in which joint-derived ECM stress signals may propagate neuroinflammation and cellular vulnerability within the central nervous system. The identified sender and receiver hubs provide candidate biomarkers and therapeutic intervention points capable of disrupting cross-tissue inflammatory circuits.
Funding: LD was supported by the University of Debrecen Program for Scientific Publication (grant number: 8XEABK00PUBT/320). CM was supported by the Young Researcher Excellence Programme (grant number: FK-134304) of the National Research, Development and Innovation Office, Hungary. CM was also supported by the Bolyai János Research Fellowship of the Hungarian Academy of Sciences.This research was supported by the EKÖP-25-4 University Research Fellowship Programme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, Hungary, awarded to RT (EKÖP-25-4-II-DE-249). CM and RT also acknowledge support from the European Cooperation in Science and Technology COST Association Action CA21110 – Building an open European Network on OsteoArthritis research (NetwOArk); https://www.cost.eu/actions/CA21110/).

NLRP3 inflammasome activation is a potential driver of motor neuron degeneration in ALS

Botond Csont[1,2]; Bernát Nógrádi[1,2]; Tamás Ferenc Polgár[1,2]; Miklós Nemes[1,2]; Orsolya Horváth[1,2]; Péter Klivényi[1,2]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Department of Neurology, Szeged
[2]Hungarian Research Network, University of Szeged, HUN-REN-SZTE Neuroscience Research Group, Szeged

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder, primarily affecting motor neurons. The classical neuropathological alteration in ALS is the mislocalization of TDP-43 protein in the degeneration motor neurons, however, neuronal cell death is driven by multiple pathophysiological processes. Among these, immune mechanisms are considered to be of primary importance, capable of inducing inflammatory cell death. Inflammasome activation is the initial step in the induction of neuroinflammatory processes, leading to robust cytokine (IL-1β) release. Here, we aimed to investigate the potential involvement of inflammasome activation in a transgenic mouse model of ALS.
We performed our experiments on late-symptomatic (12-15-week-old) male transgenic TDP-43A315T (hemizygous) mice and wild-type littermates. Nissl-staining and immunolabelling was performed on spinal cord and brain samples to quantify motor neuron loss, TDP-43 pathology, microglial and astroglial activation and IL-1β expression. The gene expression changes of inflammasome components (IL1b, NLRP3, AIM2, PYCARD) was evaluated with qPCR technique.
We observed marked motor neuron loss and TDP-43 pathology in the spinal cord of TDP-43A315T transgenic mice, compared to wild-type controls. Single-cell microglia morphometry revealed alterations indicating microglial activation in the spinal cord and motor cortex of TDP-43A315T mice. Inflammasome-related genes showed increased expression, primarily affecting the spinal cord. Immunostaining revealed robust IL-1β protein response in the spinal cord of TDP-43A315T mice, primarily expressed by astroglial cells in the ventral horn.
Our findings demonstrate marked inflammasome activation and astroglial IL-1β release around degeneration motor neurons in the spinal cord of TDP-43A315T mice. This inflammasome activation might contribute to motor neuron degeneration, supporting the potential therapeutic relevance of targeting the inflammasome pathway in ALS.

Tracking of the Transient Receptor Potential Ankyrin 1 (TRPA1) receptor protein by fluorescent JT010 ligand in wild type and mutant TRPA1 variants expressing cell lines and endogenous TRPA1 expressing cells

Katalin Szabó[1]; Géza Makkai[1,2]; Péter Ábrányi-Balogh[3]; János Konkoly[1]; Dóra Judit Kiss[3]; Balázs István Tóth[4]; Viktória Kormos[1]; György M Keserű[3]; Dóra Zelena[4]; Erika Pintér[1]
[1]University of Pécs, Medical School, Institute of Pharmacology and Pharmacotherapy, Pécs
[2]University of Pécs Medical School, Institute of Physiology, Nano-Bio-Imaging Core Facility, Pécs
[3]Research Centre for Natural Sciences HUN-REN, Institute of Organic Chemistry, Budapest
[4]University of Pécs, Medical School, Institute of Physiology, Pécs

TRPA1 is a non-selective cation channel involved in sensation to various exogenous and endogenous stimuli in primary neurons and non-neuronal cells. We aimed to generate a fluorescent ligand that detect TRPA1 protein and to validate its selective labelling. Cell-permeable JT010 (JTCy5) and its non-covalent bonder counterpart (metJTCy5) were coupled through a hexylamine linker to the cell-permeable fluorescent dye Cyanine5 (freeCy5). Labelling with fluorescent variants were monitored by flow cytometry in TRPA1-expressing cell lines with simultaneous detection of intracellular Ca2+ levels. Subcellular localization and lifetime of labelling were compared by confocal and stimulated emission depletion microscopy.
Simulateously detection of *TRPA1 mRNA* by RNAscope ISH and JTCy5, metJTCy5 or freeCy5 fluorescence revealed a functional wild-type TRPA1-dependent labelling by JTCy5 in TRPA1 variants expressing CHO cell lines and in human dental pulp cells. Comparison of JTCy5, metJTCy5 and freeCy5 labelling by flow cytometry proved that JTCy5 stained CHO cells in a TRPA1 expression-dependent manner. Labelling was modified by preincubation with TRPA1 selective antagonists and agonist. Preincubation with AITC, which also binds to the electrophilic binding pocket, desensitized the Ca-channel, whereas JTCy5 staining was further increased depending on TRPA1 expression. A similar effect of the non-electrophil agonist thymol was also demonstrated. Based on these findings, JTCy5 fluorescent dye is suitable for the selective detection of endogenous functional TRPA1 expression when used compared with appropriate control dyes. The effect of electrophilic and non-electrophilic binding pocket-reactive agonists on JTCy5 fluorescent dye labelling raised the possibility of complex regulation of TRPA1 channel opening.

Connectivity of enkephalinergic interneurons in the superficial laminae of the spinal dorsal horn

Lídia Gömöri[1,2]; Kornél Kovács[2]; Tobin Koshy[2]; Ayesha Muneeb[2]; Levente Bögös[2]; Éva Kókai[2]; Miklós Sivadó[2]; Tímea Krisztina Nánásiné Molnár[2]; Angelika Varga[2]; Péter Szücs[1,2]
[1]*University of Debrecen, HUN-REN Neuroscience Research Group, Debrecen*
[2]*University of Debrecen, Department of Anatomy, Histology and Embryology, Debrecen*

Introduction
Endogenous opioid containing interneurons in the spinal dorsal horn are known to form local modulatory networks that can gate ascending nociceptive signalling and thus may be promising targets for pharmacological interventions. To targets these neurons effectively it is imperative to identify their local connections and to avoid potential side-effects. Our aim in this work was to perform such characterization of the proenkephalin (PENK) containing spinal dorsal horn neurons.
Materials and methods
Selective targeting and stimulation of PENK neurons was achieved by using PENK::TdTomato and PENK::ChR2 hybrid mice strains. Peripheral inputs of PENK neurons were characterized by electrical stimulation of primary afferents through suction electrodes. Local connectivity was investigated by single and double whole-cell patch-clamp recordings combined with optogenetic stimulation of PENK neurons. Recorded neurons were filled with biocytin and reconstructed with Neurolucida following the recording session.
Results
Approximately two-thirds of the PENK neurons proved to be excitatory. We showed that nociceptive input to PENK neurons is subject to modulation by other PENK interneurons. Optogenetic activation of PENK neurons can facilitate primary afferent evoked feed-forward inhibition, present on some spinal dorsal horn neurons. Furthermore, PENK interneurons form excitatory and inhibitory monosynaptic connections.
Discussion
Our data suggest that PENK neurons may have a more complex role in the endogenous opioid systems than previously anticipated. Further investigation of local interactions of PENK and other endogenous opioid containing neurons will be needed for effective exploitation of the endogenous pain modulatory systems to alleviate pain.

Specialized axon initial segment enables low firing threshold and rapid action potential output in fast-spiking interneurons of the human neocortex

Emőke Bakos[1]; Ádám Tiszlavicz[1]; Viktor Szegedi[1]; Abdennour Douida[1]; Szabina Furdan[1]; Daphne Welter[2]; Jonathan Laundry[2]; Balázs Bende[1]; Gábor Hutóczki[3]; Pál Barzó[4]; Gábor Tamás[4]; Vladimir Benes[2]; Attila Szűcs[5]; Karri Lamsa[1]
[1]*Hungarian Center of Excellence for Molecular Medicine, Szeged*
[2]*EMBL, Heidelberg*
[3]*University of Debrecen, Debrecen*
[4]*University of Szeged, Szeged*
[5]*Eötvös Lóránd University, Budapest*

The mammalian brain exhibits notable interspecies variation. Microanatomical and molecular differences in homologous neurons, those with similar locations and developmental origins across species, are best characterized in the neocortical mantle, the center of complex brain functions; however, the purpose of these differences remains unclear. We performed whole-cell microelectrode recordings along with microanatomical and molecular analyses of human fast-spiking parvalbumin (pvalb)-expressing interneurons in neocortical tissue resected during brain surgery, comparing them with similar data obtained from the mouse neocortex. The action potential (AP) firing threshold was lower in human neurons than in mouse neurons. This was due to a deficiency in low-voltage–activated inhibitory Kv1.1 and Kv1.2 potassium channels in the axon initial segment (AIS), a specialized axonal region that determines AP threshold and initiation, in human cells. In contrast, Kv1 ion channels were prominent in mouse neurons. The AIS was also elongated in humans. Computational simulations of fast-spiking interneurons revealed that the human-type AIS lowers the AP threshold and shortens the time lag for AP initiation. We found that the low membrane AP firing threshold in pvalb neurons is closely linked to slow membrane potential kinetics in the soma. Thus, the human AIS supports fast in–fast out circuit function in human pvalb neurons, compensating for electrically slow somatic membrane responses. When formulating therapeutic strategies that involve fast- spiking neurons, it is crucial to take into account the molecular and functional species differences.

iGluSNFR3 imaging of individual hippocampal CA1 pyramidal cell synapses in vitro reveals heterogeneous release probability and short-term plasticity

Noemi Holderith[1]; Zoltan Nusser[1]
[1]*HUN-REN Institute of Experimental Medicine, Budapest*

Postsynaptic target cell type-dependent variation in release probability and short-term plasticity (STP) at glutamatergic synapses enhance the computational power of cortical network. Hippocampal CA1 pyramidal cells (CA1PCs) establish different synaptic connections on local GABAergic interneurons (INs), both in their efficacy and STP. However, the functional properties of synapses onto other CA1PCs are poorly understood due to technical challenges imposed by a very low connection probability. Here we used iGluSNFR3 glutamate sensor imaging with 2-photon microscopy to measure glutamate release from single CA1PC boutons with identified postsynaptic targets in adult mice.
First, we verified iGluSNFR3's ability to reveal distinct glutamate release and STP at CA1PC – local IN synapses expressing the sensor postsynaptically either in oriens lacunosum-moleculare INs (O-LM INs, Chrna2-Cre) or in parvalbumin expressing INs (PV INs, PV-Cre). Extracellular stimulation evoked iGluSNFR3 signals on O-LM IN dendrites showed characteristic short-term facilitation and a dramatic increase in response to phorbol dibutyrate (PDBU). Glutamate responses at CA1PC - PV IN synapses exhibited large amplitudes and short-term depression (paired pulse ratio ~0.9), consistent with results obtained with whole-cell patch-clamp paired recordings.
To reveal the properties of CA1PC – CA1PC connections, iGluSNFR3 was expressed in CA1PCs allowing the measurement of glutamate release from a large number of boutons per cell (~25 boutons per CA1PCs). Our results reveal that at ~30% of the boutons no detectable iGluSnFR3 signal can be measured upon a complex high-frequency train of presynaptic action potentials. Approximately 10% of boutons display depressing, whereas ~60% facilitating release properties upon 20Hz presynaptic activation.
The majority of the identified targets of the imaged boutons (75%) were IN dendrites, while 25% were CA1PCs spines. IN targeting boutons displayed glutamate transients with variable STP patterns, and 16% were silent. Spine targeting boutons were mostly silent for the 1st AP, and either remained silent for the whole train (23%) or showed short-term facilitation (65%).
Our results demonstrate the usefulness of iGluSNFR3 for studying the output synapses of CA1PCs and revealed unique properties of CA1PC – CA1PC connections.

Anatomical investigation of regions generating spontaneous population activity in the human epileptic hippocampal formation

Nour Essam[1,2]; Dorka Nagy[3]; Kinga Tóth[1]; Loránd Erőss[4]; Dániel Fabó[3]; István Ulbert[1,2,4]; Lucia Wittner[1,4]
[1]*HUN-REN Research Centre for Natural Sciences, 1117 Budapest*
[2]*Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, 1083 Budapest*
[3]*Eötvös Loránd University, Faculty of Natural Sciences, 1117 Budapest*
[4]*National Institute of Mental Health, Neurology and Neurosurgery, 1145 Budapest*

Mesial temporal lobe epilepsy – a neurological disorder with high ratio of pharmacoresistant cases – is characterized by unpredictable seizures. The hippocampal formation is closely associated with the generation of seizures, resulting from an imbalance between excitatory and inhibitory neuronal firing. This project entails a comprehensive analysis of the cellular firing and population activity in the human epileptic hippocampus, using both electrophysiological recordings and anatomical techniques. We investigated the excitatory and inhibitory synaptic input of pyramidal cells with quantitative electron microscopic technique in slices that either generate or lack spontaneous population activity (SPA) in vitro, within the CA2 and the subiculum regions.
The local field potential gradient was recorded with a 24-channel linear microelectrode in the CA2 and subicular region in hippocampal slices of epileptic patients, in vitro. Antibodies against the neuronal marker NeuN and the Ca2+-binding protein calbindin (CB) were used to stain all neurons and specific subsets of hippocampal neurons including inhibitory and excitatory cells, respectively. Regions either generating or lacking SPA in both the CA2 and subicular regions were identified and chosen for further electron microscopic investigation. The inhibitory and excitatory synaptic coverage (µm synaptic active zone/100 µm cell body perimeter) of neurons located in these regions was measured in CB-stained sections.
SPA was recorded in both examined hippocampal regions in most of the slices. The morphology of CB-positive cells did not show notable differences between regions generating or lacking SPA. Our electron microscopic analysis revealed that both CA2 and subicular neuronal somata receive predominantly inhibitory synaptic input, however, excitatory input was also observed. Further analysis of the synaptic morphology demonstrated the presence of several perforated synapses. These phenomena suggest that a synaptic reorganization takes place in the examined regions of the human epileptic hippocampus. Interestingly, we identified calbindin-positive axon terminals forming synaptic contacts on the soma of pyramidal neurons suggesting the presence of a calbindin-positive basket cell type in the human epileptic hippocampal formation. More data are needed concerning the synaptic coverage of neurons to identify possible differences between regions generating and lacking SPA.

Altered molecular composition of a specific subset of prefrontal cortical excitatory synapses in schizophrenia

Andrea Lorincz[1]; Maria Ashaber[1]; Zoltan Nusser[1]
[1]*HUN-REN Institue of Experimental Medicine, Laboratory of Cellular Neurophysiology, Budapest*

Abnormal excitatory synaptic transmission in the human prefrontal cortex has been implicated in the pathophysiology of schizophrenia based primarily on genetic evidence. However, changes in synaptic function cannot be predicted from altered gene expressions, but determining the amount, density and subsynaptic distribution of synaptic proteins is the only reliable indirect readout of function. Detecting proteins in individual synapses of human postmortem tissues has been severely constrained by technical limitations. Here we overcome this limitation by optimizing a high-resolution, quantitative localization method to facilitate antigen recognition at excitatory synapses in postmortem brains of both sexes. Using PSD-95 immunoreactivity as molecular marker of excitatory synapses, we demonstrate the lack of significant differences in synapse density and size in upper cortical layers of control and schizophrenia subjects. The synaptic densities of postsynaptic AMPA and NMDA receptor subunits, presynaptic molecules Bassoon and Munc13-1 are also indistinguishable between control and schizophrenia subjects. The number of Munc13-1 nanoclusters, marking presynaptic neurotransmitter release sites, does not differ either. Excitatory synapses on parvalbumin expressing interneurons contain similar AMPA, but significantly lower NMDA receptor densities in schizophrenia compared to control subjects. Our study provides the first comprehensive comparison of key functionally relevant synaptic proteins in individual human excitatory synapses and demonstrates that changes in the molecular composition of only a specific subset of excitatory synapses may contribute to the pathophysiology of schizophrenia.

Semi-automated workflow for the post-hoc histological characterization of neurons recorded in behaving mice with one-photon microscopy

Hanga Dormán[1]; Elja Belhadef[2]; Michael Graupner[2]; Lucia Wittner[1]
[1]*HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Saints-Pères Paris Institute for the Neurosciences, Paris*

Correlating behaviour with the firing properties of neurons and their anatomical identity helps us better understand the cellular and network mechanisms of brain regions with specific functions. In this study, we investigated the post-hoc histology of head direction cells located in the presubiculum of mice. GCaMP-expressing neurons were recorded using a one-photon miniscope in behaving animals, then identified in 40 µm-thick sections, and their cell type was determined through additional immunostainings against the interneuron markers vasoactive intestinal polypeptide (VIP), somatostatin (SST), and parvalbumin (PV). To achieve reliable results, several factors were considered, including the limited resolution and two-dimensionality of the miniscope images, the visibility of landmarks across imaging domains, and local deformations of the tissue both in vivo and during the histological procedure. We designed a semi-automated workflow in which the miniscope images were manually aligned to native confocal 3D images of brain sections based on orientation, landmarks, and cell contours. Triple immunostaining was then applied to sections containing the neurons of interest to label VIP-, SST- and PV-positive interneuron populations. Distortions in the tissue caused by re-mounting the sections were corrected by applying a thin plate spline transform with the help of ImageJ's BigWarp plugin. We found that using the maximum projections of the confocal z-stacks leads to false negative results due to brighter cells masking weakly stained ones, as well as false positives due to the overlap of multiple cells. Instead, z-stack images were examined layer-by-layer in the software GIMP. With this approach, we were able to identify the subpopulation of cells detected in the miniscope that also expressed interneuron markers. As a potential improvement, a larger proportion of the workflow could be automated. We believe that the present study provides a useful framework for the histological identification of cells recorded in vivo with limited resolution, which can be further refined to robustly tackle the methodological challenges.

Hybrid synapses: possible electrical-chemical synapse interactions in the mouse retina

Liliana Ross[1]; Gergely Szarka[1]; Boglárka Balogh[1]; Loretta Péntek[1]; Tamás Kovács-Öller[1]; Béla Völgyi[1]
[1]*University of Pécs, Pécs*

Communication between retinal cells relies on both chemical and electrical synapses. Our preliminary investigations found that Ribeye (ribbon chemical synapse protein) and Cx36 (electrical synapse gap junction - GJ - protein) are found in close proximity on a specific type of retinal bipolar cell, the type 3a bipolar cell (3aBC). This raises the question of whether an interaction between these two forms of neural communication exists, forming complex chemical-electrical contacts, also called hybrid synapses in the retina. By utilizing a computational model, we estimated the theoretical maximum of the effective inter-junctional distance (EIJD) that represents a distance between contacts at which the ion flow through the Cx36 GJ could exert a local modulatory effect on the neurotransmitter release of the nearby chemical synapse. Our model is based on known parameters, including the Ca2+ diffusion coefficient, Ca2+ channel open time, GJ space constant and Ca2+ concentration threshold for vesicle release. We found that the theoretical maximum for the EIJD value is 0.38 µm. Next, we investigated the actual distance between Ribeye and Cx36 on the 3aBC axon terminals. Using immunohistochemistry and laser scanning confocal microscopy, we found the distances to most often range between 0.5-0.6 µm; however, a significant portion of these contacts (2271/15414; 14.7%) fell below the 0.38 µm theoretical threshold, suggesting that a hybrid synapse interaction may be possible in these latter cases. Next, we aimed to determine synaptic partners contributing to these putative hybrid synapses by using the EyeWire II electron microscope database. So far, when investigating GJ-resembling subcellular structures on 3aBC axon terminals, we found 14 cases where the apparent GJ was between the axon terminals of two 3aBCs, suggesting possible homologous GJ coupling of these BCs. Out of the 14 examined cases, 4 hybrid synapses (DGJ/ribbon < 0.38 µm) were found. The smallest distances measured were 0.26 µm, 0.29 µm and 0.28 µm, and in these cases the ribbon synapse postsynaptic partner was a starburst amacrine cell, an unidentified wide-field amacrine cell, and a ganglion cell, respectively. Taken together, these results suggest that hybrid chemical-electrical synapses may be present in the retina and potentially serve a role in visual signaling.

Synapse loss in the hippocampus of depressed patients

Abigél Sebők-Tornai[1,2]; Péter Szocsics[3,4]; Zsófia Maglóczky[3,4]; Péter Gombás[5]; Craig A Stockmeier[6]; Boldizsár Czéh[1,2]
[1]*University of Pécs, Szentágothai János Research Centre, Neurobiology of Stress Research Group, Pécs*
[2]*University of Pécs, Medical School, Department of Laboratory Medicine, Pécs*
[3]*HUN-REN Institute of Experimental Medicine, Human Brain Research Laboratory, Budapest*
[4]*Semmelweis University, Department of Psychiatry and Psychotherapy, Budapest*
[5]*St. Borbála Hospital, Department of Pathology, Tatabánya*
[6]*University of Mississippi Medical Center, Department of Psychiatry and Human Behavior, Jackson MS*

BACKGROUND: synaptic changes likely to play a central role in the pathophysiology of major depressive disorder (MDD). In this post-mortem quantitative ultrastructural study, we examined putative synaptic loss in the hippocampus of depressed patients.
METHODS: hippocampal tissue from MDD subjects (n=11) and matched healthy controls (n=17) was analyzed. Synaptic densities were determined with a quantification method based on unbiased stereological principles. We studied the neuropil of dentate gyrus, CA3, and CA1 subregions.
RESULTS: overall hippocampal synaptic density did not differ significantly between groups. However, synaptic density was significantly reduced in the CA3 region of MDD patients with a single depressive episode. Age showed no correlation with density.
LIMITATIONS: small sample sizes limited statistical power. Only one rostral segment of the hippocampal body was analyzed.
CONCLUSIONS: this finding support the concept that synaptic alterations contribute to the neurobiology of MDD.

Studying neuronal autophagy in human ageing using induced neurons directly reprogrammed from adult human dermal fibroblasts

Roland Zsoldos[1,2]; Kinga Vörös[1]; Zsófia Koltai[1]; Chandramouli Muralidharan[3]; Anikó Göblös[4]; Idris János Jimoh[5]; Bence Király[2]; Anna Anoir Abbas[1]; Balázs Kis[1]; Ágnes Varga[1,2]; Csaba Kerepesi[2,6]; Johan Jakobsson[3]; Mária Judit Molnár[5]; Lajos Kemény[4]; Karolina Pircs[1,2,3]
[1]HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Institute of Translational Medicine, Semmelweis University, Budapest
[2]HUN-REN-SZTAKI-SU Rejuvenation Research Group, Office for Supported Research Groups (TKI), Hungarian Research Network (HUN-REN), Budapest
[3]Laboratory of Molecular Neurogenetics, Lund University, Lund
[4]HCEMM-Szeged University, Szeged University, Szeged
[5]Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest
[6]Institute for Computer Science and Control (SZTAKI), Hungarian Research Network (HUN-REN), Budapest

Age is the greatest risk factor for neurodegenerative diseases (NDDs), yet the molecular links between physiological aging and NDD pathogenesis remain poorly understood. Autophagy- a lysosomal degradation pathway essential for maintaining cytoplasmic homeostasis- declines with age and contributes to neuronal dysfunction. To investigate how aging alters autophagic flux in human neurons, we utilized direct reprogramming of human dermal fibroblasts to generate induced neurons (iNs), a method that retains donor-specific genetic and epigenetic signatures.
We generated iNs from a cohort of 54 healthy donors ranging in age from 24 to 86 years. Neuronal identity was verified by immunocytochemistry and high-content automated microscopy using neuronal (TAU) and autophagy-specific markers (BECLIN1, LC3, P62, LAMP1) under both basal and stress-induced conditions. Our results indicate donor specific alterations in autophagy. We identified distinct clusters of stress-induced autophagic flux in the case of both young and old donor groups. These variations were observed in autophagosome formation and turnover, as well as in the lysosomal degradation step.
These findings suggest different trajectories of age-related decline in neuronal homeostasis, which may contribute to increased vulnerability to age-related neurodegeneration. Currently, we are validating these findings across the full donor cohort. In parallel, we are conducting multi-omic autophagy profiling using a range of molecular assays including genome-wide DNA methylation arrays, bulk RNA-sequencing, mass spectrometry, and metabolomics to compare young and old iNs.
Our long-term goal is to identify key regulators of autophagy and explore rejuvenation strategies. This approach could inform future therapies for NDDs, where impaired autophagy and accelerated aging are often observed.

Rejuvenation in human derived induced neurons

Vivien Pillár[1]
[1]*Institute of Translational Medicine, Neurobiology and Neurodegenerative Diseases Research Group, Budapest*

Neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's are becoming an increasingly severe global issue and could become the leading causes of death in developed countries by 2050. Diseases associated with dementia impose a significant burden on society. Given that these conditions are currently incurable, it is essential to investigate the molecular processes of neurons, particularly mechanisms related to autophagy and synaptic connections. The main goal of our project is to study the changes occurring in the aging processes of human induced neurons and to identify therapeutic targets that may delay or even reverse the progression of neurodegenerative diseases. In our experiments, we generate induced neurons (iNs) through direct reprogramming of human fibroblast cells, preserving the genetic and epigenetic aging characteristics of the donor. We have developed lentiviruses that are able to express mCherry or TagBFP, allowing us to distinguish young and aged cells using fluorescence-activated cell sorting (FACS). By mixing fibroblasts from 2 young and 2 aged donors and reprogramming them into iNs, we aim to investigate whether the young cells and milieu can rejuvenate aged iNs. We first examine the mixed iN cultures using automated microscopy and next we will analyse them using DNA methylation arrays and RNA sequencing to determine their biological age through transcriptomic and epigenetic clocks, followed by patch-clamp electrophysiology measurements.Our experiments can significantly contribute to the identification of therapeutic targets for neurodegenerative diseases where an accelerated aging is often present and to the establishment of new treatment options aimed at neuronal rejuvenation.

Conserved Kir channel mechanisms governing intrinsic excitability in human and rodent parvalbumin neurons

Karri Lamsa[1]
[1]*Hungarian Centre of Excellence for Molecular Medicine, Szeged*

Compared to rodents, inhibitory interneurons in the human neocortex exhibit low passive ion leakage across their extracellular membranes, resulting in high input resistance and excitability. However, the regulation of excitability by voltage-gated ion channels in human interneurons remains poorly characterized. Using whole-cell patch-clamp recordings in mouse and nonpathological human neocortical slices, we demonstrate that Kir channels modulate the excitability of parvalbumin (Pvalb) neurons at subthreshold membrane potentials. Whole-cell recordings, dynamic clamp experiments, and computational modeling demonstrated that despite species differences in input resistance through passive ion leakage, activation of Kir channels causes proportional inhibition of input excitability and synaptic excitatory potentials in both human and mouse Pvalb neurons. mRNA expression analyses with patch sequencing revealed the predominant expression of Kir3.1, Kir3.2, Kir3.3, and Kir2.3 channels in Pvalb neurons across species. Immunohistochemical analyses showed these channels in the extracellular membrane of the cell soma. Our results demonstrate that Kir-mediated excitability regulation is an archetypal feature of Pvalb neurons in the mammalian neocortex.

Neuron density and inhibitory balance predict local synchrony in vitro but not in vivo in the human neocortex

Katalin Zs. Tóth[1,2]; Réka Bod[1,2]; Yossef Michaeli[1,2]; Orsolya Farkas[1,3]; Loránd Erőss[4]; László Entz[4]; Dániel Fabó[4]; István Ulbert[1,4,3]; Kinga Tóth[1]; Lucia Wittner[1,4]
[1]*Hun-Ren Research Centre for Natural Sciences, Budapest*
[2]*Semmelweis University Doctoral School, Budapest*
[3]*Péter Pázmány Catholic University, Faculty of Information Technology and Bionics, Budapest*
[4]*Semmelweis University Clinics of Neurosurgery and Neurointervention, Budapest*

The link between human cortical structure and spontaneous neuronal activity is not fully understood, particularly across in vivo and in vitro conditions. To investigate this, we combined laminar microelectrode recordings with post hoc histological analysis of neocortical tissue from epileptic patients. Chronic in vivo intracortical recordings were followed by acute in vitro recordings from neighboring resected tissue using the same 24-channel linear microelectrode system. Neuronal densities were quantified in sections stained for the neuronal marker NeuN and parvalbumin (PV) to estimate excitatory and inhibitory cell populations in tissue surrounding the microelectrode track and in the in vitro slices. NeuN density was comparable across patients ($F = 2.33$, $p = 0.10$), whereas PV density varied significantly ($F = 5.05$, $p = 0.0076$); both were higher in supragranular than in infragranular layers ($p < 0.001$). Correlation analyses revealed that in vitro firing and coupling properties reflected local structure: NeuN density correlated with theta ($r = 0.33$, $p < 0.001$) and gamma phase-locking values ($r = 0.18$, $p = 0.037$), while PV density correlated negatively with firing rate ($r = −0.45$, $p = 0.017$) and positively with gamma phase locking ($r = 0.35$, $p = 0.07$). The NeuN/PV ratio predicted firing rate ($r = 0.72$) but was inversely related to gamma synchrony ($r = −0.64$). These findings suggest that structural–functional coupling is enhanced under isolated in vitro conditions, whereas global in vivo dynamics reduce local specificity.

Katalin Zs. Tóth and Réka Bod contributed equally to this work and share first authorship.

Investigation of Autophagy Changes in Human Induced Neurons Using Live-Cell Imaging

Zsófia Koltai[1]; Roland Zsoldos[1,2]; Kinga Vörös[1]; Chandramouli Muralidharan[3]; Anikó Göblös[4]; Idris János Jimoh[5]; Bence Király[2]; Anna Anoir Abbas[1]; Balázs Kis[1]; Ágnes Varga[1]; Csaba Kerepesi[2,6]; Johan Jakobsson[3]; Mária Judit Molnár[5]; Lajos Kemény[4]; Karolina Pircs[1]
[1]HCEMM-SU, Neurobiology and Neurodegenerative Diseases Research Group, Semmelweis University, Budapest
[2]HUN-REN-SZTAKI-SU Rejuvenation Research Group, Office for Supported Research Groups (TKI), Hungarian Research Network (HUN-REN), Budapest
[3]Lund University, Laboratory of Molecular Neurogenetics, Lund
[4]HCEMM, Szeged University, Szeged; [5]Institute of Genomic Medicine and Rare Disorders, Faculty of Medicine, Semmelweis University, Budapest
[6]Institute for Computer Science and Control (SZTAKI), Hungarian Research Network (HUN-REN), Budapest

In our society, ageing and age-related diseases place an increasingly significant burden on healthcare systems and the overall population. However, investigating ageing in human neurons is challenging due to their limited accessibility. Human induced neurons (iNs), which are directly transdifferentiated from human dermal fibroblasts, retain the epigenetic and genetic characteristics of the donor. Therefore, iNs provide valuable insights into the cellular mechanisms involved in healthy and pathological ageing of human neuronal cells.
Autophagy is an evolutionarily conserved lysosomal degradation pathway that maintains cellular homeostasis by degrading and recycling damaged or unnecessary cellular components. This cellular process is particularly important during ageing, especially in highly metabolic, postmitotic cells like neurons. Our automated microscopy-based autophagy experiments suggest the presence of separate clusters based on the autophagic activity in basal and starvation-induced conditions in young and old donor-derived iNs. Clusters can be separated, regardless of the chronological age of the donor, based on their autophagic data. Moreover, our observations suggest that the regulation of autophagy undergoes alterations, including changes in autophagosome formation and lysosomal recycling.
Our current project aims to investigate the dynamic changes in autophagic flux to better understand the differences between the characterised clusters. We use a dual reporter system, LV.mCh-LC3.GFP to visualise autophagosomes before and after their fusion with lysosomes. For these experiments, human dermal fibroblasts from young and old donors were used. Following the conversion, live-cell imaging was performed to monitor the starvation-induced changes in the autophagic flux. Images were acquired at 0, 30 and 60 minutes after autophagy induction using HBSS treatment. Neuronal identification will be achieved using NeuO, a live-cell neuronal marker. Image analysis will be performed by automated microscopy to obtain a large-scale, non-biased quantification. We will assess the number, size and colocalization (yellow) of the autophagosomes (green) and autolysosomes (red) measured as puncta in both neurites and cell bodies.
The real-time observations of the autophagic flux help us better understand the difference between the previously identified clusters, which were primarily defined based on static autophagic markers.

Hunting for drugs: repurposing AMPK-targeting drugs in Huntington's disease

Balázs Kis[1]; Daniella Hühn[2]; Zsófia Márta Mező[1]; Ágnes Varga[1,3]; Anna Anoir Abbas[1]; Idris Jimoh[4]; Ulrike Uhrig[5]; Zádori Dénes[6]; Péter Klivényi[6]; Anikó Göblös[7]; A. Barker Roger[8]; Lajos Kemény[7]; Mária Judit Molnár[4]; Oscar Fernandez-Capetillo[2,9]; Karolina Pircs[1,3,10]
[1]HCEMM-Semmelweis University, Institute of Translational Medicine, HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Budapest
[2]Karolinska Institute, Division of Genome Biology, Department of Medical Biochemistry and Biophysics, Science for Life Laboratory, Stockholm
[3]HUN-REN SZTAKI-SU, Rejuvenation Group, HUN-REN Supported Research Groups, Budapest
[4]Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Faculty of Medicine, Budapest
[5]EMBL, Chemical Biology Core Facility, Heidelberg
[6]Albert Szent-Györgyi Clinical Centre, Albert Szent-Györgyi Faculty of Medicine, University of Szeged, Department of Neurology, Szeged
[7]HCEMM-USZ, University of Szeged, Human neuron physiology and therapy Core Group, Szeged
[8]Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, Cambridge
[9]Spanish National Cancer Research Centre, Genomic Instability Group, Madrid
[10]HCEMM-USZ, Univesity of Szeged, Human neuron physiology and therapy Core Group, Szeged

Neurons are among the most energy-demanding cell types in the human body; therefore, disruptions in cellular energy metabolism disproportionately affect neuronal viability and function. Neurodegenerative diseases are increasingly understood to impose chronic energetic stress on neurons, either as a downstream consequence of pathology or as a contributing driver of disease progression. In Huntington's disease (HD), using a human, patient-derived, disease-specific induced neuronal (iN) model, we identified distinct dysregulation across multiple nodes of AMP-activated protein kinase (AMPK) signaling pathway. AMPK signaling has been consistently shown to exert neuroprotective effects across multiple HD models. AMPK functions as a key cellular energy sensor by monitoring the intracellular ATP/AMP ratio and responds to energetic deficits by promoting catabolic processes such as autophagy, stimulating mitochondrial biogenesis, and suppressing energy-consuming anabolic pathways.

This project aims to repurpose FDA-approved drugs to target dysregulated components of the AMPK pathway. Using a viability assay, we identified 89 protective compounds from a 2,400-compound EMBL library screened in an HD cellular model (U2OSQ94). To identify specific AMPK activators, we screened hits with an ExRAI-AMPKAR sensor. Compounds with FDA and EMA approval, current availability, and minimal side effects were prioritized, resulting in four candidates: salsalate, olmesartan, levodopa, and acetylcysteine. These compounds will be further tested in HD-iNs to assess effects on neuronal morphology (TAU), mitochondria (MitoTracker™ Deep Red), AMPK (α-subunit) and autophagy (LC3, p62). We will also use qPCR to assess treatment-induced changes in the expression of genes dysregulated in the AMPK pathway and in *HTT* expression. Ultimately, this work aims to identify the safest and most effective AMPK-targeting drug for use in HD-iNs, with the goal of rapidly translating a repurposed compound into the clinic for treating Huntington's disease.

State-dependent recruitment of human cortical microcircuits: enhanced local synchrony and phase alignment in vitro compared to in vivo condition

Réka Bod[1,2]; Yossef Michaeli[3]; Orsolya Farkas[1,4]; Loránd Erőss[5]; László Entz[5]; Dániel Fabó[5]; István Ulbert[1,4,5]; Kinga Tóth[1]; Lucia Wittner[1,5]
[1]*HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Integrative Neuroscience, Budapest*
[2]*Semmelweis University Doctoral School, János Szentágothai Neurosciences Division, Budapest*
[3]*Semmelweis University, Faculty of Medicine, Budapest*
[4]*Péter Pázmány Catholic University, Faculty of Information Technology and Bionics, Budapest*
[5]*Semmelweis University, Clinics of Neurosurgery and Neurointervention, Budapest*

Understanding how human cortical microcircuits reorganize their activity across physiological and experimental states is essential for linking structure to function. Here, we combined chronic *in vivo* laminar recordings during wakefulness and non-REM sleep with acute *in vitro* recordings obtained from the same cortical regions of the same epilepsy patients (n=8). Recordings were performed using identical 24-channel linear microelectrode arrays (150 µm inter-contact spacing) inserted perpendicularly to the cortical surface, enabling direct layer-matched comparison of single-unit and local-field-potential dynamics across preparations. Following spike sorting and waveform classification, units were categorized as principal cells (PC) or interneurons (IN) for cell-type–specific analyses.
Across 122 *in vivo* and 385 *in vitro* single units, both cell types exhibited marked state-dependent differences in excitability and oscillatory coupling. PC firing rates were significantly higher *in vitro* than during awake *in vivo* recordings (1.78 vs 0.94 Hz; p=0.001), while INs showed a similar increase from sleep to *in vitro* (0.57 vs. 1.32 Hz; p=0.008). Spike waveform half-widths broadened *in vitro* for both cell types (PC: 0.25 → 0.31 ms; IN: 0.19 → 0.23 ms; p<0.0001), consistent with reduced synaptic input and altered ionic dynamics following isolation.
Oscillatory coupling analyses revealed a 3-4x enhancement of rhythmic entrainment *in vitro*, where PCs and INs showed markedly stronger theta (phase lock value - PLV ≈ 0.18) and gamma (PLV ≈ 0.55 and 0.40, respectively) synchronization. Preferred phases of significantly coupled units clustered near 100° for theta and 50° for gamma *in vitro*, compared to higher *in vivo* gamma phase angles (≈141° awake, ≈328° sleep), indicating that isolated networks fire earlier within the oscillatory cycle.
These results demonstrate that when long-range afferents are removed, human cortical microcircuits exhibit stronger local synchrony and cell-type-specific rhythmic alignment. Isolation thus enhances structure–function coupling and intrinsic resonance, whereas *in vivo* integration promotes decorrelation and flexible population dynamics, highlighting a fundamental trade-off between global coordination and local coherence in the human neocortex.

Characterization of an aging related ATF3 driven galectin-3 expressing microglial cell population

Tamás Lakat[1]; Tamás Kiss[1,2]; Alexandra Krisztin-Németh[3]; Anna Abbas[4]; Kinga Vörös[4]; Karolina Pircs[4]; István Pesti[3]; Yaqub Mir[5]; Ádám Légrádi[3]
[1]Data Science and Me Ltd., Kecskemét
[2]1st Department of Paediatrics, Semmelweis University, Budapest
[3]Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged
[4]Neurobiology and Neurodegenerative Diseases Research Group, HCEMM-Semmelweis University, Budapest
[5]Lund University Hospital, Department of Clinical Sciences, Epilepsy Centre, Lund

Aging is associated with chronic, low-grade inflammation within the central nervous system (CNS), leading to progressive impairment of neuronal function. Microglial cells orchestrate CNS immune responses by secreting a wide variety of pro- and anti-inflammatory mediators. Galectins, a family of β-galactoside–binding lectins, play key immunomodulatory roles; among them, galectin-3 is strongly linked to pro-inflammatory microglial activation. Here we used a publicly available single-cell mRNA sequencing dataset (Hammond et al., 2019) to identify aging related changes among an Lgals3 expressing mouse microglia subpopulation. Bioinformatic interrogation of this cluster identified Lpl (lipoprotein lipase), Ccl4, and Ccl3 as its top co-expressed genes, indicating a lipid-droplet–rich, foam-cell–like inflammatory phenotype.
Gene set enrichment analysis showed that this population exhibits increased pathway activity related to cell proliferation, mTORC autophagy inhibition and cholesterol homeostasis. Gene regulatory network analysis further demonstrated markedly elevated activity of ATF3-regulated transcriptional programs. Comparison of young and aged mouse microglia datasets revealed a global age-dependent increase in Lgals3 expression, with a particularly prominent expansion of the galectin-3–positive cluster 5. Cell-cell interaction patterns indicated that cluster 5 represents a highly communicative population, with significantly enhanced signaling strength in aged microglia. Aging predominantly increased incoming TGF-β signaling and outgoing CCL4 and CCL3-mediated communication.
For in vivo–relevant characterization of galectin-3 protein expression, long-term cultured primary rat neuronal cultures were analyzed. At DIV7, amoeboid, Ki67-positive microglia displayed markedly higher galectin-3 density compared with the more ramified microglial forms that emerged later in culture. Prolonged cultivation also led to impaired microglial autophagic function, evidenced by enlarged autophagic vesicles.
Together, these findings define an aging-associated microglial population with inflammatory and lipid-metabolic features, representing a potential therapeutic target for interventions aimed at reducing neurodegeneration-related inflammation.

Selective, genetically induced increase in synaptic vesicle priming

Mohammad Aldahabi[1]; Flora Balint[1]; Andrea Lorincz[1]; Noa Lipstein[2]; Nils Brose[3]; Zoltan Nusser[1]
[1]HUN-REN Institute of Experimental Medicine, Laboratory of Cellular Neurophysiology, Budapest
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin
[3]Max Planck Institute for Multidisciplinary Sciences, Department of Molecular Neurobiology, Göttingen

Synaptic vesicle (SV) release probability (*Pv*) is determined by two key probabilistic factors: the probability of release sites being occupied by fusion competent, well-primed SVs (*Poccupancy*), and the fusion probability (*Pfusion*) of such fusion-competent SVs. While recent studies emphasized the importance of SV priming as a major mechanism underlying functional diversity of central synapses, dissecting SV priming and fusion is notoriously challenging. Here we developed a mouse genetic approach for inducible and selective increase of SV priming. Mutation of the amino acid 576 of Munc13-1 from histidine to lysine (HK) increases Munc13-1 function. Combining this mutation with a Cre-dependent removal of the wild-type Munc13-1 allele allows a cell-type selective conditional enhancement of Munc13-1 function. Here we demonstrate that an increase in *Pv* fully accounts for the enhanced EPSC amplitude in Munc13-1HK/- hippocampal pyramidal cell to fast spiking interneuron synapses without any alteration in the number of release sites or the quantal size. A sequential, two-step priming model predicts that the enhanced *Pv* is the consequence of an elevated proportion of well-primed SVs, without altering *Pfusion*. Finally, using these mice, we provide unequivocal evidence that the postsynaptic target cell type-dependent variability in presynaptic glutamate release is mainly the consequence of variability in SV priming.

Medio-lateral gradients in the medial entorhinal cortex

Áron Olivér Kolozsvári[1]; Szilárd Szőcs[1]; Ágnes Agócs-Laboda[1]; Gergely Szarka[1]; Béla Völgyi[1]; Dominik Berényi[1]; Csaba Varga[1]
[1]*Szentágothai Research Centre, Institute of Physiology, University of Pécs, Medical School, Pécs*

The primary gateway between the hippocampus and cortical regions is the entorhinal cortex (EC). Stellate cells from the medial-, and fan cells from the lateral entorhinal cortex target the medial and the outer molecular layer of the dentate gyrus (DG), respectively. These two populations of layerII cells possess distinct electrophysiological and morphological characteristics. Are, however, stellate or fan cells homogenous populations? In this study we correlated the location and electrophysiological parameters of medial entorhinal cortical layerII stellate cells. Surprisingly, we found that the H-current changes along not only dorso-ventrally as previously described, but medio-laterally as well. Stellate cells located closer to the parasubiculum have larger sag-potential than their peers located more laterally. We have also investigated their anatomical features and found many major differences in protein expression levels and axonal targets. Above all we also found that they contribute differently to dentate spikes detected in the dorsal DG of the hippocampus.

Are we on the same page? Toward a consensus in human cortical cell-type classification using electrophysiological features

Joanna Sandle[1]; Femke Waleboer[2]; Aneta Lisowska[3]; Mark Hoogendoorn[3]; Brian Kalmbach[4]; Ed S. Lein[4]; Rachel Dalley[4]; Brian Lee[4]; Christiaan P.J. de Kock[2]; Gábor Tamás[1]; Gábor Molnár[1]
[1]University of Szeged, Department of Physiology, Anatomy and Neuroscience, Szeged
[2]Vrije Universiteit Amsterdam, Center for Neurogenomics and Cognitive Research, Amsterdam
[3]Vrije Universiteit Amsterdam, Department of Computer Science, Amsterdam
[4]Allen Institute for Brain Science, Seattle

The complexity of the human nervous system arises from its fundamental functional units, neurons, which differ in their electrophysiological, morphological, and transcriptomic properties. Although multimodal, patch-seq, characterisation provides the most accurate definition of neuronal cell types, it remains technically and financially inaccessible to most laboratories. As a result, the literature still lacks a unified framework for cell-type classification, leading to inconsistent terminology and making cross-laboratory comparison difficult.

To promote consensus and reproducibility, a data-analysis pipeline was developed to standardise the processing of human cortical patch-clamp recordings and to enable cross-modal cell-type prediction. The workflow (i) converts recordings stored in various formats into the community-accepted Neurodata Without Borders (NWB) format while accommodating differences in the widely used current step stimulus and sampling frequency, (ii) integrates the Allen Institute's IPFX analysis protocol for feature extraction, and (iii) extends it with additional parameters relevant for distinguishing neuronal subtypes.

This pre-processed electrophysiological dataset provides the substrate for a supervised machine-learning classifier trained on patch-seq reference data, in which electrophysiological and transcriptomic profiles are aligned at the single-cell level. In this framework, the electrophysiological features that best distinguish transcriptomic subclasses are learned from the reference dataset and then used to predict transcriptomic identity for neurons with electrophysiology-only recordings.

Applying this standardised pipeline to a large set of human cortical recordings obtained under diverse experimental conditions enabled the transcriptomic group assignment of neurons with only electrophysiological features, demonstrating that electrophysiological properties capture meaningful information about transcriptomic identity. Together, these results highlight the potential of unified analysis workflows to bridge differences in recording conditions, support cross-laboratory comparability, and contribute to a consensus in human cortical cell-type classification.

Beyond perisomatic inhibition: heterogenous populations of CCK-expressing interneurons in the mouse medial entorhinal cortex

Ágnes Agócs-Laboda[1]; Szilárd Szőcs[1]; Áron Olivér Kolozsvári[1]; Rashmit Kaur[2]; Zoltán Máté[3]; Zsuzsanna Erdélyi[3]; Ferenc Erdélyi[3]; Nóra Henn-Mike[1]; Klaudia Barabás[1]; Csaba Földy[2]; Csaba Varga[1]
[1]Szentágothai Research Centre, Inst. of Physiology, Medical School, University of Pécs, Pécs
[2]Brain Research Institute, Faculties of Medicine and Science, University of Zürich, Zürich
[3]HUN-REN Institute of Experimental Medicine, Medical GeneTechnology Unit, Budapest

Heterogenous populations of GABAergic interneurons differently shape the activity of local neuronal circuits. One major class is the CCK-expressing interneurons. Most CCK-expressing interneurons are thought to be basket cells that target the perisomatic regions of pyramidal cells and other interneurons. In the medial entorhinal cortex (MEC), this unique target selectivity has been mostly studied in layer II. Here, we characterize previously unknown CCK-expressing interneuron types in the layer I of the MEC. First, using VGAT-IRES-Cre/BAC-CCK-eGFP-coIN transgenic mice, we found a population of cells with narrow spikes, stuttering firing and unique, layer I-specific axonal cloud. Electronmicroscopical investigation revealed dendrite and spine specific targets of their axons, expanding widely in layer I of the medial entorhinal cortex. Second, we found that a subpopulation of neurogliaform cells also contained CCK. We also found that these CCK-expressing neurogliaform cells differ from other neurogliaform cells in terms of their electrophysiological and axon morphological properties. We also demonstrate using single-cell transcriptomics that these cell groups have a unique gene expression patterns.

Glial fibrillar acidic protein (GFAP)-positive neurons in human postoperative neocortical brain samples

Eszter Juhász[1]; Estilla Zs. Tóth[1]; Liza Szalai[1]; Rebeka Stelcz[1]; Loránd Erőss[2]; Péter Orbay[2]; Imre Fedorcsák[2]; István Ulbert[1,2,3]; Kinga Tóth[1]; Lucia Wittner[1,2]
[1]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest
[2]Semmelweis University Clinics of Neurosurgery and Neurointervention, Budapest
[3]Péter Pázmány Catholic University, Faculty of Information Technology and Bionics, Budapest

Glial fibrillar acidic protein (GFAP) is known to be selectively expressed in astroglial cells. However, it can be detected in the blood of patients suffering from several neurodegenerative diseases including traumatic brain injury (TBI) and Alzheimer's disease (AD). As in post mortem samples of TBI patients, GFAP-positive cells with neuronal morphology were observed in the human neocortex.
We made peroxidase-based immunostaining against GFAP in 145 postoperative human neocortical samples derived from tumour and/or epileptic patients and found neuron-like cells in 41 samples. They were also present in organotypic slice cultures (n=15) prepared from these tissues (n=27), and showed different morphology. Pyramidal-like neurons with dark nucleus and long dendrites, cells with inhibitory interneuron morphology, as well as cells with pale cytoplasmic staining were observed. Immunofluorescent staining combining GFAP with the neuronal markers NeuN and neurofilament-L (NFL) provided evidence for the neuronal identity of these cells. Correlation could not be demonstrated between the presence of GFAP+ neurons and gender, age, aetiology, lobe of origin, possible TBI events or AD.
The hypotheses, i.e. either neurons start to express GFAP, or the NFL changes its conformation in the way that the antibody against GFAP recognizes it, have to be answered with further experiments. The factors initiating the appearance of GFAP-positive neurons might be linked to neurodegenerative diseases and remain to be identified.

Evolution of Inhibitory Neurotransmission: GABAergic Signaling in a Numerically Simpler Nervous System

István Fodor[1]; Luis Alfonso Yañez-Guerra[2]; Bence Kiss[3]; János Schmidt[3]; Veronica Rivi[4]; Cristina Benatti[4]; Edit Pollák[5]; László Molnár[1]; Tomohiro Osugi[6]; Shin Matsubara[6]; Honoo Satake[6]; Tibor Kiss[1]; Károly Elekes[1]; Zsolt Pirger[1]
[1]*HUN-REN Balaton Limnological Research Institute, Tihany*
[2]*Institute for Life Sciences, University of Southampton, Southampton*
[3]*Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs*
[4]*Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena*
[5]*Institute of Biology, Faculty of Natural Sciences, University of Pécs, Pécs*
[6]*Bioorganic Research Institute, Suntory Foundation for Life Sciences, Seika*

Inhibitory neurotransmission is a fundamental functional property of nervous systems. However, the evolution of GABAergic signaling, as well as its functional diversity in invertebrates, has largely been unexplored. Here, we provide a comprehensive analysis of the GABAergic system in the CNS of a widely studied molluscan model, the great pond snail (*Lymnaea stagnalis*), by applying a complex experimental approach.
Using cluster analysis and phylogenetics, we mapped the complete GABAergic toolkit across prebilaterian and bilaterian lineages. Our results revealed that GABAB receptors are found even in animals without defined nerve cells, whereas GABAA receptors are exclusive for cnidarians and bilaterians. Moreover, canonical GABAA receptor subunits (α, β, and γ) are conserved, while δ, ε, π, and θ subunits represent vertebrate-specific types, highlighting a deep evolutionary divergence in inhibitory receptor architecture. Comparative analyses also showed lineage-specific differences in GABA transporters, including the absence of GAT-2 in gastropods and cephalopods, suggesting that mechanisms of synaptic clearance have evolved differently across lineages. Neurochemical assays confirmed the presence of GABA and its precursors within the CNS, and immunogold electron microscopy provided *a priori* ultrastructural evidence of GABA localization within synaptic varicosities of a gastropod CNS. The expression pattern of GABAA and GABAB receptor subunits in the CNS visualized by *in situ* hybridization, as well as electrophysiological recordings on various identified single neurons, including the cerebral giant cell (CGC), a key interneuron involved in the LTM formation after food-reward classical conditioning, seems to confirm the postsynaptic effects of GABA. Ligand-binding assays showed no interaction between GABA and *Lymnaea* GABAB receptor, indicating unique regulatory mechanisms (e.g., chaperon or auxiliary proteins) which are absent in vertebrates. Finally, machine learning-based modeling predicted the quaternary assemblies of *Lymnaea* GABAA and GABAB receptors, linking molecular evolution with functional architecture.
Together, our findings contribute to our understanding how inhibitory signaling has diversified during evolution, showing that while the fundamental principles of GABAergic transmission are deeply conserved, lineage-specific modifications may shape circuit dynamics and, consequently, behavioral complexity.

Cellular and molecular footprint of aging in a defined neuronal network encoding associative memory

István Fodor[1]; Bence Gálik[2]; Péter Urbán[2]; János Schmidt[3]; Réka Svigruha[1]; Sándor Gonda[1,4]; György Kemenes[1,5]; Ildikó Kemenes[1,5]; Zsolt Pirger[1]
[1]*HUN-REN Balaton Limnological Research Institute, Tihany*
[2]*Genomics and Bioinformatics Core Facilities, Szentágothai Research Centre, University of Pécs, Pécs*
[3]*Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Pécs*
[4]*Department of Pharmacognosy, University of Debrecen, Debrecen*
[5]*School of Life Sciences, Sussex Neuroscience, Brighton*

Due to the complexity of the central nervous system (CNS), the study of aging processes in vertebrates is not an easy task at the level of neural circuits and individually identified neurons. As a result, aging research heavily relies on invertebrate model organisms. One such model is the great pond snail (*Lymnaea stagnalis*), which has been used extensively for decades to study aging and age-related memory impairment with a characteristically integrative top-down approach.
We made the neuronal transcriptome assembly of *Lymnaea* and identified several evolutionarily conserved homolog sequences to genes involved in aging, age-related memory impairment, and neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease) of vertebrates including humans. We hypothesize that the proteins encoded by these sequences are involved in age-related impairments of learning mechanisms in *Lymnaea* by targeting the identified components (e.g., NMDA receptor) of the signalling pathways of long-term memory formation. Using young (3-4-month) and old (11-12-month) snails, we investigated the age-related cellular and molecular changes in the whole CNS and in an identified key interneuron of implicit learning, the Cerebral Giant Cell (CGC). In the whole CNS, the expression of 960 transcripts significantly changed during aging. Highlighting, the expression of several key molecules of learning, such as NMDA receptor and CREB-binding protein, showed an age-related decline. In the CGC, the expression of 143 transcripts showed an age-dependent manner. Using LC-MS-based untargeted lipidomics, we identified 291 lipids in the whole CNS. The levels of 79 lipids significantly changed during aging. Notably, polyunsaturated fatty acids increased, diacylglycerols decreased, and a phospholipid-lipophospholipid shift was observed, indicating age-related alterations in membrane fluidity and certain signal transduction pathways. Our LC-MS-based proteomics investigations also revealed proteins that significantly changed during aging (data evaluation is ongoing).
The identified cellular and molecular changes both at the system and single-cell levels during aging which may contribute to age-related memory impairment. The investigation of molecular processes underlying age-related memory decline in more detail leading to the discovery of novel mechanisms operating not just in molluscs but also in higher organisms.

Species-Specific T-Type Calcium Channel Contributions to Spike Precision in Human Parvalbumin Interneurons

Abdennour Douida[1]; Viktor Szegedi[1,2]; Attila Szucs[3]; Emőke Bakos[1,2]; Ádám Tiszlavicz[1]; Daphne Welter[4]; Jonathan Landry[4]; Szabina Furdan[1]; Gabor Hutoczki[5]; Pal Barzo[6]; Gabor Tamas[7]; Karri Lamsa[1,2]

[1]Hungarian Center of Excellence for Molecular Medicine, Research Group for Human neuron physiology and therapy, Szeged
[2]University of Szeged, Department of Physiology, Anatomy and Neuroscience, Szeged
[3]Eötvös Loránd University, Neuronal Cell Biology Research Group, Budapest
[4]European Molecular Biology Laboratory, Heidelberg
[5]University of Debrecen Clinical Centre, Department of Neurosurgery, Debrecen
[6]University of Szeged, Department of Neurosurgery, Szeged
[7]University of Szeged, Department of Physiology, Anatomy and Neuroscience, ELKH-SZTE Research Group for Cortical Microcircuits, Szeged

Parvalbumin-expressing interneurons (PVINs) play a vital role in regulating neocortical gamma oscillations, which are crucial for precise neuronal timing. Disruptions in these oscillations are linked to various disorders, including schizophrenia, epilepsy, and autism spectrum disorder. To overcome limitations of mouse models for translating findings to human biology, we examined species-specific differences in PVIN excitability and spike timing. Our research showed that human PVINs have a unique subthreshold membrane voltage deflection, absent in mice. This active conductance, driven by T-type calcium channels, enhances spike precision. By adding this conductance into mouse cells through dynamic clamp, we improved spike timing, demonstrating its critical role in neuronal temporal synchrony. Single-cell patch sequencing revealed that the gene encoding the CaV3.1 subunit of T-type calcium channels (CACNA1G) is expressed at higher levels in human PVINs. Additionally, confocal imaging showed Cav3.1 channels are enriched in the dendrites of human PVINs, a feature not present in mice. These findings emphasize the importance of T-type calcium channels in fine-tuning the excitability and spike timing of human PVINs, offering valuable insights into species-specific mechanisms with significant implications for translational brain research.

The protection of the blood-brain barrier by a small-molecule cocktail, cARLA in a cell culture model of ischemic stroke

Anikó Szecskó[1,2]; Koppány Párdi[1]; Zuhao Cui[1,2]; Gergő Porkoláb[1,3]; Zsófia Hoyk[1]; Csilla Kovács[1]; Nárcisz M. Cser[1]; Krisztina Tóth[4]; Ádám Dénes[4]; Csilla Sajben[5]; Roland Tengölics[5]; Mária A. Deli[1]; Szilvia Veszelka[1]
[1]HUN-REN Biological Research Centre, Institute of Biophysics, Biological Barriers Research Group, Szeged
[2]University of Szeged, Doctoral School of Biology, Szeged
[3]Trinity College Dublin, Smurfit Institute of Genetics, Dublin
[4]HUN-REN Institute of Experimental Medicine, Neuroimmunology Research Group, Budapest;
[5]HUN-REN Biological Research Centre, Core Facilities, Metabolomics Laboratory, Szeged

Stroke is the second leading cause of death, and 88% of all stroke cases are ischemic triggered by a vascular occlusion caused by a thrombus. During ischemic stroke, the resulting lack of oxygen and glucose and the subsequent reperfusion leads to neuronal damage and disruption of the blood-brain barrier (BBB). Currently, there are two main approaches in clinical practice for the treatment of ischemic stroke: thrombolysis and mechanical thrombectomy. However, these procedures can only be used in larger arteries, carry significant risks and have a narrow therapeutic window (4.5 h) from symptom onset. Therefore, additional effective therapeutic approaches for treatment of ischemic stroke are urgently needed.
The small-molecule combination, cARLA, simultaneously activates cAMP and Wnt/β-catenin signaling while inhibiting the TGF-β pathway of the BBB. Previously we demonstrated that cARLA robustly enhancing BBB properties by increasing barrier tightness, glycocalyx density, and shifting gene expression toward a brain endothelial phenotype under normal conditions. Here, our aim was to investigate the effect of cARLA on restoring the function of the BBB to prevent ischemic damages.
The effects of cARLA were tested on the human model of the BBB under normoxia and during a 24-hour reoxygenation (OGD/R) following a 6-hour oxygen-glucose deprivation (OGD). The viability of brain endothelial cells decreased dramatically after OGD, whereas cARLA treatment during OGD/R significantly improved cell index and metabolic activity detected by impedance-based measurement and MTT assay. The expression of the tight junction protein claudin-5 and sialic acid residues of the glycocalyx was significantly reduced after OGD, but cARLA treatment increased the levels of both during reoxygenation. Moreover, cARLA influenced BBB-related gene expression, modulated immune responses, and altered metabolomic profiles after ischemic events.
Based on our results, cARLA may be a potential therapeutic tool for the treatment of ischaemic stroke, but further research is needed to gain a deeper understanding of its mechanism of action and to develop its potential clinical applicability.

Investigation of astrocyte subtypes in the dorsolateral prefrontal cortex

Paulina Hoppa[1]; Katalin Berta[1]; Teadora Tyler[1]; Erzsébet Frank[1]; Gergely Rácz[2]; István Adorján[1]
[1]*Semmelweis Egyetem, Anatómiai, Szövet- és Fejlődéstani Intézet, Budapest*
[2]*Semmelweis Egyetem, Patológiai és Kísérleti Rákkutató Intézet, Budapest*

Astrocytes play crucial roles in maintaining brain homeostasis and modulating synaptic function. Although astroglial heterogeneity in the human cerebral cortex is increasingly recognized, its involvement in neuropsychiatric disorders remains poorly understood. We aimed to investigate different astroglial populations present in the the dorsolateral prefrontal cortex (DLPFC) with morphometric and topographic analyses.
Human postmortem brain samples from ten individuals were analyzed. Immunohistochemistry was performed to label three astrocyte populations using the following markers: glial fibrillary acidic protein (GFAP), aldehyde dehydrogenase 1 family member L1 (ALDH1L1); and dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32). Astrocytes were manually counted across cortical layers I–VI, and the longest diameter was also measured.
GFAP+ and ALDH1L1+ populations displayed partially overlapping, complementary distributions, with GFAP+ astrocytes preferentially located in layers I and VI, and ALDH1L1+ astrocytes concentrated in layers II–V. DARPP32+ astrocyte density was predominant in layer two. Preliminary qualitative observations from fluorescent double-staining reveal a strong overlap between DARPP32+ and ALDH1L1+ cell populations, in contrast to the limited overlap observed between GFAP–DARPP32+ and GFAP–ALDH1L1+ populations.
Our study indicates diverse astroglial populations distributed in the human cerebral cortex in a complementary fashion. Furthermore, our results suggest that the use of GFAP in routine pathological investigations only informs about approximately one-third of the cortical astroglia. Regional distribution of diverse astroglial populations was mapped quantitatively in the human grey matter which will allow future investigations of potential astroglial alterations in conditions such as autism spectrum disorder and schizophrenia.

Integrative transcriptomic analysis of cell-to-cell communication patterns in the human striatum

Teadora Tyler[1,2]; Gábor M. Mórotz[3,4,5]; Tamás Kovács[3,4,5]; Zoltán V. Varga[3,4,5]; Zdravko Petanjek[6]; Árpád Dobolyi[1,2]; István Adorján[2]
[1]Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest
[2]Semmelweis University, Department of Anatomy, Histology and Embryology, Budapest;
[3]Semmelweis University, Department of Pharmacology and Pharmacotherapy, Center for Pharmacology and Drug Research & Development, Budapest; [4]Semmelweis University, HCEMM-SU Cardiometabolic Immunology Research Group, Budapest; [5]MTA-SE Momentum Cardio-oncology and Cardio-immunology Research Group, Budapest
[6]University of Zagreb, Croatian Institute for Brain Research, Zagreb

The striatum plays a crucial role in action selection and higher cognitive functions, and is implicated in a range of neuropsychiatric and neurodegenerative disorders. Recent single-cell transcriptomic studies began to establish the cellular composition of the human striatum, but the molecular signaling landscape between these cell types remains poorly understood. Here, we integrated three independent human caudate nucleus single nucleus RNA sequencing datasets in R environment using Seurat into a harmonized cohort comprising over 70,000 high-quality nuclei. After quality control and batch correction, we recovered all expected major striatal cell types, including D1, D2, and hybrid D1/D2 medium spiny neurons (MSNs), striatal interneuron subtypes, oligodendrocytes, astrocytes, microglia, and vascular-associated cells. We used CellChat to infer potential ligand–receptor–based cell-to-cell signaling patterns between cell populations, providing a data-driven overview of predicted signaling networks within the human caudate. RNAscope in situ hybridization was applied to validate selected genes (*OPRD1*, *TAC3;* N=3 donors).
CellChat analysis revealed structured, cell type–specific communication networks across neuronal and non-neuronal populations, including the suggestion that calretinin-expressing (CR+) interneurons participate in opioid signaling via delta opioid receptor *(OPRD1)* expression, which we confirmed by RNAscope – providing a link between this interneuron class and opioid-related signaling in the human striatum, previously primarily attributed to MSNs. In addition, the Cellchat analysis highlighted candidate ligand–receptor pairs that exhibit marked cell type specificity, including interactions involving somatostatin interneurons (e.g., *FLRT3–UNC5D*), which may represent focal points for future functional and pharmacological studies.
The CR+ interneurons (identified by *CALB2* and *TAC3* expression) have been described as a primate-enriched interneuron population, raising the hypothesis of species-specific aspects of striatal neuromodulation, as well as potential relevance for neuropsychopharmacology. More broadly, the newly integrated single-nucleus resource provides a framework for systematically exploring cell type–resolved signaling architecture in the human caudate nucleus.

State-dependent firing of cortical non-fast-spiking and regular-spiking interneurons during natural sleep

Janos Horvath[1]; Sándor Bordé[1]; Boglárka Bozsó[1]; Robert Averkin[1]; Gábor Tamás[1]
[1]University of Szeged Faculty of Science and Informatics, Physiology, Anatomy and Neuroscience, ELKH-SZTE Research Group for Cortical Microcircuits, Szeged

Population activity of neocortical neurons is tightly linked to behavioral state; however, the distinct contributions of cortical neuron types to state-dependent dynamics remain poorly understood. Here, we used a drug-free pipette microdrive technique for juxtacellular recording and labeling to study identified cortical pyramidal neurons (PYRs), a heterogeneous population of non-fast-spiking or regular-spiking interneurons (RSIs), and parvalbumin-positive fast-spiking interneurons (PV^+ FSIs) in freely behaving rats during natural sleep. We focused on characterizing the electrophysiological properties of RSIs and comparing them with those of PYRs and FSIs across non–rapid eye movement (NREM) and rapid eye movement (REM) sleep. The recorded neurons (PYRs n=35; RSIs n=32; FSIs n=29) formed well-separated groups in both sleep states based on firing properties, including median firing rate, action potential half-width, and burst firing ($p<0.001$). Notably, the bursting index was significantly lower during REM than NREM sleep ($p<0.001$) across all three cell types. Unlike PYRs and FSIs, RSIs exhibited a broad range of distinct firing patterns in both sleep states, suggesting the presence of functionally distinct subpopulations.

To quantify sleep-state modulation, we calculated a sleep-state preference value for each neuron based on the ratio of firing rates in NREM versus REM sleep. Silhouette-optimized k-means clustering revealed two major RSI groups: NREM-preferred (NREMp; n=9/3 labeled), characterized by increased activity during NREM sleep ($p=0.004$), and REM-preferred (REMp n=12/3 labeled), characterized by reduced activity during NREM sleep ($p<0.001$).

Further analyses identified at least two subgroups within each main RSI category. Among NREMp RSIs, some neurons were strongly inhibited outside NREM sleep (n=2), whereas others showed reduced firing during REM sleep (n=5). Within the REMp group, some neurons were active exclusively during REM sleep (n=3), while others exhibited reduced firing during NREM sleep (n=7). These cells showed tonic, phasic, strongly theta phase–locked, or mixed tonic/phasic firing patterns during REM sleep.

Consistent with previous observations, most FSIs were more active during NREM sleep (4 out of 6 cells analyzed). In conclusion, RSIs exhibit a markedly greater diversity of sleep state–dependent firing modulation compared with FSIs and PYRs, highlighting their potential role in shaping cortical network dynamics across sleep states.

A versatile brain-on-a-chip system to study central nervous system diseases and brain targeting

Judit P. Vigh[1,2]; Anna E. Kocsis[1,2]; Ana R. Santa-Maria[1,3]; Nóra Kucsápszky[1,2]; Silvia Bolognin[4]; Jens C. Schwamborn[4]; András Kincses[1]; Anikó Szecskó[1,2]; Szilvia Veszelka[1]; Mária Mészáros[1]; Emese Bélai[1]; Melinda Pirity[1]; András Dér[1]; Fruzsina R. Walter[1]; Mária A. Deli[1]
[1]HUN-REN Biological Research Centre, Szeged
[2]Doctoral School of Biology, University of Szeged, Szeged
[3]Wyss Institute, Harvard University, Boston
[4]University of Luxembourg, Belvaux

The blood-brain barrier (BBB) protects the brain and provides oxygen and nutrients for the central nervous system (CNS). It also restricts the entry of neurotherapeutics into the brain. Microfluidic chip devices allow complex and physiological modelling of the BBB. Stem cell-based technologies and human brain organoids provide a new platform to study diseases, cellular interactions and drug uptake in a 3D setup. Our aim was to establish a complex dynamic lab-on-a-chip model integrating a human BBB co-culture model with cortical or midbrain organoids to study brain pathological conditions and nanoparticle penetration across the BBB.
Human stem cell derived endothelial cells and brain pericytes were used to create the BBB model. Human midbrain or cortical organoids were differentiated from healthy donor iPSCs. To mimic pathologies, the BBB model was treated with proinflammatory cytokines or tetrahydrocannabinol (THC) and the barrier integrity was investigated in a dynamic setup by the measurement of impedance and permeability for fluorescent markers. The morphology of brain endothelial cells was examined by immunostaining for tight junction associated proteins. A clinically used hyperosmolar contrast agent was also tested on the chip model to reveal the cellular mechanism of its CNS side effects. The functionality of the model was tested by the passage of nanocarriers across the BBB and by characterizing the uptake into the brain organoids.
Pro-inflammatory cytokine and THC treatments decreased the BBB integrity and altered the morphology of brain organoids. The contrast agent caused BBB dysfunction and damaged the neuronal network. BBB-targeted nanoparticles carrying a fluorescent cargo crossed and entered the brain organoids effectively. In conclusion, the new BBB-on-a-chip system can be a valuable research tool for toxicological, pharmacological and pathological investigations.

Exploring the cellular components of contralateral hippocampal connectivity using an all-optical approach

Dániel Balogh[1]; János Brunner[1]; Antónia Arszovszki[1]; Eszter Sipos[1]; János Szabadics[1]
[1]HUN-REN Institute of Experimental Medicine, Cellular Neuropharmacology, Budapest

Important components of the hippocampal circuits remained unknown despite their critical involvement in episodic memory. CA3 pyramidal cells and hilar mossy cells form extensive axonal arborization in both the ipsi- and contralateral hippocampi. Consequently, almost half of the Schaffer collateral synapses and other influential excitatory drives arrive from the opposite hemisphere. However, it was not possible to precisely map contralateral connections between the two hemispheres of the hippocampus at the level of individual neurons.
We employ optical imaging techniques to precisely measure the synaptic responses from large populations of individual hippocampal neurons evoked by contralateral hippocampal input pathways. Specifically, we explored the applicability of voltage imaging with Voltron sensor in diverse populations of CA1, CA2 and CA3 neurons while specific subsets of contralateral fibers were activated by optogenetic ChR2 actuators. These new experiments posed several technical challenges, and this poster demonstrates our current solutions. These include (1) the optimal acquisition setting for a CMOS camera based imaging, (2) improvement of the sensitivity of imaging with optimal spectral ranges of the Voltron sensor, (3) identification and characterization of direct excitatory and disynaptic inhibitory responses elicited by contralateral fibers, (4) affiliating axonal bundles to precise source region in the contralateral hemisphere, and (5) and anatomical characterization of the postsynaptic target cells which receive strong excitation and also those that are specifically avoided by these massive axonal clouds.

Neurochemical heterogeneity of corticotropin-releasing hormone–expressing neurons near the pontine midline in the mouse brain

Shima Rashidiani[1]; Viktória Kormos[2]; Balázs Gaszner[3]; , Dóra Zelena[4]
[1]1Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary, Pecs
[2]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Pécs
[3]3Department of Anatomy, Medical School and Research Group for Mood Disorders, Centre for Neuroscience, University of Pécs,, pecs
[4]1Institute of Physiology, Medical School, University of Pécs, Pécs, Pécs

Corticotropin-releasing hormone (CRH)–expressing neurons located near the midsagittal plane of the pontine tegmentum have been inconsistently assigned to the median raphe nucleus (MNR), paramedian raphe, or the reticulotegmental nucleus of the pons (RtTg). Here, we aimed to characterize the precise localization and neurochemical identity of these CRH-positive neuronal populations.

Using RNAscope in situ hybridization combined with immunohistochemistry, we examined *Crh* mRNA expression together with markers for serotonergic (*Tph*), GABAergic (*Gad1*), and glutamatergic (*Vglut1, Vglut2, Vglut3*) phenotypes in intact C57BL/6 and NMRI mice (N = 5 per group). Anatomical localization was guided by the Allen Mouse Brain Atlas and Paxinos and Franklin stereotaxic coordinates.

Crh-expressing neurons near the pontine midline segregated into two spatially and neurochemically distinct populations. Dorsally located CRH neurons (corresponds to MNR) co-expressed *Gad1* mRNA but lacked *Vglut1/2/3* expression, indicating a GABAergic phenotype. In contrast, ventrally located CRH neurons (corresponds to RtTg) did not express *Gad1* but robustly co-expressed *Vglut2* and, in a subset, *Vglut1* mRNA, consistent with a glutamatergic identity. Interestingly, some neurones co-expressed both *Vglut1* and *Vglut2*, simultaneously. CRH neurons in this region did not co-express *Tph*, excluding a serotonergic phenotype, and showed minimal co-expression with *Vglut3*.

These findings reveal pronounced neurochemical heterogeneity among CRH neurons near the pontine midline and suggest that a substantial fraction of ventral CRH neurons likely belong to the RtTg rather than the MRR with a substantially different neurotransmitter composition. This distinction has important implications for interpreting the functional role of pontine CRH circuits in stress- and arousal-related behaviors.

Cariprazine alters firing profiles and network activity of mouse primary hippocampal neurons in a cell type-dependent manner

Melinda E. Gazdik[1,2,3]; Anna A. Abbas[1]; Balázs Kis[1]; Ádám Tiszlavicz[4]; Lea Danics[1]; Kai K. Kummer[5]; Katalin Schlett[2]; Karri P. Lämsä[4]; Karolina Pircs[1,3]; Attila Szűcs[1,2,3]
[1]*Semmelweis University, Institute of Translational Medicine, HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Budapest*
[2]*Institute of Biology, Eötvös Loránd University, Department of Physiology and Neurobiology, Cellular Neurobiology Research Group, Budapest*
[3]*Hungarian Research Network (HUN-REN), Office for Supported Research Groups (TKI), HUN-REN-SZTAKI-SU Rejuvenation Research Group, Budapest*
[4]*Hungarian Centre of Excellence for Molecular Medicine, HCEMM Human Neuron Physiology and Therapy Core Group, Szeged*
[5]*Institute of Physiology, Medical University of Innsbruck, Innsbruck*

Schizophrenia is a severe, chronic psychiatric disorder characterized by heterogeneous symptom domains: positive symptoms (e.g., hallucinations, delusions), negative symptoms (e.g., diminished motivation, social withdrawal) and cognitive deficits, that together produce substantial functional impairment. Effective pharmacological management therefore requires agents that attenuate multiple symptom domains while maintaining acceptable tolerability. Cariprazine is a third generation antipsychotic drug acting on dopamine D2/D3 receptors by partial agonism with a preference on D3-receptors. This compound has demonstrated efficacy in reducing both positive and negative symptoms and is generally associated with a favorable side-effect profile relative to several commonly used drugs (e.g., aripiprazole, haloperidol, pramipexole). In this study, we investigated the effects of cariprazine on neuronal membrane properties and network activity using *in vitro* primary hippocampal cultures from mice, employing whole-cell patch-clamp recordings and multielectrode array (MEA) measurements. Patch-clamp experiments revealed that acute treatment with 1 µM cariprazine induced a shift in the firing phenotypes of hippocampal neurons, with regular-firing cells tending to adopt an irregular firing mode in the presence of the drug. This effect was more pronounced in neurons exhibiting strong voltage-dependent potassium currents, which are known to contribute to the generation and maintenance of irregular firing patterns in several neuronal cell types, including interneurons.
Consistent with these findings, MEA recordings demonstrated that acute treatment with 1 µM cariprazine reduced overall network activity in hippocampal cultures, an effect comparable to that observed with aripiprazole, haloperidol, and pramipexole. While aripiprazole and haloperidol produced a robust suppression of neuronal firing, cariprazine and pramipexole exerted a more moderate regulatory effect on network activity. Collectively, these results complement existing literature on the favorable pharmacological profile of cariprazine and provide new insights into the cellular and network-level mechanisms underlying its therapeutic actions.

Investigating the role of major cholinergic cell populations during Pavlovian conditioning

Réka Kispál[1]; Írisz Szabó[1]; Dániel Schlingloff[1,2]; Balázs Hangya[1,3]
[1]HUN-REN Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience,, Budapest
[2]University of Copenhagen, Laboratory of Systems Neuroscience, Copenhagen
[3]Medical University of Vienna, Division of Neurophysiology, Center for Brain Research, Vienna

The cholinergic system regulates numerous cognitive and behavioral functions, such as learning, memory processes and attention. Investigating less explored cholinergic cell populations may provide new insights into cognitive disorders and support the development of targeted therapies.
In this study, we investigated how five key cholinergic regions contribute to associative learning: the horizontal limb of the diagonal band of Broca (HDB), the ventral pallidum (VP), the ventral striatum (VS), the dorsal striatum (DS) and the lateral septum (LS).
ChAT-Cre mice were trained in a sound detection Pavlovian conditioning task. Animals were presented with either a reward or punishment predicting cue, followed by a delay, and the predicted reinforcement in 90% of cases and omission in 10%. To monitor cholinergic activity dynamics, we expressed GCaMP8 in cholinergic neurons and recorded bulk calcium signals using fiber photometry.
Behavioral results indicated that mice differentiated between reward- and punishment-predicting cues confirming that they successfully learned the task. Photometry recordings showed that the VP and VS responded more strongly to reward-predictive cues and reinforcements, whereas the HDB and DS exhibited faster and larger responses to punishment predicting-cues and punishment delivery. In contrast, the LS showed no cue response for either of the stimulus types; however, its feedback response resembled that of the HDB and the DS. Correlation analyses between cue and reinforcement responses indicated that the VP, VS and LS showed positive correlations for both reward- and punishment-related signals. Meanwhile, the HDB showed a negative correlation for reward responses, but a positive correlation for punishment.
All five cholinergic populations responded to both rewards and punishments, but their response timing and magnitude differed across regions. Cholinergic cells in the VP and VS showed faster and stronger reward responses, while the HDB, DS and LS were more responsive to punishments. Correlation analyses indicated that the HDB encoded reward prediction error, responses were scaled with the unexpectedness of reward, but not with the expectedness of punishment, indicating no punishment prediction error. These findings indicate that different cholinergic populations contribute differentially to reward and aversive learning through region-specific patterns of activity.

Investigating neuromodulator release during implicit learning in a stochastic alternating serial reaction time task in mice

Yahya Sulok[1]; Írisz Szabó[1]; Réka Kispál[1]; Hanga Dormán[1]; Balázs Hangya[1,2]
[1]*HUN-REN Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience, Budapest*
[2]*Medical University of Vienna, Center for Brain Research, Division of Neurophysiology, Vienna*

Introduction: The brain can use a variety of learning strategies to adapt to an ever-changing environment, which is essential for survival. The implicit sequential learning we studied is a type of learning in which we unconsciously acquire a certain action sequence. Examples of skills developed through this kind of learning include riding a bike or speaking.
Objective: One of the main goals of our research was to develop a model analogous to human studies, allowing for the comparison of characteristics measured in humans and animals. Additionally, we aimed to understand how the dopaminergic and cholinergic systems encode this process.
Methods: In our experiment, we trained mice using a water reward. The sequence was represented by the order in which four lights turned on, which the mice had to follow by nose pokes. The animals received a reward after every fourth correct choice. The sequence included fixed elements alternating with random ones, which created frequent and rare triplets. We also included test trials, where all four lights turned on at the same time and the animal had to choose the correct one. Using neuromodulator biosensors, we measured dopamine (DA) release from the ventral striatum (VS) and prefrontal cortex (PFC), and acetylcholine (ACh) release from the basolateral amygdala (BLA) through fiber photometry.
Results: The animals responded faster to frequent triplets, and they also omitted less compared to the rare ones. In the test trials the animals' accuracy was higher for frequent triplets and was also above chance level. We divided the animals into "implicit" and "explicit" learner groups based on their performance. Comparing the two groups, we found that the "implicit" learners showed higher DA release in the VS and higher ACh release in the BLA while the explicit learners showed higher DA release in the PFC.
Conclusions: The improvement in reaction time suggests that the animals were able to distinguish between frequent and rare triplets, and the better accuracy at the frequent triplets in the test trials indicates that this knowledge was accessible. The differences in brain activity patterns between the two groups imply that the dominance of certain networks depends on the type of learning involved.

Effects of post-weaning social isolation on baseline and recovery sleep and EEG patterns in male rats

Dorka Kárász[1], Tünde Hajnik[1], Attila Tóth[1]
[1]*Eötvös Loránd University, Faculty of Science, Physiology and Neurobiology, In vivo electrophysiology, Budapest*

Post-weaning social isolation (PWSI) in rats models early-life social deprivation inducing neurodevelopmental alterations. The sleep-regulating medial prefrontal cortex (mPFC) is particularly affected by PWSI. Social isolation is known to induce various behavioural alterations, but its impact on sleep and EEG remains overlooked. In this study, we aimed to characterise the effects of PSWI on active- (AW) and quiet wakefulness (QW), light sleep (LS), deep sleep (DS) and REM sleep during baseline sleep and after 6-h sleep deprivation. Male rats with PWSI or social housing were implanted with mPFC- and vertex EEG electrodes and neck EMG electrodes. PWSI rats showed more frequent and prolonged epochs of AW and QW in the light phase, while the amount of AW decreased in the dark phase. DS occurred more frequently during the dark, but with shorter episodes than in the light phase. Notably, the total amount of wakefulness and non-REM sleep did not differ between groups, as PWSI induced a temporal redistribution of vigilance states, reflected by an approximately 5-hour phase shift in the sleep-wake cycle rather than an overall change in sleep quantity. REM sleep was less frequent in both phases. After the 6-h sleep deprivation, PWSI rats exhibited pronounced DS but reduced REM sleep replacement. mPFC EEG showed reduction both in high gamma (53-98 Hz) power and density during both AW and QW in the light phase. LS and REM sleep theta (4-10 Hz) power and density was reduced during the dark phase, suggesting reduced intensity for wakefulness and REM sleep in PWSI rats. These findings indicate that PWSI shifts the sleep-wake balance toward elevated wakefulness in the light phase, enhancing the DS rebound after sleep deprivation. However, both baseline and recovery REM sleep remain reduced.

High-density spatiotemporal single-unit activity profiling and cell-type identification in the human neocortex

Orsolya Farkas[1,2]; William Munoz[3]; Richard Hardstone[3]; Brian Coughlin[4]; Irene Caprara[3]; Mohsen Jamali[3]; István Ulbert[1,2,5]; Ziv M. Williams[3]; Sydney S. Cash[4]; Angelique C. Paulk[4]; Domokos Meszéna[1,2,4]

[1]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Integrative Neuroscience Group, Budapest

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

[3]Massachusetts General Hospital, Harvard Medical School, Department of Neurosurgery, Boston, MA

[4]Center for Neurotechnology and Neurorecovery, Massachusetts General Hospital, Harvard Medical School, Department of Neurology, Boston, MA

[5]Semmelweis University, Faculty of Medicine, Department of Neurosurgery and Neurointervention, Budapest

High-density silicon probes have transformed the study of neuronal populations in animal models, yet their use in the human cortex has remained limited. Leveraging recently developed clinically adapted Neuropixels probes, we analyzed intraoperative extracellular recordings to characterize single-unit waveform properties, classify putative neuronal types, and identify spatiotemporal propagation phenomena at cellular resolution. Following motion correction and manual curation of spike-sorted units, cluster quality and template-based waveform metrics were computed using the SpikeInterface framework, including mean waveform, peak-to-valley time, half-width, and peak amplitude. Multiple clustering strategies were tested on the computed waveforms and metrics. After empirical thresholding of peak-to-valley distributions, unsupervised algorithms such as feature-based and waveform-based k-means were applied. The WaveMAP package (using nonlinear embedding and Leiden clustering) provided the most coherent results, and we therefore used its four waveform groups - two narrow, one broad, and one triphasic - as the basis for further analysis. Clusters with narrow waveforms were interpreted as putative interneurons, broad units as pyramidal cells, while triphasic waveforms likely represented mixed or axonal signals. This approach yielded an unsupervised, data-driven separation of putative cell types in the absence of histological ground truth. To characterize spatial aspects of signal propagation, we computed the spread and vertical propagation velocity of single units as multi-channel propagation metrics. A subset of units showed extracellular signatures of somatodendritic action potential backpropagation (bAP), expressed as consistent propagation across adjacent contact sites. bAP presence did not depend on spike amplitude, firing frequency, or count, but correlated with WaveMAP-defined cell-type classes. These spatiotemporal patterns matched canonical bAP signatures from rodent studies. In contrast, we observed a high-amplitude yet spatially confined “small-footprint” unit group classified as putative interneurons with narrow waveforms. Although their physiological origin remains unresolved, recent studies suggest they may instead reflect axonal signals from Ranvier nodes. Our large-scale analysis of Neuropixels single-unit recordings via waveform-based clustering offers effective classification of cell types and propagation profiles in the human neocortex.

Investigating neuromodulator dynamics with fiber photometry in mice during a reversal learning paradigm

Annamária Benke[1]; Írisz Szabó[1]; Bálint Király[1,2]; Yi Shih Lee[1]; Vivien Pillár[1,3]; Balázs Hangya[1,4]
[1]*HUN-REN Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience, Budapest*
[2]*Institute of Artificial Intelligence, Medical University of Vienna, Vienna*
[3]*Semmelweis University – HCEMM, Neurobiology and Neurodegenerative Diseases Research Group, Budapest*
[4]*Center for Brain Research, Medical University of Vienna, Division of Neurophysiology, Vienna*

Dopaminergic (DA), cholinergic (ACh), and noradrenergic (NE) systems shape learning, rapid adaptation, decision-making, and behavior, and their dysregulation contributes to disorders such as Alzheimer's and Parkinson's which can impair cognitive abilities. Although we know a lot about these neuromodulator systems, their dynamics during decision-making are still unknown.
Our aim is to investigate how an environment that requires continuous adaptation influences learning and the roles of neuromodulators in this adaptation.
In our experiment, water-restricted, freely moving, young adult female and male mice are placed in an automated training system for one week, where they are expected to choose between a left or right water dispenser port after a light stimulus, which encode water reward with different probabilities. We control the variability of the environment through changing contingencies and block lengths. We interpret similar probabilities and short blocks as variable, and distinct probabilities and long blocks as predictable. DA, ACh, and NE activity was recorded in prefrontal cortex, basolateral amygdala (BLA), and ventral striatum (VS) by measuring fluorescent signals using fiber photometry.
Animals performed better and required fewer trials to switch sides in predictable than in variable environments (performance, 76 vs. 62%, respectively). Their behavior was also influenced by the previous block: their performance was higher, and they could switch from one side to the other quicker if they had been in a short block previously. DA and ACh showed opposing cortical dynamics but similar in deep nuclei. Cortically, DA increased after rewarded or omitted stimulus, whereas ACh spiked before stimulus. In deep nuclei, DA encoded value and reward prediction error (RPE), showing increase for rewards in general, while ACh was more sensitive to reward uncertainty, it distinguished between lucky and likely rewards. NE responded to stimuli in both brain areas but differentiated between reward and omission opposingly in the cortex.
Overall, mice learn better in a predictable environment. They adapt faster after variable ones. Photometry reveals that DA encodes RPE, ACh carries RPE-related signals that may shape decision-making and, and NE may adjust arousal and attention. Together, these systems form a cooperative yet distinct mechanism enabling continuous evaluation of outcomes and adaptive behavioral updating.

Beyond Acute Imaging: Chronic 3D Mapping of Multiple Visual Areas Using Functional Ultrasound

Abel Petik[1,2]; Klaudia Csikos[1,3]; Domonkos Horvath[1,2]; Attila B. Dobos[1]; Botond Roska[4]; Daniel Hillier[1,2]
[1]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest*
[3]*János Szentágothai Neuroscience Doctoral School, Semmelweis University, Budapest*
[4]*Institute for Molecular and Clinical Ophthalmology Basel, Basel*

Functional imaging in neuroscience typically faces a fundamental trade-off between spatial resolution and the size of the accessible brain area. To understand visual information processing in the mammalian brain, however, it is crucial to resolve mesoscale structures, where the characteristic functional maps of early visual areas are organized. We present a functional ultrasound imaging approach that achieves mesoscale resolution (~150 µm) while maintaining a field of view large enough (>1500 mm^3) to simultaneously cover multiple visual cortical areas in a large animal model (cat). Using this method, we map key functional properties of the visual cortex, including retinotopy and orientation preference, and reconstruct these maps in 3D. A further advantage of our approach is its chronic applicability: we record from the same animal repeatedly over periods of months, or even years, enabling the longitudinal tracking of functional map stability and plasticity in vivo. We demonstrate the reproducibility of high-resolution 3D functional maps across sessions and compare their spatial organization over time.
To support these experiments, I developed an integrated acquisition and analysis software toolkit tailored to functional ultrasound imaging of the visual cortex. This pipeline streamlines experimental control, data processing, and 3D map reconstruction, establishing fUS as a robust tool for long-term mesoscale studies of cortical plasticity in large mammals.

Phase organization of the hippocampus- and entorhinal cortex-projecting GABAergic neurons in the medial septum

Kolos Németh[1]; Márta Jelitai[1]; Viktor Varga[1]
[1]*Institute of Experimental Medicine, Subcortical Modulation Research Group, Budapest*

Rhythmic oscillations in the brain provide a temporal framework for communication across neural circuits. These coordinated brain waves underlie diverse behaviors and cognitive functions, and their disruption—oscillopathies—is increasingly recognized as a mechanism underlying many neurological and psychiatric disorders. Among these rhythms, theta oscillations (4–12 Hz), paced by the medial septum (MS), are prominent in the hippocampus (HC) and entorhinal cortex (EC), where they organize neuronal firing during navigation, learning, and memory. **However**, the cellular mechanisms by which stable theta phase relationships arise within different parts of the episodic memory system remain unclear.

To address this, we aim to determine whether distinct MS cell groups broadcast different phases of theta signals to their respective targets, providing the temporal framework for coherent memory-related activity. **To this end**, we injected retrogradely spreading viral tracers into the HC and EC to label projection-defined MS neurons. High-density Neuropixels 2.0 silicon probes were used to record spiking activity in the MS together with hippocampal CA1 theta oscillations. **In addition**, optogenetic tools were applied to identify projection-defined septal cells and investigate their theta phase.

Retrograde viral tracers were injected into the EC and HC of 40 transgenic mice. **After establishing a reliable protocol**, we identified a dual-projecting septal population alongside previously described projection groups. **Building on this**, subsequent recording sessions in five mice allowed us to distinguish differentially projecting septal cells and compare their firing patterns and theta phase relationships.

Together, our findings further characterize the diverse septal cell populations modulating both hippocampal and entorhinal activity. They provide a basis for assessing how projection-specific septal neurons contribute to the theta-timescale coordination of activity within the episodic memory circuit and lay the groundwork for exploring the mechanisms of theta oscillopathies and the potential therapeutic relevance of medial septum stimulation in psychiatric disorders.

Directional bias of dendrites in parvalbumin-expressing wide-field amacrine cells of the rat retina

Levente Rácz[1]; Fruzsina György[1]; Ildikó Telkes[1]; Katalin Fusz[1]; Tibor Zoltán Jánosi[2,1]; Péter Kóbor[1]; Kristóf László[1]; Péter Buzás[1]
[1]*University of Pécs Medical School, Institute of Physiology, Pécs*
[2]*University of Pécs Medical School, István Ábrahám Nano-Bio-Imaging Core Facility, Pécs*

Background: Orientation and motion detection in the visual system rely on the integration of photoreceptor signals across spatially organized retinal circuits. Amacrine cells play a central role in shaping feature selectivity of retinal output. Here, we investigated whether the little-known parvalbumin-positive wide-field amacrine cells (PV-wfACs) of the rat retina exhibit systematic asymmetry in their dendritic organization and whether this asymmetry aligns with defined anatomical axes of the retina. These features are thought to be morphological signatures of orientation or direction selectivity. Furthermore, we compared this spatial bias in the albino Wistar and pigmented Long-Evans rat strains.
Methods: Retinal wholemounts from Wistar rats (n = 6) and Long-Evans rats (n = 2) were processed using immunohistochemistry for parvalbumin (PV), Prox1 and S-opsin. The distribution of S-cones was mapped to determine the ventral direction of the retina. PV-wfACs were identified by strong PV immunoreactivity and absence of Prox1 labeling. A total of 170 PV-wfACs (95 from Wistar, 75 from Long-Evans) were analyzed across 34 regions of interest (n = 19 and 15, respectively). Proximal dendrites were manually traced, and dendritic orientations were quantified relative to both the nasal axis and the radial axis extending from the optic disc.
Results: The distribution of angles relative to the nasal direction was clearly unimodal with a significant bias towards 72° (Wistar) or 66° (Long-Evans) downwards (Rayleigh test, $p = 1.3\times10-14$ and 0.035, respectively). There was no significant difference between the two strains (Watson-Williams test, $p = 0.693$). No significant directional bias of the dendrites was observed relative to the radial direction ($p > 0.05$).
Conclusions: The pronounced directional bias of PV-wfACs dendritic trees suggests a potential role for PV-wfACs in orientation or direction-selective retinal processing, which is not affected by the abnormal visual pathway development of albino animals. The preferential alignment toward the ventral direction raises the possibility of functional specialization for a single orientation or movement direction. It remains to be seen if complementary amacrine cell populations with a different neurochemical identity exist.

Characteristics of place field formation in the hippocampal CA1 and CA3 regions

Martin Blazsek[1,2]; Kata Szamosfalvi[1]; Snezana Raus Balind[1]; Balázs Lükő[1]; Gáspár Oláh[1]; Máté Sümegi[1]; Balázs Ujfalussy[1]; Judit Makara[1]
[1]*HUN-REN Institute of Experimental Medicine, Budapest*
[2]*Roska Tamás Doctoral School of Sciences and Technology, Budapest*

Hippocampal pyramidal cells (PCs) express spatially tuned activity (i.e., 'place cells' exhibiting 'place fields', PF) to support navigation. However, the cellular mechanisms of novel PF formation are elusive. Behavioral time scale synaptic plasticity (BTSP), recently described in mouse CA1PCs, is a putative mechanism of PF formation (Bittner et al., 2015). It starts with a silent PC exhibiting a large Ca2+ plateau in dendrites accompanied by somatic bursting, which initiates the emergence of tuning near the location of induction. Consequently, activity is strongest during formation and lower during subsequent visits. BTSP in CA1PCs has an asymmetric, seconds-long kernel giving rise to a backward shift in tuning after PF formation. The properties of BTSP-formed PFs in CA1 are still incompletely understood, with even less known about BTSP in CA3.

Here we recorded CA1PCs and CA3PCs using two-photon Ca2+ imaging in head-fixed Thy1-GCaMP6s mice navigating in two randomly alternating virtual environments, and classified PFs as either newly formed or established. Inspired by recent work (Priestley et al., 2022), we calculated PF formation gain (i.e., relatively strong formation activity) and initial shift (i.e., backward shift in tuning) that capture the proposed properties of BTSP-formed PFs in CA1. We show that newly formed PFs in CA1PCs exhibit higher gain and larger shift than established PFs. In contrast, newly formed PFs in CA3 do not differ from established PFs in terms of shifting. This suggests that BTSP is either less prevalent in CA3, or it manifests differently.

Wide-field amacrine cells link parallel transient OFF-alpha and ON-delayed retinal pathways

Gergely Szarka[1,2,3]; Áron Olivér Kolozsvári[3]; Csaba Varga[3]; Béla Völgyi[1,2]
[1]*University of Pécs, Szentagothai Research Centre, Pecs*
[2]*University of Pécs, Faculty of Natural Sciences, Neurobiology Department, Pecs*
[3]*University of Pécs, Medical School,, Institute of Physiology, Pecs*

The retina extracts behaviorally relevant features of the visual scene through parallel pathways; however, how/if these signaling routes interact with each other is not entirely understood. Transient OFF-alpha (tOFFα) retinal ganglion cells (RGCs) play a key role in detecting approaching objects and initiating defensive behaviors. In contrast, ON-delayed (ONdel) RGCs represent another yet uncharacterized information stream towards the brain.

Here, we characterize a wide-field amacrine cell (WF-AC) that couples to tOFFα RGCs while providing GABAergic output to ONdel RGCs, thereby forming an inhibitory circuit between these two parallel routes. We find that these WF-ACs receive excitation from two primary sources: (i) through chemical synapses via type 3a bipolar cells and (ii) through tOFFα GJs. Our morphological analyses also revealed that WF-ACs form a single, homogeneous cell population assembled into spatially organized domains. While the WF-ACs in each domain only maintain direct GJ contacts with tOFFα RGCs whose dendritic arbor spatially aligns, a second set of GJs link WF-ACs of adjacent domains into a larger network architecture. Our paired tOFFα RGC spike recordings demonstrated that this electrically coupled WF-AC network synchronizes medium timescale burst activity among tOFFα ganglion cells, thereby enhancing approach detection.

On the other hand, by utilizing the EyeWire II electronmicroscopic dataset, we found that the same WF-AC population provides robust GABAergic inhibition to ONdel RGCs, thereby indicating suppression of this signaling pathway. In fact, we found in our multielectrode recordings that ONdel RGCs receive a robust approach stimulus-initiated inhibition that is markedly reduced when GJs were locked. Together, we characterized a WF-AC population that integrates various excitatory inputs, synchronizes RGC output, and impairs signaling in another pathway, thereby serving as a communication hub in the retina. These findings suggest that parallel information channels interact at the level of the retina. In the present case, signaling through one channel suppressed activity in another. Whether this inhibitory interaction is reciprocal or whether additional forms of inter-channel interactions, such as unidirectional or reciprocal excitation, exist within the retina, remains unknown. Elucidating the nature of these interactions represents a central unresolved question in contemporary visual neuroscience.

Neural circuits involving the cholinergic lateral septum might mediate processing of aversive stimuli

Victoria Lyakhova[1,2]; Dániel Schlingloff[1,3]; Réka Kispál[1]; Ágnes Simon[1,4]; Erika Gyengesi Bilaver[5]; Balázs Hangya[1,6]
[1]*HUN-REN Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience, Budapest*
[2]*Semmelweis University, Doctoral College, Budapest*
[3]*University of Copenhagen, Department of Neuroscience, Systems Neuroscience Laboratory, Copenhagen*
[4]*University of Szeged, Albert Szent-Györgyi School of Medicine, Szeged*
[5]*Western Sydney University, School of Medicine, Penrith*
[6]*Medical University of Vienna, Center for Brain Research, Division of Neurophysiology, Vienna*

The lateral septum (LS) regulates aversive affective states like fear, anxiety and pain. Although existing literature describes the cells in the LS as predominantly GABAergic alongside minor glutamatergic neurons, we showed the existence of a neuronal subpopulation of LS cells that express choline acetyltransferase.
We set out to study the function of LS cholinergic cells (LSCNs) by manipulating and measuring their activity with optogenetics and fibre photometry, respectively. First, we optogenetically stimulated LSCNs in one chamber of a place preference test and investigated its effect on behavior. Next, we measured neuronal responses of LSCNs to noxious stimuli, such as foot shock, fox odour (FO) and air puff in GCaMP-expressing mice. Anatomical connectivity studies and stereological quantification of cFos-expressing cells responsive to the FO were also performed.
From anatomical experiments we ascertained, that LSCNs project to the amygdala and the anterior hypothalamus. Optogenetic stimulation of LSCNs induced avoidance-like behaviour in the stimulation-coupled chamber in the place preference test, while foot shock and FO increased LSCN activation, with FO producing higher LSCN activation relative to controls. Thus, LSCNs are likely to mediate processing of aversive stimuli by regulating behaviour through limbic projections.
In the future, we plan to employ chemogenetic stimulation to confirm the avoidance effect on behaviour, and further elucidate the involvement of LSCNs in emotional, social, and cognitive function. To address this, we will be using fiber photometry in both healthy and dementia mouse models.

Model driven, layer-specific analysis of top down-inherited invariances in mouse V1

Róbert Layous[1]; Ferenc Csikor[1]; Márton A. Hajnal[1]; Gergő Orbán[1]
[1]HUN-REN Wigner Research Centre for Physics, Department of Computational Sciences, Computational Systems Neuroscience Lab, Budapest

The visual cortex is organized hierarchically, with successive processing stages encoding increasingly complex features of the visual scene. Feedback (top-down) connections are ubiquitous throughout this hierarchy and have a strong influence on neural responses in lower-level layers. Despite their abundance and importance, the computational role of feedback connections is not fully understood. Recent work has provided normative arguments on the contribution of top-down connections to hierarchical inference and could predict the structure of feedback based on adaptation principles. Intriguingly, the proposed hierarchical generative model provides insights into the layer-by-layer organization of the computations in the ventral stream. Inspired by these insights, here we set out to investigate the laminar properties of visual processing, with particular emphasis on the distinction of dominantly feed-forward components, in particular layer 4, and components engaged in processing top-down information flow, namely layer 5 neurons. Using calcium imaging recordings from mice that span multiple layers across both the primary visual cortex (V1) and lateral medial area (LM), we analyze the representations emerging at different layers in response to grating stimuli. Our results show that the neural representation in V1-level processing components of the hierarchical generative model that are involved in feed-forward computations align more closely with L4. In contrast, V2-level components of the model better match the representations present in the LM region of mice. Importantly, we also found that layer 5/6 of V1, a layer innervated by top-down connections from LM, is characterized by a neural representation of the stimulus that is reminiscent of that of the higher-order visual area, indicating a functional fingerprint of top-down processing. Furthermore, we investigate how these fingerprints emerge and evolve over time within the representations of successive processing stages. Overall, these findings suggest that top-down generative models provide a useful computational framework for mapping hierarchical processing in the mouse visual system.

Stimulus-driven top-down computations in the visual cortex: a normative account

Ferenc Csikor[1]; Balázs Meszéna[1]; Katalin Ócsai[2]; Gergő Orbán[1]
[1]HUN-REN Wigner Research Centre for Physics, Department of Computational Sciences, Computational Systems Neuroscience Lab, Budapest
[2]Budapest University of Technology and Economics, Institute of Mathematics, Department of Algebra and Geometry, Budapest

Current functional models of the ventral stream in the primate visual cortex are usually built by training deep artificial neural networks (ANNs) for performing one selected visual task like object recognition or visual scene description. While these tasks are definitely relevant in the context of primate vision and deep ANNs trained to perform any of these are capable of capturing some of the hierarchical computations in biological vision, such single-goal-driven models do not explain simultaneous performance of multitudes of downstream tasks like predator species recognition (i.e., core object recognition) and behavior prediction (from e.g. postures of body parts). To deal with such multi-inference scenarios, we distanced ourselves from task-oriented modeling and created a deep hierarchical generative model of images that is only trained to account for natural image statistics but for no specific task, and compared hierarchical inference under this model to well established functional properties of early areas of the primate visual ventral stream, V1 and V2. Critically, beyond the functional considerations, such deep generative models promise unprecedented insights into the organization of cortical computations, and in particular into the organization of top-down connections in the visual cortex. To maximize the generality of the predictions of our hierarchical deep generative model, we constrained it only with mild, biologically well motivated inductive biases, and otherwise kept it as general as possible. Surprisingly, after training our model on natural image patches, it not only learned a biologically relevant hierarchical representation, but the emergent top-down inference computations were found to be biologically relevant, too. We demonstrated the latter with in-silico illusory contour and contour completion experiments, as well as by studying the dependence of noise correlations on the high level structure of the input. Importantly, we showed that these favorable properties were not the result of the chosen amortized variational approximation of the model posterior since stimulus conditioned samples from the deep generative model almost perfectly correlate with the approximate posterior. Through these results, our model demonstrates that a deep generative model of the visual environment is a viable model of the representations and bottom-up and top-down computations in lower primate vision.

Pup induced activation pattern of distinct posterior intralaminar thalamic neuron types in female mice

András Lukács[1]; Szilvia Bartók[1]; Melinda Cservenák[1]; Árpád Dobolyi[1]
[1]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*

The posterior intralaminar thalamic nucleus (PIL) has been implicated in the regulation of social and maternal behaviors. It is thought to function as an important relay area connecting somato- and other sensory inputs to cortical and subcortical brain structures. Previous studies have shown that PIL neurons are robustly activated during social interactions with pups as well as with adult conspecifics, highlighting its potential role in socially relevant information processing. The PIL is composed of a heterogeneous population of neuronal subtypes including neurons expressing calbindin, calretinin, tuberoinfundibular peptide of 39 residues (TIP39) and its receptor, the parathyroid hormone 2 receptor, as well as calcitonin gene-related peptide (CGRP) and calmodulin-dependent kinase II (CaMKII). However, the specific cell types engaged in pup-related interactions have not yet been clearly identified. To address this question, we visualized neuronal activation using c-Fos immunolabeling following pup exposure. Our results demonstrate that a distinct subset of PIL neurons is selectively activated. The co-localization pattern of c-Fos-activated neurons suggests that specific neuronal populations are specifically recruited during pup-directed interactions and may contribute to the regulation of maternal behavior. To further investigate the role of CaMKII-expressing PIL neurons, we performed stereotaxic surgeries and injected an mCherry-labeled CaMKII-containing viral vector into the PIL. To determine the neurotransmitter phenotype of CaMKII-positive and other PIL neuron populations, we conducted viral injections and visualization of different PIL cell types with immunolabeling in VGAT-ZsGreen female mice, enabling the identification of the GABAergic nature of the different neuron types. Additionally, we examined the projection patterns of CaMKII-expressing PIL neurons using anterograde tracing via mCherry fluorescence, which revealed a projection profile that differs from previously characterized PIL output pathways, suggesting the existence of a previously unrecognized neuronal subpopulation within the PIL. Together, our findings uncover an unexpected complexity in ascending PIL projections and highlight their potential relevance in the neural circuitry underlying social and maternal behaviors.

Stable Multimodal Access to Neuronal Activity in Large Animal Brains Using Functional Ultrasound Imaging

Domonkos Horvath[1,2]; Klaudia Csikos[1,3]; Abel Petik[1,2]; Alan Urban[4]; Botond Roska[5]; Daniel Hillier[1,2]
[1]*HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest*
[3]*Semmelweis University Doctoral School, Budapest*
[4]*Vlaams Instituut voor Biotechnologie, Ghent*
[5]*Institute for Molecular and Clinical Ophthalmology Basel, Basel*

Studying neuronal function in large-brained species is critical for translational neuroscience but is hindered by the limited availability of animal models. Longitudinal experiments offer a solution by enabling comprehensive analyses of brain function over extended periods, allowing for intra-subject normalization and reducing the variability inherent in cross-sectional studies, all while minimizing animal use.
We developed a multimodal protocol combining functional ultrasound imaging (fUSI) with electrophysiology for mesoscale neuronal monitoring in large-brained animals. fUSI provides ~150 µm spatial and 5 Hz temporal resolution, surpassing traditional fMRI, and is compatible with modern tools such as Neuropixels probes and optogenetics. We performed functional mapping over several months using visual stimulation, while systematically evaluating anesthesia protocols to ensure stable and reproducible data during extended sessions.
Because individual animals exhibit marked differences in anesthetic sensitivity and physiological stability, these factors can strongly impact fUSI signal quality. To address this, we applied a model-based approach to tailor anesthesia protocols to each subject. This individualized strategy improved robustness and reproducibility across longitudinal sessions and was aimed to minimize variability attributable to anesthesia-related confounds.
Our approach enables stable multimodal recordings over several months in cats, demonstrating the compatibility of fUSI with electrocorticography. Optimized anesthesia protocols supported extended mapping sessions, yielding data with high reproducibility and stability. Together, this work establishes a robust framework for longitudinal multimodal access to neuronal function in large-brained animal models. By addressing the challenge of model scarcity, our protocol provides a scalable approach for translational neuroscience studies.

Acetylcholine and dopamine track progression in the serial reaction time task in mice

Hanga Dormán[1]; Yahya Sulok[1]; Írisz Szabó[1]; Vivien Pillár[2]; Bálint Király[3]; Balázs Hangya[3]
[1]*HUN-REN Institute of Experimental Medicine, Budapest*
[2]*Semmelweis University, Budapest*
[3]*Medical University of Vienna, Vienna*

Implicit sequence learning is integral to performing many everyday activities, such as language use, social interactions, and sports. Yet, our knowledge of the neural mechanisms underlying this type of learning is scarce and mainly based on fMRI studies in humans. In the Serial Reaction Time Task (SRT), subjects are required to respond to sequences of visual stimuli in a limited time. As a measure of implicit sequence learning, performance is typically compared between sequential blocks with a deterministic, and baseline blocks with a randomized order of stimuli. Here, we conducted the SRT in mice in conjunction with dual photometry measurements to gain mechanistic insights into the learning process. We found that animals could rely on implicit learning during the task, as evidenced by higher accuracies and faster reaction times in sequential relative to baseline blocks. In the final phase of the training, subjects received a water reward after four consecutive correct trials. We found that the neuromodulators acetylcholine and dopamine tracked the progression toward reward in successful trial sequences. Namely, acetylcholine response to the stimulus in the basolateral amygdala increased monotonously over the four correct trials, while dopamine in the prelimbic area exhibited a reverse tendency, decreasing monotonously until the third step and reaching its peak at the last (rewarded) trial. Interestingly, this effect was present regardless of whether stimuli were presented in a deterministic or random order, suggesting that these activity patterns might be related to reinforcement rather than the implicit learning aspect of the task. Building on the clear behavioral differences between sequential and baseline blocks, further investigation will target a potential implicit learning-specific signature of the observed neuromodulator dynamics, as well as their oscillatory nature.

Multimodal investigation of functional map organization and stability in the primary visual cortex

Klaudia Csikós[1,2]; Ábel Petik[1,3]; Domonkos Horváth[1,3]; Alan Urban[4]; Dániel Hillier[1,2,3]
[1]*HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest*
[2]*Semmelweis University Doctoral School, Budapest*
[3]*Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest*
[4]*Neuro-Electronics Research Flanders (NERF), VIB, Department of Neuroscience KU Leuven, imec,, Leuven*

Understanding the three-dimensional organization and stability of functional maps in the primary visual cortex is essential for uncovering how sensory representations are maintained and reorganized over time. To address this, we established a multimodal experimental framework that integrates functional ultrasound (fUS), electrocorticography (ECoG), and widefield optical imaging within a modular recording chamber designed for long-term imaging and controlled developmental environments.
We employed high-resolution fUS imaging to explore the 3D organization of the cat primary visual cortex, focusing on the interplay between retinotopic and orientation maps. This approach enabled the identification of functional structures across multiple spatial scales—from the centimeter range, spanning several cytoarchitectonic areas, to the millimeter scale of pinwheel centers, and down to sub-millimeter iso-orientation domains. The unique combination of high spatial resolution and sensitivity to hemodynamic changes allowed detailed mapping of cortical architecture. Our findings suggest that local iso-orientation domains are influenced by their embedding within the global retinotopy, highlighting an interdependence between large-scale and fine-scale representations that supports efficient feature processing across the visual field.
Through repeated imaging sessions, we observed robust and reproducible hemodynamic responses, indicating stable functional organization over time. Simultaneous ECoG recordings provided electrophysiological validation and enabled assessment of fUS signal quality and stability. The environment for widefield optical imaging has been prepared and validated, and joint analyses integrating these modalities are planned for future work.
Together, this multimodal approach provides a flexible platform for examining the stability and interconnection of cortical maps across spatial and temporal scales, offering new opportunities to investigate how large-scale organization, local circuitry, and developmental or adaptive processes interact within sensory cortex.

Home cage gamma oscillation pattern in the rat prelimbic area predicts behavior in the modified successive alley paradigm

Levente Gellért[1]; Lee Qun[2]; Jiale Wang[3]; Antal Berényi[1,4,5]
[1]*University of Szeged, Department of Physiology, MTA-SZTE "Momentum" Oscillatory Neural Networks Research Group., Szeged*
[2]*Xuzhou Medical University, Research Center for Biochemistry and Molecular Biology, Jiangsu Key Laboratory of Brain Disease and Bioinformation, Xuzhou*
[3]*École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, Paris*
[4]*Neunos ZRt, Szeged, 6725, Hungary, Szeged*
[5]*University of Szeged, HCEMM-SZTE Magnetotherapeutics Research Group, Szeged*

The role of gamma oscillations in regulating fear behavior has been widely implicated. Moreover, sub-ranges in the gamma oscillatory range appear to play distinct role in regulating fear behavior in fear conditioning paradigms, where a learned fear cue is present. Excessive innate fear and behavioral avoidance are core features of anxiety disorders. Therefore, animal models that are monitored in more naturalistic environments over longer periods of time are of great translational value.
Beyond basolateral amygdala (BLA), prefrontal cortex (PFC) oscillation and PFC-BLA functional connectivity are crucial in regulating learned fear behavior. However, whether PFC and BLA gamma oscillation patterns can predict and explain innate fear behavior and voluntary risk taking under naturalistic conditions, is still elusive.
To this end, we used a modified version of the successive alley paradigm and investigated if gamma oscillatory pattern has a predictive and explanatory power for alley behavior. We repeatedly recorded LFP activity in the animals' home cage and when animals had a free choice to leave their home cage and enter the aversive successive alley.
We calculated the ratio of high (70-95) Hz and low (40-70 Hz) gamma power range, respectively. We checked also if PFC BLA functional connectivity (coherence and Granger prediction values) is predictive for behavior in these oscillatory ranges.
We found that (a) the modified successive alley paradigm can separate innate higher and lower fear level between and within individual animals on a longitudinal basis; (b) From two key nodes of the limbic system (PFC and BLA) only PFC home cage oscillatory pattern can independently predict the fear level under later aversive conditions; (c) The higher the home cage high-low gamma ratio, the better the subsequent behavioral open alley performance; (d) Two competing subranges of gamma oscillations can explain the behavioral performance in the open alley part of the paradigm; and (e) The above subrange competition in the gamma range emerges also at the level of functional connectivity between PFC and BLA. A PFC lead function in the high gamma subrange predicts the open alley performance.
We conclude that gamma oscillations in the prelimbic part of the PFC may represent a potential target for interventions aimed at modulating elevated innate fear and anxiety.

Fascicular connections of the Brodmann areas of the human cerebral cortex

Rege Sugárka Papp[1,2]; Miklós Palkovits[1,2]
[1]*Semmelweis University, Department of Anatomy, Histology and Embryology, Budapest*
[2]*Semmelweis University, Human Brain Tissue Bank, Budapest*

The gray matter of the human cerebral cortex can be divided into different anatomical and functional regions. Based on its cytoarchitectonic feature, Korbinian Brodmann differentiated several cortical areas, the so-called Brodmann areas (BAs), which show not only histological but functional as well as connectional differences. Thanks to modern techniques, the borders of these BAs were modified, or redefined; but these numbered regions still serve as functional landmarks today. The associational pathways that connect the different cortical lobes/regions are well-known, but it is important to note that the white matter is not a homogenous structure it differs in a great extent regarding the different cortical fascicles. Moreover, even a certain fascicle is not uniform in itself as it contains fibers that originate and terminate in several different cortical areas. For example, the superior longitudinal system, that is built up by three superior longitudinal fasciculi (SLF I-III) and the arcuate fasciculus, connects the frontal lobe with the parietal, occipital and temporal cortices. The SLF I connects the superior frontal gyrus with the superior parietal lobe thus its fibers can be found in several BAs such as BA5-10, 24 and 32.
In this work, our goal is to identify which known fascicle/tracts are associated with certain BAs. Since no comprehensive literature is available on this subject, the latest data from the literature were collected and the various tracts associated with the BAs were mapped. Furthermore, where data was obtainable, we attempted to refine the picture of the location of white matter tracts within a proper BA. Some of these areas have less extensive connections and are only connected to one or two pathways. For example, BA26 (the so-called ectosplenial area) is connected to the cingulum. At the same time, e.g., BA9 (dorsal prefrontal cortex) has extensive connections, as it is connected with six different associational pathways. Additionally, the BA37 (posterior fusiform gyrus) is heterogenous regarding its fiber connections as it acts as a node for two different networks; the visual recognition and the language networks; through different fascicular connections. Our results accurately reflect the functional heterogeneity of the given area. The detailed knowledge of white matter helps us to understand the functional connections of the human brain as well as to build an anatomically accurate connectivity map.
Supported by NAP2022-I-4/2022

State-dependent inter-slice synchronization in isolated cortical tissue revealed by AI-driven spectral analysis

Mihály Rámpay[1,2]; Kinga Tóth[1]; Réka Bod[1]; Nour Essam[1]; Lucia Wittner[1]; István Ulbert[1]
[1]HUN-REN RCNS, CPI, Integrative Neuroscience Research Group, Budapest
[2]Semmelweis University, János Szentágothai Neuroscience Doctoral School, Institute of Experimental Medicine, Budapest

Understanding how large-scale neural coordination emerges from local dynamics remains a central challenge in neuroscience. While global synchronization is known to play a key role in perception and integration, it is unclear whether coordinated activity can persist between neural systems that are physically isolated and lack anatomical connectivity. In this study, we investigate spontaneous population activity (SPA) and state-dependent synchronization in paired, in vitro cortical and hippocampal slices obtained from human surgical tissue and rodent preparations.
Local field potentials were recorded using 24-channel laminar multielectrode arrays from physically separated slice pairs. SPA events were identified as brief, low-amplitude, synchronized population transients. To reduce reliance on manual event labeling and to capture latent dynamical structure, we applied an AI-driven spectral analysis framework based on spectral Hidden Markov Models (HMMs). Continuous recordings were segmented into overlapping temporal windows, each represented as a low-dimensional spectral feature vector, allowing the identification of recurrent hidden network states associated with SPA-like, non-SPA, and mixed activity patterns.
Inter-slice synchronization was quantified using multichannel phase-based and amplitude-based measures, including phase-locking value, envelope correlation, and the Kuramoto order parameter computed across all 24 channels to estimate global coordination. Non-parametric permutation statistics revealed that inter-slice synchronization was significantly higher during shared SPA-like states compared to mixed or non-SPA states, exceeding levels expected by chance. These effects were frequency-specific, with dominant contributions in the alpha–beta range in human cortical slices and in the beta band in rodent hippocampal slices.
Our results demonstrate that synchronization between isolated neural tissues is not random but strongly depends on the underlying spectral network state. While no claims are made regarding causal coupling or information transfer, these findings indicate that collective neural dynamics can exhibit structured, state-dependent coordination even in the absence of direct connections. This work highlights the utility of AI-based state modeling for probing non-classical aspects of neural organization in reduced experimental systems.

The Functional Underpinnings of Performance Fluctuations in Acute Mental Fatigue: an fMRI study

Ákos Arató[1]; Barnabás Dudás[1]; Gábor Perlaki[1]; Gergely Orsi[1]; Nagy Szilvia Anett[1]; Gegely Darnai[1]; József Janszky[1]
[1]*University of Pécs, Pécs*

However, there is extensive empirical literature on the behavioural investigation of acute mental fatigue (AMF), the neural underpinnings of AMF emergance are still a matter of debate. As AMF emerges with an increase in the time spent on a cognitively demanding task, slower reaction times, higher error rates, and an increase in subjectively perceived mental fatigue could be expected through the time-on-task (ToT) effect. The investigation of the functional neural basis of AMF has been prolifelated in recent years. These studies found that altered activation in the insula, dorsolateral prefrontal cortex, and anterior cingulate cortex was associated with the decline in behavioural performance caused by the ToT effect. However, the performance changes induced by the ToT effect are often characterised by fluctuations, the functional underpinnings of which are still considered an under-researched area. In accordance with this the aim of our study was to investigate the functional background that might be responsible for the performance fluctuations that occur during AMF. We used the 15-minute long Psychomotor Vigilance Task to induce AMF and functional magnetic resonance imaging (fMRI) to detect the task perfromance fluctuations related changes in the blood-oxygen-level-dependent (BOLD) signal in healthy young people. To capture individual performance fluctuations within tasks, the standard deviation of residual reaction times (SD_RRT) based on a linear model per minute was used to model performance fluctuations in subject-level BOLD analyses for each participant. The 15-minute SD_RRT and subjective fatigue change (SFC) were used as dependent variable in post-hoc brain-behaviour regression analyses. We found that task performance fluctuations during the PVT were related to deactivation in four clusters: Cluster 1 (right middle and superior frontal gyri); Cluster 2 (left middle and inferior frontal gyri); Cluster 3 (bilateral precuneus and posterior cingulate gyri); and Cluster 4 (bilateral medial frontal gyri). Additionally, post-hoc regression analysis revealed that smaller deactivation in Cluster 3 predicted greater SFC due to the cognitively demanding task. Our findings align with previous results on AMF, and extend the literature by highlighting a novel functional mechanism: increases in subjective AMF appear to be underpinned by performance-fluctuation–related (SD_RRT) deactivations in default mode network regions.

Effects of Hormone Replacement Therapy on Resting-State Brain Network Dynamics in Postmenopausal Women

Orsolya Horváth[1,2]; Petra Csizmadia[1]; Nóra Csikós[1]; Lili Kővári[1,3]; Szabolcs Várbíró[4,5]; Dóra Gerszi[5]; Dorina Greff[5]; Zsófia Anna Gaál[1]
[1]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Doctoral School of Psychology (Cognitive Science), Budapest University of Technology and Economics, Budapest*
[3]*Doctoral School of Psychology, ELTE Eötvös Loránd University, Budapest*
[4]*Department of Obstetrics and Gynecology, University of Szeged, Szeged*
[5]*Centre for Translational Medicine, Semmelweis University, Budapest*

Menopause represents a transitional stage in a woman's life, characterized by substantial hormonal changes, primarily decreased levels of estrogen and progesterone. These hormonal shifts are associated with somatic symptoms, emotional and cognitive alterations, as well as modifications in neural dynamics. Understanding how hormone replacement therapy (HRT) modulates these neurophysiological changes is essential for promoting cognitive resilience in postmenopausal women.
In our ongoing study, we investigated the effect of HRT on resting-state brain activity and cognitive performance in 27 postmenopausal women, including 12 receiving (HRT+) and 15 not receiving HRT (HRT-). Resting-state EEG was analyzed using Multiscale Entropy (MSE) to access neural signal complexity and Spectral Power Density (SPD) to examine oscillatory activity, while cognitive performance was evaluated via the Wechsler Adult Intelligence Scale (WAIS-IV).
Compared to the HRT– group, the HRT+ group showed higher MSE values at short (1–20 ms) and medium (20–35 ms) time scales, indicating greater local neural complexity. SPD analyses revealed lower delta (0.5–4 Hz) power and increased frontal beta (13–30 Hz) and gamma (30–40 Hz) power in the HRT+ group. These neural measures positively correlated with overall IQ and subtest scores reflecting frontal lobe functions.
Our findings suggest that hormone therapy in postmenopausal women may be associated with distinct patterns of neural activity that could support cognitive resilience.

Differential association between electrophysiological sleep and depression given ADHD risk and pharmacotherapy

Vivien Reicher[1]; Boglárka Kertész[2]; Róbert Bódizs[3]; Orsolya Szalárdy[3]; János M. Réthelyi[4]; Nóra Bunford[1]
[1]*Research Centre for Natural Sciences, Budapest, Institute of Cognitive Neuroscience and Psychology, Clinical and Developmental Neuropsychology Research Group, Budapest*
[2]*Eötvös Loránd University, Institute of Psychology, Budapest*
[3]*Semmelweis University, Institute of Behavioural Sciences, Budapest*
[4]*Semmelweis University, Department of Psychiatry and Psychotherapy, Budapest*

Youth with ADHD often have sleep problems, and these are especially pronounced in those who also show difficulties with emotion regulation (ED) and internalizing problems. Most studies rely on subjective measures (questionnaires), with few using objective tools like electroencephalography (EEG). Non-REM sleep slow-wave activity (SWA), an index of cortical maturation, remains underexplored in ADHD and co-occurring affective difficulties.
We examined the association between EEG sleep variables (sleep onset latency, sleep efficiency, SWA) and affective problems (depression, ED) in adolescents aged 14 to 20 years (N=77, Mage=16.98, SD=1.37), as a function of ADHD and pharmacotherapy, controlling for age and sex. Sleep was measured during an at-home sleep assessment using the DREEM2 headband. Depression and ED were measured using the Youth Self-Report Form. ADHD risk was determined via parent-report on the ADHD Rating Scale-5. In adolescents at-risk for ADHD –but not in adolescents not at-risk for ADHD– longer sleep onset latency was associated with higher depression scores (b=4.910, p<.001). SWA decreased with age, regardless of ADHD risk and pharmacotherapy status. In ever-medicated adolescents at-risk for ADHD –but not in medication-naïve, at-risk adolescents or in adolescents not at-risk–, higher SWA was associated with higher depression scores (b=.180, p=.003).

Findings align with the broader literature showing a differential association between sleep problems and depression in at-risk and not at-risk populations, as well as a decrease in SWA with age. A main novelty here is the association between SWA and depression in ever-medicated adolescents at-risk for ADHD. SWA could be a marker for better understanding the intersection of neurodevelopment and depression in ADHD.

The effects of microgravity on visually-guided associative learning function: a human case study

Olívia Mária Huszár[1]; Noémi Harcsa-Pintér[1]; Gabriella Eördegh[2]; Kálmán Tót[1]; Ádám Kiss[3]; András Kelemen[3]; Ádám Schlégl[4]; Attila Nagy[1]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Dep.of Physiology
[2]Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theorethical Health Sciences and Health Management, Szeged
[3]University of Szeged, Department of Technical Informatics, Szeged
[4]Medical School, University of Pécs, Department of Orthopaedics, Pécs

Microgravity could affect sensorimotor and vestibular systems, yet its impact on higher-order cognitive functions, i.e. associative learning and connected memory processes remain poorly understood. These cognitive functions are essential for adaptive performance also in space missions, where the ability to acquire and flexibly apply new information is critical.
In this case study we intended to investigate whether short-duration exposure to microgravity influences associative learning, retrieval, and generalization processes in astronauts, using tasks based on the original Rutgers Acquired Equivalence Test. The paradigm was modified to be compatible to specific industrial requirements on the international space station (ISS).
Three trained astronauts participated in a repeated-measures protocol across preflight, inflight, and postflight phases. One participant completed inflight testing during a 20-day orbital mission, while two others remained on Earth and served as contextual comparators. Participants completed three associative learning tasks on a tablet device at 1–2-day intervals, yielding 21 sessions per individual. Performance was quantified using number of acquisition trials, error ratios and reaction times. Data were analyzed using non-parametric statistics, permutation tests, and linear detrending to separate practice effects from potential mission-related changes.
Across all phases (acquisition, retrieval, and generalization), performance was near ceiling and reaction times showed gradual improvement consistent with practice-related learning. No significant differences were found between the preflight, inflight and postflight phases by Friedman tests. In the space-exposed participant, a small inflight increase in retrieval error ratio was detected by the permutation test; however, this effect was isolated and not accompanied by changes in other parameters. Linear trend analyses revealed highly similar learning slopes with and without inflight data, indicating that microgravity exposure did not alter the overall learning process significantly.
These findings suggest that short-term microgravity does not substantially disrupt acquired equivalence learning and generalization in highly trained astronauts. The results could demonstrate the feasibility of conducting cognitive research and suggest a possible cognitive training method during spaceflight.

Comparative analysis of cortical electrical activity in adults with migraine and healthy controls during an audiovisual associative learning task

Noémi Harcsa-Pintér[1]; Kálmán Dr. Tót[1]; Adél Papp[1]; Olívia Mária Huszár[1]; Gabriella Dr. Eördegh[2]; Balázs Dr. Bodosi[1]; Anett Dr. Csáti[3]; János Dr. Tajti[3]; Attila Dr. Nagy[1]
[1]*Albert Szent-Györgyi Medical School, University of Szeged, Dep.of Physiology*
[2]*Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theoretical Health Sciences and Health Management, Szeged*
[3]*Univerity of Szeged, Department of Neurology, Szeged*

The SoundFace task is an audiovisual adaptation of the original visually-guided Rutgers Acquired Equivalence Test (RAET). Previous studies have shown performance alterations in migraine patients on the RAET, but surprisingly, migraine patients outperformed healthy controls in the audiovisual task. The decreased β activity found in migraine patients during performance of the RAET could contribute to these alterations. The present study aims to investigate the cortical activity of migraine patients in the audiovisual SoundFace task.
EEG was recorded from 18 migraine patients in the interictal phase and 18 age- and education-matched healthy controls using a 64-channel BioSemi system at a sampling rate of 2048 Hz. Preprocessing included notch (50/60 Hz), high-pass (>1 Hz), and low-pass (<100 Hz) filtering, average re-referencing, and ICA with ICLabel-assisted artifact rejection. Data were segmented into epochs time-locked to the button-press response (–500 to +500 ms), followed by multitaper power spectral density estimation in the θ (4–7 Hz), α (8–12 Hz), β (13–30 Hz), and γ (31–70 Hz) frequency bands, expressed as baseline-normalized decibel values. Group differences were evaluated using cluster-based permutation tests (1000 iterations) across all EEG channels, frequency bands, task phases (acquisition and test), and pre- and post-response time windows.
Although both power spectral analysis and Morlet-wavelet convolution indicated a reduction in cortical β activity in the migraine group in response to audiovisual stimuli, this decrease did not reach statistical significance. Thus, no statistically significant differences emerged between the migraine patients and the healthy controls across any EEG channels, frequency bands, task phases, or pre- and post-response time windows in the SoundFace test.
One possible explanation for our findings is that audiovisual stimuli may elicit greater cortical engagement than purely visual stimuli, thereby preserving baseline β activity levels to a larger extent, consistent with previously observed performance advantages for audiovisual learning. Alternatively, subcortical compensatory mechanisms may support superior performance in the SoundFace task in migraine patients. While the present study cannot identify these specific subcortical neural structures, prior literature suggests potential contributions from the basal ganglia or the anterior cingulate cortex, though this remains to be confirmed by future research.

Expectation alters facial sex representation

Szabolcs Sáringer[1]; András Benyhe[1]; Péter Kaposvári[1]
[1]*University of Szeged, Albert Szent-Györgyi Medical School, Department of Physiology, Szeged*

The signal-to-noise ratio of environmental information presents a significant challenge for sensory processing. One way to facilitate perception is by preparing the sensory system for the most probable input through prediction, based on expectations grounded in accumulated experience. It enables us to draw inferences from often ambiguous sensory information, resulting in behavioral benefits. Here, we focus on behavior and neural activity associated with perception regarding expectation in the case of ambiguous or dissociated input.

We employed multivariate pattern analysis (MVPA) of EEG recordings to investigate the neural mechanisms of prediction during facial sex perception. Participants were presented with face stimuli depicting male, female, and gender-ambiguous faces. Prior to the face presentation, the expected sex of the face was cued by an auditory signal. After the face stimulus, a short text (“female” or “male”) appeared on the screen. Behavioral effects were measured using a two-alternative forced-choice task, in which participants judged whether the written statement was true or false.

Behavioral results revealed a robust effect in sex-neutral trials, with participants’ decisions biased toward the cued sex. At the neural level, a linear discriminant analysis (LDA) classifier trained on male and female faces showed a significant decoding interval when cross-validated on the ambiguous faces, peaking around 170 ms after stimulus onset. This time window overlapped with the peak decoding accuracy obtained when cross-validation was performed on male and female faces themselves.

The prediction biased the decision about the same ambiguous face towards both sexes depending on the auditory cue, and shifted the neural pattern to code the expected facial sex. These findings demonstrate that expectations about facial sex alter not only behavior, but the neural representations at early stages of perceptual processing as well.

Resting Aperiodic Exponent as a Stable Individual Marker and Its Relation to Emotional Valence Use

Michelle Fitos[1]; Raissa de Oliveira Negrão[1]; Ayanna Carla de Moraes Gerôncio[1]; Renáta Cserjési[2]
[1]*Doctoral School of Psychology, ELTE Eötvös Loránd University, Budapest, Hungary, eMIND, Budapest*
[2]*Institute of Psychology, ELTE Eötvös Loránd University, Budapest, Hungary, eMIND, Budapest*

Background:
The aperiodic (1/f) exponent of resting EEG has been proposed as a marker of excitation–inhibition balance and neural "noise," while frontal alpha asymmetry (FAA) is often treated as a trait-like index of approach–avoidance tendencies. We asked whether resting aperiodic activity is (a) a stable individual characteristic across eyes-open and eyes-closed rest, and (b) related to how strongly and flexibly people use emotional valence when judging social scenes, as well as to FAA.
Methods:
Adults completed an Emotional Shifting Task in which they judged social scenes under different contextual instructions. Trial-level choices ("positive" vs "negative") were modeled with per-participant logistic regressions to derive valence sensitivity and contextual flexibility indices. Resting EEG (eyes open/closed) was preprocessed and decomposed with specparam to estimate the aperiodic exponent χ per channel. We computed ROI-level χ (frontal, central, parietal, occipital), simple front–back and left–right gradients, and open–closed deltas for participants with usable data in both states (N≈40–45). FAA (ln F4 – ln F3 alpha power) was derived from resting EEG in a separate pipeline. We related χ levels and open–closed deltas to behavioral indices and FAA.
Results:
ROI-level χ values were in a plausible range (~1.6–1.9) and showed moderate-to-high within-subject stability across eyes-open and eyes-closed (r≈0.6–0.9), indicating a robust trait-like component. Front–back and left–right gradients were small on average but showed meaningful individual variability. Across participants, associations between aperiodic measures (levels, gradients, and open–closed deltas) and both behavioral indices and FAA were generally small, with confidence intervals overlapping zero.
Conclusions:
This work establishes a reproducible pipeline linking resting aperiodic EEG, FAA, and contextual emotional decision-making, and shows that the aperiodic exponent behaves as a stable individual marker in this sample. At the same time, our estimates suggest that global resting 1/f slope is not a major driver of contextual valence flexibility or FAA here, providing useful boundary conditions and motivating more localized and task-evoked aperiodic measures in future work.

Comparison of cortical electrical activity in pediatric migraine patients and healthy controls during associative learning tasks

Bence Gyula Nagy[1]; Adél Papp[1]; Noémi Harcsa-Pintér[1]; Kálmán Tót[1]; Olívia Mária Huszár[1]; Gabriella Eördegh[2]; Jenő Kóbor[3]; Attila Nagy[1]
[1]Univerity of Szeged, Albert Szent-Györgyi Medical School, Department of Physiology, Szeged
[2]University of Szeged, Faculty of Health Sciences and Social Studies, Department of Theoretical Health Sciences and Health management, Szeged
[3]University of Szeged, Department of Pediatrics and Pediatric Health Center Albert Szent-Györgyi Health Centre, Szeged

Previous behavioral studies have demonstrated impairments in associative acquired equivalence learning in adult migraine patients without aura, whereas pediatric migraine patients typically do not show such deficits. This discrepancy may be explained by compensatory mechanisms within the cerebral cortex in pediatric patients. To check this, we aimed to investigate the cortical electrical activity of migraine patients during acquired equivalence learning and to identify possible EEG biomarkers associated with the disorder.
Sixty-four–channel EEG was recorded from 22 pediatric migraine patients and 22 matched healthy controls during completion of the modified Rutgers Acquired Equivalence Test (RAET). In the acquisition phase, participants learned visual antecedent-consequent associations based on feedback provided by the software. In the subsequent test phase, these associations were assessed without any more feedback. After preprocessing (frequency filtering, ICA-based artifact removal, epoching), cluster-based permutation analysis was applied to identify significant differences in cortical activity between groups.
Resting-state analysis revealed a significant group difference in frontal beta-band activity. The pediatric migraine patients showed altered cortical oscillations compared to those of the healthy controls. During performing the RAET test, no group differences were detected in normalized EEG activity either in the acquisition or test phases, regardless of EEG channel, frequency band, or the –500 to +500 ms time window around pressing button, that signs decision-making.
The absence of EEG differences during associative learning suggests that the comparable behavioral performance of pediatric migraine patients and healthy controls is unlikely to be explained by cortical compensation. Thus, the cortical activity changes found in adult migraine patients are not yet detectable in children with migraine. We believe these alterations develop over time, which raises the possibility of interpreting migraine as a neurodegenerative disease.

Impaired multisensory-guided acquired equivalence learnig in Tourette syndrome

Gabriella Eördegh[1]; Balázs Bodosi[2]; Noémi Harcsa-Pintér[2]; Ákos Pertich[2]; Kálmán Tót[2]; Attila Nagy[2]
[1]Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theoretical Health Sciences and Health Management, Szeged
[2]Albert Szent-Györgyi Medical School, University of Szeged, Department of Physiology, Szeged

Tourette syndrome is a neurodevelopmental disorder with motor and vocal tics as main symptoms. Several cognitive functions are affected in Tourette syndrome primarily due to altered functioning of cortico-striatal loops. Memory and learning, including both explicit and implicit processes, have been widely investigated, often yielding heterogenous results. These studies primarily using unimodal, most often visual stimuli. In our previous study, we found that children with Tourette syndrome performed worse in a type of associative learning, in visually guided acquired equivalence learning, in building pairs of stimuli (acquisition), while their recall (retrieval) and generalization performance did not differ from that of matched control children. Acquisition performance depends mainly on the function of the basal ganglia, whereas test phase performance depends primarily on the function of the hippocampi. In contrast, bimodal stimuli in Tourette syndrome children have been studied only in the context of inhibitory control. In our present study, we investigated acquired equivalence learning of children with Tourette syndrome using an audiovisual version of the original visual Rutgers Acquired Equivalence Test (RAET), in which visual consequent stimuli had to be associated with auditory antecedents. We compared the performance of 45 children with Tourette syndrome and 45 healthy control children matched for gender, age, and intelligence level. Acquisition trial number acquisition, retrieval and generalization error ratios of participants as well as their reaction times were analyzed.
Consistent with results obtained in the unimodal visually guided acquired equivalence test, children with Tourette syndrome showed weaker performance than that matched healthy control children during the acquisition phase of the audiovisual test. In contrast, no differences between the two groups were observed in the recall or generalization parts of the test phase.
Our results demonstrate that structural and functional alterations of frontostriatal loops in Tourette syndrome impair performance not only in visually guided acquired equivalence learning, but also multisensory-guided learning processes.

Exploring the potential in decoding the complex Fourier transform of human EEG data

Szabolcs Sáringer[1]; András Benyhe[1]; Péter Kaposvári[1]
[1]*University of Szeged, Department of Physiology, Szeged*

Decoding is a widely used technique in EEG research to extract cortical patterns during cognitive processes. These decoding methods, such as multivariate pattern analysis (MVPA), identify patterns in a given feature dimension, most commonly the channels, and use single-trial event-related potential (ERP) data. This results in a time series, which can identify time windows related to certain cortical mechanisms. The decoding algorithms, however, can handle higher-dimensional complex data, thus possibly increasing information extraction. In this study, we analyzed the data of two experiments and explored the potential in decoding the time-frequency representation (TFR).
In two experiments (n1=25, n2=25), healthy volunteers were presented with faces (600 trials) during EEG recording. By changing the number of presented faces and the presentation number of each face, we manipulated the face familiarity. Neural data of female and male faces were compared using MVPA with a linear discriminant analysis (LDA) algorithm on the ERP data, thereby extracting the temporal window of facial sex-related cortical mechanisms, while also employing classic TFR analysis. Subsequently, we decoded the complex Fourier spectrum along the channels and extracted the scalp distribution of the activity by decoding the data along the time dimension, in discrete windows. All decoding values were compared against chance (0.5), and significant windows were determined using threshold-free cluster enhancement (TFCE).
In Experiment 1, where face familiarity was higher, a 500-ms long time window emerged at the beginning of the stimulus presentation in the 2-17 Hz frequency range when comparing female and male faces. Less familiar faces showed more restricted time-frequency windows spanning from 6-20 Hz in the first 300 ms. Similar time windows emerged using ERP decoding, while classic TFR analysis showed no significant windows in either experiment. Spatial analysis revealed that faces with higher familiarity elicited both occipital and frontal activity in the below 30 Hz range. In contrast, low-familiarity related activity was restricted to the 10 Hz range in the occipital area.
We demonstrated the decoding of the TFR in a face-sex perception study and compared it with more commonly employed techniques, such as ERP decoding and classic TFR analysis. Our results show that TFR decoding can extract additional insights from the data, complementing time windows with frequency information.

A study in the multimodal model of statistical learning: The interference of auditory and visual stimuli

Hedvig Sütő[1]; Regina Gerencsér[1]; András Benyhe[1]; Péter Kaposvári[1]; Szabolcs Séringer[1]
[1]*University of Szeged, Department of Physiology, Szeged*

Statistical learning is the recognition and acquisition of environmental stimulus patterns, which accelerates sensory processing. These days, the exact neural background of statistical information processing has not been clarified, but previous findings suggest that these operations occur independently from modalities, in a singular centre. However, there is also proof that the sensory cortical areas work independently, processing statistical information depending on the modality. The modality-general theory allows the interference of modalities, while the modality-specific theory enables the parallel processing of information. To resolve this contradiction, we set our goal to study the interference between different modalities.
A total of 138 healthy participants were involved in our experiments (95 female, mean age(±SD): 21.99(±2.52) years). The subjects learned an artificial language based on visual and auditory information presented in parallel while they were kept engaged with a simple detection task. The participants were divided into five groups based on the relation between modalities; as a control, only unimodal statistical information was presented. Learning was monitored through the reaction time in the detection task and the sensitivity gained from a familiarity post-test. Conditions were analyzed using linear regression and variance analysis.
In the model of reaction times, the condition emerged as a significant effect ($F(4,11013)=102.91$, $p<0.001$). The parallel presentation of statistical information increased the reaction time compared to the control. The condition also presented as a significant effect in sensitivity ($F(4,120)=4.56$, $p=0.002$), which showed a decrease in comparison to the control.
Based on these results, we conclude that during the processing of multimodal information presented in a parallel way, interference occurs, hindering the effectiveness of statistical learning. Our findings further support that statistical information is processed in a modality-independent centre, which confirms the modality-general theory.

Testing a valproic acid-induced ASD model in zebrafish larvae

Alexandra Júlia Hencz, PhD[1]; Assel Kamysbayeva[2]; Dávid Szép, PhD[1]; Renáta Gajdács-Dóró[1]; Dóra Zelena, PhD[1]
[1]*Institute of Physiology, Medical School, University of Pécs, Pécs*
[2]*Institute of Biology, Faculty of Sciences, University of Pécs, Pécs*

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition. ASD is typically associated with deficits in motor, cognitive, and social abilities. ASD-like symptoms can be caused by prenatal valproic acid (VPA) exposure in rodents and zebrafish. Valproic acid (VPA) is a branched short-chain fatty acid used in humans as an antiepileptic drug. VPA exposure is used to induce behavioral changes in zebrafish to characterize ASD-like responses.
For the improvement of this ASD model in zebrafish, 4 different VPA concentrations (12.5, 25, 37.5, 50 µM) were tested. VPA is teratogenic, which can be seen in our mortality rates, increasing with concentration. In higher concentrations, the most important mortality causes are yolk and pericardial edemas. In these groups, there were more fish with underdeveloped swim bladders. To investigate the ASD-like symptoms, open field and light-dark tests (studying locomotion and anxiety) were used on 5-day-old zebrafish. The treatment with VPA caused a dose-dependent increase in movement time and velocity. The treated groups spent more time with movement in the outer zone of the observed area. In the light-dark test, fish treated with different VPA concentrations spent significantly less time in the illuminated part of the well than the control. This effect was stronger with the higher concentration groups.
Based on our results, all applied VPA concentrations induced detectable hyperactivity and anxiety-like behaviors dose-dependently. Due to lower mortality and strong significant effects, the 37.5 µM concentration seems to be the most suitable for further research. To validate the ASD-like symptoms in this concentration, we plan further behavioural tests, such as the social interaction test with older larvae.

Preserved Acquired Equivalence Learning in Children with Obsessive–Compulsive Disorder Cannot Be Explained by Cortical Compensation

Sushmita Singh[1]; Kálmán Dr. Tót[1]; Olívia Mária Huszár[1]; Noémi Harcsa-Pintér[1]; Gabriella Dr. Eördegh[2]; Gergő Égerházi[1]; Adél Papp[1]; Balázs Dr. Bodosi[1]; Ákos Dr. Pertich[1]; Laura Németh[3,4]; Péter Dr. Nagy[3,4,5]; Szabolcs Dr. Kéri[1]; Attila Dr. Nagy[1]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Department of Physiology, Szeged
[2]Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theoretical Health Sciences and Health Management, Szeged
[3]Bethesda Children's Hospital, Budapest
[4]Vadaskert Child and Adolescent Psychiatric Hospital, Budapest
[5]Doctoral College of Semmelweis University, Mental Health Sciences Division, Interdisciplinary Social Sciences Doctoral Program, Budapest

In this study we investigated acquired equivalence learning in children with obsessive-compulsive disorder (OCD) compared to healthy controls in the Visual Rutgers Acquired Equivalence Test (RAET) and its modified audio-visual version. The goal was to determine whether preserved learning abilities in children with OCD are supported by cortical compensatory mechanisms.
17 children with OCD and 17 age, gender and IQ level matched healthy controls completed a visual associative learning task involving acquisition, retrieval, and generalization of stimulus pairs. 14 children with OCD and their matched healthy controls also performed an audio-visual acquired equivalence learning task. Behavioral performance was assessed using the number of trials, error ratios, and reaction times. Electroencephalography recordings captured neural oscillatory activity across theta, alpha, beta, and gamma frequency bands during stimulus presentation and button press epochs. Data was pre-processed using ICA with ICLabel and analyzed using cluster-based permutation testing to identify group differences.
Children with OCD demonstrated comparable accuracy and reaction times across all learning phases and task modalities, with no significant differences between the groups. EEG analysis revealed similar cortical oscillatory patterns in both groups, characterized by stable spectral power distribution and modulation across all frequency bands. Statistical tests support no significant differences in baseline-normalized cortical activity in either of the tasks across any EEG channels, frequency bands, task phases, pre- or post-event time windows for stimulus presentation, or for button press.
Visual and audio-visual acquired equivalence learning is preserved in children with OCD and is not supported by detectable cortical compensatory mechanisms. However, this does not preclude the possibility of subcortical compensation. Alternatively, the dorsal cortico-striatal pathway may remain intact in children with OCD, thereby supporting acquired equivalence learning without requiring compensatory reorganization.

Comparative neural evidence for consonant-based speech segmentation in both humans and dogs

Kinga G. Tóth[1,2,3]; Boglárka Morvai[1]; Marianna Boros[1]; Dorottya S. Rácz[1,2,4]; Ivaylo Iotchev[1]; Kitti Szabó[1]; Attila Andics[1,2,5]
[1]Eötvös Loránd University, Department of Ethology, Neuroethology of Communication Lab, Budapest
[2]Eötvös Loránd University, Institute of Biology, Doctoral School of Biology, Budapest
[3]The Ministry of Culture and Innovation, National Research, Development and Innovation Office, Cooperative Doctoral Program 2023, Budapest
[4]University of Oslo, Department of Psychology, Oslo
[5]Eötvös Loránd University, NAP Canine Brain Research Group, Budapest

Efficient segmentation of continuous speech streams relies on neural mechanisms that track statistical regularities over time. During speech segmentation in humans, despite the greater acoustic salience of vowels, neural entrainment indicates more efficient word extraction when statistical regularities are carried by consonant- rather than vowel patterns. This human consonant bias is commonly attributed to the uneven distribution of lexical information across speech sounds: in most languages, consonants provide more reliable cues to word identity than vowels. Human statistical learning mechanisms therefore appear to operate economically, focusing on the more informative speech sound category for word extraction. Humans preferentially track consonant-based regularities – a bias absent in non-human primates and rodents. However, it is yet unknown whether this consonant bias reflects a prelinguistic sound processing preference in humans, which may have promoted the greater lexical informativity of future consonants, or whether it can emerge experientially in speech-exposed non-human species as well, and may therefore be the consequence, rather than the cause, of the greater lexical load of consonants. To test this, we directly compared the EEG responses of humans (N=19) and family dogs(N=24), living in the speech-rich human niche, to continuous 7-min speech streams consisting of trisyllabic nonsense words defined by either consonant or vowel patterns. Neural entrainment to the word-level frequency (1.33 Hz), reflecting speech segmentation efficiency, was assessed by inter-trial coherence (ITC), a measure of phase consistency across trials. We observed stronger word-level neural entrainment for consonant- than vowel-structured speech streams not only in humans but also in dogs. These findings provided the first neural evidence that consonant bias during speech segmentation is not unique to humans but is also present in dogs. Our results suggest that exposure to speech may thus be sufficient for the emergence of selective neural tracking of consonant patterns, even in evolutionarily distant, non-speaking species. More broadly, this raises the possibility that key speech processing mechanisms in the early human brain may have developed through rapid evolutionary adaptation to speech as an emerging socially relevant stimulus.

Cortical Beta-Band Alterations During Associative Learning: A Possible EEG Biomarker for Migraine

Adél Papp[1]; Kálmán Tót[1]; Noémi Harcsa-Pintér[1]; Olivia Mária Huszár[1]; Bence Gyula Nagy[1]; Kata Fodor[1]; Gabriella Eördegh[2]; Anett Csáti[3]; János Tajti[3]; Attila Nagy[1]
[1]Albert Szent-Györgyi Medical School, University of Szeged, Department of Physiology, Szeged
[2]Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theoretical Health Sciences and Health Management, Szeged
[3]University of Szeged, Department of Neurology, Szeged

Migraine is frequently associated with cognitive alterations that may persist during interictal periods. Prior behavioral studies using the visually guided Rutgers Acquired Equivalence Test (RAET) have shown impaired associative learning in migraine patients, suggesting possible involvement of visual-associative cortical networks. However, the electrophysiological correlates of these deficits have not been fully characterized. Our aim was to compare the cortical electrical activity of migraine patients and healthy adults during an associative learning task and to identify potential EEG biomarkers associated with altered cognitive performance in migraine.

Seventeen interictal migraine patients and seventeen age-, sex-, and education-matched healthy controls completed the RAET while undergoing 64-channel EEG recording. During the learning phase, participants associated an antecedent stimulus with one of two outcomes based on feedback; the test phase assessed previously learned associations without feedback. EEG data underwent band-pass filtering, independent component analysis for artifact removal, and epoching time-locked to the button press marking each participant's decision. Group-level differences were investigated using cluster-based permutation statistics.

Across both RAET phases, migraine patients exhibited a significant reduction in beta-band activity within parieto-occipital associative cortical regions compared to healthy controls. These alterations were present in the 500 ms time windows before and after the button press and demonstrated a strongly left-hemisphere–lateralized distribution. The spatial pattern suggests involvement of visual-associative cortices in the observed cognitive differences.

Interictal migraine is associated with distinct beta-band activity reductions during visually guided associative acquired equivalence learning, indicating altered functioning of parieto-occipital networks and hemisphere-specific processing strategies. These cortical activity differences may contribute to behavioral impairments observed in acquired equivalence learning and may serve as potential EEG biomarkers for more refined neurophysiological characterization and future diagnostic approaches in migraine.

Sensory modality-independent discrimination of human emotional cues in the dog brain

Fruzsina Eszter Herzog[1,2]; Franceska Furik[1,3,4]; Dorottya Szilvia Rácz[1,4,5]; Tamás Faragó[1,3]; Asami Nakaimuki[1,4,6]; Belén Larrán[7]; Boglárka Morvai[1]; Biagio D'Aniello[8]; Gün R. Semin[9,10]; Márta Gácsi[6]; Enikő Kubinyi[6,11]; Attila Andics[1,6]

[1]Eötvös Loránd University, Department of Ethology, Neuroethology of Communication Lab, Budapest

[2]University of Veterinary Medicine Budapest, Budapest

[3]Eötvös Loránd University, Department of Ethology, Barks Lab, Budapest

[4]Eötvös Loránd University, Doctoral School of Biology, Budapest

[5]University of Oslo, Department of Psychology, Oslo

[6]Eötvös Loránd University, ELTE NAP Canine Brain Research Group, Budapest

[7]University of Santiago de Compostela, Department of Animal Pathology, Faculty of Veterinary, Campus Terra, Lugo

[8]University of Naples Frederico II, Department of Biology, Naples

[9]William James Center for Research, ISPA – Instituto Universitário, Lisbon

[10]Utrecht University, Faculty of Social and Behavioral Sciences, Utrecht

[11]Eötvös Loránd University, MTA-ELTE Lendület "Momentum" Companion Animal Research Group, Budapest

Differentiating between emotions is essential in both intra- and interspecific interactions, as it allows for the assessment of others' internal state and motivations, thus enabling the selection of appropriate behaviour. Behavioural evidence suggests that dogs, who have been living in the human social niche for tens of thousands of years and are constantly exposed to human communicative signals, can differentiate human emotions and integrate them across multiple sensory modalities. However, to date, there is no direct evidence for abstract (i.e. sensory modality- and donor sex-independent) neural representations of allospecific emotions in any species. To investigate whether dogs possess abstract neural representations of human emotions, we recorded EEG activity from 28 family dogs while presenting human emotional cues of joy and fear expressed by adult men and women, conveyed through two sensory modalities: olfactory and auditory. Olfactory emotional stimuli were human sweat samples, whereas auditory emotional stimuli were non-verbal human vocalisations. Power spectrum analysis revealed stronger activity for fear- than for joy-conveying samples in the beta, low and high gamma frequency bands. Crucially, this effect was independent of sensory modality and donor sex. Furthermore, this emotion discrimination was heightened with the increasing perceived intensity of the emotions (i.e. stimuli perceived as more fearful or joyful by human raters were better discriminated against each other in the dog brain). This pattern indicates that the emotional content of the stimuli was the basis of the joy-fear discrimination rather than inherent low-level sensory differences between the stimulus categories. Taken together, these findings provide the first evidence for abstract, modality- and donor sex-independent neural representations of human emotions in a non-human brain.

Cortical Activity in Acquired Equivalence Learning in Children with Tourette Syndrome: An EEG Study

Kata Fodor[1]; Csongor Darvasi[1]; Noémi Harcsa-Pintér[1]; Kálmán Tót[1]; Adél Papp[1]; Bence Nagy[1]; Olívia Huszár[1]; Gabriella Eördegh[2]; Attila Nagy[1]
[1]Szent-Györgyi Albert Medical School, University of Szeged, Department of Physiology, Szeged
[2]Faculty of Health Sciences and Social Studies, University of Szeged, Department of Theorethical Health Sciences and Health Management, Szeged

Our previous study demonstrated that children with Tourette syndrome performed significantly worse than matched healthy controls during the learning phase of the visually guided Rutgers Acquired Equivalence Test (RAET), whereas their performance in the test phase did not differ significantly. The aim of this study was to determine whether detectable differences in cortical electrical activity exist between children with Tourette syndrome and healthy controls, which could help explain the impaired learning performance.
During the RAET, EEG recordings were obtained from 18 children with Tourette syndrome and 18 age-, IQ-level-, and sex-matched controls using a 64-channel BioSemi system (sampling rate 2048 Hz). Preprocessing of the raw EEG data involved filtering (50/60 Hz notch, >1 Hz high-pass, <100 Hz low-pass), average rereferencing, and ICLabel-assisted artifact removal with ICA. Then, the recordings were segmented relative to the button press (–500 to +500 ms), indicating the participants' responses. Power spectral density estimation was conducted in the θ (4–7 Hz), α (8–12 Hz), β (13–30 Hz), and γ (31–70 Hz) frequency bands, expressed as baseline-normalized decibel values. Statistical comparison between groups was performed using cluster-based permutation tests across all EEG channels, frequency bands, task phases, and pre- and post-response time windows.
In contrast to the previously observed learning deficit, the EEG analysis did not reveal any significant differences in cortical activity between the two groups. Neither the resting baseline nor the recordings from the learning or test phases showed significant differences in any EEG channels or frequency bands between Tourette syndrome patients and control children. This finding held true for both the 500 ms time window preceding the button press and the 500 ms window following it.
Based on our findings, the reduced performance in associative acquired equivalence learning in children with Tourette syndrome is not accompanied by detectable alterations in cortical activity. Additionally, the functional difference is most probably connected to that mainly subcortical structures that are altered in Tourette syndrome, the basal ganglia.

Disrupting Binocular Integration Alters Visuomotor Prediction: A VR-EEG Study

Bendeguz Fekete[1]; Harald Barzan[2,3]; Vasile Vlad Moca[2]; Raul Muresan[2]; Mirella Barboni[4]; Gabriella Trieb[1]; Aphrodite Babakhani[4]; Zoltan Zsolt Nagy[4]; Daniel Hillier[1,5]
[1]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Transylvanian Institute of Neuroscience, Department of Experimental and Theoretical Neuroscience, Cluj-Napoca*
[3]*Technical University of Cluj-Napoca, Basis of Electronics Department, Cluj-Napoca*
[4]*Semmelweis University, Department of Ophthalmology, Budapest*
[5]*Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest*

Impaired predictive processing may underlie deficits in neurodevelopmental disorders such as amblyopia. To investigate this, we present a multimodal framework combining a visuomotor task in an immersive virtual environment with high-density EEG. As a precursor to clinical application, we characterize predictive mechanisms in healthy adults before and after short-term monocular deprivation (STMD). STMD temporarily disrupts binocular integration, serving as an experimental model to induce amblyopia-like visual imbalance and isolate neural signatures associated with perturbed input.
In our paradigm, participants pedal a stationary bike through a virtual corridor, using self-motion to generate continuous visual predictions. We introduce "visual mismatches" – transient halts in visual flow – to elicit error signals. We analyze these mismatch-related EEG signatures to determine how acute monocular deprivation alters the brain's predictive hierarchy compared to a healthy baseline. These findings will validate the sensitivity of our paradigm in detecting subtle shifts in predictive processing, establishing a robust foundation for longitudinal studies on recovery in clinical amblyopia.
Results: Our analysis identified a robust negative ERP component time-locked to visual mismatches. Crucially, this error signal was modulated by deprivation, revealing significant asymmetries in amplitude and latency between the patched and non-patched visual fields. Furthermore, spectral analysis indicated broader alterations in neural state, evidenced by shifts in oscillatory power relative to the pre-patching baseline.
Conclusion: Our approach enables the objective quantification of how visual imbalance reconfigures the brain's predictive hierarchy. By successfully isolating deprivation-specific asymmetries in error signals, we confirm the framework's diagnostic utility for indexing neural plasticity. This supports its translation from experimental models to clinical assessments of amblyopia recovery.

Representation and causal dynamics in a mesoscale cortical network of visual working memory

Bálint Varga[1,2]; Marcell Stippinger[1]; Fülöp Bazsó[1]; Attila Bencze[1,3]; Zoltán Somogyvári[1]; Tamás Kiss[1]; Isao Hasegawa[4,5]; Hisashi Tanigawa[4,6]; László Négyessy[1]
[1]*HUN-REN Wigner Research Centre for Physics, Department of Computational Sciences, Theoretical Neuroscience and Complex Systems Research Group, Budapest*
[2]*Semmelweis University, Semmelweis Doctoral School, Budapest*
[3]*Óbuda University, Electrophysics Institute, Budapest*
[4]*Niigata University, Department of Physiology, Niigata*
[5]*Niigata University, Center for Transdisciplinary Research, Niigata*
[6]*Zhejiang University, Interdisciplinary Institute of Neuroscience and Technology, Hangzhou*

Working memory (WM) arises from interactions between the prefrontal cortex (PFC) and other associative cortical areas. In WM, these cortical interactions are coordinated by phase-amplitude coupling (PAC) between neuronal populations. However, the causal mechanisms underlying PAC remain unclear. Our study aims to investigate the causal cortical network involved in PAC during fronto-temporal interactions.
High-density electrocorticography (ECoG) recordings were obtained from the PFC and temporal cortex (TE) of two macaque monkeys performing a delayed color recall task, which required them to recall colors associated with grayscale images.
Task-specific oscillatory activity was dynamically modulated in both temporal and prefrontal cortices. Notably, significant PAC emerged within a localized region of the TE during the delay period, specifically between low (delta-theta) and high (beta-gamma) frequency bands. Using frequency-dependent Granger causality, we identified the PAC site in the TE as being causally linked to specific subregions of the PFC during mnemonic processes, particularly in the theta band. Neural decoding further illuminated the functional role of this causal network, revealing how specific cortical regions represented different aspects of the task.
Our findings highlight the critical role of PAC in working memory and demonstrate the PFC's regulatory function in coordinating cortical interactions.
Keywords: object-based visual working memory, fronto-temporal cortical network, electrocorticography, Granger causality, phase-amplitude coupling (PAC)

In vivo GABA and Glutamate Levels in the Human Cortex

Zsófia Kürtös[1,2]; Gergely Orsi[2,3,4]; Gábor Perlaki[3,5,6]; Boldizsár Czéh[1,7]; Szilvia Anett Nagy[3,5,6]
[1]*Structural Neurobiology Research Group, Szentágothai Research Centre, University of Pécs, Pécs*
[2]*Pécs Diagnostic Centre, Pécs*
[3]*HUN-REN-PTE Clinical Neuroscience MR Research Group, Pécs*
[4]*Department of Neurology, Medical School, University of Pécs, Pécs*
[5]*Experimental Parkinson's Disease Research Group, Szentágothai Research Centre, University of Pécs*
[6]*Department of Medical Imaging, Faculty of Health Sciences, University of Pécs*
[7]*Department of Laboratory Medicine, Medical School, University of Pécs, Pécs*

Introduction: The balance between excitatory Glutamate (Glu) and inhibitory γ-aminobutyric acid (GABA) is essential for normal brain function. Disruption of this balance has been implicated in various neuropsychiatric disorders. Proton magnetic resonance spectroscopy (1H-MRS) using the MEGA-PRESS sequence enables non-invasive quantification of GABA, Glx (the sum of Glutamine and Glutamate) in vivo. This study assessed the test-retest reliability of MEGA-PRESS measurements and examined the effects of age, sex, and education on GABA+ and Glx levels in three neocortical regions: anterior cingulate cortex (ACC), occipital cortex (OCC), and precuneus (PrC).
Materials and methods: Ten healthy adults (6 females; mean age = 33.40±6.81 years) underwent repeated scans one week apart to evaluate the reliability of GABA+ and Glx levels in ACC, OCC and PrC. Thirty participants (21 females; mean age = 27.07±7.84 years) provided data to compare metabolite references and correction methods in the ACC. Sixty-nine healthy adults (40 females; mean age 25.7±6.8 years) were analyzed to explore demographic influences using creatine-referenced GABA+ and Glx levels.
Results: Test-retest reliability was higher for Glx than GABA+, with lower coefficients of variation and higher intraclass correlation coefficients. Water- and creatine-referenced GABA+ and Glx measures in the ACC were strongly correlated ($p<0.001$), and different water-based correction methods yielded highly consistent results ($p<0.001$). No significant interactions between sex and age or education were found. However, males exhibited significantly higher uncorrected and cerebrospinal-fluid (CSF) corrected Glx/Cr levels than females ($p<0.05$), while GABA+/Cr levels showed no sex differences. Age was positively associated with GABA+/Cr ($p<0.07$) and negatively with Glx/Cr ($p<0.05$) levels in females. Years of education was negatively correlated with uncorrected and CSF-corrected Glx/Cr levels in males ($p<0.05$ and $p<0.07$, respectively) but not in females.
Conclusions: These findings highlight the robustness of MEGA-PRESS for GABA+ and Glx measurement and reveal sex-specific age and education effects on those metabolites. Creatine referencing is appropriate for quantification in healthy young to middle-aged adults, though water referencing may be preferable in multi-center or clinical studies. Understanding these demographic influences is critical for interpreting MR spectroscopy data in neurocognitive research.

A functional magnetic resonance study examining the long-term effect of formal music education on emotion processing during the Emotional Face Matching Task.

Mónika Gálber[1,2]; Szilvia Anett Nagy[2,3,4]; Elza Flóra Lakner[1]; Gergely Orsi[4,5,6]; Gábor Perlaki[2,3,4]; Boldizsár Czéh[1,7]
[1]*University of Pécs, Szentágothai Research Centre, Structural Neurobiology Research Group, Pécs*
[2]*University of Pécs, Szentágothai Research Centre, Experimental Parkinson Disease Research Group, Pécs*
[3]*University of Pécs, Faculty of Health Sciences, Pécs*
[4]*HUN-REN-PTE, Clinical Neuroscience MR Research Group, Pécs*
[5]*University of Pécs, Medical School, Department of Neurology, Pécs*
[6]*Pécs Diagnostic Centre, Pécs*
[7]*University of Pécs, Medical School, Department of Laboratory Medicine, Pécs*

Introduction and aims: Childhood music education has been associated with functional alterations across several neural systems, and individuals with musical training often show enhanced emotion-recognition performance. However, the neurobiological mechanisms of the relationship between the musical training and emotion recognition are not fully understood. Our previously developed and tested Emotional Face Matching fMRI paradigm is used for assessing the cognitive and neural processes underlying emotion recognition from facial expressions. Here, we investigated the influence of formal music training on brain activity in an emotional processing fMRI task.
Materials and methods: Participants with musical training (Musical training group, N=30) and healthy controls (Control group, N=30) were studied with fMRI while performing a block design facial emotion matching task with images portraying fear, anger and sadness. The groups were matched based on age and gender. Group differences were assessed using the FEAT (v6.00) tool of the FMRIB Software Library, with age, gender and years of education included as covariates. Statistical maps were considered to be significant at $Z > 2.3$.
Results: After controlling for age, gender and years of education, the Musical training group had significantly increased activity in the precentral gyrus and middle frontal gyrus of the frontal lobe, as well as in the supramarginal gyrus, middle temporal gyrus, superior and inferior lateral occipital cortex and posterior cingulate gyrus. Bilateral precuneus had increased activity in women than in men, while the opposite effect was found in some cerebellar areas. Age was negatively correlated mainly with the activity of the posterior cingulate cortex and precuneus, while years of education had no effect during the Emotional Face Matching paradigm.
Conclusion: Our results confirm that the blood-oxygen-level dependent (BOLD) signal differed significantly between participants with childhood musical training and those without formal musical education in brain regions involved in emotion processing and emotion recognition. In addition, age and gender influenced the activity of some of these regions.

Single Trial Source-resolved EEG Analysis of a Free Viewing Neuroaesthetic Task with Independent Component Analysis

Iffah Syafiqah Suhaili[1]; Zoltan Nagy[1,2]; Zoltan Juhasz[1]
[1]*University of Pannonia, Department of Electrical Engineering and Information Systems, Bioelectric Brain Imaging Laboratory, Veszprem*
[2]*Semmelweis University, Heart and Vascular Centre, Budapest*

Neuroaesthetics seeks to understand how the brain responds to art appreciation, yet traditional event-related potential (ERP) approaches are limited in free-viewing paradigms with long epochs, where ERP averaging may conceal dynamics of ongoing neural oscillations. Due to volume conduction, scalp electroencephalography (EEG) reflects linear mixtures of multiple cortical sources, limiting anatomical interpretability at the sensor level. Independent Component Analysis (ICA), although commonly used for artifact removal, also able to separate statistically independent cortical sources from mixed EEG signals. Here, we investigate the feasibility of using ICA, beyond artifact removal, as a source/component-based analysis approach.
Participants viewed 80 paintings, each preceded by a 1-second cue and presented for 8 seconds, followed by a 4-second blank black screen during which they indicated their preference (“like” or “dislike”). EEG activity was recorded using a 128-channel BioSemi ActiveTwo system at a sampling rate of 2048 Hz. Preprocessing included filtering, ICA decomposition, classification of brain-related components using ICLabel, and dipole fitting (DIPFIT) to estimate source locations. For group-level analysis, components were clustered across subjects based on scalp maps, dipole locations and activation time courses, showing representative source-level clusters for further analyses, such as event-related spectral perturbation (ERSP) and power analyses across conditions.
Applying this pipeline to a free-viewing neuroaesthetic experiment with long epochs revealed physiologically interpretable and reproducible component clusters. Particularly, a strong occipital cluster corresponding to the lambda wave (associated with saccade-related visual processing) was consistently identified across participants, alongside additional occipital and motor-related component clusters. These components showed clear time-frequency dynamics at the single-trial level that are typically flattened in conventional ERP averaging. ERSP and power analyses further demonstrated condition-related modulations within source-resolved activity.
Overall, these findings demonstrate the feasibility of ICA-based source clustering for neuroaesthetic EEG data acquired under naturalistic viewing conditions. This approach enables more direct access to cortical dynamics than scalp-level ERPs and provides a promising approach for studying brain activity during art perception.

The effects of CMDT intervention on U8-U12 soccer players' multifocal attention and multisensory integration: a longitudinal study.

Szabolcs Székely[1,2]; Anna Gergely[1]; József Topál[1]
[1]*Institute of Cognitive Neuroscience and Psychology, Budapest*
[2]*Institute of Psychology, Adaptation Research group, Budapest*

A well-established principle of human executive functioning is that cognitive and physical efforts operate within a coupled system. This shared energy demand is a crucial factor affecting athletes' performance across various sports. However, research faces a methodological challenge: while cognitive processes can be measured under controlled laboratory conditions, these often fail to mirror the complex demands of real-life sports situations. Consequently, traditional screen-based cognitive training may show limited transfer to the field. To address this, cognitive-motor dual training (CMDT) integrates cognitively demanding tasks with sport-specific motor actions. The present study evaluated the effects of a 10-month CMDT intervention on two key cognitive abilities in football: multifocal attention, and multisensory integration.
The study involved 34 school-age soccer players (mean age 9.2 SD: ± 1.82 years) from a Hungarian football talent centre, representing three age divisions (N=10 U12; N=14 U10; N=10 U8). Participants were assigned to either a CMDT intervention group or a control group using a matched-control design. Cognitive performance was assessed through the Multiple Object Tracking (MOT) task for multifocal attention, and the Sound-Induced Flash Illusion (SIFI) task for multisensory integration.
In the MOT task, the control group showed significantly better baseline performance than the CMDT group ($p < 0.05$); however, this difference disappeared following the 10-month intervention (GLMM, group × session interaction: $p = 0.038$). Regarding the SIFI task, binomial GLMM analysis revealed significant group × session interactions for both fission errors ($p = 0.003$) and fusion errors ($p = 0.006$). These results indicate that the intervention group achieved superior improvements in multisensory processing and attentional tracking compared to the control group.

Our findings demonstrate that CMDT intervention effectively enhances multifocal attention and multisensory integration in youth athletes. Participants in the intervention group showed significant developmental gains compared to those following traditional training protocols. This suggests that integrating cognitive load into motor practice is a potent method for developing the specialized executive functions required for elite-level sports performance.

Progressive ratio paradigm in macaques - probing the effects of reward value and pharmacological challenges

Judit Inkeller[1,2]; Balázs Knakker[1,2]; Rafaella Mínea Riszt[1,2,3]; Anna Padányi[1,2]; Antonietta Vitális-Kovács[1,2]; Evelin Kiefer[1,2]; István Hernádi[1,2,4]
[1]*University of Pécs, Grastyán E. Translational Research Centre, Pécs*
[2]*University of Pécs, Centre for Neuroscience, Pécs*
[3]*University of Pécs, Medical School, Pécs*
[4]*University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs*

The progressive ratio (PR) task was designed to assess motivation and goal-directed behaviour by escalating the effort required for obtaining successive rewards, and it has become the benchmark for evaluating the reinforcing efficacy of drugs of abuse in animals. In our study we aimed to extend the PR paradigm to measure motivation and effort using natural reinforcers (food rewards) in non-human primates.
Ten cynomolgus macaques (*Macaca fascicularis*; six males) participated in the experiments conducted in custom-developed operant chambers equipped with the Monkey CANTAB (Cambridge Neuropsychological Test Automated Battery) system. We employed a lever-press-based PR task wherein the number of responses required to obtain the same amount of reward (2 pellets) increased exponentially in each consecutive trial until the subject stopped responding i.e., reached the breakpoint. We set task parameters so that the breakpoint occurred well before satiation could influence motivation. To determine the effects of reinforcer magnitude on PR performance, the reward value was modified to 1 or 4 pellets, and also to 0 pellets (extinction). Furthermore, we tested the effect of adjusting the response requirement progression by altering the exponential increment parameter. Finally, in a pharmacological validation, dopamine depleting agent tetrabenazine (TBZ) was administered at 0.3 and 1 mg/kg doses to assess effort-related performance changes.
Higher reward magnitude increased operant responding while schedule manipulations had no significant impact on performance. Extinction sessions led to a gradual, learning-based decline in performance. TBZ caused a significant dose-related decrease in lever pressing, interpreted as an anhedonia-like state driven by reduced incentive value of reward and/or increased effort cost.
Our results suggest that, in NHP experiments, using food reinforcement provides a well-suited approach to examine motivation and effort-based decision making, because the breakpoint is more strongly influenced by “wanting”, or the incentive salience of the food stimulus. This paradigm is particularly relevant for the investigation motivational aspects of psychiatric disorders, including anhedonia associated with depression and schizophrenia, as well as addiction. Furthermore, the model offers high translational value making it an effective tool for assessing the neuronal background and pharmacological modulation of effort-related behaviour in primates.

Evaluating cholinergic modulation in a cognitive battery paradigm in non-human primates

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

In human cognitive research, test batteries provide a multidimensional assessment of different cognitive functions. In preclinical studies, however, comparable paradigms are often applied as separate tasks analyzed independently. In our study, we employed a cognitive battery paradigm closely aligned with established human protocols and aimed to pharmacologically validate this multi-task approach in non-human primates, strengthening translational links between clinical and preclinical cognitive research.
In our study, 7 adult male rhesus macaques were introduced to our paradigm, consisting of 4 different touchscreen tasks covering object memory (delayed matching to sample, DMTS), location memory (self-ordered spatial search, SOSS), associative object-location memory (paired associates learning, PAL) and visual discrimination and attentional set shifting (intra-extra dimensional set shifting, IDED). We also added a fifth task (SOSS based motivation measurement task, SOMM) with three trials of 12 identical, task-specific stimuli that had to be touched to disappear, eliminating mnemonic demands and only focusing on motivational drive. This task was administered before and after the session, as well as in the intervals between the tasks.
Results indicate that in the within-session multi-task setup, an 8µg dose of the muscarinic acetylcholine-receptor antagonist scopolamine induces similar degree of transient cognitive impairment across the PAL, SOSS and DMTS tasks, but for IDED, these impairments were less prominent. The acetylcholinesterase inhibitor donepezil showed a tendency to reverse the temporary amnestic effects of scopolamine and again, this compensatory trend was less clearly expressed in the IDED task.
Our results suggest a domain-specific pattern of pharmacological sensitivity within the cognitive battery, with tasks relying more heavily on memory-related processes showing greater susceptibility to cholinergic modulation than a task with low memory demands engaging attentional control and cognitive flexibility. This contrast emphasizes the functional heterogeneity of the battery and supports its interpretability at the level of cognitive domains rather than individual tasks. Altogether, our cognitive battery paradigm offers a multi-task framework for evaluating pharmacological efficacy in a preclinical translational setting. Furthermore, the paradigm also provides a basis for future integration with non-invasive stimulation techniques.

Transcranial magnetic stimulation based cortical excitability measure in awake non-human primates

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

Transcranial magnetic stimulation (TMS) is a widely used non-invasive brain stimulation technique in both basic and clinical neuroscience. Non-human primates (NHPs) constitute highly valuable translational models owing to their close anatomical and functional similarity to humans. In human studies, the obligatory initial TMS protocol—determining the motor threshold (MT) as defined by the International Federation of Clinical Neurophysiology (IFCN)—provides an index of cortical excitability (CE) and is well established; however, it remains considerably less characterised in awake NHPs. In the present study, we therefore implemented both the traditional MT (tradMT) method (Rossini et al., 1994, 2005) and SAMT, a recently developed adaptive MT determination approach originally validated in humans (Wang et al., 2023).
Using neuronavigation-guided single-pulse TMS targeting the primary motor cortex (M1), motor-evoked potentials (MEPs) were recorded from the right abductor pollicis brevis muscle using surface electromyography in awake rhesus macaques. Traditional MT was first determined using larger intensity steps (1–4% of maximum stimulator output, %MSO) in eight subjects. Although MT estimates were reasonably reliable (n = 8; within-subject SD = 2.41% MSO; ICC = 0.821), the proportion of the suprathreshold responses (>100 μV) at the estimated MT varied considerably and did not consistently approximate the expected 50% criterion. Therefore, subsequent MT measurements were performed using smaller intensity steps (1% MSO) in four subjects, where the proportion of suprathreshold responses at the estimated MT was more stable and consistently fell within the expected 40–60% range.
A recently developed MT measurement technique with adaptive stepping was implemented in 4 subjects as well, which converged successfully within 25 pulses, as prescribed by the protocol. Additionally, at the finalised MT the IFCN-defined criterion was also fulfilled.
In summary, we demonstrate that human-relevant MT determination protocols can be successfully implemented in awake NHPs, yielding valid and reliable measures of cortical excitability. Although the adaptive approach substantially reduces the number of required stimuli, it did not confer a clear temporal advantage over the traditional method. Nevertheless, both approaches provide robust foundations for subsequent, bidirectionally translatable and clinically relevant neuromodulation protocols.

Proactive interference in object-location short-term memory - a comparison between humans and rhesus macaques

Balázs Knakker[1]; Anna Padányi[1,2]; Viktória Pál[1]; Evelin Kiefer[1]; István Hernádi[1,2,3]
[1]*University of Pécs, Grastyán E. Translational Research Centre, Pécs*
[2]*University of Pécs, Medical School, Pécs*
[3]*University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs*

Interactions between multiple items to remember are usually detrimental to memory performance – that is, they cause interference. Proactive interference is a key limiting factor of short-term memory in humans and animals alike. Here we show that proactive interference, conceptualised as a "misbinding" error, can be effectively studied in rhesus macaques and humans in a custom version of the (MonkeyCantab) PAL object-location short-term memory task.
In each trial of the Paired Associates Learning task (PAL) the subject has to recall the locations of several distinct sequentially presented schematic visual stimuli. After extensive training, 12 young male adult rhesus macaques were able to perform the task on a 67-stimulus set with up to 3-7 stimuli per trial. A new large stimulus set (n=670) was introduced to better control between-trial interference, and in a subset of trials, we introduced stimulus recurrence from the previous trial. We found a very strong between-trial proactive interference effect leading to 20-30% performance decrement for the affected memory item, which gradually tapered off with increasing distance, i.e. if the interfering memory came from earlier trials. Importantly, this interference effect was very stable even after months of daily training in the task, indicating that the interference effect measured here represents an important inherent limitation in the animals' memory performance.
Then, we went on to study the same phenomenon in young adult university volunteers. Using a smaller stimulus set (n=120), controlling for gradual between-session and within-session familiarization with the stimuli, we show that though task performance gradually increases with task/stimulus exposure, the interference effect is stable across the studied conditions. Interestingly, in human volunteers the interference effect did not taper off with distance.
Proactive interference can be robustly quantified as object-location misbinding error in the PAL task in both macaques and humans. Interference effects were robust to learning effects in both macaques and humans, implying that interference is an evolutionarily conserved inherent limitation of short-term memory. Species differences in the temporal profile of interference suggest distinct control or decay mechanisms. This paradigm provides a tool for assessing how neuromodulatory, pharmacological, or disease-related factors impact memory binding and interference.

ERP indices of initial response to reward: redundancy, unique variance, reliability across 3 measurement occasions and generalization of reliability across clinical and demographic groups

György Hámori[1]; Nóra Bunford[1]
[1]*HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Clinical and Developmental Neuropsychology Research Group, Budapest*

Where data are available on stability of indices of response to reward, explicit tests of generalizability across groups are scarce and little is known about measurement properties of less commonly derived indices. We examined, in adolescents (*N*=269), whether 16 behavioral and ERP (RewP) indices of response to reward are (1) redundant and (2) account for unique variance. Also, in a subsample of adolescents and young adults (*n*=140), whether ERP indices are stable across 3 assessments/ 30 months and whether stability generalizes across demographic and clinical groups. Findings indicated the indices (1) are not redundant; *r*s adjusted for age, birth sex, and depressive problems range: -.307-.779, 75th percentile:.242. (2) Account for unique variance; EFA suggested *f1* (gain and loss): amplitude, peak-to-peak amplitude and intra-subject variability of latency; *f2* (gain) and *f3* (loss): intra-subject variability of amplitude and jitter of latency; *f4* (gain and loss): intra-subject variability of latency; *f5* behavioral indices. (3) Are stable; ICCs were good-excellent (.629-.860). Stability generalized across n=81 boys (.599-859) and n=59 girls (.610-.862) but less so across clinical groups. In low ADHD (n=88), ICCs were good-excellent (.593-.881; 50% in excellent range). In high ADHD (n=52), ICCs were fair-excellent (.464-.819; 25% in excellent range). Results suggest examined indices 1) reflect different but related processes and 2) are stable across late adolescence and young adulthood, irrespective of birth sex but variably across ADHD levels.

Oxytocin receptor expressing neurons in the medial preoptic area inhibit aggression via ventromedial hypothalamic projection

Botond Drahos[1]; Tamás Láng[1];; Valery Grinevich[3] , Árpád Dobolyi[1,2]
[1]*Semmelweis University, Department of Anatomy, Histology and Embryology, Laboratory of Neuromorphology, Budapest*
[2]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*
[3]*University of Heidelberg, Department of Neuropeptide Research in Psychiatry, Mannheim*

The medial preoptic area (MPOA) is a key neural hub integrating hormonal homeostasis and social behavior. While its role in maternal and reproductive functions is well established, its involvement in aggression is less clear. Previously, we found that the posterior intralaminar thalamic nucleus (PIL), through projections to the MPOA, reduces aggression and increases positive valence behaviors. We also found that PIL neurons containing parathyroid hormone 2 (PTH2) projecting to the MPOA promote prosocial actions.
We hypothesized that oxytocin-receptor (OTR) expressing MPOA neurons mediate rodent aggression and prosocial behaviors. While oxytocin is central in maternal functions, its influence on positive valence and aggression remains unclear. This study examined the role of MPOA OTR neurons in social interaction in male rats. We used OTR-Cre transgenic rats to selectively express receptors (DREADDs) and mCherry fluorescent proteins in OTR-positive neurons through viral vectors. Chemogenetic manipulation was achieved by injecting clozapine-N-oxide (CNO), and behavioral changes were assessed using the resident-intruder test. Inhibiting MPOA OTR neurons increased aggression and reduced positive valence behaviours, while stimulating these neurons increased behaviours with positive valence but did not alter aggression, likely due to low baseline aggression in the stimulation group.
Next, we visualized OTR neuron projections using mCherry and immunohistochemistry, revealing connections to regions like the ventromedial hypothalamic nucleus (VMH) and the medial amygdala (MeA). Also, double immunolabelling showed dense PTH2 fiber terminals near MPOA OTR neurons. Chemogenetic inhibition of MPOA OTR neurons during male social interaction increased c-Fos expression in the VMH, suggesting their involvement in aggression regulation via this neuronal pathway. Targeted inhibition of the MPOA OTR-VMH pathway by local CNO injection through an intracerebral cannula increased aggression and decreased positive valence behaviors.
In conclusion, OTR-positive MPOA neurons may act as a central hub suppressing aggression by inhibiting the VMH and increasing prosocial behaviors. PTH2-containing PIL neurons may target this population, potentially conveying somatosensory inputs, highlighting the OTR-positive neurons as a key relay integrating PIL inputs and influencing social behavior.

Dynorphin signaling drives adolescent shifts in social reward

Klaudia Misiołek[1]; Maria Kaczmarczyk-Jarosz[2]; Łukasz Szumiec[1]; Zofia Harda[1]; Barbara Ziółkowska[1]; Jan Rodriguez Parkitna[1]
[1]*Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Molecular Neuropharmacology, Krakow*
[2]*Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Physiology, Krakow*

Adolescence is a period of extensive brain remodeling, accompanied by changes in reward-related behaviors that reflect maturation of neural systems. The endogenous opioid system, particularly dynorphin (DYN) and κ opioid receptor (KOR) signaling, plays a critical role in shaping reward processing and stress responsivity. However, the developmental trajectory of DYN/KOR signaling, and its influence on age-dependent reward behaviors, is underexplored.
We investigated developmental changes in endogenous opioid gene expression in the medial prefrontal cortex, nucleus accumbens, and dorsal striatum of male C57BL/6 mice at early (~postnatal day 32; pubertal onset), mid (~P38; peripubertal), and late adolescence (~P43; sexual maturation). We then tested whether these developmental shifts are associated with reward-related behavioral changes using social and cocaine conditioned place preference (CPP) following pharmacological or genetic disruption of opioid signaling. qPCR and RNAscope *in situ* hybridization analyses revealed region- and cell-type-specific developmental changes: basal *Pdyn* expression decreased in medial prefrontal cortex and dorsal striatum, *Pdyn*/KOR mRNA co-expression increased in dorsal striatum and nucleus accumbens, and the proportion of cells co-expressing *Pdyn*/somatostatin mRNA increased in mPFC, indicating a shift toward circuits that are potentially less tonically active but more contextually responsive. Behavioral assays showed that late adolescent mice exhibited selective reductions in social, but not cocaine CPP following administration of the long-acting KOR antagonist norbinaltorphimine or in complete *Pdyn* knockout mice. These results indicate that as the DYN/KOR system matures, it becomes increasingly essential for mediating acquisition of social, but not cocaine CPP.
Overall, these findings highlight adolescence as a window of heightened plasticity in social reward circuits, during which DYN/KOR systems critically shape the emergence of mature social motivation. Our work provides mechanistic insight into how developmental changes in opioid signaling support the transition from early- to late-adolescent social reward processing, while raising the question of whether, and how, these changes continue to evolve into adulthood.

Critical role of basal amygdala output to dorsomedial striatum in Pavlovian fear learning

Zsófia Reéb[1,2]; Bíborka Bruzsik[1,3]; Gergő Attila Nagy[1,3]; Dániel László Magyar[1,3,4]; Orsolya I. Papp[1]; Judit M. Veres[1]; Erzsébet Gregori[1]; Cecília Pardo-Bellver[1]; Máté Tóth[1]; Manó Aliczki[1]; Éva Mikics[1]; Norbert Hájos[1,4,5]

[1]HUN-REN Institute of Experimental Medicine, Laboratory of Network Neurophysiology, Budapest
[2]ELTE Eötvös Loránd University, Institute of Biology, Doctoral School of Biology, Budapest
[3]Semmelweis University, János Szentágothai Doctoral School of Neurosciences, Budapest
[4]The Linda and Gill Institute for Neuroscience, Indiana University, Bloomington
[5]Indiana University Bloomington, Program in Neuroscience, Department of Psychological and Brain Sciences, Bloomington

The lateral amygdala is known to serve as the central node of associating a conditioned stimulus with an unconditioned stimulus during Pavlovian fear conditioning. In the prevailing model, signals from the lateral amygdala are relayed to downstream nuclei - the basal and central amygdala - which mediate fear expression/memory storage and the activation of midbrain defensive circuits, respectively. Recent studies have further divided the basal amygdala (BA) into two distinct regions: the anteromedial (BAam) and posterolateral subdivisions. Using Arhgef6-Cre mice, which allow for the selective targeting of BAam neurons, we found that inhibition of BAam markedly reduced freezing during fear conditioning as well as during both contextual and cued fear memory recall. Strikingly, these behavioral effects were fully recapitulated by inhibiting BAam neurons projecting to the dorsomedial striatum (DMS), but not those projecting to the medial prefrontal cortex (mPFC). Accordingly, in vivo electrophysiological recordings revealed that footshocks evoked significantly stronger spiking responses in DMS-projecting BAam neurons than in mPFC-projecting neurons. Together, these results demonstrate that the BAam contributes directly to fear acquisition during Pavlovian conditioning through its projections to the DMS. We propose that the BAam–DMS pathway represents a critical output channel of the amygdala that regulates defensive behavior.

Chemogenetic modulation of the vasopressinergic system influences social interactions

Kristóf László[1]; Dávid Vörös[1]; Jan Bakos[2]; Barbora Bugár Bodorová[2]; Bettina Réka László[1]; Erika Kertes[1]; Tamás Ollmann[1]; Barnabás Bálint[1]; Vivien Fodor[1]; László Lénárd[1]; Dóra Zelena[3]
[1]Medical School, University of Pécs, Pécs, Hungary., Physiology Department, Neuropeptides, Cognition, Animal Models of Neuropsychiatric Disorders Research Group, Pécs
[2]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava
[3]Medical School, University of Pécs, 7624 Pecs, Hungary., Physiology, Pécs

Arginine vasopressin (AVP) is a hypothalamic neuropeptide hormone that, beyond its well-known peripheral effects (e.g. vasoconstriction and antidiuresis), plays an important role in the regulation of complex behaviors, including social interactions. AVP-expressing neurons in the paraventricular nucleus of the hypothalamus (PVN) project to several limbic regions, including the central nucleus of the amygdala (CeA), a key structure involved in emotional and social processing.
The aim of the present study was to investigate how selective chemogenetic manipulation of vasopressinergic neurons projecting from the PVN to the CeA affects social interaction and related behavioral patterns.
AVP-Cre transgenic rats were used to selectively target AVP-expressing neurons. Adeno-associated viral vectors were stereotaxically injected into the PVN, carrying either a control construct (AAV8-hSyn::DIO-mCherry), an excitatory DREADD (AAV8-hSyn::DIO-hM3Dq-mCherry), or an inhibitory DREADD (AAV8-hSyn::DIO-hM4Di-mCherry). Prior to behavioral testing, clozapine-N-oxide (CNO) was microinjected into the central nucleus of the amygdala to selectively modulate the activity of PVN-derived vasopressinergic projections. Social behavior was subsequently assessed using a standardized social interaction test.
Activation of excitatory DREADDs resulted in a significant increase in the sociability index, whereas activation of inhibitory DREADDs led to a marked reduction in social interaction. The control viral construct had no detectable effect on social behavior.
These findings suggest that vasopressinergic neurons within the PVN–amygdala pathway play a facilitatory role in the regulation of social behavior and that these processes can be selectively modulated using chemogenetic approaches. Our results contribute to a deeper understanding of the neural mechanisms underlying social behavior and may help identify novel therapeutic targets for disorders characterized by social dysfunction.

Memory consolidation correlates with synchronized astrocyte activity

Márton Péter[1]; László Héja[1]
[1]HUN-REN Research Centre for Natural Sciences, Institute of Organic Chemistry, Chemical Biology Research Group, Budapest

One of the major breakthroughs of neurobiology was the identification of distinct ranges of oscillatory activity in the neuronal network that were found to be responsible for specific biological functions, both physiological and pathological in nature. Astrocytes, physically coupled by gap junctions and possessing the ability to simultaneously modulate the functions of surrounding synapses, are perfectly positioned to introduce synchronised oscillatory activity into the neural network. While astrocytes are generally considered to be slow responders in terms of Ca2+ signalling, we were previously able to reveal fast Ca2+ oscillations in the soma of astrocytes in the delta (0.5–4 Hz) and theta (4–8 Hz) frequency bands *in vivo* in the rat cortex using high frequency 2-photon imaging under ketamine–xylazine anaesthesia, which is known to induce permanent slow-wave activity (SWA). These oscillations appear to be linked to the induced SWA, as they were not observed under fentanyl anaesthesia. The signals are present in a large number of astrocytes and are synchronised at the network level (Péter & Héja 2024). Recently, we have investigated the link between astrocytes and SWA on the cellular, network, and behavioural level. Astrocytic synchronization was modified by activating gap junctions using trimethylamine (TMA), or by blocking them with an astrocyte-specific Cx43 antibody. High frequency 2-photon imaging of astrocytes and neurons shows that synchronization of both cell types at frequencies characteristic of SWA (0.5 –2 Hz) is strongly diminished following gap junction blockade. In contrast, gap junction activation resulted in increased synchronization in both cell networks. Since SWA is known to be involved in memory formation, we investigated whether the activation or inhibition of gap junctions influences memory performance in the novel object recognition memory test. We demonstrated that the working memory of rats can be enhanced by TMA, while treatment with the Cx43 antibody causes memory impairment. To validate our findings, we have also conducted a series of experiments under fentanyl anaesthesia to demonstrate that baseline memory performance correlates with naturally occurring (not ketamine–xylazine-induced) SWA. We believe that large-scale synchronization in the astrocyte network through gap junctions plays a previously unrecognized, essential role in higher cognitive functions and may open up new avenues in the therapy of cognitive disorders.

Social Touch Suppresses Aggression via Thalamic Mechanisms

Tamás Láng[1]; Botond Drahos[1]; Fanni Dóra[1]; Dávid Keller[1]; Vivien Szendi[2]; Gina Puska2,3, Valery Grinevich4, Árpád Dobolyi[3]; Valery Grinevich[4]; Árpád Dobolyi[2]
[1]Semmelweis University, Department of Anatomy, Histology and Embryology, Laboratory of Neuromorphology,, Budapest
[2]Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest
[3]University of Veterinary Medicine Budapest, Department of Zoology, Budapest
[4]University of Heidelberg, Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health,, Mannheim

Introduction: Aggression is a core social behavior however, unnecessary escalation of aggression is maladaptive, necessitating a mechanism for suppression.
Aim: We aim to validate that the posterior intralaminar thalamic nucleus (PIL) suppresses aggressive behavior by integrating tactile signals.
Methods: First, we investigated whether the lack of tactile inputs led to increased aggression. Rodents were housed in three groups: complete isolation, barrier housing (no tactile stimulus), and control pairs. To assess how tactile stimuli affect PIL neurons, we used fiber photometry to measure neuronal activity. Functional investigations employed chemogenetics in resident-intruder tests. We expressed DREADDs and manipulated them with clozapine-N-oxide (CNO) on socially-tagged neurons in the PIL through the vGATE system. Additionally, we performed optogenetic activation of PIL neurons, using CRh2 during fighting. We also examined the PIL-medial preoptic area (MPOA) pathway by administering CNO locally to the MPOA using an intracerebral cannula. Finally, we investigated the role of the ventromedial hypothalamic nucleus' (VMH) projection to the PIL using selective pathway manipulation.
Results: Both isolated animals and those housed without tactile input displayed a significant increase in aggression compared to pair-housed controls. Neuronal activity of the PIL increased immediately upon social touch, which decreased thereafter. Stimulation of the vGATE-tagged neurons significantly decreased aggression, while inhibition increased it. The optogenetic stimulation of the PIL neurons decreased the individual duration of the attack. Selective stimulation of the PIL-MPOA pathway significantly decreased aggression, while inhibition exerted the opposite action. In contrast to the PIL-MPOA pathway, chemogenetic stimulation of the VMH-PIL pathway increased aggression.
Conclusion: The PIL neurons process social tactile stimuli and, by their projections to the MPOA, decrease aggressive behaviour, while the VMH can override this mechanism by its projection to the PIL, increasing the animal's aggression if necessary. These results suggest that the PIL could serve as a potential relay center for integrating tactile social signals to prevent and terminate aggression.

Behavioral consequences of the chemogenetic silencing of the ventral tegmental area in rats II.: Memory, locomotor activity and anxiety

Zsolt Kristóf Bali[1,2]; Angelika Bodó[1,2]; Nóra Bruszt[1]; Zsóka Mária Ábel[1,2,3]; Fruzsina Kovács[1,2,3]; István Hernádi[1,2,3]
[1]*University of Pécs, Szentágothai Research Centre, Translational Neuroscience Research Group, Pécs*
[2]*University of Pécs, Grastyán Translational Research Center, Pécs*
[3]*University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs*

Here, we applied the DREADD (designer receptors exclusively activated by designer drugs) chemogenetic technic in the ventral tegmental area (VTA) of rats, and tested the effects of VTA-silencing on different aspects of rat behavior. We injected adeno-associated viral vectors (AAV5) into the VTA bilaterally during stereotactic surgery under general anesthesia. Ten animals were injected with vectors carrying the gene of the hM4Di designer receptor after a synapsin promoter, and 8 other animals were injected with vectors carrying a control plasmid containing no designer receptor gene. After several weeks, rats were subjected to different behavioral tasks. The locomotor activity was measured in the open field (OF) test; anxiety level was measured both in the OF and in the elevated O-maze (EOM) tasks; intermediate-term explicit memory was assessed in the novel object recognition (NOR) task, while long-term spatial memory was assessed in the Morris water maze (MWM). To activate the designer receptors and consequently silence the targeted neurons deschloroclozapine (DCZ) was subcutanously injected approx. 30-40 min before the behavioral tests at 0.3 mg/kg dose. In the OF test, rats expressing the hM4Di receptor showed a tendency to move more and to spend more time in the inner zone of the arena. These suggest higher locomotor activity and lower level of anxiety as a result of VTA silencing. Furthermore, the modulation of VTA also decreased anxiety in the EOM task: rats expressing the designer receptor spent marginally significantly more time in the open zone of the maze than control rats. On the other hand, there was no difference between control and DREADD expressing rats in the NOR and in the MWM tasks, and the DREADD expressing rats showed no cognitive impairment.
In summary, the silencing of the VTA influenced mainly the general activity and anxiety of rats without any detectable change in the memory performance. The histological analysis of the targeted region of the VTA is in progress, the assessment of the exact localization and the neurochemical nature of the transfected neurons (i.e., GABAergic, dopaminergic or glutamatergic cells) will provide more insight into the mechanism underlying the presently described behavioral alterations.

Behavioral effects of guanfacine in a triple-hit schizophrenia rat model

Szonja Bianka Plesz[1]; Gabriella Kekesi[1]; Gyongyi Horvath[1,2,3]
[1]*University of Szeged, Albert Szent-Györgyi Medical School, Department of Physiology, Szeged*
[2]*University of Szeged, Albert Szent-Györgyi Medical School, Department of Medical Physics and Informatics, Szeged*
[3]*University of Tokaj, Sztárai Institute, Sárospatak*

Introduction: Schizophrenia is a multifactorial disorder with complex pathogenesis, including neurochemical disruptions. Aside from the classical dopaminergic hypothesis, impairments in the noradrenergic system also play a significant role in its etiology. Human studies proved that the α2-adrenoreceptor agonist drugs (e.g. clonidine, guanfacine) are beneficial for the treatment of positive signs, but the data about their effects on the negative and cognitive symptoms are controversial. The goal of this study was to investigate the effects of guanfacine on schizophrenia-related behavioral phenotype in the triple-hit (postweaning social isolation, ketamine exposure, and selective breeding) rat model of schizophrenia (named Lisket).
Methods/animals: In the study, starting at the age of 11 weeks, male Long Evans (control, n = 25) and Lisket (n = 26) rats were involved. Intraperitoneal guanfacine in three doses (0.02-0.1-0.5 mg/bwkg) or its vehicle (physiological saline) was administered daily for 11 days. Before and during the days 8-11 of the pharmacological treatment, a food-rewarded test (Ambitus) was performed to assess locomotion, exploration, and cognition-related parameters.
Results: The pretreatment Ambitus test revealed significant group differences: Lisket rats showed decreased exploration, exhibited motivational deficit, produced less adequate exploration, and made higher omission errors. The locomotor and exploratory activities decreased significantly by the highest dose of guanfacine, and this was more pronounced in the Lisket animals. All doses of guanfacine impaired the learning capacity in Lisket group, while only the highest dose caused this phenomenon in the control group. In contrast, neither the working nor the reference memory-related parameters were influenced. The only beneficial effect of guanfacine was observed in the exploratory latency into the non-rewarded boxes, i.e. both groups showed prolonged value.
Conclusion: The study demonstrated that while the α2A-adrenoreceptor agonist treatment significantly decreased the behavioral activity and learning ability, it did not influence memory functions and even improved the selection between the rewarded and non-rewarded sites. These data suggest that guanfacine might not be an ideal treatment for the improvement of negative and cognitive symptom domains, but further studies are required to explore its effects as an add-on treatment with antipsychotic drugs.

Medial preoptic oxytocin receptor-expressing neurons control social behavior in rats

Máté Egyed[1]; Lilla Radvan[1]; Vivien Szendi[1]; Gina Puska[1,2]; Valery Grinevich[3]; Árpád Dobolyi[1]
[1]Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest
[2]University of Veterinary Medicine Budapest, Department of Zoology, Budapest
[3]Central Institute of Mental Health, University of Heidelberg, Department of Neuropeptide Research in Psychiatry, Mannheim

Oxytocin is released in the brain in response to social interactions, acts through oxytocin receptor (OTR) and plays a key role in regulating social behavior in rodents. The medial preoptic area (MPOA), located in the anterior hypothalamus, contains a dense population of OTR-expressing neurons and acts as a central hub for social behavioral control. While the role of the MPOA in reproductive behaviors has been well established, emerging evidence indicates that it also contributes to affiliative social processes and the maintenance of social homeostasis.
In this study, we sought to ascertain the functional contribution of MPOA OTR-expressing neurons to interfemale social behavior using chemogenetic manipulation. We selectively activated and inhibited OTR+ neurons in the MPOA of transgenic female Sprague–Dawley rats expressing Cre recombinase under the OTR promoter. Excitatory or inhibitory DREADD construct was delivered to the MPOA via a Cre-dependent adeno-associated viral vector. Behavioral results on the treatment day were compared with the preceding and subsequent vehicle-injection control days.
The chemogenetic activation of MPOA OTR+ neurons led to significant increase in the frequency and duration of various components of social behavior, including allogrooming, body sniffing, mounting, and chasing. Conversely, there was a decline in moving-away behavior and non-social behaviors. A control group lacking the DREADDs demonstrated behavioral patterns similar to the vehicle days. We subsequently ascertained that DREADDs activation did not influence sociability, social preference, anxiety- and depression-like behaviors. Upon inhibition of MPOA OTR+ neurons, we observed significant decrease in the duration and frequency of body sniffing, mounting, chasing and approaching.
With c-Fos immunohistochemistry we showed that OTR+ neurons in the MPOA are activated by social contact. Using fiber photometry, we proved that these neurons are activated during anogenital sniffing. We established that the anogenital sniffing is a starting point of a behavioral sequence significantly followed by direct contact behaviors.
Finally, we revealed that MPOA OTR+ neurons project to several brain regions, including the periaqueductal grey matter, and the lateral septum, both known to be involved in the control of social behavior. These data suggest that MPOA OTR+ neurons control specific aspects of social interactions between adult female conspecifics.

The effects of fifth-generation mobile phone-like radiofrequency exposure on general well-being and declarative memory of adolescent rats

Nóra Bruszt[1]; Zsóka Mária Ábel[1,2]; Zsolt K. Bali[1,3]; György Thuróczy[4]; Zsuzsanna Vecsei[4]; Philippe Leveque[5]; István Hernádi[1,2,3]
[1]*Szentágothai Research Centre, Translational Neuroscience Research Group, Pécs*
[2]*University of Pécs, Department of Neurobiology, Pécs*
[3]*Grastyan Translational Reserach Center, University of Pécs, Pécs*
[4]*National Center for Public Health and Pharmacy, Budapest*
[5]*Université de Limoges, CNRS, XLIM, Limoges*

Telecommunication has become an essential part of our modern life. Environmental radiofrequency electromagnetic field (RF-EMF) levels produced by emerging telecommunication technologies continue to rise with the widespread use of mobile phones. Consequently, public concern about the potential adverse health effects of especially the 5th generation (5G) RF-EMFs has increased. Research on the potential neurocognitive effects — particularly in children and adolescents — has become a priority, as these groups are considered more vulnerable to RF-EMF-induced impacts than adults.
To investigate the effects of 5G RF-EMF on general well-being and cognitive performance, 48 adolescent (6 to 10-week-old during 5G exposure period) male Lister Hooded rats were used. The rats were divided into 4 groups (n=12) and were continuously exposed to 3500 MHz 5G RF-EMF with a specific absorption rate (SAR) of 0 W/kg (sham), 0.08 W/kg (low-dose), 0.4 W/kg (mid-dose), or 4 W/kg (high-dose) for 1 hour per day for 4 weeks. To assess motor activity and anxiety-like behavior the Open Field (OF) and Elevated-O-Maze (EOM) tasks were used. Two domains of declarative memory were assessed: object (semantic) memory was tested in the Novel Object Recognition (NOR) task, while spatial and navigation (episodic) memory was evaluated using the Morris Water Maze (MWM) task.
Results showed a dose-dependent decrease in activity among the exposed groups in the OF task, however, by the end of the exposure period, the animals' performance returned to normal levels. The EOM task, conducted during the third week of exposure, revealed no significant differences between groups in anxiety-like behavior.
According to the results of the NOR task, only the mid and the high-dose groups did not discriminate between the objects after two weeks of exposure, which remained unchanged in the mid-exposure group till the end of the entire exposure period. Two weeks after the exposure all groups except the mid-dose group showed successfully recovered NOR performance. No significant changes were observed in spatial memory performance in the MWM task.
Our results indicate that 4-week chronic exposure to 3500 MHz 5G EMF induces reduced general motor activity together with recognition memory impairment in a dose-dependent manner, presumably caused by a potential delay in neuronal development in young rats and however, these effects are mainly observed during the exposure period.

Distinct roles of two types of medial prefrontal cortical projection neurons in the social behaviour of rats

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

The medial prefrontal cortex (mPFC) has a role in social behaviour. Its dysfunction has been linked to several neuropsychiatric disorders, including autism spectrum disorder and schizophrenia. Neurons in the mPFC project to multiple subcortical regions, allowing this area to exert broad influence over behaviour. The aim of this study was to map the projection patterns of two distinct populations of mPFC projection neurons and to assess their involvement in social behaviour using chemogenetic techniques.
To investigate neurons projecting to the medial preoptic area (MPOA), a retrograde, Cre-expressing adeno-associated virus (AAV) was injected into the MPOA, followed by a Cre-dependent AAV expressing excitatory or inhibitory DREADDs in the mPFC. These MPOA-projecting mPFC neurons gave collateral projections to several subcortical regions, including the nucleus accumbens, ventral pallidum, lateral septum, several hypothalamic nuclei, and medial amygdala, but did not project to the thalamus. In a separate experiment, AAV expressing DREADDs driven by the calcium/calmodulin-dependent protein kinase II (CaMKII) promoter was injected into mPFC using viral gene transfer. CaMKII-positive neurons projected selectively to thalamic nuclei, including the paratenial, mediodorsal, submedius, ventral reuniens and reticular nuclei, without projecting to other cortical or subcortical areas. Double retrograde tracing revealed that MPOA-projecting neurons were primarily located in layer V of the mPFC, whereas mediodorsal thalamus–projecting neurons were mainly found in layer VI.
Chemogenetic activation of MPOA-projecting mPFC neurons reduced sociability in the three-chamber test but did not affect direct social interactions between freely moving animals. In contrast, activation of CaMKII-expressing mPFC neurons decreased both sociability and the duration of several direct social interactions, while inhibition of these neurons evoked the opposite effects.
In conclusion, these findings suggest that the mPFC exerts an inhibitory influence on social behaviour, with distinct mPFC projection neuron populations contributing differently to this regulatory function.

Isoform-specific and age-dependent behavioural roles of Caskin scaffold proteins

Daniel Kimsanaliev[1]; András Török[1]; Norbert Bencsik[1]; Katalin Schlett[1]
[1]Eötvös Loránd University, Department of Physiology and Neurobiology, Cellular Neurobiology Group, Budapest

Neuronal scaffold proteins are essential for the spatial organisation of signalling complexes at excitatory synapses, thereby linking synaptic architecture to circuit-level function and behaviour. Members of the Caskin family, Caskin1 and Caskin2, interact with core postsynaptic density (PSD) proteins like Shank2. Caskin1 has been implicated in synaptic and behavioural functions, whereas the structurally similar Caskin2 isoform's potential role remains poorly understood. Previous studies have reported spatial learning and memory deficits following deletion of both Caskin isoforms, as well as behavioural alterations in Caskin1-deficient mice. However the isoform-specific behavioural contributions of Caskins and their potential functional interactions remain unclear.
To address this, we systematically examined behavioural phenotypes of Caskin1 knockout (KO), Caskin2 KO, and Caskin1/2 double knockout (dKO) mice at 2, 4, and 6 months of age. Animals were tested for anxiety-like behaviour, social interaction, repetitive behaviour, and hippocampal-dependent spatial learning, with wild-type C57BL6/J and double heterozygous (dHz) littermates serving as control.
Caskin deficiency resulted in isoform-specific and age-dependent alterations in behavioural measures. Anxiety-like behaviour and sociability remained largely intact across all genotypes, indicating preserved emotional reactivity and social behaviour. Repetitive behaviour showed genotype- and age-dependent changes. Older Caskin1 KO and dKO mice displayed reduced grooming duration and shorter grooming bouts, accompanied by increased marble-burying activity. Spatial learning was likewise affected in a genotype-dependent manner. Caskin1 KO and dKO mice exhibited spatial learning deficits in the Morris water maze, with impairments being most pronounced in dKO animals, while Caskin2 KO mice performed similarly to controls, suggesting partial functional compensation between isoforms.
Together, these findings demonstrate that Caskin scaffold proteins exert isoform-specific and age-dependent effects on behaviour. Repetitive behaviours are selectively altered with age, whereas spatial learning critically depends on Caskin1, with limited functional compensation by Caskin2. These results underscore the importance of synaptic scaffold integrity in supporting hippocampal-dependent learning and the proper regulation of repetitive behavioural output.

Chemogenetic and pup-induced activation of a thalamo-preoptic pathway suggests its maternal function

Lilla Radvan[1]; Vivien Szendi[1]; Tamás Láng[2]; Máté Egyed[1]; Gina Puska[1,3]; Árpád Dobolyi[1,2]
[1]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*
[2]*Semmelweis University, Department of Anatomy, Histology and Embryology, Laboratory of Neuromorphology, Budapest*
[3]*University of Veterinary Medicine Budapest, Department of Zoology, Budapest*

The medial preoptic area (MPOA) is a hypothalamic brain region known to be the major regulatory site of maternal behaviour. However, its somatosensory inputs and regulatory cells are not well established. The neurons of the posterior intralaminar thalamic nucleus (PIL) in the lateral thalamus, send projections to the MPOA, and have been shown to receive somatosensory inputs during suckling. In this study, we aimed to investigate the PIL-MPOA pathway in the aspect of maternal care. We confirmed the involvement of the PIL-MPOA pathway in maternal regulation using a retrograde tracing technique as most of the neurons projecting from the PIL to the MPOA were activated, and their majority expressed the neuropeptide parathyroid hormone 2 (PTH2). We also confirmed the functional importance of PIL neurons in maternal behaviour through chemogenetic manipulation. Following the activation of an excitatory designer receptor expressed in the PIL by injection of a viral vector, the time rat dams spent with their pups, grooming and licking them, as well as the duration of nest building increased significantly. Next, we investigated the extent of neuronal activation within the PIL in rat dams by using the c-Fos technique. Three groups were examined followed by pup separation: mother rats either received their pups back with or without physical touch, or did not receive their pups back at all. Using triple immunolabelling, we measured the amount of cells that expressed PTH2, calbindin (Cb+), and c-Fos, to investigate the distribution of activated cell types. An elevated number of double-labelled c-Fos-activated Cb+ neurons were found in the presence of pups not only in the medial part of the PIL, where PTH2+ cells are located, but in the lateral part, too. The activation of these neurons was the highest when mothers could freely interact with their pups. Moreover, we found that nearly all PTH2+ neurons were also Cb+ in the PIL, and were activated to unrestrained pup exposure following separation. However, triple-labelled neurons were almost completely absent in the absence of pups, and greatly reduced when direct touch with the pups was not allowed. These findings imply that PTH2-expressing neurons in the PIL relay pup-related somatosensory touch information via their projection to the MPOA in rat dams.

Lateral thalamic input to the dorsomedial hypothalamic nucleus as a possible regulatory pathway of enhanced food intake in rodent mothers

Bereniké Dorka Kovács[1,2]; Vivien Szendi[2]; Lilla Radvan[2]; Gina Puska[1,2]
[1]*University of Veterinary Medicine Budapest, Department of Zoology, Budapest*
[2]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*

The dorsomedial hypothalamic nucleus (DMH) plays a crucial role in the termination of food intake following satiation. During pregnancy and lactation, hyperphagia can be observed in mother animals as they must provide energy sources for the developing offspring in the form of lactation. Despite previous efforts to reveal the background of these changes, the neural regulatory pathways and the participating cell types have not been established yet. In the present study, we focused on the DMH as a potential regulatory centre of maternal food intake. First, we mapped the inhibitory and excitatory inputs of the DMH with retrograde tracing in VGAT-ZsGreen mice using cholera toxin beta (CTB) subunit. We explored numerous brain areas that contribute to the afferent pathways of the DMH, among which we found regions that take part in maternal adaptation of the brain, such as the medial preoptic area (MPOA) in the hypothalamus, and the posterior intralaminar thalamic nucleus (PIL) in the lateral thalamus. We showed that the PIL provides excitatory input to the DMH. This thalamic nucleus expressing a maternally induced neuropeptide, parathyroid hormone 2 (PTH2) in its glutamatergic projection neurons, has been shown to transmit suckling-related sensory cues towards forebrain centres. To address the possible function of the DMH-projecting PIL neurons in the feeding behaviour of mothers, CTB was injected into the DMH of female mice, then the animals were mated and perfused on the 9th day after parturition. By using c-Fos immunolabeling, we observed that the majority of the DMH-projecting PIL neurons were activated in mothers. Next, we investigated the possible target cells of this pathway. We revealed that PTH2-containing fiber terminals closely surround GABAergic and calbindin-positive neurons in the DMH. Fiber photometry in VGAT-Cre mice revealed that GABAergic cells are activated during food intake. To gain more information about the activating cell types, we used the c-Fos technique combined with calbindin staining in female mice after 16-hour starvation and subsequent refeeding. We showed that a high number of DMH GABAergic cells express calbindin, and calbindin neurons show c-Fos positivity in response to refeeding. Our findings suggest that the PIL-DMH pathway may affect food intake in mother mice through PTH2-containing projections to DMH calbindin neurons.

AI-based analysis of social behaviour in rodents: development and validation of an automated workflow using DeepLabCut and Emerenka

Vivien Szendi[1]; Bereniké Dorka Kovács[2,1]; Szilvia Bartók[1]; Attila Bartók[3]; Tamara Hajdu[4]; Tamás Láng[4]; Gina Puska[2,1]; Árpád Dobolyi[1]
[1]Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest
[2]University of Veterinary Medicine Budapest, Department of Zoology, Budapest
[3]Eyenet Ltd, Atkár
[4]Semmelweis University, Department of Anatomy, Histology and Embryology, Laboratory of Neuromorphology, Budapest

Social behaviour plays a fundamental role in the survival and adaptation of many vertebrate species and is a key focus in neuroscience and neuropsychiatric research. Understanding the neural basis of social interactions requires accurate and objective behavioural analysis. However, traditional methods often rely on manual scoring, which is time-consuming and subject to bias.
To address these limitations, we developed and validated a reliable and efficient AI-based methodology for analysing direct social interactions in rodents. Our workflow integrates DeepLabCut (DLC), an open-source pose estimation deep learning software that tracks user-defined body parts, and Emerenka, a custom-developed software tool for extracting behavioural elements from the DLC output matrix. Emerenka identifies specific behaviours based on the spatial relationships between the animals' body parts, using a set of predefined criteria, allowing the identification of a high number of behavioural elements. The software evaluates each frame sequentially, assigns the most fitting behavioural category, and outputs both frame-by-frame annotations and summary statistics. Its adjustable parameters allow users to refine detection rules and adapt the system to additional behavioural tests.
The method was validated using test videos made with rats and mice. In both cases, previously fur-coloured animals underwent a 10-minute social interaction test with a familiar conspecifics in an open field apparatus while being video-recorded. DLC was trained on selected frames from recordings before all videos were analysed. Emerenka parameters were iteratively adjusted to optimise the accuracy of the detection of 11 behavioural elements. Automated and manual scoring matched very well from frame to frame. Consequently, Comparison of the time spent with each behavioural elements revealed no significant differences, confirming the reliability of the automated method.
The newly developed automated pipeline provides a fast, objective, and user-friendly solution for analysing complex social behaviours in rodents, contributing to more efficient workflows in behavioural neuroscience. Importantly, our approach enabled the differentiation of multiple interaction types surpassing previous automated methods that identified only a few categories.

IntelliCage assessment reveals cognitive deficits intensified by ovariectomy in 3xTg-AD mice

Tamara Kállai[1]; Melinda Cservenák[1]; Luca Darai[1]; Dóra Zelena[2]; László Détári[3]; Árpád Dobolyi[1]
[1]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*
[2]*Medical School, University of Pécs, Institute of Physiology, Pécs*
[3]*Eötvös Loránd University, Department of Physiology and Neurobiology, Budapest*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that occurs about twice more frequently in women, especially post-menopausal women than in men. The 3xTg-AD mouse line is a widely used model that develops β-amyloid (Aβ) plaques and hyperphosphorylated tau tangles. Sex-related hormones with neuroprotective effects, such as estrogen, can reduce Aβ levels and tau hyperphosphorylation. Besides Aβ and tau pathology, alterations in cholinergic neurons and calcium buffering mechanisms also contribute to AD.
In this study, we investigated cognitive changes induced by ovariectomy (OVX) in wild-type (WT) and 3xTg-AD female mice using the IntelliCage system, an automatized apparatus for long-term behavioural monitoring. Thirty-two female mice were divided into four groups: WT-SHAM, WT-OVX, 3xTg-SHAM, and 3xTg-OVX. Behavioral tests included adaptation, impulsivity, and fixed ratio tasks. For histological assessment, choline acetyltransferase (ChAT) was used to label cholinergic neurons in relation to calbindin (CB), a neuronal calcium-binding protein.
During free adaptation, 3xTg-AD mice adapted more slowly to the new environment. In the impulsivity test, increasing delay significantly impaired the performance of 3xTg-OVX mice. Performance in the fixed ratio task was significantly impaired in 3xTg-AD mice, with the strongest deficit observed in 3xTg-OVX animals.
Immunohistochemical analyses of the medial septum and the horizontal limb of the diagonal band of Broca (HDB) showed no differences in ChAT or CB labeling among WT, 3xTg-SHAM, and 3xTg-OVX groups. Amyloid labeling confirmed that 3xTg-AD mice developed Aβ plaques, validating the AD model.
Overall, we showed that 3xTg mice exhibit normal place learning but impaired contextual adaptation while the structure of their cholinergic system remains intact. The results also indicate that OVX, a model of postmenopausal, further worsens cognitive performance in 3xTg-AD mice, supporting the protective role of sex-related hormones in cognition.

Screening social behaviour in non-human primates within an open field environment: A novel AI-based technical approach for preclinical research in behavioural pharmacology.

Evelin Kiefer[1,2]; Balázs Knakker[1,2]; István Hernádi[1,2,3]
[1]*University of Pécs, Grastyán E. Translational Research Centre, Pécs*
[2]*University of Pécs, Centre for Neuroscience, Pécs*
[3]*University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs*

Non-human primates (NHPs) are essential in translational pre-clinical research, especially for modelling complex cognitive functions in healthy and diseased states. While computerized tasks have been successfully employed with NHP neurocognitive research, recent technological advancements permit the design of novel paradigms that are based on accurate quantification of the behaviour of freely moving animals. Here, we specifically aim to directly translate an arena-based rodent behavioural assay - the open field test (OF) - to non-human primates.
Open field test (OF) is a rodent test used to measure general locomotor activity, anxiety and willingness to explore. In this task, rodents are placed in a large test apparatus and their spontaneous behaviour is observed via a camera while they may explore the arena in a simple OF test, however it is not a methodology widely used with non-human primates. First our aim was to create a similar setting with NHPs where their activity could be observed undisturbed in a familiar environment, on further analysis we aimed at quantifying social behavioural in a dyadic social environment by placing two familiar animals in the arena and observing their affiliative behaviours, like grooming, physical contact, playful behaviour and proximity.
Six young (age 4-5 y) adult macaque monkeys (Macaca fascicularis) were grouped in pairs and were placed in a large familiar environment (approx. 3,3mx1,85mx3m arena) for 40 minutes for 5 consecutive days. The arena was equipped with a climbing tree, and a resting area. The animals' behaviour was observed for 4x10 min using 4 digital cameras. Body marker points were defined and tracked offline, followed by the estimation of the animals' 3D position using DeepLabCut. As a next step, the animals' spontaneous behaviour was perturbed with pharmacological agents in the open field arena.
Preliminary results suggest that the animals' social behaviour can be reliably measured by analysing the 3D pattern of their movement trajectories and that this activity pattern can be altered via different challenges, validating the test for later use in preclinical drug development for psychiatric diseases.

Assessment of motivational anhedonia in rats for preclinical psychiatric drug evaluation

Zsófia Rita Hernádi[1,2,3]; Eszter Bálint[2]; Viktor Román[2]; Erika Pintér[3]; István Hernádi[1]
[1]*University of Pécs, Szentágothai Research Center, Translational Neuroscience Research Group, Pécs*
[2]*Gedeon Richter Plc., Department of Pharmacology and Drug Safety Research, Budapest*
[3]*University of Pécs, Department of Pharmacology and Pharmacotherapy, Pécs*

Psychiatric conditions such as depression and schizophrenia are frequently characterized by negative symptoms, including anhedonia and diminished motivation which still pose an unmet medical need. In light of the limited predictive validity of preclinical rodent behavioral assays, a modified version of the Effort Expenditure for Reward Task (EEfRT), a translational paradigm, was developed and validated. The EEfRT aims at assessing motivation and effort-based decision-making, where subjects choose between a high-effort/high-reward (HEHR) option and a low-effort/low-reward (LELR) alternative, enabling the quantification of motivational states and the evaluation of pharmacological manipulations. Young adult, food-restricted Lister Hooded rats (N = 30) were initially trained to lever-press for palatable reward pellets under a fixed-ratio 3 (FR3) operant schedule. Then, animals were presented with a choice between exerting effort to obtain reward pellets (HEHR) or consuming standard laboratory chow placed adjacent to the feeder unit (LELR). To validate our EEfRT task we clustered the animals into two groups - high preference (HP) and low preference (LP) - based on their baseline performance. In the HP group, pharmacological anhedonia was successfully induced with dopamine-depleting agent tetrabenazine and then reversed with dopamine-increasing agent bupropion. In the LP group, natural anhedonia was reversed with bupropion alone. Subsequently, we cross-validated the EEfRT paradigm using the Elevated O-Maze (EOM) task to examine potential associations between reward preference and anxiety-related behavior. Lastly, we investigated the impact of various antidiabetic medication GLP-1 agonists on choice performance in the validated EEfRT task. GLP-1 receptor agonists consistently reduced food intake, confirming their expected appetite-suppressing effects. Exenatide decreased the effort animals were willing to exert for food rewards, while liraglutide and semaglutide did not alter effort expenditure, indicating distinct effects among GLP-1 agonists on motivation. We successfully established and pharmacologically validated our rat EEfRT paradigm. Cross-validation revealed no significant correlation between task performance and anxiety traits. Findings highlight that GLP-1 agonists differ in their impact on reward-related behaviors, beyond appetite suppression. Further studies are needed to examine whether these agents differentially modulate general affective states.

Contrasting foraging and reinforcement learning computations in the prefrontal cortex of macaques

Zsombor Ungvarszki [1, 2], Clement Goussi-Denjean[2], Anna Szekely [1],Gergo Orban[1], Matteo di Volo[2], Emmanuel Procky[2] [1]*Computational Systems Neuroscience Lab, Wigner Research Center for Physics, Budapest, Hungary, Budapest, Hungary* [2]*Univ Lyon, Université Lyon 1, Inserm, Stem Cell and Brain Research Institute U1208, Bron, France, Lyon, France*

Flexible behavior relies on computations that weigh alternatives, integrate new evidence, commit to options, and abandon courses of action when evidence no longer supports them. These processes are typically studied using economic choice tasks, in which subjects make discrete choices between simultaneously presented options. The dominant framework for explaining such behavior is reinforcement learning (RL), which assumes that decisions are made by comparing the values of available alternatives. By contrast, ethology emphasizes more naturalistic behaviors such as foraging, where the forager encounters reward options sequentially and decides when to leave an option to search for alternatives. Although this foraging account is gaining popularity in neuroscience, it remains unclear to what extent it can explain decisions in classical economic choice tasks. Because the prefrontal cortex (PFC) is key to flexible behavior—it computes value estimates and translates them into action policies—we address this question at the level of neural computation by testing whether PFC dynamics reflect RL-like compare-alternatives or foraging-like stay-or-leave computations. We recorded 5,000 neurons across 113 sessions simultaneously from the midcingulate cortex (MCC) and the lateral prefrontal cortex (LPFC) in macaques performing a multi-armed bandit task, a classic economic choice paradigm. Using behavioral modeling, we inferred trialwise value signals and decision policies under both frameworks. However, despite the fundamentally different underlying algorithms (distinct value definitions and control policies), behavior alone was insufficient to dissociate them. In contrast, population activity revealed a clear dissociation. We identified a simple linear neural subspace in MCC that maintained a value signal and updated it by integrating recent outcomes into this signal. This low-dimensional representation showed that neural dynamics encoded the value of staying with the current strategy versus switching—consistent with foraging-style computations and inconsistent with RL-style action-value requirements. We propose a mechanistic account in which MCC tracks value and compares it to a switching threshold to determine when abandoning the current strategy is beneficial, while recruiting LPFC to implement the strategy indicated by MCC. Our results provide a computationally efficient, ecologically motivated neural account of stay-or-leave decisions in the primate frontal cortex.

Dual effects of antidepressant fluoxetine treatment on central corticotropin-releasing hormone neurons

András Varga[1]; Bence Pytel[1]; Zsombor Márton[1]; Viktória Kormos[2]; Balázs Gaszner[1]; László Ákos Kovács[1]
[1]*University of Pécs, Medical School and Centre for Neuroscience, Research Group for Mood Disorders,, Pécs*
[2]*University of Pécs, Department of Pharmacology and Pharmacotherapy, Pécs*

Introduction: An estimated 5.7% of adults globally suffer from depression. Major depressive disorder (MD) causes the second-greatest number of years lived with disability.
One common factor leading to MD is stress. Stress is controlled by the hypothalamic-pituitary adrenal (HPA) axis, where corticotropin-releasing hormone (CRH)-secreting cells in the hypothalamic paraventricular nucleus (PVN) play a crucial role in the glucocorticoid response. Disturbance in glucocorticoid feedback or hyperactivity of the HPA axis is a common observation in patients with MD. Standard antidepressant therapy (fluoxetine-a serotonin reuptake inhibitor) usually decreases the activity of the HPA axis, but its effectiveness is less than 40%.
We hypothesized that fluoxetine treatment modifies the neuropeptide-ergic system of the HPA axis, which may cause a limited response.
Methods: Male Wistar rats were divided into four groups: control, chronic variable mild stress (CVMS) group, in which animals were exposed to three weeks of stress, CVMS group treated with fluoxetine (CVMS-FLUO) for three weeks. We monitored body weight loss and the animals' sucrose preference during the study. At the end of the experiment, blood samples were collected, and adrenal gland weights were measured. We performed CRH-FOSB/ΔFOSB immunohistochemistry on coronal sections of the PVN and central nucleus of the amygdala (CeA). In the PVN, we performed *crh* detection using RNAscope technique.
Results: Serum corticosterone (CORT) levels were elevated in CVMS animals. Adrenal gland weights were higher in both stress-exposed groups. The sucrose preference in CVMS animals was less than that in the control groups. The number of CRH-positive neurons increased in fluoxetine-treated animals, with a concomitant increase in FOSB. C*rh* mRNA was decreased in fluoxetine treated animals. CRH immunoreactivity was decreased in the CeA in both the CVMS and CVMS-FLUO groups.
Discussion: The CVMS paradigm was successful, and according to the results, the therapy was effective, while it had the opposite effect on CRH neuron subpopulations. In the PVN, the fluoxetine may have induced the accumulation of CRH, with the possibility of decreased release (according to the *crh* data). In the CeA, this CRH immunoreactivity was decreased. The model can be used for further investigation of the neurobiology of neuropeptides in the context of MD and its therapy. Additional studies are needed to explore the effects of antidepressant therapy.

Investigation of pituitary adenylate cyclase-activating polypeptide (PACAP) in patients undergoing transcatheter aortic valve implantation (TAVI)

Kata Anna Toller[1]; Balázs Magyari[2]; Bálint Kittka[2]; Tünde Tóth[1]; Dóra Reglődi[1]; Beata Polgár[3]; Andrea Tamás[1]
[1]*University of Pécs, Medical School, Department of Anatomy, Pécs*
[2]*University of Pécs, Medical School, Heart Institute, Pécs*
[3]*University of Pécs, Medical School, Institute of Medical Microbiology and Immunology, Pécs*

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide with well-known cardiac and cardioprotective effects. Our research group previously demonstrated that plasma PACAP levels change significantly in various cardiovascular conditions, and during pulmonary vein isolation procedures we detected higher PACAP levels in the atria compared with those measured in peripheral blood vessels.

In our current study, we collected blood samples from patients undergoing transcatheter aortic valve implantation from the jugular vein (n = 20), the aorta (n = 15), and the left ventricle (n = 15). We defined four sampling times: first, before the artificial valve was inserted; then immediately after rapid cardiac stimulation and valve insertion; next, at the end of the procedure, when samples were taken from all three sites; and finally, on the day after the procedure, when samples were taken from the jugular vein. Endogenous PACAP levels were determined in 5 ml anticoagulated blood samples using the ELISA method.

During the analysis of plasma samples, the lowest PACAP levels were measured in the jugular vein, whereas significantly higher levels were observed in samples from the aorta and left ventricle. Immediately after rapid stimulation, we measured significantly higher PACAP levels in the jugular vein than before the procedure, which returned to the original level on the day after the procedure. We also measured significantly higher PACAP levels in the aorta at the end of the procedure compared with pre-procedure levels.

Similar to our previous studies, the higher PACAP levels measured in the left ventricle and aorta confirmed that locally, a greater amount of PACAP can be detected in the heart, presumably originating from myocardial cells and/or neural elements. The elevated PACAP levels measured in the jugular vein during the intervention may indicate central nervous system release, which could be a consequence of the acute intervention, but further studies are necessary to elucidate the exact mechanism of action.

Cell-cell communication in the human arcuate nucleus based on single cell sequencing data

Fanni Dóra[1,2]; Teadora Tyler[3,2]; Éva Renner[1]; Alán Alpár[1]; Miklós Palkovits[1]; Árpád Dobolyi[3,2]
[1]*Semmelweis University, Human Brain Tissue Bank, Budapest*
[2]*Semmelweis University, Department of Anatomy, Histology and Embryology, Laboratory of Neuromorphology, Budapest*
[3]*Eötvös Loránd University, Department of Physiology and Neurobiology, Laboratory of Molecular and Systems Neurobiology, Budapest*

The arcuate nucleus (ARC) of the hypothalamus is a key center coordinating metabolic, endocrine, and reproductive functions, yet its neuronal diversity and signaling architecture are incompletely defined. We performed single-nucleus RNA sequencing on microdissected ARC tissue from eight neurologically healthy adult donors, yielding 8,832 filtered nuclei representing all major ARC cell classes. Integration with a curated human hypothalamus reference, restricted to the arcuate manifold via scArches, identified 916 neurons with validated ARC identity. Reclustering resolved 31 transcriptionally distinct neuronal subtypes. Rank-weighted marker overlap showed that 30 clusters corresponded to human ARC groups previously identified, while one rare OTX2-high population represented a molecularly distinct, previously unrecognized subtype. Higher-order analysis revealed two mesoscale transcriptional modules. Neurotransmitter mapping indicated a predominantly GABAergic architecture, with additional glutamatergic, mixed, cholinergic, and dopaminergic groups. To characterize intra-arcuate ligand-receptor signaling, we quantified neuropeptide co-detection within transcriptionally defined clusters, delineating cluster-specific peptide repertoires that represent supported ligand pools. In parallel, CellChat applied to the full secreted signaling repertoire decomposed the arcuate connectome into four outgoing communication patterns derived from ligand-receptor topology. Overlaying the independently defined peptide repertoires onto these network-derived communication patterns revealed reproducible peptide enrichments within each pattern. The first pattern enriched in CCK, SST, PACAP, and NPY corresponded to clusters with broad peptidergic output, a second pattern showed preferential association with NPPC, NKB, and ENK. A third was linked to clusters expressing GHRH, GAL, TRH, NTS and POMC, and a fourth involved mixed POMC-, PACAP- and KISS1-positive groups. These peptide-pattern associations do not define the patterns themselves but provide molecular profiles for communication states that were identified independently of peptide expression. The data provide a high-resolution, regionally validated molecular and signaling atlas of the human arcuate nucleus. They also outline how secreted signaling and neuropeptide specialization organize ARC circuitry and highlight avenues for targeting endocrine and metabolic disorders.

Fasting activates proglucagon-producing neurons of the posterior hypothalamic area

István Várkonyi[1]; Csaba Fekete[1]; Zsolt Liposits[2]; Gábor Wittmann[1]
[1]*HUN-REN Institute of Experimental Medicine, Laboratory of Integrative Neuroendocrinology, Budapest*
[2]*HUN-Ren Institute of Experimental Medicine, Laboratory of Endocrine Neurobiology, Budapest*

Introduction
Glucagon-like peptide-1 (GLP-1) analogues are widely used to treat type 2 diabetes and obesity. Endogenous GLP-1 is a hormone released from the gut, but it also serves as a peptide neurotransmitter in the brain. GLP-1 is synthesized from its precursor, proglucagon, encoded by the glucagon gene (*Gcg*). Classic GLP-1 neurons are localized in the medulla oblongata, primarily in the nucleus of the solitary tract. In rodents, these neurons are activated in response to feeding, and GLP-1 release from these cells inhibits food intake. We recently identified another proglucagon and GLP-1-producing neuron population in the mouse brain, in the posterior hypothalamic area (PH). In these cells, however, *Gcg* mRNA, proglucagon protein and GLP-1 syntheses are markedly increased during fasting.
Objective
We tested the hypothesis that proglucagon neurons in the PH are regulated oppositely to medullary GLP-1 neurons, by examining the expression of the cellular activation marker, c-fos, in these cells in *ad libitum* fed, fasted, and refed mice.
Methods
We used adult female *Gcg*-Cre;tdTomato mice, in which *Gcg* neurons are easily identified by tdTomato fluorescence. Three experimental groups were compared (n=4 mice in each group): *ad libitum* fed mice, mice fasted for 30 hours, and mice fasted for 30 hours and then allowed to feed unrestricted for 3h (refed mice). The c-fos protein was detected by immunofluorescence, and c-fos positive tdTomato neurons were counted. The percentage of c-fos positive tdTomato neurons were compared between groups with one-way ANOVA and Tukey's multiple comparison test.
Results
In *ad libitum* fed control mice, 30.3 ± 10.4 % of the tdTomato neurons contained c-fos. In fasted mice, 85.3 ± 4.8 % of the tdTomato neurons were c-fos positive. Three hours after the refeeding started, the ratio of c-fos positive tdTomato neurons returned to control levels, 21.3 ± 4.6 %. Fasted mice had significantly more c-fos containing tdTomato neurons than *ad lib* fed ($p=0.001$) or refed mice ($p=0.0003$), while there was no statistically significant difference between fed and refed mice ($p=0.66$).
Conclusions
Food deprivation robustly activates proglucagon neurons in the PH, indicating that feeding status oppositely regulates the activity of these cells compared to medullary GLP-1 neurons.

Silencing Nesfatin-1/NUCB2 expression in the supraoptic nucleus modulates renal function

Klaudia Sípos[1]; Miklós Geiszt[2]; Zsuzsanna E. Tóth[1]
[1]*Semmelweis University, Department of Anatomy, Histology, and Embryology, Laboratory of Neuroendocrinology and In Situ Hybridization, Budapest*
[2]*Semmelweis University, Department of Physiology, Budapest*

An increase in blood osmolality triggers the axonal release of arginine vasopressin (AVP) from neurons in the supraoptic nucleus (SON) into the systemic circulation through the pituitary gland. AVP then increases water reabsorption in the kidneys by enhancing the expression and activity of aquaporin-2 (AQP2) water channel proteins, thereby restoring the osmotic balance. The release of AVP from the axons is tightly controlled within SON. Dendritic release of different neurotransmitters, such as AVP and oxytocin is one of the key elements of the regulatory mechanism. Nesfatin-1, the biologically active fragment of the NUCB2 prohormone, is known to act by dendritic release, and has previously been implicated in the regulation of fluid homeostasis. Given the strong coexpression of nesfatin-1 and AVP, we hypothesized that nesfatin-1 may modulate the activity of AVP-producing neurons and influence kidney function.
To examine this, we silenced Nesfatin-1/NUCB2 expression in the SON of male rats by adeno-associated virus (AAV) mediated delivery of small hairpin RNA (shNUCB2) into the nucleus. Controls received AAVs expressing scrambled shRNA (shSCR). Four weeks after the virus injections, the effects of water deprivation (24 hours) and subsequent rehydration on the osmolality of urine and plasma were determined. Plasma copeptin (a molecule released in equimolar amounts with AVP) concentrations were quantified by ELISA. Nesfatin-1 and AVP expression in the SON and phosphoAQP2 (the activated form of AQP2) expression in the kidney were assessed by immunohistochemistry.
NUCB2 deficiency in the SON resulted in inadequate release of AVP, which adversely affected phAQP2 channel expression in the kidneys in response to WD and rehydration. Plasma and urine osmolality measurements revealed a disturbance in osmoregulatory function in SON-NUCB2-deficient rats. Our results suggest that NUCB2/nesfatin-1 in the SON plays an important role in the regulation osmotic balance and kidney function.

Novel proglucagon (Gcg)-expressing neuron populations in the mouse brain include fasting-responsive GLP-1-producing neurons in the posterior hypothalamic area

Gábor Wittmann[1]; Andrea Kádár[1]; István Várkonyi[1]; Csaba Fekete[1]
[1]*HUN-REN Institute of Experimental Medicine, Laboratory of Integrative Neuroendocrinology, Budapest*

Objective
Glucagon-like peptide-1 (GLP-1), a peptide neurotransmitter in the brain, is synthesized from proglucagon, the protein product of the glucagon gene (*Gcg*). Besides medullary GLP-1-producing neurons that regulate feeding behavior, *Gcg* expression was confirmed only in the olfactory bulb and basolateral amygdala. However, several lines of evidence suggest that additional *Gcg*-expressing neuron populations might exist.
Methods
We conducted a brain-wide fluorescent *in situ* hybridization (FISH) study to identify *Gcg* mRNA-expressing neurons in male and female C57Bl6/J mice, and male FVB/Ant mice. Proglucagon and GLP-1 expression was studied with immunofluorescence using monoclonal antibodies directed against the midportion or the amidated C-terminus of GLP-1, respectively. To identify proglucagon-producing neuron populations involved in the regulation of energy homeostasis, we examined the effect of 30h fasting on *Gcg* mRNA and proglucagon/GLP-1 expression.
Results
Besides medullary GLP-1 neurons, we identified *Gcg*-expressing neuron populations in the olfactory bulb, claustrum, piriform cortex, basolateral amygdala, posterior hippocampus, posterior hypothalamic area, periaqueductal grey/dorsal raphe, and dorsal nucleus of the lateral lemniscus. These neurons generally express low *Gcg* mRNA levels, and a subset contains detectable levels of proglucagon protein and GLP-1. Fasting markedly upregulated *Gcg* mRNA, proglucagon protein and GLP-1 expression in the posterior hypothalamic area. We also demonstrated by FISH that posterior hypothalamic *Gcg* neurons expressed the mRNA of prohormone convertase 1 (*Pcsk1*), the enzyme that converts proglucagon to GLP-1.
Conclusions
The brain proglucagon/GLP-1 system is more complex than previously understood. Our results demonstrate that at least 9 *Gcg* neuron populations exist in the brain. Fasting robustly regulates *Gcg* mRNA and GLP-1-production in the posterior hypothalamic area, suggesting that this novel *Gcg* neuron population is involved in the regulation of energy homeostasis and feeding behavior.

Characterization of the energy balance and glucose homeostasis of nesfatin-1 knockout mice

Katalin Könczöl[1]; Rege Sugárka Papp[1]; Klaudia Sípos[1]; Zsuzsanna E. Tóth[1]
[1]*Semmelweis University, Department of Anatomy, Histology, and Embryology, Laboratory of Neuroendocrinology and In Situ Hybridization, Budapest*

Nesfatin-1 is the N-terminal fragment of the NUCB2 prohormone that was described as an anorexigenic peptide in 2006. It is strongly expressed in hypothalamic nuclei related to the regulation of the energy balance and glucose homeostasis. We have previously revealed that chronic intracerebroventricular administration of nesfatin-1 improves glucose tolerance and insulin sensitivity in rats. Therefore, we have generated a nesfatin-1 knockout (KO) mouse line to investigate the exact role of nesfatin-1 in the central regulation of energy balance and glucose homeostasis.
We measured the bodyweight (BW) and the FI of adult male KO, heterozygote (HZ) and wild type (WT) mice on a weekly basis for a period of three weeks. To assess glucose homeostasis in mice, we performed intraperitoneal glucose, insulin, and pyruvate tolerance tests on weeks 4-5. In a separate experiment, the animals were fasted for 24 hours and then allowed to eat for 2 hours. The BW changes after fasting, the consumed food during the refeeding, and the neuronal activity (c-Fos) in the hypothalamus after refeeding were determined.
The BW and FI of mice did not differ between genotypes under normal conditions. The tolerance tests indicated that the glucose homeostasis of the KO animals was superior to that of the other groups. Furthermore, KO mice exhibited a reduced rate of weight loss during fasting and consumed greater amounts of food during the refeeding period when compared to the other groups. Neurons activated by fasting were counted in the FI related hypothalamic nuclei, like the paraventricular, supraoptic and arcuate nuclei. A significant difference between groups was detected in the arcuate nucleus only, where reduced number of cells was activated in KO mice relative to HZ and WT animals.
Our data suggest a complex role of nesfatin-1/NUCB2 in the regluation of the energy balance and glucose homeostasis.

A Systematic Review and Meta-analysis of Metabolic Biomarker Alterations in Preclinical Post-Traumatic Stress Disorder Models

Prabhat Kumar[1]; Imola Plangár[1]; Arie Arizandi Kurnianto[2]; Dóra Zelena[1]
[1]*Institute of Physiology, Medical School, Centre for Neuroscience, Szentágothai Research Centre, University of Pécs, Pécs*
[2]*Centre for Health Technology Assessment and Pharmacoeconomic Research, Faculty of Pharmacy, University of Pécs, Pécs*

Background: Post-traumatic stress disorder (PTSD) is increasingly recognized as a systemic disorder with prominent metabolic alterations. Preclinical rodent models offer essential mechanistic insights; however, metabolic biomarker findings are still disjointed across various paradigms and tissues. We conducted a systematic review and planned meta-analysis to synthesize evidence on metabolic biomarker alterations in rodent models of PTSD, following a prospectively defined PROSPERO protocol. **Methods:** Included a thorough search of Ovid/MEDLINE, Embase, Scopus, and Web of Science from their inception to 1st October 2025. Eligible studies involved in vivo controlled trials in mice or rats exposed to various PTSD paradigms, such as fear conditioning and chronic unpredictable stress. Outcomes assessed were metabolic biomarkers related to energy and lipid metabolism, stress-axis activity, neuroinflammation, oxidative stress, and neurotransmitter metabolites. Data was independently extracted by two reviewers who also evaluated bias risk with SYRCLE and study quality per CAMARADES criteria. Suitable studies underwent random-effects meta-analyses using standardized mean differences, along with subgroup and sensitivity analyses. **Results**: Many eligible studies have been found in a variety of PTSD paradigms and species. Initial synthesis reveals convergent trauma-related modifications in stress hormones (particularly corticosterone), glucose regulation, lipid profiles, and inflammatory mediators, although effect sizes differ across models, tissues, and post-trauma timing. A lot of different methods are used, which supports the use of random-effects models and structured subgroup analyses. **Conclusions:** The study elucidates reproducible metabolic signatures of trauma exposure by synthesizing results across various paradigms and biomarker domains, while also identifying gaps that hinder translational relevance. The final meta-analytic results will guide subsequent mechanistic investigations and the formulation of metabolism-focused therapeutic approaches for PTSD.

Keywords: Post-traumatic stress disorder; Mice; Rats; Biomarkers; Fear conditioning; Single Prolonged Stress; Metabolic biomarkers; Corticosterone; Glucose

Alterations in urocortin-1 and corticotropin releasing hormone systems in the rotenone model of Parkinson's disease and their response to levodopa/benserazide and fluoxetine treatments

Bence Pytel[1]; János Kocsa[1]; Zsombor Márton[1]; Bernadett Kovács[1]; József Farkas[1]; László Á. Kovács[1]; Nóra Füredi[1]; Viktória Kormos[2]; Balázs Gaszner[1]
[1]*University of Pécs Medical School, Department of Anatomy, Research Group for Mood Disorders, Pécs*
[2]*University of Pécs Medical School, Department of Pharmacology and Pharmacotherapy, Pécs*

Parkinson's disease (PD) is a neurodegenerative disorder with motor (tremor, rigor, hypokinesia) and non-motor (e.g. depression, anxiety) symptoms. We have previously found a correlation between damage to urocortin-1 (UCN1)-containing cells of the centrally projecting Edinger-Westphal nucleus (EWcp) and mood disorders in the rotenone model of PD, in the rat. An inverse correlation between corticotropin-releasing hormone (CRH) and UCN1 expression levels has been previously found, which also raises the question whether there are deficits of the CRH-containing systems in the PD rotenone model.
Therefore, we aimed to investigate functional morphological changes in the urocortinergic neurons of the EWcp, and the CRH cells of the hypothalamic paraventricular nucleus, central amygdala and the bed nucleus of stria terminalis upon rotenone treatment, and upon anti-PD treatment consisting of levodopa/benserazide with or without fluoxetine adjuvant antidepressant medication.
Six weeks of subcutaneous rotenone treatment was applied to induce PD-like state. Control rats received vehicle injections. One third of the treated rats also underwent levodopa/benserazide anti-PD therapy, whilst another third received fluoxetine antidepressant on top of the anti-PD medication. The animals' locomotion was analyzed by rotarod test, the anhedonia by sucrose preference. Morphological changes were assessed by a combination of RNAscope *in situ* hybridization and immunofluorescence. The motor deficit was alleviated by levodopa/benserazide treatment, in contrast to non-motor symptoms. Anhedony improved upon SSRI treatment. We reproduced the previously observed UCN1/EWcp neuron loss, which was not affected by the therapy. Surviving cells exhibited higher UCN1 peptide content and lower *Ucn1* mRNA expression, which remained unaffected by drug treatment. Rotenone treatment did not induce remarkable CRH neuron loss in any of the regions studied, but *Crh* mRNA levels decreased, which remained unaffected by the treatment.
The inverse change in UCN1 peptide and mRNA content suggests inhibited neuropeptide release. Our results indicate that the anti-PD treatment is ineffective in treating mood-related symptoms and should be administered in conjunction with SSRI treatment. We provide further indirect evidence that the impairment of the EWcp contributes to the mood disorder in PD because no significant CRH neuron death occurred in the rotenone model of PD.

Obligatory peptidergic neurons of the Edinger-Westphal nucleus show transient glutamatergic phenotype in utero

Zsófia Havasi[1]; Dániel Hegedüs[1]; Viktória Kormos[2]; Balázs Gaszner[1]
[1]*University of Pécs Medical School, Department of Anatomy, Research Group for Mood Disorders, Pécs*
[2]*University of Pécs Medical School, Department of Pharmacology and Pharmacotherapy, Pécs*

The centrally projecting Edinger-Westphal nucleus (EWcp) expresses urocortin1 (UCN1) and cocaine- and amphetamine-regulated transcript peptide (CART). It plays an important role in the regulation of stress adaptation. A special feature of its cells is that they are obligate peptidergic neurons: they do not possess classical, fast-acting neurotransmitters. Since the developmental background of this feature is unknown, our goal was to characterize the neurochemical characteristics of the EWcp during its development. We previously established that *Ucn1* mRNA appears in peptidergic cells in mice only two weeks after birth, while *Cart* mRNA is detectable as early as 14 days after fertilization, making it suitable for identifying embryonic EWcp cells developing and migrating in the mesodiencephalic basal plate that express the *Nkx6.1* and *Nkx6.2* homeobox genes. In our present work, we aimed to determine whether embryonic peptidergic cells express markers of any of the fast-acting, classical neurotransmitters. We applied RNAscope in situ hybridization on sections of the mesencephalon of 14-day-old mouse embryos. Histological preparations were assessed by confocal microscopy and we also performed morphometrical analyses.
We did not detect tyrosine hydroxylase, tryptophan hydroxylase, choline acetyltransferase, or vesicular GABA transporter mRNA expression in EWcp *Cart* neurons, suggesting that these cells do not exhibit dopaminergic, serotonergic, cholinergic, or GABAergic phenotype. In contrast, when testing the glutamatergic markers, we found that most peptidergic EWcp cells embryonically express mRNAs for the vesicular glutamate transporter (Vlgut) type 2 (*Vglut2*) and type 3 (*Vglut3*), but not *Vglut1*.
Since the expression of glutamatergic markers decreased to a nearly undetectable level to the postnatal age, our results suggest that EWcp/*Cart* cells exhibit a transient glutamatergic phenotype. We plan to conduct further studies to investigate how stress experienced in utero affects the neurochemical development and function of the peptidergic cells.

TAVAE: A VAE with Adaptable Priors Explains Contextual Modulation in the Visual Cortex

Balázs Meszéna[1]; Keith Thomas Murray[2]; Márton A. Hajnal[1]; Julien Corbo[3]; O. Batuhan Erkat[3]; Pierre-Olivier Polack[3]; Gergő Orbán[1]
[1]*HUN-REN Wigner RCP, DCS, CSNL, Budapest*
[2]*Princeton Neuroscience Institute, New Jersey*
[3]*Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark*

The brain interprets visual information through learned regularities, a computation formalized as performing probabilistic inference under a prior. The visual cortex establishes priors for this inference, some of which are delivered through widely established top-down connections that inform low-level cortices about statistics represented at higher levels in the cortical hierarchy. While evidence supports that adaptation leads to priors reflecting the structure of natural images, it remains unclear if similar priors can be flexibly acquired when learning a specific task. To investigate this, we built a generative model of V1 that we optimized for performing a simple discrimination task and analyzed it along with large scale recordings from mice performing an analogous task. In line with recent successful approaches, we assumed that neuronal activity in V1 can be identified with latent posteriors in the generative model, providing an opportunity to investigate the contributions of task-related priors to neuronal responses. To obtain a flexible test bed for this analysis, we extended the VAE formalism so that a task can be flexibly and data-efficiently acquired by reusing previously learned representations. Task-specific priors learned by this Task-Amortized VAE were used to investigate biases in mice and model when presenting stimuli that violated the trained task statistics. Mismatch between learned task statistics and incoming sensory evidence showed signatures of uncertainty in stimulus category in the posterior of TAVAE, reflecting properties of bimodal response profile in V1 recordings. The task-optimized generative model could account for various characteristics of V1 population activity, including within-day updates to the population responses. Our results confirm that flexible task-specific contextual priors can be learned on-demand by the visual system and can be deployed as early as the entry level of the visual cortex.

Mathematical modelling of intracellular Ca2+ concentration changes in Deiters cells using ATP induction

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

Hearing is an important sense on which our society relies heavily, yet its disorders affect a large proportion of the population. The hair cells, which are responsible for the sensation of sound waves, are the most studied cells of the Organ of Corti. However, growing evidence suggests that their neighbouring cells, known as the supporting cells, may also play an important role in the proper sensation. One such type of supporting cells are the Deiter's cells. Due to their direct contact with the outer hair cells, they are hypothesized to participate in the formation of the auditory threshold and in the development and regeneration of the outer hair cells.
The investigation of the cells in the Organ of Corti requires strong technical skills, as it is a part of the spiral and bony inner ear. Consequently, mathematical modelling can serve as a tool for investigating these cells outside of a laboratory setting. To the best of our knowledge, no mathematical models have been established for Deiter's cells to date. Therefore, our aim was to assess whether a model originally developed for a different cell type could accurately describe their intracellular calcium handling mechanisms. The selected model was published by Taheri *et al.* (2017) and was created for astrocytes. The model validation was performed using functional calcium imaging recordings (15 day old BALB/c strain mice, n = 34 cells, prepared either from the basal, middle or apical turns of the cochlea, induced with 100 µM ATP). Approximately 800,000 parameter combinations were screened to identify the settings that best describe the calcium handling mechanisms of Deiter's cells. The results indicate that this model serves as a suitable foundation for developing a Deiter's cell-specific model.

Separate or share? Orthogonalization and alignment in hierarchical cascade computations

Andrea Albert[1]; Gergő Orbán[1]; Márton A. Hajnal[1]
[1]HUN-REN Wigner Research Centre for Physics, Department of Computational Sciences, Computational Systems Neuroscience Lab, Budapest

Sequential neural learning faces two challenges with contradicting goals: separating and sharing. First, networks will forget previously learned tasks as their globally stored memories are overwritten during updates to the current task. However, various mechanisms such as targeted inhibition or orthogonal weight updates explicitly separate representations into orthogonal subspaces in a population of neurons preserving earlier memories. Second, shared features between tasks should be stored once and reused, a primitive form of transfer learning, facilitated by optimal energy and wiring resource consumption. Although these two contradicting learning principles are evidenced to be computationally similar in animal and artificial networks, deeper understanding of the relation between continual and transfer learning is lacking. To answer how networks develop together orthogonal and shared representations, we examined hierarchically structured composite data such as classification in a visual scene or solving a complex cognitive problem. These tasks can be tackled with a hierarchical neural network that builds computations and features step by step, famously discovered in deep learning. Hierarchical networks, a characteristic element in the structure of the mammalian brain, are particularly well suited to examine local feature-orthogonalization and -sharing in a stepwise, controlled manner on realistic data. Here we show first that repeating the tasks sequentially, orthogonalized representations gradually develop while currently irrelevant memory is preserved. In particular, during training lower layers of the hierarchy orthogonalize feature representations early which helps orthogonalize higher level category layers. Catastrophic forgetting is thus overcome in hierarchical networks by spontaneous cascade orthogonalization in the order of features unfolding throughout the hierarchy. Second, common features align and collapse into reusable shared abstractions for the higher layers. Using varying class combinations and tunable overlap between tasks in the handwritten MNIST dataset, we show that if data complexity and algorithmic computational capabilities are matched, a combination of complementary orthogonalization and sharing of representations spontaneously solves catastrophic forgetting within a behaviourally relevant duration. These results should contribute to understanding early visual neurodevelopment and cognitive computations in the prefrontal cortex.

Investigation of the molecular mechanisms of long-term postsynaptic plasticity using detailed, optimized computational models

Gábor Farkas[1,2]; Máté Mohácsi[1,2]; Luca Tar[1,2]; Szabolcs Káli[1,2]
[1]*HUN-REN Institute of Experimental Medicine, Cerebral Cortex Research Group, Theoretical Neuroscience Research Laboratory, Budapest*
[2]*Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest*

A sophisticated network of intracellular signalling pathways in the spine heads of postsynaptic dendrites remarkably takes part in shaping synaptic plasticity, the cellular-level basis of learning and information storage in the brain. Using computational modelling combined with experimental results, it is possible to investigate sophisticated networks of biochemical cascades.
Optimized computational models were used to examine the underlying molecular mechanisms of postsynaptic LTP and LTD in a hippocampal CA1 pyramidal cell spine head. Such models can be used to study the mechanisms of different forms of plasticity, the roles and contributions of molecular pathways, individual molecular species, and the effects of different induction protocols and various types of neuromodulations. The goal of the present study is to describe the molecular changes underlying different forms of synaptic modification and to explain a diverse set of experimental data in a unified framework.
The detailed model contains the main postsynaptic signalling pathways that take part in the formation, maintenance, and expression of hippocampal LTP and LTD: the CaMKII, the PKA, and the PKC cascades. The activation of the subcellular cascades results in altered total AMPA receptor conductance, which can be used as a measure of synaptic changes. Parameters of the model were fit to electrophysiological data upon plasticity induction from hippocampal Schaffer collateral synapses. Furthermore, we aimed to have a realistic, steady-state baseline by fitting quantitative biochemical data regarding the resting state, such as intracellular calcium concentration; ratios of phosphorylated and membrane-bound subunits, and AMPAR tetramers.
In addition to modelling and studying simple LTP and LTD induction, different kinase inhibition experiments were modelled and fitted to experimental data. In these kinds of experiments, the effects of the kinase inhibition on LTP induction can be investigated.

After an in-depth analysis of the fitted models, changes in the numbers and properties of AMPA receptor subunits and tetramers were identified that shape altered synaptic strength. These changes act on different timescales using various mechanisms mediated by the interactions of the biochemical cascades. According to our predictions, the baseline ratio of different AMPA receptor subunits has a large influence on the molecular mechanisms that are utilized by the synapse to express LTP and LTD.

Structural determinants of gap junction formation and function

Agnes Simon[1]; László Héja[1]; Julianna Kardos[1]
[1]*Research Centre for Natural Sciences, HUN REN, Institute for Organic Chemistry,, Budapest*

Rapid progression of Ca2+ signals plays a vital role in synchronized neuronal activities, like slow wave sleep (Szabó et al., 2017, Péter & Héja, 2024). This activity propagates through the network of astrocytic gap junction channels (GJC). However, the lack of subtype-specific inhibitors for GJCs prevents their targeting in pharmacological strategies. To explore the structural features responsible for GJC coupling, we applied a theoretical approach to understand GJC formation by simulating the coupling of two adjacent hemichannels (HCs) in silico. We paid special attention to understand the role of the three disulphide bonds per chain that are able to open depending on the redox environment.
We used the recently determined Cx43 HC structure (Qi et al., 2023) as a base and positioned two membrane-embedded Cx43 HCs to simulate HC-HC docking. The role of disulfides was examined by in silico opening and closing of the disulfide bonds. To explore HC-HC coupling, we determined stabilization centers (hubs of structurally important residues) and the minimal channel diameter size to assess the functional (conducting or non-conducting) state of the channel throughout the 200-300 ns molecular dynamics simulations.
Results show that in the open-Cys solo HC model, canonical disulfide bonds are reformed during the simulation as S-S distance between 54C-198C, 65C-187C and 61C-192C -SH groups are arranged at <4.5 Å distance. The two extracellular loops (EL1 and EL2) play a significant role in the coupling process. The original GJC structure is kept together through EL1-EL1 interactions: 56T, 57Q acting as stabilization centers and 55N, 58Q participating in trans-junctional H-bonds. In contrast, the coupling HC-HC model was found to be connected by both EL1-EL1 and EL2-EL2 interactions (the latter involving 196V-195Q-194H).
When the solo HC was pre-equilibrated for 100 ns in open or closed disulfide conditions followed by simulating the HC-HC coupling in open or closed disulfide conditions different binding modes were found. Namely, weak EL1-EL2 and strong EL2-EL2 coupling for the closed-closed HC-HC model. and weak EL1-EL1 and strong EL2-EL2 interactions for the open-closed and open-open models. Analysis of minimal channel diameter data revealed that the kinetics and extent of channel opening can be also correlated to the above coupling mechanisms.
In summary, the high number of disulfide bonds play an essential role in HC-HC docking and in channel function.

Computational and Electrophysiological Evidence for Kv1-Dependent Firing Pattern Changes Induced by Cariprazine

Ádám Tiszlavicz[1]; Melinda E. Gazdik[1,2,3]; Anna A. Abbas[1,2]; Balázs Kis[1,2]; Lea Danics[1,2]; Katalin Schlett[3]; Karolina Pircs[1,2]; Attila Szűcs[1,2,3]; Karri Lamsa[1]
[1]*Hungarian Centre of Excellence for Molecular Medicine, Szeged*
[2]*Semmelweis University, Budapest*
[3]*Eötvös Loránd University, Budapest*

Cariprazine, a dopamine D3/D2 receptor-targeting antipsychotic, has been suggested to modulate neuronal excitability, yet its impact on subthreshold and spike-generating potassium conductances remains insufficiently characterized. In this study, we examined the effects of Cariprazine on the D-type potassium current mediated by Kv1 family ion channels in hippocampal neurons. Using whole-cell patch-clamp electrophysiology combined with quantitative PCR analysis, we assessed both functional and molecular consequences of 1 µM Cariprazine administration.
Cariprazine induced pronounced alterations in neuronal firing patterns. Notably, treated neurons exhibited stuttering or intermittent action potential generation, indicating a disruption of normal spike-timing precision. qPCR measurements revealed an upregulation of transcripts encoding D-type Kv1 channels, suggesting that changes in channel expression contribute to the observed electrophysiological phenotype.
To further delineate the mechanistic basis of these effects, we implemented computational models of hippocampal neurons to investigate their physiological properties and excitability. The simulations demonstrated that Cariprazine-induced stuttering behavior can be explained by modifications in D-type potassium conductance and by shifts in the steady-state activation threshold of Kv1 channels. We also investigated the how compartmental distribution of the Kv1 channels and their interactions with other intrinsic voltage-gated currents shape the firing responses of the model neurons. Together, these findings highlight a previously underappreciated mechanism through which Cariprazine may influence hippocampal circuit activity and provide new insights into how modulation of Kv1-mediated currents shapes neuronal firing dynamics.

Divisive normalization underlying efficient inference in a deep generative model account of V1

Domonkos Martos[1]; Josefina Catoni[2]; Ferenc Csikor[1]; Balázs Meszéna[1]; Enzo Ferrante[2]; Diego H. Milone[2]; Rodrigo Echeveste[2]; Gergő Orbán[1]
[1]HUN-REN Wigner RCP, Department of Computational Sciences, Computational Systems Neuroscience Lab, Budapest
[2]Research Institute for Signals, Systems and Computational Intelligence sinc(i), FICH-UNL/CONICET,, Santa Fe

Divisive normalization (DN), the division of a neuron's activity by a pool of connected neurons, has been recognized as a general computational motif in the cortex. Arguments support that in the visual cortex, DN acts as a canonical computation in generative models, which delivers contextual effects and contributes to shaping response variability. Deep generative models (DGMs) have recently been adopted by neuroscience from machine vision to embrace nonlinear computations in the visual cortex. Yet, it remains unclear whether DGMs effectively integrate DN as a core computational element. We investigated Variational Autoencoders (VAEs), a powerful family of DGMs, to understand the conditions under which this hallmark neural computation, DN, appears in a model of V1. VAEs are trained through learning a pair of models, the generative model that describes how latent factors are combined to produce an image, and the recognition model that approximates probabilistic inference in the generative model. When training a standard VAE on natural images, we found limited evidence for DN. Inspired by computer vision, we introduced a subtle inductive bias in the generative component of the VAE that designates one latent variable as a scaling variable that can collectively scale the output of other latents. We found that this Scale-Mixture VAE learned a representation in which the scaling variable correlated with contrast, making the rest of the latent space more invariant to contrast-changes. More importantly, the learned recognition model displayed multiple signatures of DN. Moreover, recent arguments about the concomitant increase in DN and reduced response variability (known as quenching) in neural activity were confirmed in the Scale-Mixture VAE but not in standard VAE. Interestingly, lack of quenching in standard VAE corresponded to flawed inference. In addition to empirical results, we provide a mathematical derivation that demonstrates the computational benefits of this ubiquitous motif. Thus, our results highlight that DN contributes to accurate inferences in VAEs, indicating a potentially more general role in machine learning applications.

Enhancing AAV Neutralization Assay Sensitivity for Patient Eligibility Assessment Using the coreTIA Platform

Petra Réka Molnár[1,2]; Beatrix Kovács[1,3,4]; Fanni Somogyi[1,3]; Viktória Szabó[5]; Domonkos Horváth[1,6]; Attila Balázs Dobos[1]; Zoltán Zsolt Nagy[5]; István Hernádi[7]; Ferenc Mátyás[8]; Wim Vanduffel[9]; Zsuzsanna Szemlaky[10]; Áron Szepesi[4,11]; István Ulbert[1,3,6]; Balázs Rózsa[4,11]; Dániel Hillier[1,3,6]

[1]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest*

[2]*Faculty of Science, Eötvös Loránd University, Budapest*

[3]*János Szentágothai Neurosciences Division, Semmelweis University Doctoral School, Budapest*

[4]*Laboratory of 3D Functional Network and Dendritic Imaging, HUN-REN Institute of Experimental Medicine, Budapest*

[5]*Semmelweis University, Department of Ophthalmology, Budapest*

[6]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest*

[7]*Grastyán Translational Research Center, and Szentágothai Research Center, Center for Neuroscience, University of Pécs, Pécs*

[8]*Neuronal Network and Behavior Research Group, HUN-REN Institute of Experimental Medicine, Budapest*

[9]*Laboratory for Neuro-and Psychophysiology, Leuven Brain Institute, KULeuven, Leuven*

[10]*South-Pest Central Hospital, National Institute for Infectology and Hematology, Department of Hematology and Stem Cell Transplantation, Budapest*

[11]*BrainVisionCenter, Budapest*

Adeno-associated virus (AAV) gene therapy efficacy is often limited by pre-existing neutralizing antibodies (NAbs), yet current assays lack standardization, suffer matrix-induced artifacts, and rarely quantify uncertainty, complicating cross-study comparisons and regulatory assessment. We present coreTIA (core Transduction Inhibition Assay), a modular cell-based protocol paired with a statistically rigorous analysis pipeline that incorporates two major innovations: (1) constant serum concentration across all dilutions to stabilize assay baselines, enhance sensitivity, and avoid matrix artifacts; and (2) explicit uncertainty quantification for neutralization titers (ND_{50}), enabling precise, reproducible measurements even with incomplete dilution series. Using human sera against multiple AAV serotypes, coreTIA reclassified up to 21.7% of samples deemed non-neutralizing with a conventional assay format, improved seronegative control pool selection, and extended detection of persistent seropositivity by up to one year in preclinical seroreversion models. Its robust framework reduces repeat testing, minimizes sample volumes, and ensures consistent performance across serotypes. By providing both protocol and analysis tools as open resources, coreTIA facilitates harmonized, transparent NAb measurement, ultimately supporting optimized vector administration timing, refined patient stratification, and streamlined regulatory evaluation in gene therapy development.

Long-term functional AAV-mediated brain transduction in large animal models

Fanni Somogyi[1,2]; Beatrix Kovács[1,2]; Klaudia Csikós[1,2]; Ábel Petik[3,2]; Domonkos Horváth[3,2]; Attila Balázs Dobos[2]; Lucia Wittner[2,4]; Dániel Hillier[2,3,5]
[1]Semmelweis University, János Szentágothai Doctoral School of Neurosciences, Budapest
[2]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest
[3]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest
[4]Institute of Mental Health, Neurology and Neurosurgery, Budapest
[5]Eötvös Loránd University, Department of Physiology and Neurobiology, Budapest

Long-term safe and homogeneous neuronal transduction in large-animal brains via minimally invasive AAV delivery remains a critical unmet need in translational neuroscience. We engineered a set of AAV variants and evaluated three administration routes—intravenous, intrathecal and a novel localized delivery—in rats and cats, aiming to maximize sustained functional expression while limiting any immune activation to transient, subclinical levels. Functional gene expression was assessed longitudinally using all-optical “read/write” methods that combine fluorescence-based neural activity imaging with optogenetic stimulation to capture both static expression and dynamic transgene functionality over time. Our results identify AAV construct-delivery route pairings that achieve high levels of neuronal transduction and maintain stable expression over extended periods. These findings support durable, functional expression and establish a versatile platform for both fundamental neuroscience studies and future therapeutic development.

Validation of highly flexible, ultra-long SEEG probes realized using compact bond interfaces for deep brain recording

Levente Víg[1,2]; Balázs Barkóczi[1]; Marc Keller[3,4]; Patrick Ruther[3,4]; Domonkos Horváth[1,5]; Klaudia Csikós[1,6]; Rebeka Stelcz[1,6]; Eszter Juhász[1]; Dániel Hillier[1,5]; István Ulbert[1,5,7]; Richárd Fiáth[1,5]
[1]Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest
[2]Pázmány Péter Catholic University, Roska Tamás Doctoral School of Sciences and Technology, Budapest
[3]University of Freiburg, Department of Microsystems Engineering (IMTEK), Freiburg
[4]University of Freiburg, University Center BrainLinks-BrainTools, Freiburg
[5]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest
[6]Semmelweis University, János Szentágothai Neuroscience Doctoral School, Budapest
[7]Semmelweis University, Department of Neurosurgery and Neurointervention, Faculty of Medicine, Budapest

Intracortical recordings are key for understanding brain function and advancing brain–computer interfaces, but conventional rigid probes suffer from poor long-term stability due to mechanical mismatch with soft brain tissue, leading to inflammation, glial scarring, and signal loss. Flexible polymer-based devices offer a more biocompatible alternative. Here, we validate a novel modular polymer–metal SEEG (stereoelectroencephalography) probe with an extended shank designed to access deep brain regions while minimizing tissue damage. Probes were microfabricated using polyimide as the substrate, iridium oxide for recording sites, and gold as bonding juncture between modular parts. The tip, middle cable section, and zero-insertion force interface were assembled via flip-chip thermocompression bonding. Before implantation, probes were temporarily stiffened using either (1) a tungsten shuttle or (2) Polyethylene Glycol (PEG). Implantation feasibility and recording performance were evaluated in anesthetized rats (mainly acute, partly chronic) and one cat (acute). Validation included electrochemical impedance spectroscopy (EIS), electrophysiology, and histology (track and target verification). After reaching deep structures, wideband signals were recorded from the cortex (CTX), hippocampus (HPC), and thalamus (THAL). Data analysis involved KiloSort, Phy and SpikeInterface for single-unit isolation.
EIS confirmed functional sites with suitable impedances (~200 kΩ). The tungsten shuttle enabled more reliable deep insertions (up to 8 mm), while PEG stiffening showed promise for reducing insertion artifacts. High-quality LFP and MUA were recorded at depths of 3–7 mm, revealing clear physiological patterns (cortical and thalamic slow waves and hippocampal gamma oscillation). Single units were isolated in CTX, HPC, and THAL for both species, with THAL neurons showing sensory-evoked responses (somatosensory in rats, visual in a cat). Histology confirmed accurate targeting with minimal deviation. In chronic recordings, single-unit activity was maintained for several weeks.
The modular, ultra-long flexible probe was successfully validated in in vivo experiments, yielding high-quality recordings across multiple deep structures. This design may overcome limitations of rigid probes and enable minimally invasive deep-brain access even in larger animal models. Future work will address long-term stability and performance more.

Polymer-based flexible multishank probes for simultaneous intracortical microstimulation and two-photon calcium imaging

Balázs Barkóczi[1]; Eszter Nguyen[1]; Csaba Horváth[1]; Melinda Rácz[1]; Linda Judák[2,3]; Frederik Ceyssens[4,5]; Peter Janssen[6]; Lucia Wittner[1]; Maarten Schelles[4,5]; Richárd Fiáth[1,7]
[1]*Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest*
[2]*Laboratory of 3D Functional Network and Dendritic Imaging, HUN-REN Institute of Experimental Medicine, Budapest*
[3]*BrainVisionCenter Research Institute and Competence Center, Budapest*
[4]*ReVision Implant, Haasrode*
[5]*Micro- and NanoSystems, Department of Electrical Engineering, Leuven*
[6]*Laboratory for Neuro- and Psychophysiology, Leuven*
[7]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest*

Electrical stimulation is one of the most widely used neuromodulation techniques, that is utilized by implants for restoring reduced sensory functions. Intracortical multielectrode microstimulation strategies, such as bipolar stimulation, and current steering, can shape the resulting electric field, enabling more selective activation of neuronal populations, compared to monopolar stimulations.
For evaluating the effects of these stimulation protocols, two-photon (2P) calcium imaging was performed. A polymer-based flexible multishank probe was developed with densely spaced microelectrodes on each shank, enabling the study of stimulation-evoked neuronal responses in the primary visual cortex (V1) of anesthetized or awake, head-fixed transgenic GCaMP6 mice during 2P microscopy. Probes were implanted with an angle of 55° to a depth of ~300 µm into the superficial layers of V1, and cortical activity was imaged within a 550 µm × 550 µm field of view (FOV) at a frame rate of 31 Hz.
According to our results, only a few active neurons were detected during baseline periods, both in anesthetized and in awake mice. As expected, higher monopolar stimulation parameters (current, pulse length, frequency) evoked more robust activation, the number of activated neurons and the mean calcium response also increased, in addition recruited more neurons located further from the stimulation site. Bipolar stimulation was performed between electrode pairs located on different shanks. The populations of activated neurons were markedly different from session to session. The number of activated neurons decreased with increasing distance of stimulation from the imaged cortical area. By applying current steering, where the total stimulation current was divided between two microelectrodes, the neuronal activation could be shifted systematically within the FOV, with more neurons responding closer to the electrode injecting higher current. Similar to anesthetized conditions, increasing of monopolar stimulation current recruited a larger number of neurons and increased the mean calcium response amplitude in awake, head-fixed animals.
Moreover, comparing sessions on separate days using identical stimulation parameters, the temporal stability of stimulation-related responses was similar between sessions, only substantial variability is observable.
The above mentioned results may contribute to the development of new medical devices, that can also be used in human vision restoration.

A Novel Miniature tFUS Device for Neural Modulation: From Cellular Mechanisms to Proof-of-Concept Applications

Veronika Házi[1,2]; Édua Édes[2,3]; Barnabás Kovács[4]; Aletta Mészáros[2,5]; Zoltán Somogyvári[2]; Eszter Sipos[6]; Attila Szűcs[7]; Tamás Kiss[2]; Marcell Stippinger[2]
[1]*Semmelweis University, Doctoral School, Budapest*
[2]*HUN-REN Wigner Research Centre for Physics, Computational Sciences, Theoretical Neuroscience and Complex Systems, Budapest*
[3]*Eötvös Loránd University, Biology, Budapest*
[4]*Veres Péter High School, Budapest*
[5]*Eötvös Loránd University, Physics and Astronomy, Budapest*
[6]*HUN-REN Institute of Experimental Medicine, Virus Technology Unit, Budapest*
[7]*Eötvös Loránd University, Physiology and Neurobiology, Budapest*

Introduction: Transcranial focused ultrasound (tFUS) neuromodulation (NM) is an emerging, noninvasive technique capable of modulating neural activity and has shown promise as a potential therapy for central nervous system disorders. Despite this potential, the underlying mechanisms, the safety profile, and the full spectrum of tFUS NM applications require further investigation. This study aims to advance the field by designing and testing a miniature tFUS NM device for use in pre-clinical studies.
Methods: We developed a custom ultrasound device and conducted both in vitro and in silico studies to assess its effects at the cellular level. Experiments used hippocampal neuron cultures, where current-clamp electrophysiological measurements were recorded on synaptically isolated cells using a ramping-current stimulation protocol. Neuronal voltage responses to current steps were measured before and after brief ultrasound stimulation, and controlled with sham stimulation (Fig 1A). In parallel, computational models based on the Hodgkin-Huxley formalism simulated the potential mechanisms underlying observed changes. Furthermore, we designed an in vivo experiment (Fig 2A) in rats using our miniature tFUS transducer to modulate brain states during sleep. We implemented and tested real-time as well as offline sleep spindle detectors, supported by dedicated custom hardware, for guiding ultrasonic stimulation at spindle occurrence.
Results: Electrophysiological recordings revealed that brief ultrasound exposure cause long-term effects on membrane properties including increased voltage noise, increased resting potential, decreased spike latency and action potential amplitude (Fig1B). In silico modeling supported the hypothesis that observed electrical changes may be attributed to modifications in membrane permeability or capacitance. Development and offline validation of sleep spindle detectors proved effective, and hardware for real-time sleep monitoring and stimulation was successfully established for planned rodent studies (Fig 2B).
Conclusions
Our results demonstrate the feasibility of miniaturized tFUS NM for basic research, and provide evidence that besides the acute modulation of ion channel activity, longer-term effects also emerge following transient US stimulation. The integration of real-time signal processing and custom hardware lays the groundwork for future studies exploring the neuromodulatory impact of tFUS NM on brain state and learning in animal models.

Cell- and Layer-Specific Modulation of Somatosensory Cortex by Focal Infrared Neurostimulation: Evidence for TRPV1 Ion Channel Involvement from High-Density Laminar Recordings

Zsófia Balogh-Lantos[1]; Ágoston Csaba Horváth[1]; Katalin Kovács-Rozmer[2,3,4]; Richárd Fiáth[1,5]; Zsuzsanna Helyes[2,3]; Zoltán Fekete†[1,6]
[1]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Research Group for Implantable Microsystems, Budapest
[2]University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, HUN-REN-PTE Chronic Pain research Group, Pécs
[3]National Laboratory for Drug Research and Development, Budapest
[4]University of Pécs, Faculty of Pharmacy, Institute of Pharmaceutical Chemistry, Pécs
[5]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest
[6]HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Sleep Oscillation Research Group, Budapest

Infrared neurostimulation (INS) has emerged as a promising, minimally invasive approach for modulating neural activity, with potential therapeutic applications in neurological disorders such as epilepsy. Although temperature-sensitive ion channels like TRPV1 are known to influence neuronal excitability, their contribution to infrared-induced modulation has not been fully characterized in vivo.
In this study, we investigated the role of TRPV1 in INS-evoked cortical responses using high-density laminar Neuropixels recordings in the somatosensory cortex of anesthetized wild-type and TRPV1 knockout (KO) mice. Pulsed (500 Hz) and continuous-wave (CW) infrared light at 1550 nm was delivered via an optical fiber, and more than 3000 single units were recorded across 10 animals. Recorded neurons included putative principal cells and narrow- and wide-waveform interneurons, enabling analysis of cell- and layer-specific modulation.
Both CW and pulsed INS produced robust changes in neuronal firing, with roughly half of all units showing increased or suppressed activity. Modulatory effects were observed across cortical layers and neuron types, although CW stimulation generally induced greater changes in firing rates and network dynamics than pulsed illumination.
Importantly, responses to INS were markedly reduced in TRPV1 KO mice, demonstrating a key contribution of TRPV1 channels to infrared-evoked activity. Differences in firing-rate modulation across layers and cell types between genotypes further supported this role. Histological analyses confirmed that TRPV1-expressing neurons are distributed throughout cortical layers.
Together, these findings provide direct in vivo evidence that TRPV1 channels contribute to INS-induced cortical modulation and advance our understanding of the biophysical mechanisms underlying infrared neuromodulation. This work lays a foundation for the future development of infrared light as a precise and minimally invasive tool for manipulating cortical circuits.

A multistep analysis workflow for the classification of cortical LFP events

Sándor Bordé[1]; János Horváth[1]; Boglárka Bozsó[1]; Robert G. Averkin[1]; Gábor Tamás[1]
[1]University of Szeged, Department of Physiology, Anatomy and Neuroscience, HUN-REN-SZTE Research Group for Cortical Microcircuits, Szeged

Cortical local fields and oscillatory events are usually analyzed by estimating their spectral characteristics, but these approaches have limited ability to extract all the information carried by the signal. Applying dimensionality reduction methods such as principal component analysis can improve classification performance, help discover hidden patterns, and create new features. We aimed to assemble a multi-step analysis workflow that can transform oscillatory LFP activity into a statistical representation. After filtering and downsampling the LFP signal, waveform segments for the events of interest were collected. The data was projected from the original space to the low-dimensional principal component space. After that, a Self-Organizing Map was trained and used to cluster the segments. Finally, a 2D probability distribution (SOM profile) was calculated for related LFP segments using cluster labels. We applied the workflow to cortical slow wave, theta and spindle oscillations recorded from juxtasomal position of pyramidal cells and interneurons in freely moving rodents. The application of the workflow on the juxtacellular LFP events data set recorded near pyramidal cells (n=31), regular spiking (n=37), and fast spiking (n=28) interneurons (n > 21000 down state events) revealed that the down states express a significant difference in their SOM profiles depending on the type of recorded cell. We conclude that juxtacellular LFP convey cell-type specific information. This suggests that field potentials in the network can be highly compartmentalized and can retain identities of cellular units in space and time, even if neuronal populations are in a silent state.

PS00.01

Firing rate suppression of the medial septum during spatial working memory maintenance without navigation

Malek Aouadi [1,2*], Nicola Solari[1]*, Johanna Petra Szabó[1,2,3], Bálint Király[1], Balázs Hangya[1]
[1]*MTA–HUN-REN KOKI Lendület "Momentum" Laboratory of Systems Neuroscience, HUN-REN Institute of Experimental Medicine, H-1083 Budapest, Hungary* [2]*János Szentágothai Neurosciences Program, Semmelweis University School of PhD Studies, H-1085 Budapest, Hungary* [3]*Epilepsy Center, Clinic for Neurosurgery and Neurointervention, Semmelweis University, H-1085 Budapest, Hungary*
**These authors contributed equally*

The medial septum (MS) was shown to participate in working memory (WM) processes: MS lesions led to deficits in spatial WM performance, and hippocampal theta oscillations, known to depend on the MS, were linked to WM maintenance. However, what activity features of the MS may support working memory has not been explored. Therefore, we directly compared MS single neuron activity in mice performing a 2-alternative forced choice task with and without a WM component. Importantly, we minimized the locomotion component of the task to dissociate spatial navigation and WM processes. We found that while firing rates were generally higher throughout the task, the dominant response during stimulus processing and the subsequent delay period was a suppression of activity. WM engagement was characterized by stronger responsiveness of MS neurons compared to control, and especially a stronger firing rate suppression in the second half of the delay period. Moreover, we found fewer strongly theta-rhythmic neurons in the MS during WM performance, and these neurons were more suppressed during the delay period when WM was engaged. Based on these results, the MS may have an enabling rather than an active role in hippocampal WM processing.

Acknowledgements:
We thank Katalin Lengyel and the FENS-Kavli Network, and acknowledge support from NAP3.0 (NAP2022-I-1/2022), Lendület LP2024-8/2024, NKFIH K147097, and the EU AI National Laboratory (project RRF-2.3.1-21-2022-00004)

PS00.02

Top-down cortical control of the paraventricular thalamic nucleus

Péter Márk Vízi[1]; László Bíró[1]; Gergely Komlósi[1,2]; György Buzsáki[2]; László Acsády[1]
1 Thalamus Research Group, HUN-REN Institute of Experimental Medicine, Budapest
2 Neuroscience Institute, Langone Medical Center NYU, New York, NY, USA

The paraventricular thalamic nucleus (PVT) is a critical node in the brainstem-cortex communication. The PVT integrates multitudes of stress and arousal related subcortical information and distributes these to widespread forebrain areas. Its main cell type expresses calretinin (CR). PVT/CR+ cells are critical in stress induced alteration of behavior. How this system is governed by top-down cortical control is presently little understood.

The two main sources of cortical inputs to PVT arise from the medial prefrontal cortex, (mPFC) and the ventral subiculum (vSub) of the hippocampal formation. To identify connections between the PVT and these cortical regions we used anterograde and retrograde viral tracing methods in CR-Cre and vGlut1-Cre mice. For functional relevance we recorded the activity of optogenetically tagged CR+ neurons that project to the vSUB and/or the mPFC.

We found that while the mPFC innervated the entire antero-posterior extent of PVT the vSub axons were restricted to the anterior PVT (aPVT). The distribution of mPFC and vSUB projecting PVT neurons displayed identical patterns. Co-injection of anterograde and retrograde tracers to vSUB demonstrated that the vSUB-aPVT connection is strictly reciprocal. Antidromically activated PVT neurons from the vSUB were localized in the aPVT. Their activity displayed sharp wave ripple (SWR) modulations. Antidromically activated cells from the mPFC were found along the entire antero-posterior extent of PVT but these cells displayed SWR modulation only in they were localized to aPVT.

These data disclose aPVT as a thalamic hot spot that process top-down hippocampal electrical signals and transfers it to mPFC during electrical activity patterns important in memory formation.

Acknowledgement: The work was supported by MTA–2023 Lendület “Momentum”Thalamus Research Group