# 9th Hungarian Neuroscience Doctoral Conference HuNDoC 2026

# Program and Abstract Booklet

9th HUNDOC

28th of January, 2026, Budapest

# Invitation

Dear Students and Young Researchers,

We are happy to invite you all the 9th Hungarian Neuroscience Doctoral Conference (HuNDoC 2026), which will be held on January 28, 2026, at the HUN-REN TTK in Budapest. The conference welcomes undergraduate, graduate, and junior postdoctoral researchers interested in all areas of neuroscience.

HuNDoC provides an opportunity for early-career researchers from across Hungary to share their work, exchange ideas, and meet others in the field.

This year's program will include:

- Mini-poster (group presentation) and short talk sessions highlighting ongoing research in neuroscience
- Opportunities for informal discussion and networking with fellow students, mentors, and colleagues
- Sessions focused on topics such as career development, creativity in science, and networking.

For the first time, we also invite BSc and MSc students who are not yet involved in research but are interested in pursuing it for their diploma work or TDK projects. For them, submitting an abstract is optional, and instead of mini-posters, we will organize a round table discussion, where research directions-related questions will be addressed (e.g. finding suitable laboratories and institutes, and planning the next steps toward a research career).

Come meet fellow students, share ideas, and get inspired for your next steps in neuroscience!

9th HUNDOC
BUDAPEST 2026

# General information

## Date: 28th of January 2026

## Organizing Committee

- Réka Bod (HUN-REN RCNS)
- Nour Essam (HUN-REN RCNS)
- Eszter Juhász (HUN-REN RCNS)
- Rebeka Stelcz (HUN-REN RCNS)
- Katalin Zsófia Tóth (HUN-REN RCNS)
- Vivien Szendi (ELU FNS)

## Acknowledgement

The HuNDoC Conference 2026 was founded by the Hungarian Neuroscience Society.

# Website:

https://hundoc2026.mitt.hu/invitation

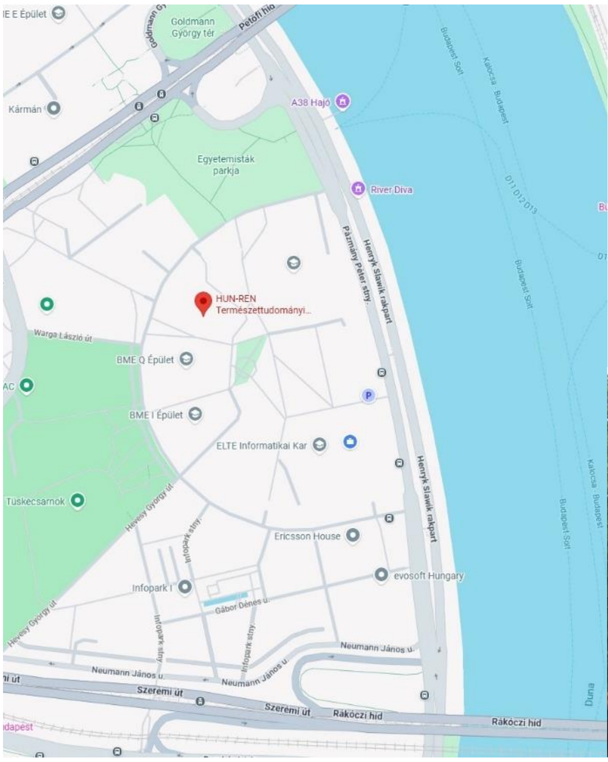
# Contact us:

hundoc2026@gmail.com

# Venue

## Research Centre for Natural Sciences

## 1117, Budapest, Magyar Tudósok Körútja 2.




## Approaching the venue by public transport

Tram 4/6: Petőfi Bridge, Buda end

Tram 1: Infopark

Bus 212: Petőfi Bridge, Buda end

Bus 107/153/154: A38 Hajóállomás

If you come by car, please note, that every street is paid parking area near the building.

## Scientific Program

Our scientific program features **a plenary lecture by Dr. Karolina Pircs**, leader of the Neurobiology and Neurodegenerative Diseases Research Group, HCEMM–Semmelweis University, followed by one **session of short talks** (7 minutes + 3 minutes Q&A) delivered by selected speakers. After lunch, we host a MiniPoster session, with presenters and discussion groups assigned on site. In parallel with the **MiniPoster session**, we run a **Student Forum for BSc/MSc students** who did not submit an abstract — an open discussion and Q&A with the HuNDoC Conference Organizers about getting started in research, choosing a lab, and what to consider when joining one. The afternoon continues with **two interactive workshops**: a **Career Roundtable** with professionals working in diverse fields with a scientific degree, and a **Grant Roundtable** focusing on real experiences of applying for and winning grants, together with university grant coordinators. The **official language of the conference is English**, and all presentations must be prepared accordingly.

### Short talks

- Format: Slide-based presentation (MS PowerPoint).
- Timing: 7 minutes presentation + 3 minutes audience Q&A (please keep strictly to time).
- Please send your slides to hundoc2026@gmail.com by 19:00 on Tuesday, 27 January 2026
- Please check your presentation in the morning or at least 10 minutes before the session starts
- The necessary tools for the presentation will be provided

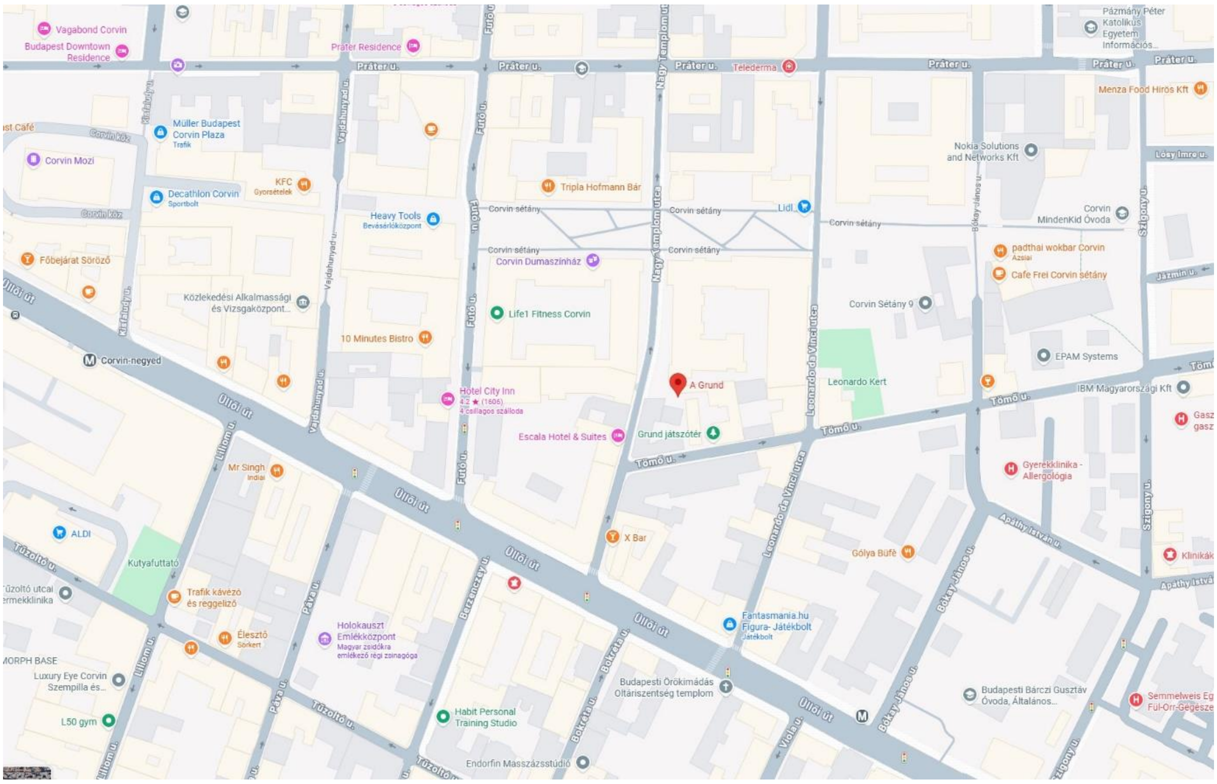
## Social event

The social event will take place at **Grund** in a reserved space for HuNDoC 2026 Conference attendees only.

**Start of the event:**

**Venue website:** https://agrund.hu/

**Address:** 1082 Budapest, Nagy Templom utca 30



# Detailed program

## 8:00-8:30: Registration

## 8:30-8:45: Opening and Welcome Speech

## 8:45-9:45: Plenary Lecture

**Dr. Karolina Pircs (Institute of Translational Medicine, Semmelweis University)**

Direct Neural Reprogramming as a Cellular Model of Human Brain Aging and Rejuvenation

## 9:45-10:15: Coffee break I.

## 10:15-12:00: Short presentations (7 min talk + 3 min Q&A)

1. **Anna Padányi (Grastyán E. Translational Research Centre, University of Pécs)**
   Transcranial magnetic stimulation based cortical excitability measure in awake non-human primates

2. **Ildikó-Beáta Márton (Faculty of Physics, Babeș-Bolyai University)**
   Comparing graph properties of axon and dendrite trees in the Drosophila connectome

3. **Lilla Radvan (Department of Physiology and Neurobiology, Eötvös Loránd University)**
   Histological and chemogenetic characterization of a thalamic input to the medial preoptic area underlying maternal function

4. **Roland Zsoldos (HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Semmelweis University)**
   Studying neuronal autophagy in human ageing using induced neurons directly reprogrammed from adult human dermal fibroblasts

5. **Máté Egyed (Department of Physiology and Neurobiology, Eötvös Loránd University)**
   Medial preoptic oxytocin receptor-expressing neurons regulate affiliative social behaviors in rats

6. **Orsolya Farkas (Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences)**
   High-density spatiotemporal single-unit activity profiling and cell-type identification in the human neocortex

7. **Sára Vida (Laboratory of Neuroimmunology, HUN-REN Institute of Experimental Medicine)**
   Selective neuronal and microglial changes in SORL1-dependent Alzheimer's disease in human and mouse brain tissue

8. **Ádám Szentes (George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș)**
   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

9. **Adél Papp (Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged)**
   Comparative Analysis of Cortical Electrical Activity in Migraine Patients and Healthy Adults During an Associative Learning Task

10. **Éva Zsuzsanna Nagy (Department of Biochemistry and Molecular Biology, University of Szeged)**
    Changes in sleep patterns and circadian clock gene expression in a Drosophila Alzheimer's disease model

## 12:00-13:00: Lunch break and informal networking

## 13:00-14:30: PARALLEL SESSIONS

### Mini-poster presentation for presenters

– For participants who submitted abstracts
– Groups will be sorted on site

### Student Forum with HuNDoC Organazing Committee

– Starting out as BSc and MSc students
– Educational backgrounds
– How we chose our labs
– What to consider when joining a lab
– Open discussion & Q&A

## 14:30-15:00: Coffee break II

## 15:00-16:00: WORKSHOP I. – Career Roundtable

- András Ecker (Research Manager at Cytocast)
- Eszter Papp (Postdoctoral Researcher at Eötvös Loránd University and Science Communicator)
- Gergely Márton (Co-Founder & CEO of MindRove and Senior Researcher at HUN-REN Research Centre for Natural Sciences)
- Rebeka Fekete (Research Fellow at the HUN-REN Institute of Medicine)
- Viktor Kis (Application Manager and Product Owner for micro-CT at 3DHISTECH Ltd.)

## 16:00-17:00: WORKSHOP II. – Grant Roundtable

- Ágnes Kandrács (Postdoctoral Researcher at the HUN-REN Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology)
- Klaudia Csikós (Research Assistant at HUN-REN Research Centre for Natural Sciences and PhD Student at Semmelweis University)
- Nóra Jeney (Chief EU Research Funding Advisor at Faculty of Science, Eötvös Loránd University)
- Tamás Zsoldos (Medical Student at Semmelweis University and Undergraduate Researcher at HUN-REN Institute of Experimental Medicine)

## 17:00-17:15: Closing remarks

## From 17:30: Social event at Grun

# Plenary lecture abstract

## Dr. Karolina Pircs - Direct Neural Reprogramming as a Cellular Model of Human Brain Aging and Rejuvenation

*[1] Institute of Translational Medicine, Semmelweis University, Budapest, Hungary*
*[2] Hungarian Centre of Excellence for Molecular Medicine - Semmelweis University, HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Budapest, Hungary*
*[3] HUN-REN-SZTAKI-SU Rejuvenation Research Group, Office for Supported Research Groups (TKI), Hungarian Research Network (HUN-REN), Budapest, Hungary*

Direct reprogramming of human fibroblasts into induced neurons (iNs) and induced astrocytes (iAs) provides a powerful model to study human brain aging and age-related neurodegenerative diseases. This method bypasses pluripotency, therefore iNs and iAs retain the donor's genetic, epigenetic, and biological aging signatures, making them uniquely suitable for investigating age-dependent cellular pathways and patient-specific disease mechanisms.

In our laboratory, this platform is integrated with automated high-content imaging, quantitative morphology, and multi-omics analyses to characterise aging and autophagy in human neurons and astrocytes. These approaches have revealed marked cell-type-specific differences in autophagy regulation, highlighting distinct neuronal and glial vulnerabilities. Through transcriptomic, proteomic, phosphoproteomic, DNA-methylation-based aging clocks, we aim to identify molecular networks that drive neuronal decline and define potential rejuvenation targets.

Beyond fundamental research, directly reprogrammed iNs enable reverse translational approaches in which patient-derived neurons are used to investigate clinically applied or candidate therapeutics. Studies performed in the context of felodipine and cariprazine treatment have shown donor-dependent cellular responses, linking autophagy, neuronal complexity and electrophysiological properties to clinical variation. These findings underscore the potential of iNs for precision medicine and for identifying patient subgroups most likely to benefit from specific interventions.

Building on these efforts, our MiND+ framework aims to establish a human neuron-based platform for drug discovery, target validation and patient stratification in cognitive and neurodegenerative disorders. Together, these approaches demonstrate how direct cellular reprogramming can bridge fundamental aging biology with translational applications and support the development of future therapeutic strategies.

# Short presentation abstracts

# 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*

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.

# Comparative Analysis of Cortical Electrical Activity in Migraine Patients and Healthy Adults During an Associative Learning Task

**Adél Papp**[1], Noémi Harcsa-Pintér[1], Kálmán Tót[1], Olívia Mária Huszár[1], Bence Gyula Nagy[1], Kata Fodor[1], Gabriella Eördegh[2], Anett Csáti[3], János Tajti[3], Attila Nagy[1]

*[1] Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary*
*[2] Department of Theoretical Health Sciences and Health Management, Faculty of Health Sciences and Social Studies, University of Szeged, Szeged, Hungary*
*[3] Department of Neurology, University of Szeged, Szeged, Hungary*

The cooperation of the basal ganglia and the hippocampus plays a key role in associative acquired equivalence learning. Using the visually guided Rutgers Acquired Equivalence Test (RAET), cognitive alterations have been demonstrated in migraine patients even during the interictal phase. The aim of our study was to investigate whether these alterations may be associated with changes in cortical activity and to explore potential EEG biomarkers underlying these differences.
We examined seventeen adult migraine patients in the interictal phase and seventeen age- and sex-matched healthy control participants. During a computerized RAET, 64-channel EEG was recorded continuously. In the learning phase, participants were required to associate an antecedent stimulus with one of two consequent stimuli based on trial-by-trial feedback. In the test phase, previously learned associations were reassessed in the absence of feedback. EEG preprocessing included band-pass filtering and independent component analysis for artifact removal, followed by epoching time-locked to the button press marking each participant's decision. The cleaned data were compared between groups using cluster-based permutation statistics.
In both the learning and test phases, we observed a significant reduction in beta-band activity in the migraine group within parieto-occipital associative cortical regions. This reduction was present in the 500 ms intervals both preceding and following the button press, and showed a strongly left-hemisphere–lateralized distribution.
These findings suggest that altered cortical electrical activity contributes to the performance deficits observed in migraine patients during visually guided associative learning tasks. The left-lateralized parieto-occipital differences may reflect modified functioning of visual-associative networks and altered hemisphere-specific processing strategies in migraine. After further validation, these cortical activity patterns may serve as EEG-based biomarkers to improve the diagnostic precision of migraine.
Acknowledgements:
This work was supported by the SZAOK-KKA-SZGYA Grant No. 2023/5S479.

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

**Anna Padányi[1,2]**, Balázs Knakker[1], Riszt Rafaella Mínea[1,2], Inkeller Judit[1], Evelin Kiefer[1], István Hernádi[1,2,3,4]

[1]*Grastyán E. Translational Research Centre, University of Pécs*
[2]*Medical School, University of Pécs*
[3]*Department of Neurobiology, Institute of Biology, Faculty of Sciences, University of Pécs, Hungary*
[4]*Szentágothai Research Centre, University of 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.

# Changes in sleep patterns and circadian clock gene expression in a Drosophila Alzheimer's disease model

**Éva Zsuzsanna Nagy [1]**, Dr. László Bodai[1]

*[1] Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary*

Alzheimer's disease (AD) is known as the leading cause of dementia. It is a neurodegenerative disease that causes changes in the circadian rhythm in humans, as well as in Drosophila models of the disease. These changes lead to disturbed sleep patterns and increased neuronal damage. Since the disturbed sleep patterns lead to further decline in the patients' condition, it isimportant to know the molecular reasons of these negative changes. The circadian rhythm is the management of physiology, controlled by an internal multicomponent oscillator system, that shows an about 24 hours long periodicity. The operation of this system is strongly conserved, which means that similar processes control the sleep rhythm in humans and in fruit flies. Considering this fact, we used Drosophila melanogaster as a suitable model to study the changes of the sleeping pattern in AD. In the disease model, neurodegeneration was induced by human Amyloid beta peptide, expressed in neurons. We investigated the sleeping patterns in relation to age, in both male and female AD model and control flies. We measured their activity with TriKinetics DAM2 activity monitors and described the changes from many aspects. We tested the expression of the clock genes of the core feedback loop of circadian rhythm (Drosophila Clock; period; timeless). We also plan to measure the expression of the secondary feedback loop genes (vrille, PAR-domain protein 1 and clockwork orange) to better understand the underlying molecular processes of the changes. We hope that the uncovered molecular changes may contribute to the treatments of the symptoms of this yet incurable disease.

# Comparing graph properties of axon and dendrite trees in the Drosophila connectome

**Ildikó-Beáta Márton[1,2]**, Balázs Péntek[1,2], Mária Ercsey-Ravasz[1,2]

[1] *Babeș-Bolyai University, Faculty of Physics, Cluj-Napoca, Romania*
[2] *Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania*

Studying the correlation between structure and function in brain networks has been a central and compelling topic in neuroscience. The FlyWire project achieved a major milestone by completing a fully digitized brain map of the Drosophila melanogaster. This dataset includes a complete connectome of 139,255 validated neurons and 34,153,566 synapses, which has been made publicly available [1].

In this study, we analyze this dataset by separating the axonal and dendritic trees of individual neurons. There have been some previous studies comparing morphological properties of axons and dendrites [2]; however, the results presented here represent a first-of-its-kind, whole-brain comparison of axonal and dendritic structural features in the Drosophila connectome. The extraction and quantification of various metrics, such as node count, maximum height, maximum width, cable length, leaf node count, and fractal dimension; reveal important differences in connectivity and cellular architecture that are difficult to capture using single-neuron sections or small-scale analyses.

We found that, in most cases and for nearly every property measured, dendritic trees were larger and more complex than their axonal counterparts. The distribution of tree heights follows an exponential rule. This is somewhat expected, as in the Drosophila brain, the Exponential Distance Rule (EDR) [3] applies to axonal length, so it is expected to affect and apply to the height of both axonal and dendritic trees. What is even more intriguing is that, while the EDR describes the height of the trees, the histogram of their maximum width follows a power-law distribution. This may reveal important insights into the organizational principles and scaling laws that govern neuronal growth and connectivity.

When comparing axons and dendrites across different functional classes, dendritic properties remain larger for almost every group. The sensory class is a notable exception, these intrinsic neurons exhibit very low fractal dimensions and small axonal and dendritic trees. This large-scale analysis highlights how the Drosophila brain balances general structural rules with class-specific exceptions, offering new insights into the physical constraints and functional requirements of neural architecture.

## References

[1] Dorkenwald et al., Nature, 634(8032), 2024, 124–138

[2] Nanda et al., Brain Structure & Function, 223(4), 2018, 1107–1120

[3] Péntek, B., & Ercsey-Ravasz, M. Network Neuroscience, 9(3), 2025, 869–895

# Histological and chemogenetic characterization of a thalamic input to the medial preoptic area underlying maternal function

**Lilla Radvan[1]**, Vivien Szendi[1], Tamás Láng[2], Máté Egyed[1], Gina Puska[1,3], Árpád Dobolyi[1,2]

*[1] Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*
*[2] Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary*
*[3] Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary*

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.

Grant support: NKFIH OTKA K146077, NKKP National Research Excellence program 151425, and MTA NAP2022-I-3/2022 (NAP 3)

# Medial preoptic oxytocin receptor-expressing neurons regulate affiliative social behaviors in rats

**Máté Egyed[1]**, Lilla Radvan[1], Vivien Szendi[1], Gina Puska[1,2], Valery Grinevich[3], Árpád Dobolyi[1]

*[1] Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*
*[2] Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary*
*[3] Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany*

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.

Grant support was provided by EKÖP-25-2-40 University Research Scholarship Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, NAP2022-I-3/2022, NKFIH OTKA K146077 and NKKP OTKA Excellence 151425.

# 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, H-1117, Hungary*
[2] *Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, H 1083, Hungary*
[3] *Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA*
[4] *Center for Neurotechnology and Neurorecovery, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA*
[5] *Semmelweis University, Faculty of Medicine, Department of Neurosurgery and Neurointervention, Budapest, H-1145, Hungary*

*Presenting first author contact: farkas.orsolya@ttk.hu

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.

# 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, Semmelweis University, Budapest, HU*
*[2] HUN-REN-SZTAKI-SU Rejuvenation Research Group, Budapest, HU*
*[3] Laboratory of Molecular Neurogenetics, Lund University, Lund, SE*
*[4] HCEMM-Szeged University, Szeged, HU*
*[5] Institute of Genomic Medicine and Rare Disorders, Faculty of Medicine, Semmelweis University, Budapest, HU*
*[6] Institute for Computer Science and Control (SZTAKI), Hungarian Research Network (HUN-REN), Budapest, Hungary.*

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.

# Selective neuronal and microglial changes in SORL1-dependent Alzheimer's disease in human and mouse brain tissue

**Sára Vida[1]**, Balázs Pósfai[1], Krisztina Tóth[1], Zoltán Máté[2], Ferenc Erdélyi[2], Zsolt Lele[3], István Katona[3], Henne Holstege[4], Olav M. Andersen[5], Ádám Dénes[1]

[1] *Laboratory of Neuroimmunology, HUN-REN Institute of Experimental Medicine, Budapest, Hungary*
[2] *Medical Gene Technology Unit, HUN-REN Institute of Experimental Medicine, Budapest, Hungary*
[3] *Molecular Neurobiology Research Group, HUN-REN Institute of Experimental Medicine, Budapest, Hungary*
[4] *Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands*
[5] *Department of Biomedicine, Aarhus University, Aarhus, Denmark*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, yet its pathomechanism remains unclear. Recently, increasing attention has been given to the role of microglia—the brain's primary immune cells—in different forms of neurodegeneration. Our study investigates disease-associated changes in microglia and neurons, particularly in the context of SORL1 (sortilin-related receptor) loss-of-function mutations that cause hereditary early-onset AD.
The aim of our translational study is to identify selective neuronal and microglial changes in SORL1-dependent AD, both resembling and differing from changes in sporadic AD.
We performed single-cell RNA sequencing on human temporal cortex samples from controls and AD patients with or without SORL1 mutations. Data were analysed in R using the Seurat package. In mice, we developed a CRISPR/Cas9 targeting strategy combined with in utero electroporation to achieve deletion of SORL1 in a subpopulation of cortical neurons in PSEN1//App_swe//tauP301L triple transgenic male AD model mice. Histological analyses were conducted using immunohistochemistry and confocal microscopy.
RNA profiling identified distinct human microglial and neuronal subtypes linked to both AD and SORL1 mutations, marked by synaptic protein loss, disrupted microglial mitochondrial function, and other key differences between the two groups. In our porated AD model mice, microglia interacting with SORL1-KO neurons showed morphological changes, including fewer but 21% longer processes, while microglia-neuron contact surface was also reduced by 55%.
Overall, the pathomechanism of SORL1 loss-of-function mutations differs from Braak-matched AD cases and involves microglial actions. Our study suggests that AD development could have several, presently unexplored links with microglia, warranting further research.
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# Mini-poster abstracts

# A paraventricular thalamic hub integrating brainstem and stress signals to regulate REM sleep

**Aletta Magyar**[1,2,3,4], Péter Berki[1,3], Sándor Borbély[1], Boglárka Barsy[1,2], Anna Virág Bakacsi[1,2,3], Judit Berczik[1,2,3], Magor L Lőrincz[5], Danqian Liu[6], Ferenc Mátyás[1,2,7]

[1] *Institute of Experimental Medicine, Budapest, Hungary*
[2] *Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary*
[3] *János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary*
[4] *Institute of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary*
[5] *Department of Physiology, University of Szeged, Szeged, Hungary*
[6] *Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, China-Hungary Belt-and-Road Joint laboratory on Brain Science, Shanghai, China*
[7] *Department of Anatomy and Histology, University of Veterinary Medicine, Budapest, Hungary*

Rapid eye movement (REM) sleep is a distinct and well-characterized sleep stage controlled by brainstem circuits. However, the network mechanism underlying their regulation of REM sleep and the associated cortical theta oscillations remains elusive.

Here, we reveal that a subpopulation of paraventricular thalamic neurons (projecting to the prefrontal cortex and nucleus accumbens, PVTNAc) transfers the medullary REM signal to the PFC in an activity- and firing mode-dependent manner, while bidirectionally modulates cortical theta oscillations. This brainstem-thalamocortical pathway is also recruited stress-induced disturbances of REM sleep. In these alterations, progressively increasing PVTNAc activity underlies the transition from REM enhancement following acute stress to REM suppression after repeated stress. Frequency-dependent thalamic stimulation that mimics elevated PVTNAc activity induces corresponding shifts in cortical excitatory/inhibitory balance and replicates the bidirectional alterations in REM-related theta oscillations.

Together, these findings reveal a brain-wide circuit that integrates theta rhythm-generating and stress-related signals into the PVTNAc activity, enabling it to dynamically regulate REM sleep under homeostatic and stressful conditions. Through this mechanism, PVTNAc neurons reshape prefrontal cortical network activity, reorganize sleep architecture, and influence cognitive function.

# Maternal metabolic risk and neonatal maturation shape large-scale functional brain connectivity in newborns

**András Ambrus[1],** Péter Nagy[1], István Winkler[1], Brigitta Tóth[1], Gábor Háden[1]

*[1] Sound and Speech Perception Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*

Early brain connectivity is crucial for healthy neural development; however, little is known about how clinical and perinatal factors influence functional networks in newborns. We investigated resting-state brain networks in full-term infants (N = 455) within 1–3 days after birth, using high-density EEG recorded during quiet sleep. Functional connectivity was estimated in the delta, theta, and alpha bands using multiple metrics, and averaged across and within five canonical networks: the default mode network (DMN), visual network (VIS), frontoparietal network (FPT), salience network (SAL), and dorsal attention network (DAT). Infants were grouped according to perinatal risk factors, pregnancy complications, delivery mode, maternal metabolic risk (defined by existing endocrine or metabolic diagnoses), and maturation scores (derived from gestational age, birth weight, and birth length).

Analyses of relative connectivity revealed robust associations with clinical factors. Infants with maternal metabolic risk showed significantly stronger connectivity within the SAL and between DMN–FPT, DMN–DAT, FPT–SAL, and SAL–DAT networks. Maturation scores were associated with network-specific patterns: more mature infants exhibited stronger relative connectivity within the DMN, VIS, and DAT, as well as between the DMN–VIS, DMN–SAL, VIS–FPT, and VIS–DAT networks. By contrast, pregnancy complications and delivery mode showed no significant effects after correction.

These findings highlight that both maternal metabolic risks and early maturation levels systematically influence large-scale brain connectivity in newborns. Resting-state EEG thus provides a powerful, non-invasive tool to identify early markers of developmental trajectories and potential vulnerability to adverse conditions.

# The impact of prior systemic inflammation on neuroinflammation and neuropsychiatric outcomes following perinatal asphyxia

**András Buzás-Kaizler[1]**, Hanga Kelemen[1], Gyula Balla[1], Kornél Demeter[1], Zsolt Borhegyi[1], Éva Mikics[1]

*[1]HUN-REN Institute of Experimental Medicine*

Introduction: Hypoxic-ischaemic encephalopathy developing after perinatal asphyxia (PA) contributes to the emergence of numerous neurodevelopmental disorders. When accompanied by a pre-existing intrauterine systemic inflammation, the long-term neurological outcome of PA may worsen significantly. The exact neurobiological processes involved in the pathomechanism of PA, intrauterine inflammation, and their synergism are not fully understood, hindering the development of specific therapies. However, it is hypothesized that the enhanced activation of shared inflammatory signaling pathways involving microglia cells plays an important role in the development of this conjoined effect, which may influence the functioning of higher-order brain networks in the long term.

Objective: To investigate the long-term, brain region-specific effects of PA, both individually and in interaction with pre-existing systemic inflammation, on behavior and microglial populations, with an emphasis on assessing sex-dependent differences.

Methods: Mice were administered s.c. IL-1β cytokine on postnatal days 2–6, after which a subset of the animals was subjected to a PA insult at 7 days of age by inhalation of a asphyxia-inducing gas mixture (4% O2, 10% CO2), establishing the following treatment groups: IL-1β+PA, PA, IL-1β, and control. Following comprehensive behavioral testing assessing both cognitive and emotional functions, microglia cells were labeled on the animals' wholebrain slices via immunohistochemistry. A mouse brain atlas was aligned to the images captured with microscope, and microglia were quantified in nearly 500 regions. Based on our results, microglia morphology analysis was performed in relevant regions to assess functional alterations.

Results: PA insult induced long-term, sex-dependent alterations in emotional and cognitive functions, which were markedly exacerbated when combined with a prior inflammatory insult. A significant increase in microglia density was observed in brain areas associated with the observed behavioral changes (e.g., mediodorsal thalamus, nucleus reuniens, amygdala nuclei), accompanied by long-term morphological changes in several regions.

Conclusions: A prior systemic inflammation induced by IL-1β increased the nervous system's susceptibility to PA, worsened its clinical outcome in a sex-dependent manner, and caused substantial, long-term alterations in microglial functionality in brain regions closely linked to the observed behavioral changes.

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# 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]Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest 1117, Hungary*

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.

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# Using state-transition networks for EEG analysis

**András Rusu[1,2]**, Zsolt I. Lázár[1,2], Mária Ercsey-Ravasz[1,2], Bulcsú Sándor[1,2]

*[1] Babeş-Bolyai University, Cluj-Napoca, Romania*
*[2] Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania*

When analyzing the time-evolution of large complex systems, one rarely has access to the equations of motion. Thus, extracting essential information from the observed time series (for example electrophysiological recordings of the human brain) such as complexity and changes in the dynamics becomes crucially important.

When it comes to analyzing EEG signals, entropic methods have proven themselves useful in various experimental settings, completing the toolbox of more standard (for example spectral) methods [1]. For instance, measures like permutation entropies, sample entropy or dispersion entropy have all been used to characterize complexity and irregularity, discriminating different brain states. These usually require a so-called symbolization procedure through which the observed time series gets coarse-grained, restricting the possible observations to a finite number of states. The relevant quantities derive from statistics of them, making use of probabilities of states and symbolic sequences. The weighted directed network of states that appeared during a certain observational timeframe is called a state-transition network, where weights correspond to transition probabilities. Through the framework of Markov processes, it becomes possible to approximate probabilities of (infinitely long) symbolic sequences which is a requirement of several different dynamic entropy rates such as Kolmogorov-Sinai entropy. This can be useful in itself, as its upper limit is the largest Lyapunov exponent which measures chaoticity. Another related measure, the variance of the logarithmic probability of trajectories (here called the Lyapunov measure) has potential utility in predicting dynamical phase transitions from intermittent, near-critical time series but also in characterizing the complexity of the underlying behavior. For the latter, we also offer a computationally efficient closed-form expression, which can be used after the state-transition network was extracted from the physiological signal [2].

We show here a possible application of the Markov-based entropic measures on human EEG signals recorded during an object recognition task [3]. We compare pre-stimulus state to post-stimulus state, with special attention to brain areas relevant to visual information processing in order to study differences in the dynamics when the task was successful vs. when it wasn't (corresponding to different levels of image distortion).

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# Behavioral consequences of the chemogenetic silencing of the ventral tegmental area in rats: 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 [1,2,3,4]

*[1] University of Pécs, Szentágothai Research Centre, Translational Neuroscience Research Group, Pécs, Hungary*
*[2] University of Pécs, Grastyán Translational Reserach Center, Pécs, Hungary*
*[3] University of Pécs, Faculty of Sciences, Department of Neurobiology, Pécs, Hungary[24]University of Pécs, Medical School, Institute of Physiology*

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

**Annamária Benke**[1], Írisz Szabó[1], Yi Shih Lee[1], Bálint Király[1,2], Vivien Pillár[1,3], Balázs Hangya[1,4]

[1] *HUN-REN Institute of Experimental Medicine, Lendület Laboratory of Systems Neuroscience, Budapest, Hungary*
[2] *Institute of Artificial Intelligence, Medical University of Vienna, Vienna, Austria*
[3] *Neurobiology and Neurodegenerative Diseases Research Group, Semmelweis University – HCEMM, Budapest, Hungary*
[4] *Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, Vienna, Austria*

Dopaminergic (dopamine - DA) and cholinergic (acetylcholine - ACh) systems play key roles in learning and rapid adaptation, influence decision-making and regulate behavior. Their dysregulation can be observed in neurodegenerative diseases such as Alzheimer's and Parkinson's, which can severely impair cognitive abilities. Although we know a lot about these neuromodulator systems, their dynamics during decision-making are still unknown.

Our goal is to investigate how an environment that requires continuous adaptation influences learning. What role do neuromodulator systems play in these adaptive mechanisms?

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 and ACh biosensors were injected to specific brain regions - the prefrontal cortex, basolateral amygdala - BLA and ventral striatum - VS and their activity was recorded by measuring fluorescent signals using fiber photometry.

Animals performed better and needed less trials to switch sides in predictable compared to 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. In cortex, DA increased after rewarded or omitted stimulus, whereas ACh spiked before the stimulus. In deep nuclei, DA encoded value and reward prediction error (RPE - difference between the expected reward and the actual reward received), showing elevated release for rewards in general, while ACh was more sensitive to reward uncertainty, showing a larger difference between lucky and likely rewards.

Mice perform better in a stable environment but adapt quicker in a variable one. The photometry results show that DA encodes RPE. ACh plays a decisive role in decision-making while also encoding RPE suggesting a cooperative yet distinct mechanism for evaluating outcomes and updating strategy.

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# 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, 1117 Budapest, Hungary*
[2]*Laboratory of 3D Functional Network and Dendritic Imaging, HUN-REN Institute of Experimental Medicine, 1083 Budapest, Hungary*
[3]*BrainVisionCenter Research Institute and Competence Center, 1094 Budapest, Hungary*
[4]*ReVision Implant NV, 3053 Haasrode, Belgium*
[5]*Micro- and NanoSystems, Department of Electrical Engineering, KU Leuven, 12 3000 Leuven, Belgium*
[6]*Laboratory for Neuro- and Psychophysiology, KU Leuven, Leuven 3000, Belgium*
[7]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, 1083 Budapest, Hungary*

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.

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# 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, Hungary*
[2] *Karolinska Institute, Division of Genome Biology, Department of Medical Biochemistry and Biophysics, Science for Life Laboratory, Stockholm, Sweden*
[3] *HUN-REN SZTAKI-SU, Rejuvenation Group, HUN-REN Supported Research Groups, Budapest, Hungary*
[4] *Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Faculty of Medicine, Budapest, Hungary*
[5] *EMBL, Chemical Biology Core Facility, Heidelberg, Germany*
[6] *Albert Szent-Györgyi Clinical Centre, Albert Szent-Györgyi Faculty of Medicine, University of Szeged, Department of Neurology, Szeged, Hungary*
[7] *HCEMM-USZ, University of Szeged, Human neuron physiology and therapy Core Group, Szeged, Hungary*
[8] *Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, Cambridge, United Kingdom*
[9] *Spanish National Cancer Research Centre, Genomic Instability Group, Madrid, Spain*
[10] *HCEMM-USZ, Univesity of Szeged, Human neuron physiology and therapy Core Group, Szeged, Hungary*

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 (U2OS$^{Q94}$). 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.

# Functional network analysis of EEG data for capturing transitions between perceptual states in object recognition

**Balázs Péntek**[1,2], Vasile V. Moca[2], Levente Varga[1,2,3], Raul C. Mureșan[2,4], Mária Ercsey-Ravasz[1,2]

[1]*Faculty of Physics, Babeș-Bolyai University, Cluj-Napoca, Romania*
[2]*Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania*
[3]*Faculty of Mathematics and Computer Science, Babeș-Bolyai University, Cluj-Napoca, Romania*
[4]*STAR-UBB Institute, Babeș-Bolyai University, Cluj-Napoca, Romania*

Functional networks (FNs) provide a useful toolset for the analysis of brain activity. In these networks, the edge weights are defined based on functional connectivity measures, one example being scaled correlation analysis (SCA). This method allows identifying neuronal synchronizations happening between two distinct sites of recordings, with temporal delays and across multiple timescales as well. Here we use SCA to extract FNs from an EEG experiment with 10 participants engaged in free visual exploration objects presented as lattices of deformed dots. To examine how brain networks change after the "Eureka" moment of recognition, we adopted a method introduced in our recent work: instead of averaging FN edge weights across trials, we compute and statistically analyze their distributions. Our approach provides a more robust way of studying FNs, avoiding the risk of information loss in the averaging process. It also led to the discovery of a dynamic yet stable network architecture consistent across datasets: a mostly bimodal edge weight distribution (EWD) with few strong 0-lag correlations (backbone) and many weaker links at various time delays. In this study, we extract FNs around stimulus onset and before subject response, separately for seen and unseen conditions to distinguish the perceptual states. Using the pre-stimulus period as baseline and Cliff's $\delta$ as effect size metric, we statistically compare EWDs. Because the distributions are bimodal, positive and negative modes are compared separately. We find no major differences between conditions in EWD changes immediately after stimulus onset. The differences are more pronounced before the response: on average, occipital and frontal scalp regions show greater change in their interaction patterns when object recognition is successful. Further investigation of the roles of correlated and anticorrelated interactions may clarify the contribution of dynamic network architecture to cognition.

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# Moculus: an immersive virtual reality system for mice incorporating stereo vision

Linda Judák[1,2], Gergely Dobos[3], Katalin Ócsai[4], Eszter Báthory[2], Huba Szebik[2], **Balázs Tarján[2,5]**, Pál Maák[6], Zoltán Szadai[1,2], István Takács[1], Balázs Chiovini[1,7], Tibor Lőrincz[1,2], Áron Szepesi[1,2], Botond Roska[8,9], Gergely Szalay[1,2] & Balázs Rózsa[1,2,7]

*[1]Laboratory of 3D functional network and dendritic imaging, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary*
*[2]BrainVisionCenter, Research Institute and Competence Center, Budapest, Hungary*
*[3]Bay Zoltán Nonprofit for Applied Research, Budapest, Hungary*
*[4]Department of Algebra and Geometry, Institute of Mathematics, Budapest University of Technology and Economics, Budapest, Hungary*
*[5]Doctoral School, Semmelweis University, Budapest, Hungary*
*[6]Department of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary*
*[7]Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary*
*[8]Department of Ophthalmology, University of Basel, Basel, Switzerland*
*[9]Institute of Molecular and Clinical Ophthalmology Basel, Basel, Switzerland*

Due to technical roadblocks, it is unclear how visual circuits represent multiple features or how behaviorally relevant representations are selected for long-term memory. Here we developed Moculus, a head-mounted virtual reality platform for mice that covers the entire visual field, and allows binocular depth perception and full visual immersion. This controllable environment, with three-dimensional (3D) corridors and 3D objects, in combination with 3D acousto-optical imaging, affords rapid visual learning and the uncovering of circuit substrates in one measurement session. Both the control and reinforcement-associated visual cue coding neuronal assemblies are transiently expanded by reinforcement feedback to near-saturation levels. This increases computational capability and allows competition among assemblies that encode behaviorally relevant information. The coding assemblies form partially orthogonal and overlapping clusters centered around hub cells with higher and earlier ramp-like responses, as well as locally increased functional connectivity.Text of your abstract (max 2500 characters)

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# 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, Dept. of Computational Sciences, Budapest, Hungary*
*[2] Semmelweis University, Semmelweis Doctoral School, Budapest, Hungary*
*[3] Óbuda University, Electrophysics Institute, Budapest, Hungary*
*[4] Niigata University, Department of Physiology, Niigata, Japan*
*[5] Niigata University, Center for Transdisciplinary Research, Niigata, Japan*
*[6] Zhejiang University, Interdisciplinary Institute of Neuroscience and Technology, Hangzhou, China*

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)

This work was supported by the Hungarian Scientific Research Fund (OTKA), grants NN118902 and K135837.

# 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] Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary*
*[2] Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

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 9$^{th}$ 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.

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

**Bettina Rákóczi**[1,2], Zsófia Ruppert[1,2], Ákos Diósdi[3], Enikő Csapó[3] László Vígh[1], Zsolt Török[1], Melinda E. Tóth[1]

*[1] Laboratory of Molecular Stress biology, HUN-REN Biological Research Centre, Szeged*
*[2] Doctoral School in Biology, University of Szeged, Szeged*
*[3] Lendület Laboratory of Microscopic Image Analysis and Machine Learning, HUN-REN Biological Research Centre, Szeged*

Heat shock proteins (HSPs) are evolutionarily conserved chaperones essential for maintaining protein homeostasis, which is disrupted in Alzheimer's disease (AD). Previously, our group observed that overexpression of HSPB1 ameliorated certain AD-related symptoms in mice, such as normalizing neuronal hyperexcitability, which is a key driver of epileptic seizures. However, the underlying mechanisms remain unclear. Over the past decade, a growing body of evidence has suggested that HSPs have several moonlighting functions, including the regulation of inflammatory processes. Indeed, we have shown that in acute brain injury HSPB1 increases the expression of pro-inflammatory cytokines without elevating cell death in the mouse brain.

In the present study, we aimed to investigate the role of HSPB1 in chronic neuroinflammation and glial activation in a mouse model of AD. To this end, the AD model (APP/PS1) mouse strain was crossed with an HSPB1-overexpressing line. The expression pattern of HSPB1 was assessed using immunofluorescence staining. Neuroinflammation and glial activation in the hippocampus and cortex were evaluated by qPCR and immunohistochemistry.

Due to a high incidence of epileptic seizures, female APP/PS1 mice exhibit increased mortality, which was markedly reduced by HSPB1 overexpression. Immunofluorescence staining confirmed HSPB1 accumulation and the presence of activated glial cells in proximity to Aβ plaques. Furthermore, immunohistochemistry revealed that the staining intensity of microglia cells in female APP/PS1/HSPB1 mice increased, thereby indicating a stronger activation of these cells. Moreover, a significant increase in the expression of inflammatory, astrocytic, and microglial marker genes was observed in both the cortex and hippocampus of APP/PS1 mice compared to wild-type controls. However, HSPB1 overexpression decreased cortical TNFα levels in male APP/PS1 mice and increased the expression of M2-associated anti-inflammatory microglial marker genes in APP/PS1 mice brain. These findings suggest a complex fine-tuning role for HSPB1 in the regulation of chronic inflammatory processes.

Taken together, our results indicate that HSPB1 exerts protective effects in the APP/PS1 model, as reflected by reduced mortality in APP/PS1/HSPB1 mice. Furthermore, HSPB1 overexpression may modulate microglial activation toward a tissue-repair phenotype.

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# 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] *Department of Neurology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged*

[2] *HUN-REN-SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged, Szeged, Hungary*

**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-43$^{A315T}$ 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-43$^{A315T}$ 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-43$^{A315T}$ 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.

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

**Botond Drahos[1]**, Tamás Láng[1], Valery Grinevich[2], Árpád Dobolyi [1,3]

*1 Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest 1094, Hungary*
*2 Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, University of Heidelberg, Mannheim 68159, Germany*
*3 Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest 1117, Hungary*

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 inhibion of MPOA OTR neurons during male social interaction increased c-FOS expression in the VMH, suggesting their involment in aggression regulation via this neuronal pathway. Targeted activation of the MPOA OTR-VMH pathway by local CNO injection through intracerebral cannula increased aggression and decreased positive valence behaviors.

In conclusion, OTR-positive MPOA neurons likely act as a central hub suppressing aggression by inhibitng the VMH and increasing prosocial behaviors. PTH2-containing PIL neurons may target this population, conveying somatosensory inputs, highlighting the OTR-positive neurons as a key relay integrating PIL inputs and influencing social behavior.

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# Syncing Minds: Exploring Interbrain Synchrony in Virtual Reality and the Real World

**Cornelia Pachner**[1,2,3], Anna Keil[3], Silvia Erika Kober[3]

[1] *Neuroethology of Communication Lab, Department of Ethology, Eötvös Loránd University, Budapest, Hungary*
[2] *Doctoral School of Biology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary*
[3] *Department of Psychology, University of Graz, Graz, Austria*

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# Investigation of persistent firing in the rodent and human cerebral cortex

**Csongor Bence Ludányi**[1], Gábor Molnár[1], Gábor Tamás[1]

[1]*HUN-REN-SZTE Research Group for Cortical Microcircuits, University of Szeged, Szeged, Hungary*

Communication between the neurons that form cortical neural networks occurs through complex patterns of electrical signals. These signals appear as rapid changes in membrane potential, known as action potentials. During my research in the HUN-REN-SZTE Cortical Microcircuits Research Group, I investigate the fundamental mechanisms underlying the generation and propagation of action potentials. The focus of my work has been on the properties of so-called persistent firing in neurons. This phenomenon consists of prolonged, high-frequency firing and plays a role in memory encoding and goal-directed decision-making. It can also be induced in brain slice preparations by a brief series of high-frequency stimuli; however, its exact mechanism of formation and function are not fully understood.

For my electrophysiological investigations, I applied the whole-cell patch clamp technique. To induce persistent firing, I established a somatic patch configuration on excitatory pyramidal neurons using microelectrodes with a resistance of 3–5 MΩ. Positive current steps were injected into the cells to trigger the phenomenon. For morphological identification and visual observation of the cells, I used DIC microscopy.

The presence of persistent firing has previously been described in certain interneuron subtypes in human samples, but it is less well known in pyramidal neurons. I conducted experiments on pyramidal neurons in layers II–III and V of the rodent cerebral cortex. With the application of a cholinergic agonist (carbachol) at a concentration of 10 µM, persistent firing could be induced in 31% of the experiments in layer V, whereas no persistent firing could be elicited at a reduced carbachol concentration (2 µM) or in experiments without the agonist.

In human samples, I performed experiments on layer V pyramidal neurons with the addition of 10 µM carbachol, in which persistent firing was observed in 1 out of 8 experiments.

Based on my results, the phenomenon of persistent firing can be induced in both rodent and human excitatory pyramidal neurons in layer V in the presence of a cholinergic agonist. No previous data have been found in the literature regarding persistent firing in human pyramidal neurons; therefore, my results offer a novel approach to the investigation of this phenomenon.

## All-optical measurements of synaptic responses evoked by contralateral hippocampal projections

**Dániel Balogh**[1], János Brunner[1], Antónia Arszovszki[1], Eszter Sipos[1], János Szabadics[1]

*[1] HUN-REN Institute of Experimental Medicine, Budapest*

Synaptic connections between individual hippocampal neurons underlie various episodic memory functions. However, studies almost exclusively focused on the local hippocampal connections, while anatomical tracing indicated that some of the major excitatory pathways (such as Schaffer collaterals) are similarly extended in the opposite hemispheres. Therefore, important components of the hippocampal circuits remained unknown.

To address this knowledge gap, we employ an all-optical approach where synaptic responses from individual hippocampal neurons are measured with voltage imaging while contralateral hippocampal projections are stimulated with light-sensitive optogenetic actuators. However, several methodological obstacles needed to be resolved for efficient measurements of these responses. Specifically, my experiments helped to find the optimal acquisition setting for a CMOS camera-based imaging and to improve the sensitivity of imaging with optimal spectral ranges of the Voltron sensor. Furthermore, we adapted our previous analysis methods to these experiments with timed stimulations allowing the identification of direct excitatory and disynaptic inhibitory responses elicited by contralateral fibers.

Thus, with this novel experimental approach we can map the synaptic connectivity between the two hemispheres of the hippocampus at unprecedented insights.

# Caskin scaffold proteins modulate mouse behaviour in an isoform and age-dependent manner

**Daniel Kimsanaliev**[1], András Török[1], Norbert Bencsik[1], Katalin Schlett[1]

*[1] Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

Neuronal scaffold proteins of the Caskin family play key roles in organising intracellular signalling pathways within synaptic regions. Caskin1 is highly enriched in postsynaptic densities, where it interacts with Shank2, a major organiser of the postsynaptic density (PSD). Previous studies have shown that the absence of both isoforms impairs spatial memory. Although Caskin1 and its isoform Caskin2 share structural similarity, their specific neuronal roles and whether they have compensatory actions are not yet clarified.

To investigate the behavioural roles of these isoforms, we tested Caskin1 KO, Caskin2 KO, and Caskin double knockout (dKO) mice at 2, 4, and 6 months of age in a behavioural battery assessing anxiety, sociability, repetitive actions, and hippocampal dependent learning. Double heterozygous (dHz) and wild-type C57BL/6J mice served as controls.

Caskin-deficient mice, especially older Caskin1 KO and Caskin dKO animals, showed reduced grooming time and shorter bouts in self-grooming test, suggesting impaired repetitive behavioural regulation, while their increased marble-burying activity indicated enhanced compulsive-like behaviour. Elevated plus maze results showed no significant genotype differences, similarly the three-chamber test which revealed intact sociability and social novelty preference. In contrast, the Morris water maze test confirmed spatial learning deficits in Caskin dKO mice while Caskin1 KO animals demonstrated milder impairments.

These findings indicate that Caskin scaffold proteins differentially influence behaviour. Repetitive actions are affected in an age-dependent manner, whereas spatial learning shows isoform-specific and compentasory regulation, with emotional and social behaviours remaining largely unaffected by the lack of Caskin isoforms.

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**Keywords:** Caskin1, Caskin2, knockout mice, repetitive behaviour, spatial learning and memory, scaffold proteins

# Probing laminar ensemble dynamics in the human neocortex using high-density intraoperative multielectrodes

**Domokos Meszéna[1,2,3#],** William Muñoz[4#], Richard Hardstone[4#], Rishab Jain[4], Mila Halgren[5], Charlie Windolf[6], Douglas J. Kellar[4], Brian Coughlin[1], Mohsen Jamali[4], Irene Caprara[4], Lucia Wittner[2,3], Réka Bod[2], Boglárka Zsófia Hajnal[7], Johanna Petra Szabó[7], Dániel Fabo[7], Loránd Erőss[7], Leigh R. Hochberg[1,8], Jeffrey S. Schweitzer[4], István Ulbert[2,3,7], Ziv M. Williams[4], Sydney S. Cash[1], and Angelique C. Paulk[1]

*[1] Center for Neurotechnology and Neurorecovery, Department of Neurology, Massachusetts General Hospital, Boston, MA, USA; Department of Neurology, Harvard Medical School, Boston, MA, USA.*
*[2] Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary.*
*[3] Faculty of Information Technology and Bionics, Pazmany Peter Catholic University, Budapest, Hungary.*
*[4] Department of Neurosurgery, Harvard Medical School and Massachusetts General Hospital, Boston, MA, USA.*
*[5] Brain and Cognitive Sciences Department and McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, USA*
*[6] Department of Statistics, Zuckerman Institute, Columbia University, New York City, NY, USA*
*[7] Department of Neurosurgery and Neurointervention, Faculty of Medicine, Semmelweis University, Budapest, Hungary.*
*[8] VA RR&D Center for Neurorestoration and Neurotechnology, Rehabilitation R&D Service, Providence VA Medical Center, Providence, RI, USA; School of Engineering and Carney Institute for Brain Science, Brown University, Providence, RI, USA.*

# These authors contributed equally

The layered architecture of the neocortex forms the fundamental basis for complex neural computations. However, identifying these layer-specific subcircuits and deciphering their functional contributions in the human brain remains challenging. Recent advances in high-resolution neural recording technologies now permit investigation of human cortical layers with unprecedented detail. We pioneered a rare opportunity to test state-of-the-art silicon probes (called Neuropixels) in the operating room setting during resection surgeries in the cases of tumors or epilepsy, or right before the implantation of a deep brain stimulator (DBS) in patients with Parkinson's disease. These probes feature hundreds of closely packed active contact sites, enabling high-density spatiotemporal sampling of extracellular single-unit activities. Spatiotemporally resolved morpho-electric properties of single units allow for sophisticated clustering of neural cell types. Using Neuropixels probes (N=21 participants) and histologically verified "thumbtack" laminar microelectrodes (N=7 patients) in human frontal and temporal cortex, we identified robust patterns of neurophysiological activity, including local field potential co-modulation and spectral features, that consistently segment the cortical column into its distinct layers. We found that neurons within these physiologically defined layers possess distinct electrophysiological profiles (waveforms, firing patterns, spatiotemporal dynamics), reflecting diverse underlying cell types. These cell types form layer-specific ensembles exhibiting characteristic oscillatory coupling patterns within and across laminae. Furthermore, these layer- and cell-type-specific subcircuits demonstrate distinct engagement profiles during

baseline activity, across fluctuations in arousal state, and during task performance, including visual perception. This work provides a validated framework for mapping laminar circuits *in vivo* and reveals layer-specific cellular dynamics in the human cortex, offering new insights into the neural basis of cognition and neurological disorders.

# The effect of chemogenetic manipulation of the medial septal cholinergic cell 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, Hungary*

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.

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

**Dorottya Várkonyi[1]**, Erika Eliza Kvak[1], Szidónia Farkas[1], Choi Muyong[1], Dóra Zelena[1,3]

[1]*University of Pécs, Medical School, Institute of Physiology*
[2]*University of Pécs, Medical School*
[3]*University of Pécs, Szentágothai Research Centre*

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.

# 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 Varga[1], Dóra Zelena[1]

*[1]Institute of Physiology, Medical School, University of Pécs, Centre for Neuroscience, Szentágothai Research Centre, Pécs, Hungary*

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.

# The role of AgRP neurons in the developing and adult brain

**Eszter Balkó**[1,2], Bence Rácz[1], Ferenc Mátyás[2], Boglárka Barsy[2]

[1] *University of Veterinary Medicine, Budapest, Hungary*
[2] *HUN-REN Institute of Experimental Medicine, Budapest, Hungary*

The regulation of food intake and metabolism is an essential function of every living animal. There is a delicate balance between energy intake and expenditure, which is regulated on multiple levels to ensure survival in constantly changing environments. The melanocortin system (built up by AgRP and POMC neurons) plays an important role in the regulation of food intake, energy expenditure, and adaptive behaviour.

AgRP neurons are the only known cell group so far that is able to sense peripheral hunger signals, integrate them, and induce feeding as well. While the neonatal loss of AgRP neurons leads to late-onset metabolic alterations, the same intervention causes profound anorexia in adult animals.

Using the $AgRP^{DTR}$ mouse model, we investigate the differential effect of AgRP neuronal loss on food intake in peripubertal, young adult, and middle-aged mice. We also try to characterize the changes in neuronal activity - which ought to compensate for the loss of AgRP neurons - using cFOS immunostaining in a fasted state. It is also known that AgRP neurons influence feeding and non-feeding related behaviours, so we perform different types of behaviour tests (EPM, OF, novel object recognition, cliff avoidance) after the induction of AgRP cell loss to parallel the appetitive and non-feeding related behavioural changes.

Our findings could help to understand the neural underpinnings of food intake and energy balance regulation, which is of crucial importance in order to find new therapeutic targets for metabolic diseases or to discover the connections between mental disorders and metabolism.

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# 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

**Evelin Szabó**[1], Katalin Várallyai[1], Kristóf László[1], Dóra Zelena[1]

[1]*Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary*

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.) $AVP^{Cre/Cre}$ 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 *V1*a 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.

# Long-term functional AAV-Mediated brain transduction in large animal models

**Fanni Somogyi[1,2],** B. Kovács[1,2], K. Csikós[1,2], Á. Petik[1,3], D. Horváth[1,3], A. B. Dobos[1], L. Wittner[1,4], D. Hillier[1,3,5]

[1] *Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
[2] *János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary*
[3] *Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary*
[4] *Neurology and Neurosurgery, Institute of Mental Health, Budapest, Hungary*
[5] *Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

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.

# Repetitive behavior of Cntnap2 KO mice and its changes by serotonin receptor agonist treatment

**Fanni Seres**[1], Vivien Szendi[1], Gina Puska[1,2], Bereniké Kovács[2], Árpád Dobolyi[1]

[1] *Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*
[2] *Department of Zoology, University of Veterinary Medicine Budapest, Budapest, Hungary*

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by repetitive behavior while social interactions are also affected. We previously described decreased expression of serotonin 2 receptor C subtype (5HT-2Rc) in the medial prefrontal cortex of adult rats kept isolated. Since these isolated rats also showed impaired social behavior resembling ASD-like behavior, we hypothesized that ASD symptoms can be reduced with serotonin agonist treatment. Several genes have been implicated in ASD including *Cntnap2* (contactin-associated protein-like 2) as genetical knocking out of this gene in mice causes autistic-like behaviors, such as repetitive behavior and social deficits. Thus, we used Cntnap2 KO mice as the autistic model animal to establish the effects of 5HT-2Rc agonist treatment. A total of 21 mice were used in the study, eleven of them were treated with a 5HT-2Rc agonist injected in the abdominal cavity, and ten of them were given vehicle solution. We assessed differences in social and repetitive behavior between serotonin-agonist-treated and control Cntnap2 KO mice.

To study behavioral differences between the groups, we used marble burying and social interaction tests. For the marble burying test, each animal was left undisturbed for 30 minutes in a box with 20 marbles placed on top of clean bedding of 5 cm. We assessed the number of marbles buried out of 20 by the two groups. Significant improvement was found in repetitive behavior using serotonin agonist treatment. Significantly fewer marbles were buried by the treated mice compared with the untreated animals, which compulsively buried the marbles.

To determine the sociability of the examined mice, we used the three chamber apparatus in which the mouse can choose between a familiar mouse and the empty side of the apparatus. In the next step, the social novelty preference was examined, the mice had to choose between a familiar and an unfamiliar conspecific. To find differences in direct social interaction, mice were placed in an open field apparatus with a familiar conspecific No significant changes in social interactions were detected in either of the social tests.

Overall, serotonin treatment markedly improved repetitive behavior in the Cntnap2 KO mouse strain, without having any effect on social deficits.

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# 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] *Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary*
[2] *HUN-REN Institute of Experimental Medicine, Budapest, Hungary*

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 different kinds of 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.

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# 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], Desdemona Fricker[2], Michael Graupner[2] and Lucia Wittner[1]

[1] *HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
[2] *Saints-Pères Paris Institute for the Neurosciences, Paris, France*

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.

# Can Independent Component Analysis Provide More Insights Into EEG-based Source Activation Patterns?

**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, Hungary*
[2] *Semmelweis University, Heart and Vascular Centre, Budapest, Hungary*

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. Moreover, scalp EEG reflects linear mixtures of multiple cortical sources, making source-level interpretation ambiguous. Independent Component Analysis (ICA), commonly applied for artifact removal, also able to separate statistically independent cortical sources from mixed EEG signals, which motivate us to explore this method on neuroaesthetics.

Participants viewed 80 paintings, each preceded by a 1-second cue and presented for 8 seconds, followed by a 4-second blank black screen for indicating their response. EEG activity was recorded using a 128-channel BioSemi ActiveTwo system at 2048 Hz. Preprocessing included filtering, ICA decomposition, classification of brain-related components using ICLabel and dipole fitting (DIPFIT). 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, e.g., event-related spectral perturbation (ERSP) and power analyses across conditions.

Applying this approach revealed both promising results and challenges. An occipital component cluster corresponding to the lambda wave (associated with saccade-related visual processing) was consistently identified across participants, alongside additional occipital and motor-related clusters, suggesting that meaningful neural sources can be recovered even in long, free-viewing epochs. Importantly, ICA enabled inspection of source activity at the single-trial level, preserving temporal dynamics otherwise flattened by averaging.

Consistent with the exploratory nature of ICA-based source separation, clustering quality remains a limitation. While anatomically plausible, component clusters often spanned broad cortical regions and varied across participants, raising questions whether weaker but functionally important sources are reliably detected, while dominant components are preferentially recovered under ICA constraints. These issues are particularly relevant for neuroaesthetics, where overlapping processes are expected. Ongoing work aims to improve component clustering strategies and expand condition-based analyses, with future directions including connectivity between source clusters.

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# 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, Hungary*
[2] *Department of Sports Biology and Kinesiology, Faculty of Sciences, University of Pecs, Pecs, Hungary*

[3] *Department of Sports Biology and Kinesiology, Faculty of Sciences, University of Pecs, Pecs, Hungary*

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.

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# *FRAG'N'FLOW*: Automated workflow for large-scale quantitative proteomics in high performance computing environments

**Istvan Szepesi-Nagy**[1,2], Roberta Borosta[1,3], Zoltan Szabo[4], Gabor Tusnady[1], Lorinc S. Pongor[5], Gergely Rona[1,6]

*[1]Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
*[2]Semmelweis University Doctoral School, Budapest, Hungary*
*[3]Doctoral School of Biology, ELTE Eötvös Loránd University, Budapest, Hungary*
*[4]Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary*
*[5]Cancer Genomics and Epigenetics Core Group, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary*
*[6]NYU Grossman School of Medicine, Department of Biochemistry & Molecular Pharmacology, New York, USA*

Although numerous databases exist for various disease contexts, a dedicated and comprehensive resource focused on the proteogenomics of nucleotide repeat expansion disorders (NREDs) — a major class of inherited neurodegenerative diseases including Huntington's disease, spinocerebellar ataxias, and ALS/FTD — remains unavailable. These disorders are characterized by progressive neuronal dysfunction, synaptic failure, and selective vulnerability of specific brain regions
Given the substantial variability inherent in proteomics data, arising from differences in instrumentation, experimental protocols, and technical biases, rigorous meta-analytical strategies are required to ensure meaningful interpretation of disease-relevant protein alterations. Two primary approaches are employed: (1) re-analysis of raw mass spectrometry (MS) data followed by normalization and statistical integration, and (2) aggregation of differential protein expression results across studies, retaining only consistently altered proteins. Our approach integrates both strategies by reprocessing raw MS data to generate differential expression profiles and applying predefined selection criteria to ensure inclusion of proteins associated with neural vulnerability and progressive neurodegeneration in NREDs.
A central component of our work is the deployment of the FragPipe (FP) proteomics suite within a high-performance computing (HPC) environment to enable efficient processing of large-scale brain and neural tissue datasets. To address the limitations of full-scale automation in FP's command-line interface, we developed FragFlow, a Nextflow-based pipeline that automates MS data processing and integrates downstream statistical analysis via the FragPipe-Analyst platform.
This workflow enables reproducible and scalable proteomic analysis of diverse proteomics datasets, facilitating large-scale meta-analyses of protein alterations associated with neuronal degeneration in NREDs. By systematically characterizing disease-associated changes in neural proteomic networks, our work provides a valuable resource for understanding molecular mechanisms driving neurodegeneration and identifying potential targets for therapeutic intervention.
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# The effect of Targeted Memory Reactivation in family dogs

**Írisz Legéndy**[1,2], Antónia Balogh[1,2], Dr. Anna Vargáné Kis[1], Dr. Dorottya Ujfalussy[1]

[1] *ELTE–HUN-REN NAP Comparative Ethology Research Group, Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Magyar Tudósok krt. 2. H-1117 Budapest, Hungary*

[2] *Department of Ethology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary*

While the exact role of sleep during the memory consolidation process is still debated, sleep and memory appear to be connected in humans. Dogs, due to their human-analogous socio-cognitive skills, have been proposed as suitable model species for research on certain human cognitive functions and behaviours. Non-invasive sleep EEG results of family dogs have been found to show similarities to human sleep EEG, suggesting that testing family dogs in similar paradigms could offer more insight into human sleep research.

The Targeted Memory Reactivation (TMR) paradigm can be used to enhance the effects of sleep on memory consolidation in a laboratory environment by selectively reactivating specific memories during sleep via presenting a previously linked sensory cue. The goal of our current study is to investigate the TMR paradigm in family dogs during a label-learning task.

We are currently recruiting family dogs, who are able to reliably execute 4 actions by commands and hand signs. During the training phase we assign new command words to these previously known actions, and for half of the actions we strengthen these associations via repeatedly presenting a sound cue during sleep. Our aim is to compare the differences in performance between the reactivated and the non-reactivated commands during a behaviour test.

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# 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] *Department of Physiology, Anatomy and Neuroscience, University of Szeged, 6720 Szeged, Hungary*
[2] *Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, the Netherlands*
[3] *Department of Computer Science, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, the Netherlands*
[4] *Allen Institute for Brain Science, Seattle, WA 98109, USA*

# equal contribution, § senior authors

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.

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# Exploring excitability changes in *in vitro* prefrontal cortical networks in a rodent model of autism

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

*[1] Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

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

To investigate alterations in the layer II- III prefrontal cortical cells and networks during early development, which result in a modified state in adulthood, we aim to develop an organotypic slice culture model. Organotypic prefrontal slices from 6-day-old mice were prepared for experimentation. By analysing active and passive membrane properties of neurons, as well as the spontaneous network activity using patch-clamp technique, the cellular parameters can be monitored. Recordings of slices at different ages in vitro demonstrate parallel development with the behaviour of acute slices from young animals. Our results indicate that the model recapitulates the trends seen in acute brain slices, indicating that its suitable for characterising the VPA-induced ASD-related changes during early development stages.

To investigate changes induced by VPA, cultures were treated on DIV 7 with 500µM VPA for 2 days. This time frame of treatment induced significant changes in neuronal properties and network activity as well. By week 3 in vitro, principal cells exhibit increased excitability, related to active and passive property changes. In addition, network activity is significantly boosted, showing an almost constantly active network of neuron in the mPFC.

The newfound hyperactivity and increased excitability of the layer II-III pyramidal cells may amplify the hyperexcitabiliy linked to mPFC in ASD. Our findings show that these principal cells based on their electrophysiological properties possibly contribute to the ASD phenotype in this brain region.

# 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], László Szapáry[2], Tünde Tóth[1], Dóra Reglődi, Beáta Polgár[3], Andrea Tamás[1]

*[1] Faculty of General Medicine, Institute of Anatomy, ELKH–University of Pécs PACAP Research Group*
*[2] University of Pécs, Heart Clinic, Electrophysiology Research Group*
*[3] University of Pécs, Faculty of General Medicine, Institute of Medical Microbiology and Immunology*

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.

Supervisor: Dr. Andrea Tamás, associate professor, Dr. Balázs Magyari, assistant professor

Support: University of Pécs, Romhányi College of Advanced Studies

## Molecular details of cell-cell communication between human stem cell-derived neural progenitor cells and microglia

**Khloud Gamal Abdelsalam**[1,2], Márk Gere[1], Krisztina Toth[3], Ádám Denes[3], Ágota Apáti[1], and László Homolya[1]

[1] *Institute of Molecular Life Sciences, HUN-REN Research Center for Natural Sciences, Budapest 2 Doctoral School, Semmelweis University, Budapest 3 HUN-REN Institute of Experimental Medicine, Budapest*

Neural progenitor cells (NPCs) play a pivotal role in neural development and tissue regeneration and thus hold great potential for stem cell-based therapies for conditions affecting neural cells or tissues. These include various neurodegenerative diseases, ischemic stroke, as well as traumatic brain and spinal cord injuries. A major obstacle of stem cell-based therapies is the limited integration of transplanted cells into neural tissue. Application of human induced pluripotent stem cell (iPSC)-derived NPCs or partially differentiated neural cells may alleviate these difficulties. The initial step of NPCs' differentiation is the outgrowth of neurites, which is determined by a combination of internal and external factors. Microglia, the brain-resident macrophage, patrolling the central nervous system, may also affect NPC differentiation. However, cell migration is key to the establishment of the NPC-microglial interaction. Our aim is to elucidate the molecular mechanisms that determine microglial migration and the neurite outgrowth and mobilization of NPCs.

We have previously demonstrated that BV2 cells (murine microglia) stimulated either toward pro- or anti-inflammatory direction enhanced elongation of neurites of human iPSC-derived NPCs, and that pro-inflammatory microglia even promoted NPCs' proliferation. As a continuation of this work, we now performed morphometry analysis using high content screening and analysis (HCS) and GFP-expressing NPCs to divulge the effect of pro- (IFN-γ, LPS) and anti-inflammatory (IL-4, IL-13) stimulated microglia on neurite morphology.We found that both the number and branching of neurites were stimulated in a similar way to what we observed in the case of neurite elongation. We also examined how pro- and anti-inflammatory stimulations influence BV2 microglia motility and how supernatants from stimulated microglia affect NPCs migration, which we evaluated using analyses combined with wound healing assay. In addition, we attempted to identify the main cytokines secreted by stimulated BV2 cells using a flow cytometry-based method. In addition, we attempted to identify the main cytokines secreted by stimulated BV2 cells using a flow cytometry-based method and tested the most prominent ones for their effect on neurite development of NPCs. Our ultimate goal is to understand the molecular details of cell-cell communication between NPCs and microglia to facilitate future developments that will make the therapeutic use of NPCs more effective by improving cell integration.

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**Key Words:** microglia-NPC interaction, iPSC-derived neural progenitor cells, neuroinflammation, cytokine signaling, neural regeneration

# 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[4, 5], 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[3], Szabolcs Takáts[5], Csaba Kerepesi[4,5], Roger A. Barker[3], Karolina Pircs[1,3,6]

[1]*HCEMM-Semmelweis University, Budapest, Hungary*
[2]*University of Cambridge, Cambridge, UK*
[3]*HUN-REN SZTAKI, Budapest, Hungary*
[4]*HUN-REN-SZTAKI-SU Rejuvenation Research Group*
[5]*Eötvös Loránd University, Budapest, Hungary*
[6]*Lund University, Lund, Sweden.*

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.

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

**Klaudia Csikos[1,2],** Abel Petik[1,3], Domonkos Horvath[1,3], Attila B. Dobos[1], Alan Urban[4], Daniel Hillier[1,2,3]

*[1] Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
*[2] János Szentágothai Neuroscience Doctoral School, Semmelweis University, Budapest, Hungary*
*[3] Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary*
*[4] Neuro-Electronics Research Flanders (NERF), VIB, Department of Neuroscience KU Leuven, imec, Leuven, Belgium*

Understanding the three-dimensional organization and stability of functional maps in the primary visual cortex is key to revealing how sensory representations are maintained and reorganized over time. We developed a multimodal experimental framework integrating functional ultrasound (fUS), electrocorticography (ECoG), and widefield optical imaging within a modular recording chamber optimized for long-term imaging under controlled developmental conditions.

High-resolution fUS imaging was used to examine the 3D organization of the cat primary visual cortex, focusing on interactions between retinotopic and orientation maps. This approach enabled identification of functional structures across spatial scales—from centimeter-level cytoarchitectonic regions to millimeter-scale pinwheel centers and sub-millimeter iso-orientation domains. The combined spatial resolution and hemodynamic sensitivity of fUS allowed detailed mapping of cortical architecture. Our results suggest that local iso-orientation domains are shaped by their embedding within global retinotopy, revealing interdependence between large- and fine-scale representations that supports efficient feature processing across the visual field.

Repeated imaging sessions produced stable and reproducible hemodynamic responses, indicating durable functional organization. Simultaneous ECoG recordings validated these findings and provided concurrent electrophysiological assessment of fUS signal quality. The system for widefield optical imaging has been successfully prepared and validated, paving the way for future multimodal integration.

This platform establishes a versatile approach for probing the stability and interconnection of cortical maps across spatial and temporal scales, offering new opportunities to investigate how large-scale organization, local circuitry, and adaptive processes shape sensory cortex function.

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# 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]*Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Biophotonics and Biomicrofluidics Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[4]*Department of Experimental Physics, Institute of Physics, University of Szeged, Szeged, Hungary*
[+]*Present address: Smurfit Institute of Genetics, Trinity College Dublin, Ireland,*
*FutureNeuro Research Ireland Centre, Smurfit Institute of Genetics, Trinity College Dublin*
[++]*Present address: University of Bergen, Bergen, Norway*

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.

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# A High-Content Screening Platform for Evaluating Immunogenicity of Antisense Oligonucleotides in hiPSC-Derived Microglia

**Laura Colar Zanjko**[1,2], Andrea Balogh[1], Anna Meller[1], Máté Lengyel[1], János Farkas[1], Melinda Zana[1], András Dinnyés[1,2]

*[1]BioTalentum Ltd, Aulich L u 26, 2100 Gödöllő, Hungary.*
*[2]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Hungary.*

Antisense oligonucleotides (ASOs) represent a rapidly advancing therapeutic modality for rare neurodegenerative disorders. These short, synthetic single-stranded nucleic acids selectively bind target pre-mRNA or mRNA sequences to modulate gene expression via RNase H-mediated degradation, steric blockade of splicing elements, or inhibition of translation. In the central nervous system, ASOs can also interact with microglia through phagocytic or receptor-mediated uptake, potentially eliciting cytotoxic or immunogenic responses.

In this study, we introduce an in vitro platform utilizing hiPSC-derived microglia for screening cytotoxic and immunogenic effects after ASO treatment. Microglial activation is accompanied by characteristic morphological changes that reflect shifts in surveillance, inflammatory signalling, toxicity, and phagocytic behaviour, making morphological profiling a valuable readout of their functional state. To enable objective and scalable quantification of these phenotypes, we developed a machine–learning–based classifier in CellProfiler Analyst. Based on TLR9 and p65 immunostainings, 261 intensity- and morphology-derived parameters were extracted for individual cells and used to train a Random Forest classifier model.

Precursor macrophages were induced from human iPSCs, after which microglia were differentiated by adding growth factors IL-34, M-CSF, and GM-CSF. Microglia were characterized with flow cytometry, immunocytochemistry, cytokine profiling, and phagocytosis assays.

Differentiated microglia were exposed for 24 hours to a panel of ASOs at varying concentrations, including the TLR9-activating ASO ISIS518477, the clinically approved nusinersen (Spinraza) used for spinal muscular atrophy, and atipeksen, an investigational therapeutic for ataxia telangiectasia. Lipopolysaccharide (LPS) served as a positive control for robust inflammatory activation.

The trained classifier successfully distinguished branching, rod-like, rounded, and amoeboid microglial morphotypes with F1-scores exceeding 0.9. By integrating structural and signalling-associated features, this approach provides a robust framework for identifying microglial activation states at the single-cell level. To conclude, we established a viable toxicity platform to screen hiPSC-induced microglia after ASO treatment.

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# 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, Hungary*
[2] *University of Pécs Medical School, István Ábrahám Nano-Bio-Imaging Core Facility, Pécs, Hungary*

**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 identityexist.

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# 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, Hungary*
*[2] Pázmány Péter Catholic University, Roska Tamás Doctoral School of Sciences and Technology, Budapest, Hungary*
*[3] University of Freiburg, Department of Microsystems Engineering (IMTEK), Freiburg, Germany*
*[4] University of Freiburg, University Center BrainLinks-BrainTools, Freiburg, Germany*
*[5] Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary*
*[6] Semmelweis University, János Szentágothai Neuroscience Doctoral School, Budapest, Hungary*
*[7] Semmelweis University, Department of Neurosurgery and Neurointervention, Faculty of Medicine, Budapest, Hungary*

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.

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

**Luca Darai[1]**, Tamara Cecília Kállai[1], Dávid Keller[2,3], Melinda Vitéz-Cservenák[1], Árpád Dobolyi[1]

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

The medial prefrontal cortex (mPFC) 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.

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# 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[1,4]

*[1] 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*
*[4] Division of Neurophysiology, Center for Brain Research, Medical University of Vienna, 1090 Vienna, Austria*

# These authors contributed equally.

Lesion studies demonstrated that the medial septum (MS) participates in working memory (WM) processes: MS lesions lead to deficits in WM performance of rats in a spatial alteration task. However, what activity features the MS show that may support WM has not been explored. To reveal how MS activity might support WM, we directly compared MS single neuron activity in mice performing a 2-alternative forced choice task with and without a WM component. We found that there is heightened MS engagement during the WM task, characterized by increased firing rates, sustained inhibition during the delay period, and selective suppression of theta-rhythmic neurons, suggesting an enabling role for the MS in hippocampal WM processing.

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# A locus coeruleus morfológiai változásai öregedő vad-típusú és PACAP-génkiütött egerekben

**Marcell Schmidt**[1], Dr. Dániel Pham[1], Dr. Balázs Dániel Fülöp[1], Dr. Balázs Gaszner[1], Dr. Tünde Tóth[1], Dr. Adél Jüngling[1], Dr. Dóra Reglődi[1], Dr. Andrea Tamás[1]

*[1] Department of Anatomy,HUN-REG-PTE PACAP Research Team, Centre for Neuroscience, University of Pecs Medical School, Pécs, Hungary*

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.

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

**Melinda E. Gazdik**[1,2,3,4], Anna A. Abbas[1,2], Balázs Kis[1,2], Ádám Tiszlavicz[5], Lea Danics[1,2], Kai K. Kummer[6], Katalin Schlett[4], Karri P. Lämsä[5], Karolina Pircs[1,2,3], Attila Szűcs[1,2,3,5]

*[1] Institute of Translational Medicine, Semmelweis University, Budapest, Hungary*
*[2] Hungarian Centre of Excellence for Molecular Medicine - Semmelweis University, HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Budapest, Hungary*
*[3] HUN-REN-SZTAKI-SU Rejuvenation Research Group, Office for Supported Research Groups (TKI), Hungarian Research Network (HUN-REN), Budapest, Hungary*
*[4] Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary*
*[5] Hungarian Centre of Excellence for Molecular Medicine, HCEMM Human Neuron Physiology and Therapy Core Group, Szeged, Hungary*
*[6] Institute of Physiology, Medical university of Innsbruck, Innsbruck, Austria*

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.

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# Investigating Information Transfer in EDR-Constrained Multi-Map simulations

**Mihály László[1]**, Ercsey-Ravasz Mária[1,2], Vasile Vlad Moca[2]

*[1]Babeș-Bolyai University, Cluj-Napoca, Romania*
*[2]Transylvanian Institute Of Neuroscience, Cluj Napoca, Romania*

Efficient cognitive function depends on the precise coordination of activity across distributed cortical areas. Impairments in this coordination are often linked to atypical structural connectivity, characterized by local hyper-connectivity and long-range deficits. This work employs a multi-map simulation environment to investigate how these structural constraints impact information transfer. Central to the approach is the organization of neuronal populations into 2D lattices where inter-map projections are governed by the Exponential Distance Rule (EDR) [1], allowing for biologically grounded axon decay rates and spatially realistic delay distributions.

One of our objectives is to evaluate and refine analytical methods for quantifying both coordination and information flow. We specifically examine the Oscillation Score (OS) [2] to assess synchrony, alongside Transfer Entropy and directed connectivity measures to identify signaling pathways between modular maps. By systematically comparing random map-to-map connectivity with EDR-governed projections, we examine how distance-dependent wiring, as opposed to stochastic coupling, influences inter-map synchronization and the emergence of gamma-frequency oscillations. We also evaluate how local membrane dynamics shape global information flow by contrasting inhibitory populations of either resonator or integrator types. Our long term goal is to examine the interactions between separately hypersynchronized neural maps to explore the functional consequences of such network states, which are theorized to underlie the coordination impairments observed in disorders like Autism Spectrum Disorder (ASD). We use multiple conductance-based neuron models, including Izhikevich-type and Hodgkin-Huxley models, to simulate realistic population dynamics. To ensure the robustness of the information transfer metrics, simulations incorporate stochastic miniature synaptic events (MINIs) and structured current injections, emulating biological background noise and controlled external inputs.

This methodology bridges mesoscale circuit dynamics and macroscale communication, providing a flexible platform to determine how structural organization shapes emergent coordination and to identify reliable methods for quantifying information propagation in complex neural systems.

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# Functional brain network hubs exhibit altered connectivity patterns in patients with temporal lobe epilepsy

**Noémi Gyüre**[1], Milán Márton Tüske[1], Gyula Gyebnár[1], Lajos Rudolf Kozák[1]

[1]*Medical Imaging Centre, Department of Neuroradiology, Semmelweis University*

**Purpose/learning objective**

Our long-term goal is to characterize individual functional brain networks in epilepsy patients. Here we investigated connectivity of hubs in resting-state functional MRI based networks of 28 healthy controls and 11 patients with left temporal epileptogenic foci.

**Methods/background**

In our framework nodes are defined as parcellation-derived regions of interest (ROIs) and edge weights as temporal correlation coefficients. We identified ROIs as hubs based on graph-theory metrics, and examined how hub-identification depended on five parcellation-schemes and density-based thresholding (1-40%). Group-level networks among hubnodes and individual seed-to-voxel connectivity maps were calculated. For group-level comparisons we used the F-statistics and false discovery rate (FDR) correction ($\alpha 1=0.05$) to compare networks, and two-sample T-test ($\alpha 2=0.001$) between connectivity maps.

**Findings**

Hubnodes of patients skewed to the left hemisphere in all parcellation schemes. Our multithreshold approach minimized arbitrariness of hub-identification. Sensorimotor areas that appeared central at low densities, were replaced by association areas as networks became denser. ROIs corresponding to default-mode areas appeared as hubs in all parcellations at most densities.

Group-level comparison of hub networks after FDR correction showed differences only at finer parcellations. We found differences in the connections of the left retroinsular area. The left posterior cingulate cortex showed stronger connections with the ipsilateral retroinsular and bilateral intraparietal regions in patients.

Analysis of connectivity maps showed altered connectivity between the left retroinsular cortex and bilateral prefrontal motor and opercular regions at finer parcellations. Similarly connectivity between left ventral visual complexes, ipsilateral parietal opercular regions and bilateral auditory areas showed differences even at lower resolutions.

**Conclusions**

Two different methods revealed connectivity changes of the left insular cortex in our patients. Comparing networks highlighted the altered connections of the posterior default-mode network, while correlation map analysis featured sensory areas.

**Acknowledgements**

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# Bidirectional control of generalized absence epilepsy networks via real-time direct depolarization of thalamocortical neurons

Tatiana P. Morais[1], Hannah L. Taylor[1], **Olivér Nagy[2]**, Ferenc Mátyás[3], Francois David[4], Vincenzo Crunelli[1], Magor L. Lőrincz[2]

*[1]Neuroscience Division, School of Bioscience, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK*
*[2]Department of Physiology, University of Szeged, Szeged 6720, Hungary*
*[3]Institute of Experimental Medicine, Budapest, Hungary*
*[4]Center for Interdisciplinary Research in Biology, College de France, Paris, France*

Absence seizures (ASs), characterized by bilateral spike-and-wave discharges (SWDs), are a hallmark of idiopathic generalized epilepsies. We investigated the role of thalamocortical (TC) neurons in the generation and termination of ASs using optogenetic techniques in freely behaving GAERS or WAG/Rij rats, a well-established AS model. We demonstrate that direct depolarization of ChR2-transfected TC neurons in the ventrobasal thalamic nuclei during quiet wakefulness (QW) reliably elicits ethosuximide- sensitive ASs that have similar duration and frequency to those of spontaneous ASs, while showing little and no eMect during active wakefulness (AW) and slow wave sleep (SWS), respectively. Light-stimulation of TC neurons fails to elicit ASs during AW, QW and SWS in non-epileptic control (NEC) rats, whereas it could evoke short ASs in Wistar rats, prevalently during QW. Notably, brief light stimulation eMectively halted ongoing spontaneous ASs in GAERS rats (i.e. both SWDs and immobility),immediately altering thalamic multi-unit activity from rhythmic to irregular firing, irrespective of the SWD phase at which it was delivered. These findings support the view that the excitability of cortico-thalamic-cortical network is highly behavioural state-dependent, with increased susceptibility to the induction of ASs during QW, thus questioning the necessity of low- threshold burst firing of TC neurons in the generation of these seizures. Moreover, they highlight the dual control of ASs by TC neurons, underscoring their potential as therapeutic targets for AS modulation.

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# Tuning of median raphe neurons during behavioural adaptation

**Péter Földi**[1,3], Norbert Hájos[2], Viktor Varga[1]

*[1] HUN-REN - Institute of Experimental Medicine, Subcortical Modulation Laboratory, Budapest, Hungary,*
*[2] HUN-REN - Institute of Experimental Medicine, Laboratory of Network Neurophysiology, Budapest, Hungary,*
*[3] Semmelweis University, János Szentágothai Doctoral School of Neurosciences, Budapest, Hungary*

The median raphe nucleus (MRN) modulates forebrain circuits via serotonergic and GABAergic outputs, yet single-unit dynamics during behavioural adaptation remain unclear. We hypothesize that distinct MRN neurons exhibit task- and state-dependent tuning that evolves as animals adjust to changing contingencies. To test this, we performed chronic multi-channel silicon probe recordings of single units in freely moving mice during adaptive behaviour. This approach enables stable tracking across sessions to disentangle sensory, motor, and outcome variables and reveal the computational motifs encoded in MRN activity.

Our preliminary results focus on optogenetically identified vGlut3+ MRN neurons projecting to the dorsal hippocampus. We found that some of these neurons exhibit learning-related changes in firing rate and temporal patterning, consistent with mixed selectivity for multiple task features. At the population level, MRN neuronal responses to external stimuli are highly heterogeneous, with pronounced cell-to-cell variability in tuning profiles and temporal dynamics. Additionally, based on our current results neighbouring MRN neurons tend to show low pairwise correlations and limited coactivation with vGlut3+ neurons, suggesting largely independent coding rather than strongly synchronized local ensembles. Ongoing analysis aims to quantify assembly dynamics, including the formation, reconfiguration, and coordination of functional neuronal groups during behavioural adaptation.

# 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, H7624 Pécs, Hungary.*
*[2] Centre for Health Technology Assessment and Pharmacoeconomic Research, Faculty of Pharmacy, University of Pécs, H-7624 Pécs, Hungary*

**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*

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

**Rafaella Mínea Riszt**[1,2,4], Balázs Knakker[1,4], Anna Padányi[1,4], Judit Inkeller[1,4], Antonietta Vitális-Kovács[1,4], Evelin Kiefer[1,4], István Hernádi[1,2,3,4]

[1]*Grastyán E. Translational Research Centre, University of Pécs*
[2]*Medical School, University of Pécs*
[3]*Faculty of Sciences, Department of Neurobiology, University of Pécs*
[4]*Centre for Neuroscience, University of 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.

# Investigating the role of the cholinergic lateral septum during Pavlovian conditioning

**Réka Kispál**[1], Írisz Szabó[1], Dániel Schlingloff[1,2], Balázs Hangya[1,3]

*[1]Lendület Laboratory of Systems Neuroscience, HUN-REN Institute of Experimental Medicine*
*[2]Laboratory of Systems Neuroscience, University of Copenhagen, Copenhagen, Denmark*
*[3]Division of Neurophysiology, Center for Brain Research, Medical University of Vienna, Vienna, Austria*

The lateral septum (LS) is a central integrative hub involved in processes such as feeding, anxiety, sociability and memory. Traditionally it is associated with the regulation of stress and emotional responses, however growing evidence indicates that the LS contributes to learning and processing context-dependent information. An often overlooked neuronal population is its cholinergic cells, which are important regulators of local circuit dynamic, yet their contribution to associative learning remains poorly understood.

ChAT-Cre mice (n=13) were trained in a probabilistic sound detection Pavlovian conditioning task. Animals were presented with either a likely reward or likely punishment predicting cue, followed by a delay, and the adequate reinforcement according to its specific probability distribution. To monitor cholinergic activity dynamics, we expressed GCaMP8 in cholinergic neurons of the LS and recorded bulk calcium signals using fiber photometry. Additionally, we assessed LS activity through its projection by expressing an acetylcholine sensor in the hypothalamus.

Behavioral results indicated that mice differentiated between reward- and punishment-predicting cues confirming that they successfully learned the task. In the LS, cholinergic activity showed robust reinforcement-evoked responses, differentiating between rewards and punishments, with stronger responses following punishments. In addition, cholinergic activity showed stronger responses to unexpected punishments than to expected, but it did not differentiate between expected and unexpected rewards. In contrast, hypothalamic acetylcholine showed higher responses to reward-predicting stimuli and did not differentiate between expected and unexpected outcomes. However, consistent with the LS, punishment evoked a stronger response than reward.

In summary, our findings show that lateral septal cholinergic neurons are actively engaged during associative learning and respond differently to rewarding and aversive events. The distinct response patterns observed in the LS and its hypothalamic projection indicate that cholinergic modulation within this circuit is not uniform. These results highlight the importance of understanding lateral septal cholinergic signaling in associative learning.

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

**Róbert Layous**[1], Ferenc Csikor[1], Márton Hajnal[1], Gergő Orbán[1]

[1] *Department of Computational Sciences, HUN-REN Wigner Research Centre for Physics, Budapest 1121, Hungary*

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 secondary visual areas (V2), 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 V2 region of mice. Importantly, we also found that layer 5/6 of V1, a layer innervated by top-down connections from V2, 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.

Acknowledgements

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# 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, Hungary*

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.

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# Investigation of cortical pyramidal cell cooperation in awake mice using two-photon microscopy

**Szandra Szedlacsek**[1]

[1] *HUN-REN Institute of Experimental Medicine*

The pharmaceutical and surgical treatment of temporal lobe epilepsy both have limited efficiency and can have different side effects. Our goal is to develop complementary therapeutic approaches that support the brain's natural functions and have no side effects. We hypothesize that in epileptic patients, attentional engagement with meaningful, information-rich stimuli, such as a visual stimulus, enhances the natural activity of the brain. Our earlier electrophysiological and two-photon microscopy experiments done on kainate-induced epileptic mice showed that in epileptic mice the efficiency of pyramidal cells' cooperation decreases in the sensory and primary association areas of the cortex. Our aim is to increase this cooperation by natural, information-bearing visual stimulation, that we achieve by projecting visual patterns (vertically moving horizontal black and white gratings) to mice to activate the visual cortex. In the current phase of our project, we used two-photon microscopy to examine the pyramidal cells' patterns in time and plane in healthy mice, comparing activity during the projection of a visual stimulus to a control state measured in the dark. Comparing these results with the experimental results of the kainite-induced model, among others, we found that, in the epileptic mice the overall activity decreases to a statistically significant extent during visual stimulation (and in some extend after projection), from a pathologically elevated baseline level, while in the healthy group, projection of the visual stimuli had no statistically significant effect. In addition, in the epileptic group, during visual stimulation, both the proportion of cells active across multiple frames and the number of frames where at least three cells were active, increased, while the coordination of cells in time was much stronger in healthy mice. We also found that in epileptic mice there was no frame in which more than three cells were active simultaneously, whereas in healthy mice, frames with more than three active cells occurred. In epileptic mice, the number of such triadic frames was higher than in controls and further increased during visual stimulation. In both groups, pivoting of the triangles formed by simultaneously active cells was observed, but whole-triangle shifts were only seen in epileptic mice. These findings indicate that visual stimulation can reduce epileptic severity during the projection and, in some extent, after projection.

# Cognitive decline detected with aotomatized long-term monitoring in ovarectomised Alzheimer model mice

**Tamara Cecília Kállai[1]**, Melinda Cservenák[1], Luca Darai[1], Dóra Zelena[2], László Détári[3], Árpád Dobolyi[1]

*[1] Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*
*[2] Institute of Physiology, Medical School, University of Pécs, Pécs Hungary*
*[3] Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

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.

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# Transcriptomic alteration of the medial preoptic area in socially isolated rats

**Tamara Hajdu**[1], Tamás Láng[2], Fanni Dóra [1,2], Botond Drahos[1], Kovács Réka[1], Árpád Dobolyi[1,3]

[1]*Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest 1094, Hungary*
[2]*Human Brain Tissue Bank, Semmelweis University, Budapest 1094*
[3]*Laboratory of Molecular and Systems Neurobiology Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest 1117, Hungary*

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 effects. The medial preoptic area (MPOA), a key hypothalamic hub for social motivation, however show poorly understood molecular plasticity 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 (elevated social sniffing, stronger preference for familiar conspecifics), but reduced time with novel animals and 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 ($|\log2 \text{ fold change}| > 0.5$, 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), neuromodulatory, peptide receptor activity and inhibitory components (Gpha, Cck, Gabrd, F2rl2), synaptic organization-related genes (Ntng1, Cpne9), and reduced cholinergic signaling capacity through 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 drive but reduced novelty preference, accompanied by coordinated transcriptional reorganization in the MPOA.

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# Social Touch Suppresses Aggression via Thalamic Mechanisms

**Tamás Láng**[1], Botond B. Drahos[1], Fanni Dóra[1], Dávid Keller[1], Ingrid Csordás[1], Vivien Szendi[2], Gina Puska[2,3], Valery Grinevich[4], Árpád Dobolyi[1,2]

*[1]Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University; Budapest, 1094, Hungary.*
*[2]Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Eötvös Loránd University; Budapest, 1117, Hungary.*
*[3]Department of Zoology, University of Veterinary Medicine Budapest; Budapest, 1078, Hungary.*
*[4]Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, University of Heidelberg; Mannheim, 68159, Germany.*

Aggression is a core social behavior that facilitates resource gathering, territory defense, and reproduction. However, unnecessary escalation of aggression is maladaptive, necessitating a mechanism for suppression. Using a rodent model, we propose that the posterior intralaminar thalamic nucleus (PIL) suppresses aggressive behavior by integrating tactile signals.
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, but visual and olfactory remained), and control pairs. Both isolated animals and those housed without tactile input displayed a significant increase in aggression compared to pair-housed controls. To examine the effect of tactile stimuli on PIL neurons, we employed fiber photometry, in which we expressed GCaMP receptors and implanted an optic fiber above the PIL to measure neuronal activity. The results showed increased neuronal activity immediately upon social touch, which decreased thereafter.
Functional investigations were conducted using chemogenetics (DREADDs) with resident-intruder tests. We expressed DREADDs and activated them with clozapine-N-oxide (CNO) on socially-tagged neurons in the PIL through the vGATE system. The stimulation of these neurons decreased aggression, while inhibition increased it. We also examined the PIL-medial preoptic area (MPOA) pathway by administering CNO locally to the MPOA using an intracerebral cannula. Selective stimulation of the PIL-MPOA pathway decreased aggression, while inhibition exerted the opposite action. Additionally, optogenetic activation of PIL neurons, using the opsin Channelrhodopsin 2 expressed through viral vectors, resulted in the immediate suspension of attacks. Finally, we investigated the role of the ventromedial hypothalamic nucleus's (VMH) projection to the PIL using selective pathway manipulation. In contrast to the PIL-MPOA pathway, chemogenetic stimulation of the VMH-PIL pathway increased aggression.
We concluded that PIL neurons process social tactile stimuli and, by their projections to the MPOA, decrease aggressive behaviour, while the VMH can override this mechanism, 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 or terminate aggression.
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# The role of the median raphe in learning

**Tamás Zsoldos**[1], Áron Orosz[1], Gábor Nyiri[1]

[1] *Institute of Experimental Medicine, Cerebral Cortex Researchgroup*

## Introduction

The median raphe region (MRR) is a key region of the brainstem that plays a role in regulating mood, particularly negative experiences. However, the precise function of MRR cells expressing vesicular glutamate transporter 3 (vGluT3) remains unknown. Since MRR activity increases during negative experiences, it can be assumed that vGluT3 cells are involved in the processing of negative emotional experiences.

## Objective

The aim of the present study was to investigate the role of the MRR vGluT3 pathway projecting to the hippocampus (HIPP) in the regulation of learning processes.

## Methods

Histological examinations were supplemented with immunohistochemical procedures and visualized using epifluorescence and laser confocal microscopy. Behavioral experiments were performed using an optogenetic approach.

## Results

During negative experiences (electric foot shock), the activity of MRR vGluT3 neurons projecting to the HIPP showed a significant increase, based on their c-Fos protein expression. Inhibition of these same cells prevented the formation of negative memory traces, as the animals not only failed to learn the association between the neutral stimulus and the aversive electrical stimulus used above, but also failed to recognize the background context.

## Conclusions

Our results show that MRR vGluT3 neurons are activated by aversive experiences and play a key role in linking negative experiences to environmental stimuli.

# A Miniaturized Closed-Loop tFUS Neuromodulation System for Rodent Studies: Acoustic Field Characterization and In Vivo Validation

**Veronika Házi**[1,2], Édua Édes[2,3], Barnabás Kovács[2,4], Mészáros Aletta[2,5], Somogyvári Zoltán[2], Eszter Sipos[6], Attila Szűcs[7], Tamás Kiss[2], Marcell Stippinger[2]

[1]*Semmelweis University, Doctoral School, Budapest, Hungary*
[2]*HUN-REN Wigner Research Centre for Physics, Department of Computational Sciences, Theoretical Neuroscience and Complex Systems Research Group, Budapest, Hungary*
[3]*Eötvös Loránd University, Biology, Budapest, Hungary*
[4]*Veres Péter High School, Budapest, Hungary*
[5]*Eötvös Loránd University, Physics and Astronomy, Budapest, Hungary*
[6]*HUN-REN Institute of Experimental Medicine, Virus Technology Unit, Budapest, Hungary*
[7]*Eötvös Loránd University, Physiology and Neurobiology, Budapest, Hungary*

**Introduction**
Transcranial focused ultrasound (tFUS) neuromodulation is a promising noninvasive technique for modulating neural activity with high spatial precision. While its therapeutic potential is increasingly recognized, reliable pre-clinical application requires careful characterization of the acoustic field and dedicated experimental systems enabling controlled in vivo stimulation. This work focuses on the development, characterization, and in vivo implementation of a miniature tFUS system for animal studies.

**Methods**
A custom miniature ultrasound transducer was developed and its acoustic pressure field was systematically characterized in an aquarium using a calibrated hydrophone. Spatial pressure and intensity distribution was assessed to ensure controlled and reproducible stimulation conditions. For in vivo application, a closed-loop neuromodulation system was designed and implemented in rats, integrating real-time EEG acquisition, sleep spindle detection, and ultrasound triggering. A custom 3D-printed headgear was developed to stably position the transducer on the animal's skull and allow stimulation. Both real-time and offline sleep spindle detection algorithms were implemented and validated to enable spindle-locked ultrasonic stimulation during sleep. Additionally, first proof-of-concept tFUS experiments were performed by targeting the motor cortex to assess immediate behavioral responses.

**Results**
The closed-loop system reliably detected sleep spindles and successfully triggered ultrasound stimulation in vivo. The 3D-printed headgear provided stable transducer placement without impeding animal movement or EEG quality. Initial proof-of-concept motor cortex stimulation elicited observable movements of the hind limbs and tail demonstrating effective ultrasonic neuromodulation in vivo.

**Conclusions**
This work establishes a fully characterized and experimentally validated miniature tFUS neuromodulation platform for animal studies. By combining precise acoustic field characterization, custom hardware, real-time signal processing, and closed-loop stimulation, the presented system provides a robust foundation for future investigations into state-dependent neuromodulation, sleep-related plasticity, and learning mechanisms using tFUS in vivo.

# Word learning biases in family dogs – a new ERP paradigm

**Viktória Bakos**[1,2,#], Marianna Boros[3,#], Kata Zoller[2], Anasztázia Gróf[2], Attila Andics[3,4], Dorottya Júlia Ujfalussy[1,2]

[1] *ELTE–HUN-REN NAP Comparative Ethology Research Group, Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Magyar Tudósok krt. 2., H-1117 Budapest, Hungary*
[2] *Department of Ethology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary*
[3] *Neuroethology of Communication Lab, Department of Ethology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary*
[4] *ELTE NAP Canine Brain Research Group, Eötvös Loránd University, Pázmány Péter sétány 1/C,, H-1117 Budapest, Hungary*

#These authors contributed equally

Dogs, immersed in the human social and linguistic environment, are exposed to speech on a daily basis, and much of human-dog interaction is mediated through the speech signal. With the recent surge of studies on dogs' speech processing and word learning abilities and the development of sensitive neuroscientific methods able to catch subtle differences in implicit knowledge, the debate about the nature of word representations in non-human animals has resurfaced.

Limited evidence and trainer/owner experience suggest that dogs' capacity to learn action labels far exceeds their abilities in learning object-label associations. To investigate whether such bias towards action label learning is rooted in dogs' greater intrinsic attention to movements than the shape of the objects, we designed a study in which novel object-label-movement triads are demonstrated to family dogs and formed associations are tested in a violation of expectation paradigm while measuring their brain activity with electroencephalograph (EEG).

In this novel ERP (event-related potential) paradigm aimed to test label learning in dogs establish new word-object-movement associations by using unknown objects and labels coupled with an assigned, arbitrary action (movement) performed with the introduced object. After demonstration trials (20 per triad, 60 over 3 sessions, 180 in total) the dog is presented with test trials (40 over 3 sessions, 120 in total) in which either the object or the movement or both are violated. Non-violation and violation trials are presented in an 80:20 ratio. This mismatch ERP response is compared to baseline responses where no violation occurs.

We predict that if actions are more attended and thus more probably associated with the label than objects then action violations will elicit a stronger mismatch ERP than object violations.

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# 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] Lendület Laboratory of Systems Neuroscience, HUN-REN Institute of Experimental Medicine, 1085, Budapest, Hungary*
*[2] Doctoral College, Semmelweis University, Budapest, Hungary*
*[3] Systems Neuroscience Laboratory, Department of Neuroscience, University of Copenhagen, DK-2200, Copenhagen, Denmark*
*[4] Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged, 6720, Hungary*
*[5] School of Medicine, Western Sydney University, Penrith NSW, 2751, Australia*
*[6] Division of Neurophysiology, Center for Brain Research, Medical University of Vienna, 1090 Vienna, Austria*

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.

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## The role of brain pericytes in the blood-brain barrier damage after ischemic injury

**Virág Fodor**[1], Koppány Párdi[1], Anikó Szecskó[1,2], Mária A. Deli[1], Szilvia Veszelka[1]

*[1] Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
*[2] Doctoral School of Biology, University of Szeged, Szeged, Hungary*

During ischemic stroke, blood flow is reduced due to the blockage of cerebral arteries leading to a decrease in oxygen and nutrient supply. The cytotoxic and inflammatory substances released from the injured cells also damaged the blood-brain barrier (BBB). Our previous results demonstrated that the histone deacetylase inhibitor suberanilohydroxamic acid (SAHA) has protective effects on brain endothelial cells in the human stem cell-based model of ischemic stroke. However, during ischemia, pericytes also suffer significant damage, so the aim of our current research was to investigate the role of pericytes in BBB impairment and the effect of SAHA on pericytes after ischemic injury.

We created BBB model using monoculture of human endothelial cells (hEC) and co-culture together with brain pericytes (bPC). To model stroke conditions, cells were kept in glucose-free medium under 5% $CO_2$ and 1% $O_2$ for 6 hours. After oxygen-glucose deprivation, normal cell culture environment was restored for 24 hours, and one group of cells was treated with SAHA during this reoxygenation. The viability of the cells was measured by impedance-based analysis and MTT assay, and the functions of the BBB were followed by transendothelial electrical resistance (TEER) and permeability measurements.

Under normoxia, SAHA had no significant effect on the BBB monoculture model. In ischemic model the co-culture of endothelial cells and pericytes proved to be more sensitive to the oxygen-glucose deprivation. Moreover, just in the co-culture system SAHA could increase the TEER and decrease the penetration of both permeability marker molecules indicating better BBB properties. In cell viability measurements, pericytes were more sensitive against ischemic damages than brain endothelial cells, and the lower concentration of SAHA had higher protective effect on pericytes.

Our results shows that the damages of pericytes is more remarkable than the injuries of endothelial cells after ischemic conditions. In addition, SAHA has a protective effect not only on endothelial cells but also on brain pericytes, and these results suggest that SAHA may useful as a therapeutic drug in the treatment of ischemia-reperfusion injury.

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# Rejuvenation in human derived induced neurons

**Vivien Pillár[1, 2],** Ágnes Varga[1], Kinga Vörös[1], Anna Abbas[1], Roland Zsoldos[1], Balázs Kis[1], Anikó Göblös[4], Lajos Kemény[4], Csaba Kerepesi[2,3], Karolina Pircs[1, 2, 5]

*[1]HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Institute of Translational Medicine, Semmelweis University, Budapest, HU*
*[2]HUN-REN-SZTAKI-SU Rejuvenation Research Group, Budapest, HU*
*[3] HUN-REN-SZTAKI BioAgeAI Research Group, Budapest, HU*
*[4]HCEMM-Szeged University, Szeged, HU*
*[5]Laboratory of Molecular Neurogenetics, Lund University, Lund, SE*

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.

# Generation and characterization of ALS mouse models via serum transfer from patients with clinically distinct disease progression

**Zalán Kádár**[1,2,3], Bernát Nógrádi[2,3], Rebeka Kristóf[1], Tamás Bellák[1], Krisztián Pajer[1], Zoltán Fekécs[1], József Engelhardt I.[2], Péter Klivényi[2,3], László Siklós[4], Antal Nógrádi[1]

[1] *Department of Anatomy, Histology and Embryology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged*
[2] *Department of Neurology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged*
[3] *HUN-REN-SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged, Szeged, Hungary*
[4] *Research Group of Neuronal Plasticity, Department of Biophysics, Biological Research Centre, Szeged*

Amyotrophic lateral sclerosis (ALS) is a degenerative disease affecting motor neurons, clinically characterized by a progressive loss of muscle strength. Both the pathomechanism and the clinical manifestations of the ALS show high heterogeneity, which cannot be fully replicated by transgenic animal models of the disease. However, novel phenotype-specific animal models may facilitate a better understanding of the ALS. Our research aimed to generate two ALS mouse models exhibiting motor phenotypes with different rates of disease progression. To this end, motor neuron degeneration was induced in Balb/c mice by passive transfer using blood serum from ALS patients with clinically slow or fast disease progression. Following four weeks of serum transfer (via ip. injections every other day), pathological alterations in the spinal cord and hindlimb skeletal muscles were examined by immunolabeling, with particular focus on motor neuron degeneration, denervation of the neuromuscular junction (NMJ), and leukocyte infiltration. In the fast-progressing group, marked NMJ denervation developed, whereas the slow- progressing group showed moderate endplate pathology. More pronounced leukocyte infiltration accompanied NMJ degeneration in the fast-progressing group. The fast-progressing group also showed higher presence of the chemokines CCL5 and CCL11 in the skeletal muscle, further supporting the importance of immune responses in ALS. The number of motor neurons did not differ significantly between the two groups; however, cytoplasmic mislocalization of the ALS-associated TDP-43 protein was observed. Our results demonstrate that passive serum transfer can induce various motor degeneration phenotypes in mice. Serum transfer primarily causes NMJ denervation, suggesting that the immune response primarily targets the NMJ. These models could be useful for mechanistic studies and testing therapies in the future.

# Emotional, molecular, and cognitive indicators underlying comorbid depression and anxiety in a mouse model

**Zsofia Eckert**[1,2], Diana Pejtsik[1,3], Zoltan K. Varga[1], Olga Wronikowska[4], Mate Toth[1], Mano Aliczki[1], Zsolt Borhegyi[1], Kornel Demeter[1], Eva Mikics[1]

[1] *Insitute of Experimental Medicine, Translational Behavioural Neuroscience research group*
[2] *University of Veterinary Medicine Budapest*
[3] *Semmelweis University*
[4] *Medical University of Lublin*

Depression and anxiety are highly prevalent mental disorders in today's society. Almost 50% of people diagnosed with major depression are also diagnosed with an anxiety disorder, and this co-occurrence (CDA) results in more severe symptoms and less effective pharmacotherapy. Despite these serious problems, the neurobiological background of CDA is not fully understood, partly due to the lack of translationally valid animal models. By developing a behavioral sampling protocol to identify stable behavioral traits we strived to examine the underlying emotional, cognitive and neurobiological factors of CDA. Mice underwent repeated assessments using commonly used anxiety and depression tests. Averaging outcomes across sessions allowed the detection of stable harm-avoidance and passive-coping traits, endophenotypes of anxiety and depressive disorders. These traits were then related to stress vulnerability using the Learned Helplessness (LH) test. This behavioral framework revealed two distinct subpopulations: a resilient group showing low trait anxiety and active coping, and a comorbid group with high trait anxiety, passive coping, and learned helplessness. Machine learning predictions enabled us to reduce the protocol to one anxiety-, the light dark test, one coping-, the forced swim test and the LH test. Applying this simplified model in female mice yielded similar results: the helpless animals showed higher anxiety and passive coping even before the LH test. Based on the human literature, higher trait anxiety can contribute to the development of depression through its effects on cognitive processes. For this reason, we examined the cognitive factors of the subpopulations via an automated home-cage system, we found that the comorbid group exhibited cognitive inflexibility and worse spatial learning. Finally, we performed RNA sequencing of the vHC and mPFC of both the female and male groups to examine the molecular background of comorbidity We found that the comorbid group in both sexes showed differences in synaptic plasticity, synaptic potentiation, learning, cognition and mitochondrion organization, while only the male cohort showed upregulation in cytoplasmic translation, and only the females in neuron axogenesis. In summary, we developed a translational model of CDA, enabling us to examine its molecular background. We identified novel targets, and we aim to investigate how their modulation plays a role in developing new therapeutic agents.

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# Investigation of the Changes in Autophagy 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, HU*
*[2] HUN-REN-SZTAKI-SU Rejuvenation Research Group, Office for Supported Research Groups (TKI), Hungarian Research Network (HUN-REN), Budapest, Hungary.*
*[3] Laboratory of Molecular Neurogenetics, Lund University, Lund, Sweden*
*[4] HCEMM, Szeged University, Szeged, HU*
*[5] Institute of Genomic Medicine and Rare Disorders, Faculty of Medicine, Semmelweis University, Budapest, HU*
*[6] Institute for Computer Science and Control (SZTAKI), Hungarian Research Network (HUN-REN), Budapest, Hungary*

In our society, aging and age-related diseases place an increasingly significant burden on healthcare systems and the overall population. However, investigating aging in human neurons are challenging due to their limited accessibility. Human induced neurons (iNs) which are directly transdifferentiated from human dermal fibroblast, retain the epigenetic and genetic characteristics of the donor. Therefore, iNs provide valuable insights into the cellular mechanism 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 aging 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 condition 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 characterized clusters. We use a dual reporter system LV.mCh-LC3.GFP to visualize autophagosomes before and after their fusion with lysosomes. For these experiments human dermal fibroblast from young and old donors were used. Following the converison, live-cell imaging was performed to monitor the starvation-induced changes in the autophagic flux. Images were acquired at 0, 30, 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 quaintification. We will asses the number, size and colocalization (yellow) of the autophagosomes (green) and autolysosomes (red) measured as punctas 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 primarly defined based on static autophagic markers.

# 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]*Translational Neuroscience Research Group, Szentágothai Research Center, University of Pécs, Hungary*
[2]*Department of Pharmacology and Drug Safety Research, Gedeon Richter Plc., Budapest, Hungary*
[3]*Department of Pharmacology and Pharmacotherapy, University of Pécs, Hungary*

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.

# 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 Mureș*
[2] *University of Debrecen, Debrecen, Hungary*

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.