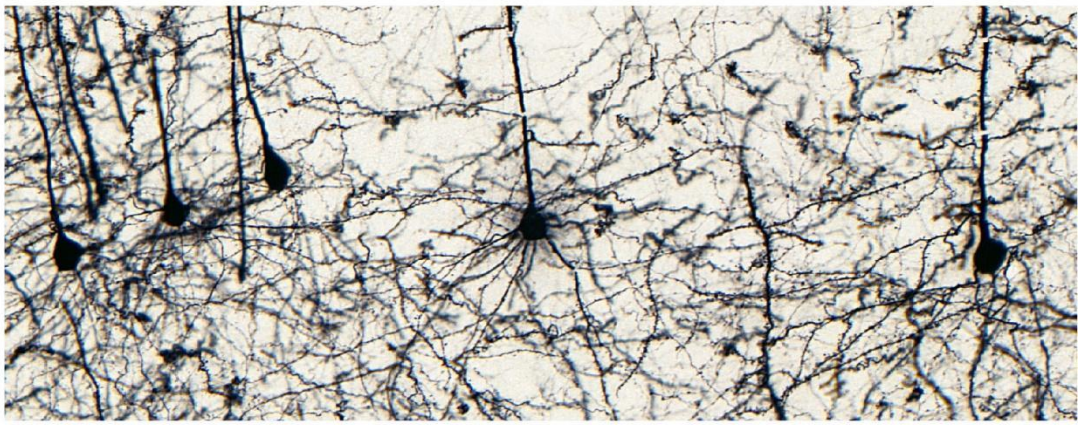
HuNDoC

5th Hungarian Neuroscience Meeting for Undergraduate Students, Graduate Students and Young Postdocs

OFFICIAL CONFERENCE BOOKLET



Hungarian Neuroscience Meeting for Undergraduate Students, Graduate Students and Junior Post-Docs

2022

ELTE, Lágymányosi Campus, North Building
Pázmány Péter sétány 1/A Budapest

facebook.com/hundocconference

hundoc2022.mitt.hu

2022 JAN 26

OUTLINE

Invitation

Dear Colleagues and Fellow Students,

We proudly invite you to participate in our 5th Hungarian Neuroscience Doctoral Conference for Undergraduate Students, Graduate Students and Junior Post-Docs. The meeting is to be held on the 26th of January 2022 in Budapest, as a satellite event preceding the IBRO Workshop 2022.

Come and share your research with other young investigators in a friendly relaxed atmosphere! It is a great opportunity to discuss the latest news in your scientific field with others walking in the same shoes, and to establish new connections with possible collaborative partners.

We are looking forward to a fruitful and successful meeting!

We hope to see you on this exciting event,

The Organizing Committee

General information

ORGANIZING COMMITTEE

Ákos Babiczky, Research Centre for Natural Sciences/BME Doctoral School of Psychology

Attila Ignácz, Eötvös Loránd University

Dávid Keller, Semmelweis University

Melinda Vitéz-Cservenák, Eötvös Loránd University

Acknowledgement

HunDoc 2022 is supported by the Buzsáki Fund.

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Website:

https://hundoc2022.mitt.hu/invitation

Contact us:

hundoc2022@gmail.com

PANDEMIC RESTRICTIONS

To attend the HUNDOC 2022 Conference:

A „COVID-19 Certificate" (Hungarian Vaccination Certificate or EU Vaccination Certificate) is mandatory.

In addition, verification of 3 vaccinations will be needed, which is possible e.g. by showing the plastic COVID-19 card (immunity certificate), or also by a vaccination document. The date of the third vaccination cannot be later than 9 January, 2022 unless the second vaccination was given within 4 months.

Furthermore, wearing an FFP2 face mask is mandatory on the site of the conference except for places designated for lunch and coffee break. If the pandemic situation requires, coffee breaks will be cancelled.

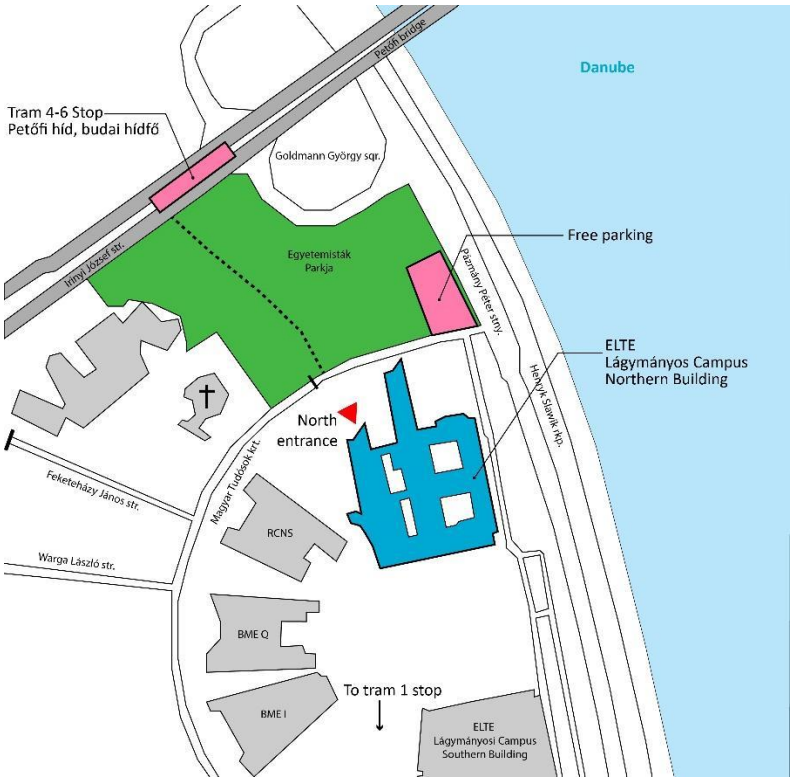
COVID-19 tests will not be mandatory for attending the meeting, but we strongly advise every participant to perform a self COVID-test (PCR of antigen) one day before the meeting for the safety of the community.

Venue

ELTE Lágymányosi Campus, Northern Building

Address: 1117 Budapest, Pázmány Péter Sétány 1/A

The building has several entrances. Conference participants are asked to use the entrance indicated in the map, where the registration will take place.



Approaching of the venue

Public Transportation

Tram 4/6: Petőfi Bridge, Buda end (stop Goldmann György tér)

Tram 1: Info park stop

Bus 12: Petőfi Bridge, Buda end (stop Goldmann György tér)

VENUE

Approach By Car from The Highways

M1 (E60, E75), M7 (E71): M1-M7 - Budaörsi út - Nagyszőlős u. - Bocskai út - Október 23. u. - Irinyi József u.

M3 (E71): M3 - Hungária körút - Könyves Kálmán körút - Lágymányosi híd - Pázmány Péter sétány

M5 (E75): M5 - Nagykőrösi út - Gyáli út - Könyves Kálmán körút - Lágymányosi híd - Pázmány Péter sétány

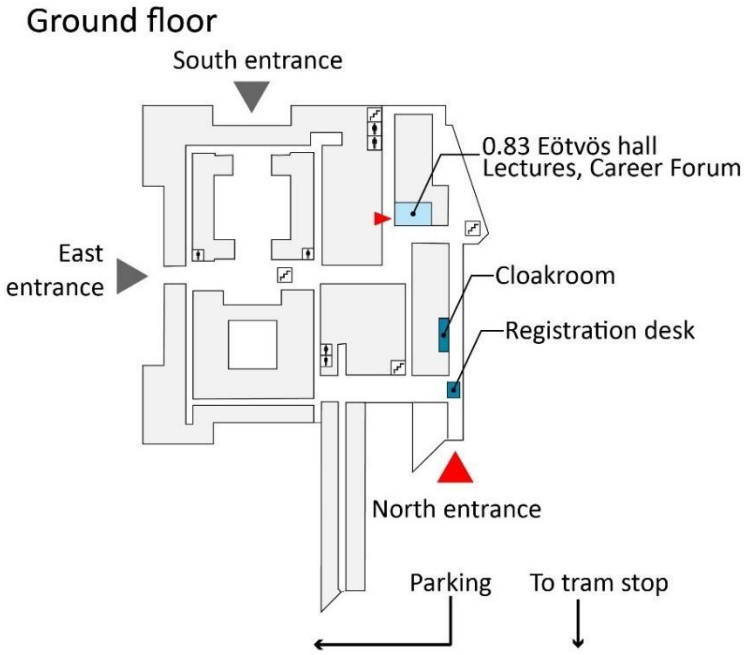
Parking

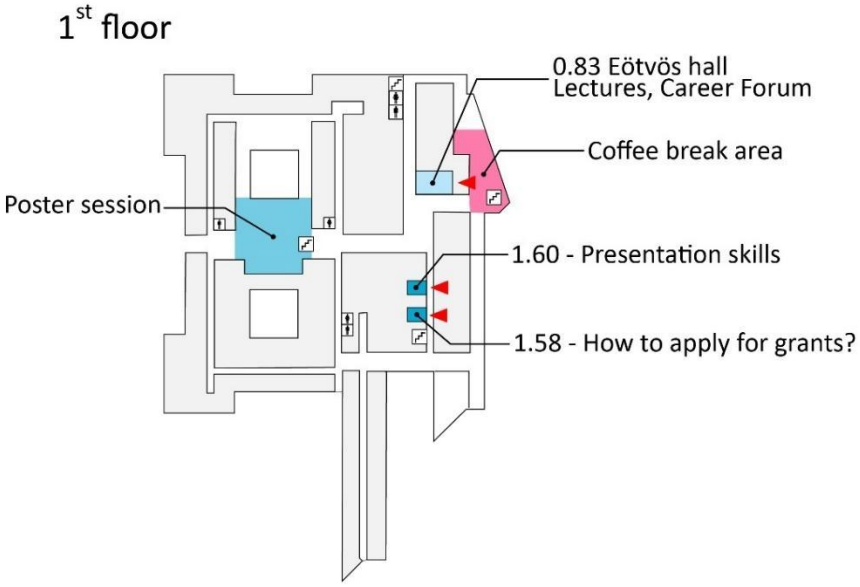
Available in the university parking lot. Those, interested in this option, should indicate the type of car and license plate beforehand via email.

Furthermore, there is a free parking area near the venue (see map).

VENUE MAP

Plenary lectures will be presented in the Eötvös Hall (0.83), while the poster presentation will take place in the Gallery of Sphere Hall (Gömb aula). Afternoon workshops will be held in the Eötvös Hall/0.83 (Career Forum) and rooms 1.58 (How to apply for grants?) and 1.60 (Presentation skills).





Scientific program

Our program includes one plenary lecture by Dr. Nóra Bunford, two sessions of oral presentations by selected speakers and a midday poster session. In the late afternoon, each registered attendee can participate in one of the thematic workshops (Career forum, Presentation skills, How to apply for grants?). All sessions and workshops will take place at the ELTE Lágymányosi Campus, Northern Building (Pázmány Péter stny. 1/A, Budapest).

Please note that the official language of the conference is English. All presentations must be prepared accordingly.

Oral presentations

Presenters selected for oral presentation must prepare a 10-minute presentation with slide show (preferred format: PowerPoint). For the lecturers, computer presentation facilities will be provided. Lecturers are kindly asked to give their presentations on USB stick to the technician before the morning or the afternoon session. Any special needs (e.g. the use of own laptop) should be discussed in time with the technician.

We ask the presenters to keep the time limits strictly.

An audience award for the best presentation will be assigned at the end of the conference.

Poster session

All registered participants, who submitted an abstract, must prepare a mini-poster (A4 size), except for those who were selected for oral presentation. Poster presenters will be assigned to groups of 4-6 and each participant will present their work to the others in the group in a less formal manner.

Ideal mini-posters are not shrunk versions of regular scientific posters. Keep text as little as possible, present the most essential data only. Keep in mind that there might be significant differences in scientific background and career stage among group members within each group.

Poster session will take place after lunch for 60 minutes. During this time, all participants should present their poster in the group. Please respect other group members' time: try to save time for others to present their work and leave room for discussion.

Digital posters are permitted, but technical support (e.g. tablets) for these will not be provided.

Workshops

There will be three different thematic workshops in the afternoon. For detailed description of each workshop, please visit the Workshops page.

After the registration is closed, each registered participant will be asked to choose a workshop.

Please note that, due to the limited number of available places, not everyone will be assigned to the workshop they preferred.

Please respect the organizers' decision and attend the adequate workshop only.

Social event

The evening social event will take place at Grund in a reserved space for HUNDOC 2022 conference attendees only. The event starts at 20:00.

Please note that the space reserved for us is a closed/heated terrace. Dress accordingly 😊

The HUNDOC 2022 Covid-19 restrictions are all applicable for the social event.

Venue website:

https://agrund.hu/

Address:

1082 Budapest, Nagytemplom str. 30.

PROGRAM OVERVIEW

09:00 – 09:45	Registration (North Entrance)
09:45 – 10:00	Welcome speech (Eötvös Hall)
10:00 – 10:45	Invited speaker: Dr. Nóra Bunford (Eötvös Hall)
10:45 – 11:00	Coffee break
11:00 – 12:30	Student symposia I. (Eötvös Hall)
12:30 – 13:00	Lunch break
13:00 – 14:00	Poster session (Gömb Aula)
14:00 – 15:30	Student symposia – II. (Eötvös Hall)
15:30 – 15:50	Coffee break
15:50 – 17:00	Thematic workshops Career Forum (Eötvös Hall) How to apply for grants? (1.58) Presentation skills (1.60)
17:00 – 17:15	Closing remarks (Eötvös Hall)

20:00 -	Social event (at GRUND)[1*]

[1*] For details, see page 11

Detailed program

09:00 – 09:45 – Registration (North Entrance)

09:45 – 10:00 – Welcome speech (Eötvös Hall)

10:00 – 10:45 – Invited speaker (Eötvös Hall)

Dr. Nóra Bunford (Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences)
Heterogeneity in adolescent attention-deficit/hyperactivity disorder (ADHD): In search for bio-behavioral risk and protective factors

10:45 – 11:00 – Coffee break

11:00 – 12:30 – Student symposia I. - Learning and connectivity (Eötvös Hall)

11:00 – **Bence Fogel**: Contribution of synaptic and intrinsic currents to in vivo-like neuronal activity
11:15 - **Camila Miranda**: Morphological and neurochemical characterization of glycinergic neurons in laminae I to IV of the mouse spinal dorsal horn
11:30 - **Vivien Szendi**: Lateral septum affects maternal adaptation via a parathyroid hormone 2 neuropeptide-containing pathway arising from the thalamus
11:45 - **Anna Virág Bakacsi**: The cellular and synaptic connectivity of the colliculo-thalamic network
12:00 - **Krisztián Zichó**: Brainstem can recall fear memory via hippocampal somatostatin interneurons
12:15 - **Kata Szamosfalvi**: Functional imaging of hippocampal CA1 pyramidal neurons during virtual navigation in mice

12:30 – 13:00 – Lunch break

13:00 – 14:00 – Poster session (Gömb Aula)[2*]

14:00 – 15:30 – Student symposia II. - Diseased brain (Eötvös Hall)

14:00 - **Barbara Asbóth**: Progenitor cells in the adult human retina
14:15 - **Evelin Patkó**: The protective effects of PACAP1-38 on the retinal vasculature and hypoxic molecules in rat glaucoma model
14:30 - **Diána Pejtsik**: Neurobiological correlates of trait anxiety
14:45 - **Péter Sere**: Cortical and subcortical neural dynamics during absence epilepsy
15:00 - **Panna Hegedüs**: Immunhistochemical characterization of the human and mouse septal area
15:15 - **Tibor Nánási**: Deep plasma proteomics reveal age-related molecular pathways modulated by GRF6019 treatment in Alzheimer's disease patients

[2*] For poster groups, see page 17

Detailed program

15:30 – 15:50 – Coffee break

15:50 – 17:00 – Thematic workshops

Career Forum (Eötvös Hall)
Dr. Éva Mikics (Institute of Experimental Medicine)
Dr. László Grand (Appercell Biotech Kft. CEO)
Dr. Ferenc Mátyás (Institute of Experimental Medicine, University of Veterinary Medicine, Research Centre for Natural Sciences)

How to apply for grants? (1.58)[3**]
Nóra Jeney (MSCA NCP, NKFIH)
Dr. Viktor Varga (Institute of Experimental Medicine)

Presentation skills (1.60) [4**]
László Róbert Zsiros (freelance science communicator)

17:00 – 17:15 – Closing remarks (Eötvös Hall)

[3**] Preregistered participants only!
[4]

Thematic workshops

Career Forum

Am I prepared for a career in neuroscience? What are the ups and downs of this life? Should I choose academic or industrial research? How much will I earn? Do I have the chance to have a family? Should I consider working abroad?

If You ever wondered about any of these questions, then the career forum is for You. In this workshop, invited speakers of different scientific backgrounds and at different career stages will share their insight and experience in a less formal manner.

Participants are encouraged to ask relevant questions directly from the speakers during the forum.

Presenters:

Dr. Éva Mikics (Department of Behavioural Neurobiology, Institute of Experimental Medicine)

Dr. László Grand (Appercell Biotech Kft. CEO)

Dr. Ferenc Mátyás (Institute of Experimental Medicine, University of Veterinary Medicine, Research Centre for Natural Sciences)

How to apply for grants?

In this workshop, we would like to offer some insights on grant proposals, especially postdoctoral fellowships, and we also aim to give advice about planning and writing a proposal. We will talk about how to identify potential funders and develop ideas into winning proposals tailored for their expectations. We will give some clues about the steps of preparing and submitting the proposal as well as about the review process. We will discuss what you can do during your graduate years to be prepared for a postdoc position, what are the advantages of networking and what you can earn from a grant apart from the financial support?

One of our presenters will be Nóra Jeney (National Research, Development and Innovation Office), who is the National Contact Point of the Marie Sklodowska-Curie Actions (MSCA). She will be accompanied by Dr. Viktor Varga (Institute of Experimental Medicine), who is a former recipient of the Postdoctoral Fellowship of MSCA and can share valuable experiences.

Presenters:

Nóra Jeney (MSCA NCP, NKFIH)

Dr. Viktor Varga (Institute of Experimental Medicine)

THEMATIC WORKSHOPS

PRESENTATION SKILLS

Some particularly enjoy delivering presentations, some would do anything to avoid them while most will simply resign themselves to the inevitable. The academic career path is littered with inexorable public speaking opportunities / obligations (underline as appropriate). These can range from seminars to conferences or even to unexpected TV or radio interviews.

In this workshop we will help each other to prepare for future public speaking possibilities / challenges / nightmares. Please, be prepared that this is NOT a frontal presentation on how to do a presentation. Instead, expect many lightweight communication games and exercises.

Laszlo Robert Zsiros is an award-winning Hungarian science journalist and edutainer with nearly 15 years of experience in stage science shows and online content creation. He has helped hundreds of STEM professionals to reach their science communication goals from college programs to FameLab Masterclasses.

Presenter:

László Róbert Zsiros (freelance science communicator)

Poster session

Poster group – I.

- Domonkos Nagy-Herczeg - Comparison of popular fluorescent actin markers to measure actin dynamics in dendritic spines
- Ádám Szatai - Optical recording of unitary synaptic connections using Voltron
- Ágnes Szabó - Shape memory polymer based transparent electrode array for long-term multimodal neuroimaging
- Beáta Barabási - Immunocytochemical and functional characteristics of cultured mouse brain endothelial cells in inflammatory conditions
- Balázs Barkóczi - Sensitivity study of two-photon laser scanning in mouse retina samples ex vivo

Poster group – II.

- Kristóf Furuglyás - Modelling of Neuronal Responses to Rotating Extracellular Electric Field Gradients
- Noémi Kis - Unique properties of dendritic Ca^{2+} spikes in hippocampal CA_3 pyramidal neurons
- Melinda Rácz - Saliency-map-based feature selection for electrocorticography-based brain–computer interfaces
- Domokos Meszéna - Spatio-temporal membrane potential and resistive current reconstruction from parallel multielectrode array and intracellular measurements in single neurons
- Louise Moysan - Mathematical modeling of ATP-evoked Ca^{2+} signaling in the Deiters' cells along the tonotopic axis of the cochlea

Poster session

Poster group – III.

- Lea Danics - RetaiN: Neuroimmunology and stress resistance in human ageing
- Zsófia Varga-Medveczky - Investigation of the nasal barrier function, inflammatory processes and brain morphology in healthy and diseased mice
- Jason Sparks - Role of PACAP in age-related systemic amyloidosis
- Dorottya Várkonyi - Can locomotor impairments and anxiety-like behaviour alter the measurable memory-decline in the triple transgenic mouse model of Alzheimer's disease?
- Anna Kellermayer - Microglia-neuron interactions in an animal model of Alzheimer's disease
- Attila Gáspár - Effects of intracerebroventricularly injected streptozotocin treatment on the cognitive performance of aged, experienced rats

Poster group – IV.

- Judit Berczik - Age-dependent role of midline thalamus in learning
- Ágnes Kandrács - Information flow between the dentate gyrus and CA_3 regions during sharp wave-ripple complexes in rat hippocampal slices
- Atilla Botond Kelemen - Simultaneous representation of environmental variables in the hippocampus
- Csaba Horváth - Travelling slow waves in the thalamus of anesthetized rodents
- Hunor Sebők - An alternative cholinergic innervation of the hippocampus
- Daniel Pham - Examination of the PAC1 receptor colocalization with Ca_{2+}-binding proteins and cochlea-efferent markers in the auditory pathway of pituitary adenylate cyclase-activating polypeptide - knock out (PACAP KO) and wild type (WT) mice

Poster session

Poster group – V.

- Brigitta Tekla Tajti - Using appetitive motivation to train mice for spatial learning in the Barnes maze
- Madhansai Narisetty - Computerized socio-behavioral analysis in color coded rodents
- Violetta Bartos - Comparison of anxiety tests presenting different amounts of novelty: The introduction of the Elevated Circular-Maze
- Timea Csorvási - Serotonergic anxiolysis in zebrafish requires novel or previously aversive experience
- Ábel Petik - Functional ultrasound imaging of deep visual cortex and beyond in awake cats
- Ward Fadel – Surface Laplacian based motor imagery images classification using deep learning

Poster group – VI.

- Ágota Vass - Unusual Perceptual Experiences and Beliefs Are Associated with Amplified Mnemonic Discrimination and Attenuated Generalization
- Péter Nagy - Reliability of functional connectivity estimation and modularity detection in resting-state EEG networks
- Fanni Dóra - Comparative transcriptome analysis of the dorsomedial prefrontal cortex associated with suicidal behavior
- András Adolf - Imaginary movement classification for Brain-Computer Interface Systems using 3D and 2D Convolutional Neural Networks
- Vivien Pillár - Simultaneous examination of neuromodulatory systems by fiber photometry and electrophysiology
- Csilla Lea Fazekas - Median raphe region serotonergic neurons regulate depressive-like behaviour related changes in body temperature during forced swim test

Poster session

Poster group – VII.

- Anikó Szecskó - Examination of viral peptide-targeted nanoparticles on a culture model of the blood-brain barrier
- Evelin Szabó – Use of glucose oxidase-based electrode (amperometric biosensor) in animal experiments
- Bence Tamás Varga - Attempt to transfer a pharmacological neurovascular uncoupling model from mice to rats
- Adrienn Szabó - Abnormal hypothalamic–pituitary–thyroid axis might influence the outcome of food-motivated learning tests in the triple transgenic Alzheimer's disease model mice
- Alex Horanszky - The impact of environmental exposures on the neuronal differentiation of pluripotent stem cells
- Emese Kincső Páli - Testing modified cyclodextrins on cell culture models of the blood-brain barrier

Poster group – VIII.

- Éva Pichner: Tophographical mapping of the frontal cortex related thalamic circuits
- Klaudia Sípos - Examination the role of nesfatin-1 in the supraoptic nucleus of rats
- Tamás Láng - Chemogenetic evidence that posterior intralaminar thalamic neurons stimulate maternal behavior in rats
- Dóra Keserű - Sleep effect of bromocriptine-evoked prolactin release suppression during the reproductive cycle
- Szidónia Farkas - Investigating the effect of female hormone depletion on the progression of Alzheimer's disease
- Roland Zsoldos - Analysis of ultrasonic vocalizations (USV) in mice

Poster session

Poster group - IX.

- Júlia Puskás - Excitability changes in prefrontal cortical networks in a rat model of autism
- Estilla Zsófia Tóth - Perisomatic inhibition and its relation to epilepsy and to synchrony generation in the human neocortex
- Péter Szocsics - Investigation of microglia's morphological changes in human post-mortem and surgical removed focal cortical dysplasia type 2 samples
- Anna Jász - Post-stress activity of calretinin positive cells in the paraventricular thalamic nucleus is required for long term, stress induced disturbance of sleep behavior
- Anna Velencei - Age-related changes in the activity of basal forebrain cholinergic neurons during Pavlovian conditioning

Poster group – X.

- Zsolt Buday - The role of calretinin positive midline thalamic neurons in stress induced behavioural changes
- Nikolett Arrasz - The role of PTH2 neuropeptide in social function – a study using PTH2 receptor KO mice
- Áron Orosz - A new pathway from basal forebrain somatostatin neurons to cortical areas
- Aletta Magyar - The dorsal midline thalamus effect over prefrontal cortex by different parallel pathway
- Anna Hegyi - Investigation of a pentapeptide carrier on culture models of biological barriers

Abstracts

Imaginary movement classification for Brain-Computer Interface Systems using 3D and 2D Convolutional Neural Networks

András Adolf[1], Csaba Márton Köllőd[1], Gergely Márton[1,2], István Ulbert[1,2]

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

2 Eötvös Loránd Research Network, Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary

"When designing a Brain-Computer Interface system, the classification of EEG signals is an essential task. In this work, we designed two architectures to recognize EEG patterns corresponding to the imagined motoric movements, using neural networks based on 3-dimensional and 2-dimensional convolutions. In the preprocessing step, we have reorganized the 2D EEG channel matrix related to their spatial arrangement, resulting in a tensor with 3 dimensions: SX x SY x Time. As signals have high variance between certain people, we applied Transfer Learning (TL) in the way of pretraining deep-learning models over 50 subjects, and fine-tune weights with the data of the subject we want to test on. The networks were tested on the EEG recordings of the Physionet database; 2-way and 4-way classification of motor imaginary movements were performed. Without TL, with 2D convolution, we gained higher accuracy – 2 classes: 75.7%, 4 classes: 46.5%, but after the application of it the 3D network performed better – 2 classes: 80.5%, 4 classes: 63.8%.

We have tested if the application of the FASTER artifact-rejection algorithm improves the accuracy or not, it has turned out that the effect highly depends on the subject we test on: in some cases, we have gained around 10% performance improvement, however, in some other cases the algorithm declined results by the same amount.

Finally, we examined how much does the result depend on the shift of the signal in time. The models were trained and tested with fixed time offsets, using 2D and 3D convolutional networks and Transfer Learning. The results of both networks showed that when the first 1 second of the original 4-second-long epoch is included, the accuracy is higher (around 60%), but when that part is not presented it declines to 30%. An interesting observation is that if the first second after the epoch is involved, accuracy starts increasing again, implying the recognizable information is presented not just at the beginning, but even at the stopping of the imagination."

This project was prepared with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program and the FK_19 funding scheme (FK132823) of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund. This research was also funded by the Hungarian Brain Research Program (2017_1.2.1-NKP-2017-00002) and TUDFO/51757-1/2019-ITM grant by the Hungarian National Research, Development and Innovation Office.

The role of PTH2 neuropeptide in social function – a study using PTH2 receptor KO mice

Nikolett Arrasz[1], Árpád Dobolyi[1]

1 Eötvös Loránd University, Department of Physiology and Neurobiology

Social behaviour is important for a variety of different species. In humans – one of the most sociable species – the impairment of social abilities can lead to neuropsychiatric disorders, which is an evidence of their significance. The role of parathormon 2 neuropeptide in social interactions was demonstrated in zebrafish (Anneser et al., 2020). Therefore, the role of the mammalian homologue of PTH2 neuropeptide, tuberoinfundibular peptide of 39 residues and its receptor, PTH2 receptor (PTH2R) in mammals is a relevant question.

Our objective was to examine the role of the TIP39-PTH2R neuromodulator system in social behaviour. We investigated the role of PTH2R in social tests, which were performed with PTH2R knock-out (KO) and wild type mice, in order to compare their behaviour. We carried out several supplementary tests, too, to examine the anxiety- and depression-like behaviour of the mice.

We found significant differences in the social novelty preference between wild-type (WT) and PTH2R KO mice. The latter spent more time with familiar (their previous cagemates) rather than with an unfamiliar mouse, which is in turn a characteristic of wild type mice. Another finding is that knockout mice had an increased latency of sniffing behaviours in social environment, which is an introductory behaviour between mice. In addition, knockout mice's general activity in open field was lower than the wild type mice's, which suggests the possibility of a generally increased stress level in mice lacking the PTH2 receptor. This is known to cause social stress in the animals.

In conclusion, the data suggest that different aspects of sociability are affected in the absence of the PTH2R suggesting a function of the TIP39-PTH2R system of the brain.

Reference:

Anneser, L., Alcantara, I. C., Gemmer, A., Mirkes, K., Ryu, S., & Schuman, E. M. (2020). The neuropeptide Pth2 dynamically senses others via mechanosensation. Nature, 588(7839), 653–657."

Grant support: NKFIH-4300-1/2017-NKP_17 (National Brain Research Program), OTKA K134221, and TKP2020-IKA-05.

Progenitor cells in the adult human retina

Barbara Asbóth[1], Lili Gerendás[1], Dániel Magda[1], Ferenc Kilin[1], Sándor Lovas[1], Zoltán Zsolt Nagy[2], Arnold Szabó[1]

1 Semmelweis University, Department of Anatomy, Histology and Embryology, Retina Laboratory, Budapest, Hungary

2 Semmelweis University, Department of Ophthalmology, Budapest, Hungary

The retinas of lower vertebrates have a certain degree of regenerative capacity. The main element of regeneration is the Müller cell which can also generate new glia and neurons by entering the cell cycle and transforming into a multipotent progenitor stem cell. Müller cells in the mammalian retina were thought to lose their progenitor nature and their ability to divide. However, recent studies have indicated that division and neurogenesis of Müller cells in adult rodents can be induced under specific conditions. There is insufficient data on the regenerative capacity of Müller cells in the human retina. Eyes of adult multi-organ donors without known ocular disease were enucleated within an hour of cardiac arrest and were subjected to immunohistochemistry on oriented frozen sections and whole mount preparations after fixation. The Ki-67 proliferation marker detected a significant amount of dividing cells. A subset of Ki-67 positive cells expressed Pax6 protein, indicating their retinal origin. A small fraction of dividing cells colocalized the Müller cell-specific Sox9 protein. The expression of Sox2, Pax6 and S100β proteins showed a significantly decreasing gradient from the periphery to the centre. The anti-S100β antibody labelled a subgroup of Müller cells. Labelling with the Müller cell-specific Sox9 protein revealed co- expression of Sox2 and Pax6 proteins in a fraction of peripheral Müller cells. To our knowledge, we are the first to demonstrate that Müller cell division occurs in vivo in the original, intact, three-dimensional environment, without addition of growth and other stimulatory factors. Contrary to previous concepts, our studies suggest that human Müller cells are composed of several functionally distinct populations. Furthermore, a subset of peripheral Müller cells exhibits properties of retinal progenitor cells, and in the adult human retina at least a fraction of Müller cells retains the ability to divide. Our findings suggest that selective, vector-mediated transduction of Müller cells could be used to induce their division in a targeted and controlled manner. Based on the results of experiments in rodent models, it may be feasible in the future to replace lost neurons by genetic reprogramming the generated cells.

The cellular and synaptic connectivity of the colliculo-thalamic network

Anna Virág Bakacsi[1,2], Kinga Kocsis[1,3], Ferenc Mátyás[1,3,4,5]

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

2 Eötvös Loránd University, Budapest, Hungary

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

4 University of Veterinary Medicine, Department of Anatomy and Histology, Budapest, Hungary

5 Institute of Experimental Medicine, Budapest, Hungary

Associative learning is essential for adaptation in a constantly changing environment. When a neutral (conditioned) stimulus (e.g. sound; CS) appears along with an affective (unconditioned) stimulus (e.g. pain; US), a memory trace about the conditioned signal is formed. Then, at a later time-point, a fear response is elicited even if only the CS (the former neutral stimulus) is presented. This phenomenon is called associative fear learning. The anatomical background behind CS-US pairing contains a network, where these two pieces of information are able to converge on a single neuron. Our recent data suggests that this occurs before the conventionally accepted brain area, the lateral amygdala (LA), in the tecto-thalamic circuit, at the level of the LA-projecting calretinin-expressing lateral thalamic (CR+LT) cells. However, the exact anatomical evidences for this theory are missing. The tectal part consists of the midbrain's paired structures, the inferior (IC) and the superior colliculus (SC). They can transmit uni- and multimodal information (auditory, visual, somatosensory, etc.) to the thalamus. To investigate the organization principles of dual tecto-thalamic circuits, we used classical and viral tracing combined with immunohistochemical approaches in mice. We demonstrate that, although the colliculo-thalamic innervations have different topography, both are positioned to form synaptic contact on the same CR+LT cell. This convergence is likely to be present both between glutamatergic and the GABAergic collicular cell populations. Our findings show that multisensory information mediated by the SC and the IC can be converged on the same CR+LT cell. In summary, the presented tecto-thalamic pathways can jointly inhibit or excite the CR+LT cells and these complex synaptic transmissions can shape thalamic signal integration during the associative fear learning process.

Acknowledgement: This work was supported by the Hungarian Brain Research Program (KTIA-NAP-13-2-2015-0010; 2017-1.2.1-NKP-2017-00002), by the National Office for Research and Technology (K138836 and KKP126998), by the New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-19-4-ÁTE-8; ÚNKP-20-5-ÁTE-3). FM is a János Bolyai Research Fellow.

IMMUNOCYTOCHEMICAL AND FUNCTIONAL CHARACTERISTICS OF CULTURED MOUSE BRAIN ENDOTHELIAL CELLS IN INFLAMMATORY CONDITIONS

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Hypertriglyceridemia and atherosclerosis are linked to inflammatory processes and increased production of pro-inflammatory cytokines including interleukin-6 (IL-6), which may alter blood-brain barrier (BBB) function.In the present work we examined primary brain endothelial cells isolated from wild type and transgenic mice which overexpress the human APOB-100 protein and show chronic hypertriglyceridemia. The cells were analyzed under control conditions and following pro- and anti-inflammatory cytokine treatments (IL-6, IL-10 and IL-6+IL-10) mimicking different inflammatory signals. Our experiments focused on functional and immunohistochemical characteristics of the BBB measuring paracellular permeability, transendothelial electric resistence (TEER), P-glycoprotein (P-gp) activity, and fluorescence intensity of tight junction proteins (claudin-5, occludin, ZO-1) and P-gp immunolabeling.Cultured brain endothelial cells from APOB-100 transgenic mice showed an increased paracellular permeability compared to wild types under control conditions, which was further increased after IL-6 and IL-6+IL-10 treatment. TEER was lower in transgenic endothelial cells than in wild-type cells without cytokine treatment. This difference was enhanced following IL-6, IL-10 and IL-6+IL-10 administration. In accordance with these observations an increased whole cell occludin and ZO-1 and a decreased P-gp immunofluorescence intensity was detected in transgenic cells compared to wild types in control conditions. Following different cytokine treatments the observed changes were partially enhanced. P-gp function assay showed a decreased activity in APOB-100 transgenic cells compared to wild types without cytokine treatment, and all cytokine application resulted in an increase in P-gp activity compared to control cells in the transgenic group. Our results suggest that inflammatory conditions linked to hypertriglyceridemia may damage BBB function by altering P-gp activity and the expression of some crucial proteins in brain capillary endothelial cells.

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Sensitivity study of two-photon laser scanning in mouse retina samples ex vivo

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Two-photon microscopy (TPM) is an important fluorescence imaging technique in biological sciences which provides high penetration depth, inherent three-dimensional sectioning and good detection sensitivity. However two-photon laser scanning (TPLS) of the eye is not well studied up to now. Previous calculations have shown that the required optical resolution can be achieved in the eye in vivo, and the aberrations can be corrected by adaptive imaging techniques. Therefore TPM seems to be a very useful method to scan the different layers of retina, although the harmful effects of TPLS have remained elusive. Using TPM, the whole mount retina samples of B6 wild type or Thy1-GCaMP6f transgenic mice were investigated ex vivo. The feasibility of visualizing cellular structures was tested, and the harmful effects of TPLS were specified. According to our results the living, native retina samples of B6 mice contained only few autofluorescent molecules, which are excitable by TPM. In contrast the ganglion cell layer of Thy1-GCaMP6f transgenic animals represent green, clearly visible and functioning ganglion cells. The imaging of B6 mice retina can be facilitated by fluorescent dyes, such as DAPI, and ethidium bromide, however these dyes has strong negative effect on cell viability. TPLS alone can easily damage any retinal tissue. The extent of destruction robustly depends on the following scanning parameters: duration, intensity, and wavelength. Duration and intensity dependence of demolition seems to be linearly increasing and additive. Moreover increased intensity can affect the retina tissue more powerful compared to the TPLS duration. Over 80 mW scan intensity the destruction is visible to the naked eye in every cases. The wavelength of scanning laser can also act differently in the retina, which may due to the sensitivity and adsorption unconformity of biomolecules. 10 minutes 30mW or 1 minute 300mW line scanning at 880nm can destroy almost all layers of retina. The eye is the only part of the body where optical examination of the nerve tissue can be directly performed. TPM will be a promising method for the three-dimensional sectioning of retina samples, if the limitations of TPLS are well characterized. Our results will facilitate the preparation of a functional two-photon ophthalmoscope that can be used for scanning the human retina. Thereby the research of such central nervous system diseases will be available as Alzheimer's and Parkinson's disease.

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Comparison of anxiety tests presenting different amounts of novelty: The introduction of the Elevated Circular-Maze

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Anxiety disorders are causing enormous problems worldwide while their background mechanisms and risk factors are poorly understood. The best way to research such illnesses is by developing animal models. However, a critical limitation of these is that anxiety tests measure transient states of animals, but the individuals' experiences would bias a trait-focused repeated sampling. Interestingly, in tests like the most popular Elevated Plus-Maze (EPM), avoidance increases with experience, implying enhanced anxiety. Alternatively, it is possible that as novelty decreases, animals lose their motivation for exploration, meaning that repeated measures are no longer providing information of an anxious state. In the present study, we aimed to investigate these potentially overlapping hypotheses and validate measures of repeated sampling designs. We created a novel anxiety test, the Elevated Circular-Maze (ECM), that offers a classical approach-avoidance conflict as the EPM but presents an enhanced amount of novelty, aiming to sustain explorative motivation in a repeated experimental design. The ECM is a circular platform consisting of twelve wall-separated hiding areas and an exposed interconnecting edge. We conducted a systematic comparison with the EPM through three pilot experiments in mice to investigate the short- and long-term impact of prior experiences of different frequencies and adversity. In the ECM, mice were more explorative than in the EPM through all experiments, as indicated by several measures. However, similar to EPM, ECM exploration also decreases after more time or occasions spent in the test. Interestingly, in contrast to EPM, ECM exploration is negatively correlated with the number of previous repeats and positively correlated with the length of intervals between test occasions. In addition, animals that experienced previous adversity did not change their behaviour in response to test repetitions. Overall, first-day behaviour predicted the outcome of the following tests, regardless of experimental conditions. In summary, we found that novelty and prior adversity are both elements of experience-based decrease of exploration in these tests. However, ECM triggers more explorative motivation in mice and is slightly less sensitive to previous repeats, implying its potential in preclinical experimenting.

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Age-dependent role of midline thalamus in learning

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It is well-documented that arousal–dependent cognitive functions like memory processes, sleep-wake cycles and stress management are age-dependent. The thalamo-frontal network goes through age-related changes, which could be responsible for these behavioral alterations. However, the exact causal neuronal mechanism is not fully understood. Previously, the calretinin (CR)-expressing thalamocortical cells in the dorsal midline thalamus (DMT) were identified as an important neuronal network element for cortical arousal. Furthermore, DMT cells were also shown to modulate associative learning. Thus, we aimed to clarify the age-dependent role of the CR+DMT neurons in fear learning processes. First, we found that the bi-directionally control of CR+DMT activity differently altered associative fear learning in young (<6 months old) and aged (>18 months old) mice. DMT (most probably the CR+ cell population) provides most of the thalamic brain derived neurotrophic factor (BDNF) for the cortex, which is a key factor in many cognitive functions. Furthermore, the BDNF level in the brain shows age-dependent alteration; thus, we measured the control and the fear conditioning evoked thalamic BDNF levels in young and old mice. We found that the evoked BDNF levels were also changed by age. Notably, both the mature- and the pro-BDNF levels also increased in the thalamo-frontal circuit of the young but not the old mice. Taken together, our preliminary research proposes that the dorsal midline thalamic BDNF can be a key regulator for age-dependent changes in learning and in other arousal-related behavior. Currently, we are investigating the age-dependent effect of DMT-selective BDNF deletion in various cognitive functions.

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The role of calretinin positive midline thalamic neurons in stress induced behavioural changes

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Exposure to severe stress could lead to the emergence of stress-related psychiatric disorders, which pose a considerable socioeconomic burden to society, however its underlying neuronal mechanisms remained unresolved. The paraventricular thalamic nucleus is one of the midline thalamic nuclei encompassing calretinin expressing neurons (PVT/CR+) which play fundamental roles in sleep, fear and anxiety regulating circuit operations. We tested how the post-stress activity of PVT/CR+ neurons contributes to stress-related behavioural dysfunctions induced by an exposure to a natural stressor (fox odour, 2MT). We examined how optogenetic inhibition of the PVT/CR+ cells after the stress event affects nesting behaviour, locomotion, sleep, stress hormone levels, and c-Fos activity at the projection areas of the PVT/CR+ cells. PVT/CR+ neurons were inhibited using the inhibitory step-function opsin SwiChR. After five pre-stress days both inhibited (SwiChR) and control (EYFP) mice were subjected to 2MT (10 min). Immediately after the termination of stress exposure mice were photo inhibited in their homecage. During the stress exposure, both the control and SwiChR groups showed similar levels of defensive and escape behaviours. Following the stress exposure, the control group exhibited increased EMG activity, disturbed behaviour in the nest, altered slow-wave sleep, elevated corticosterone levels and increased c-Fos expression in the PVT/CR+ cells. The behavioural changes were altered for five days following the stress exposure. With the exception of corticosterone levels, photoinhibition of PVT/CR+ cells after the stress exposure (1 hour) prevented all these changes, behaviour of the SwiChR group remained unaltered for 5 days after the stress event. This suggests that acute photoinhibiton of PVT/CR+ neurons did not affect the hypothalamic-pituitary-adrenal stress response but had long-term effects on post-stress behaviour. We also tested if post-stress photoinhibtion of PVT/CR+ cells is able to reverse elevated c-Fos expression in its major postsynaptic targets such as nucleus accumbens (NAc), central amygdala (CeA), basolateral amygdala (BLA) and prelimbic cortex (PrL).Collectively, our findings indicate that post-stress activity of PVT/CR+ neurons plays an instrumental role in the emergence of stress induced behavioural changes, and post-stress photoinhibition of PVT/CR+ cells is sufficient to prevent these changes.

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Serotonergic anxiolysis in zebrafish requires novel or previously aversive experience

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Anxiety, manifested in an abnormal form or extent, is a core symptom of many psychiatric illnesses that are a serious burden at both the individual and societal levels. The neural background of anxiety-related diseases can be most effectively studied by developing animal models. Despite their advantages, preclinical animal testing suffers from certain limitations. According to our previous results, the currently used animal models measure time-fluctuating state anxiety, instead of stable traits. As a consequence, the effectiveness of certain anxiolytic agents decreases or completely disappears by repeated testing, a phenomenon that is called "one-trial tolerance"(OTT). Despite extensive investigation, the neurobiological background mechanisms of OTT are poorly understood. We use zebrafish (Danio rerio) to unravel the background of OTT, due to the availability of high-throughput pharmacological screening and the accessibility of whole-brain imaging techniques in this model. In the present study, we aimed to i) describe OTT in larval zebrafish, and ii) investigate what type of stimuli are able to exert such phenomena. To reach this goal, we submitted 3 weeks old, wildtype zebrafish to different test experiences and measured their anxiety-like responses under anxiolytic treatment (buspirone) on the following day. Behavior was measured by the swimming-plus maze (SPM) and showjump (SJ) tests both developed by our group. We demonstrated OTT in zebrafish in both tests, because buspirone has not produced it's anxiolytic effect after repeated testing. OTT only occurred in response to repeats, but not in response to different previous tests, implying the importance of specific experience. Given these results, we pretreated zebrafish with cyclohexidine, a protein synthesis inhibitor, which rescued the effect of buspirone, indicating that OTT is based on memory formation. Finally, we examined the significance of the nature of OTT-inducing memory, and found that additional aversive cues in the preliminary experience are able to diminish OTT. In summary, buspirone able to exert it's effect only if the animal is naive to the experience or if it has a previous aversive memory of it, indicating the necessity of an alarmed internal state.

RetaiN: Neuroimmunology and stress resistance in human ageing

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There is an urgent need to understand aging, since it affects all and has an enormous impact on our life and health. Changes of the immune functions and stress-protection pathways are detected in most of the diseases including age-related disorders, like neurodegeneration. The role and importance of the altered protective mechanisms in these disorders are still not completely understood. In this project we will investigate the immunological and stress-protection-related mechanisms of the neurons in differently aged healthy individuals, using the induced neuron (iN) model. iNs represent a novel tool to investigate human aging by direct reprogramming of fibroblasts into neurons. Uniquely, iNs retain the aging signatures of the donor, thus providing us an excellent tool to perform large scale and high throughput in vitro experiments on human samples. The RetaiN project will us help to understand the role of immune- and stress-protection mechanisms in human aging, through which we can identify novel targets to prevent age-related diseases and achieve healthy aging.

Comparative transcriptome analysis of the dorsomedial prefrontal cortex associated with suicidal behavior

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There is a scarcity of data on the potential contribution of gene expressional changes to depression and suicidal behavior. Recent studies revealed that human brain networks, among which the resting state network (RSN) is outstanding, are affected in psychiatric disorders. However, expressional alterations related to depression have not been reported in the dorsomedial prefrontal cortex (DMPFC), a major and functionally significant component of the RSN network. We used RNA sequencing to investigate the molecular changes in suicide victims without any medication for chronic depression as compared to control subjects without identified psychiatric disorders. More than 1000 genes differed between the 2 groups using log2FC > ±1 and the p-value < 0.05 criteria, and RT-PCR validated 15 of them. In order to identify patterns in the transcriptome data, gene set enrichment analysis was used and identified functional pathways enriched in up- and down-regulated genes. The glutamatergic synapse, growth factor receptor signaling and cytokine receptor pathways were over-represented in suicide victims suggesting that these processes are involved in suicidal behavior. One of the validated differentially expressed genes were the neuronal Ca(2+) -binding protein 2 (NECAB2). Since this gene may have great and previously not fully characterized its importance in modulating neuronal function, we aimed to further characterize the NECAB2 expression by performing in situ hybridization and immunohistochemistry to describe the distribution in different layers of the DMPFC. The NECAB2 location, together with a comparison to cell type-specific gene expressional data of the Allan Brain Atlas suggest that it is located mainly in layer I-IV and VI in two different interneuron subtypes. Our results imply extensive gene expressional alterations in the DMPFC related to suicidal behavior. Some of these genes may contribute to the altered mental state and behavior of suicide victims.

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SURFACE LAPLACIAN BASED MOTOR IMAGERY IMAGES CLASSIFICATION USING DEEP LEARNING

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Motor-Imagery based brain-computer interface (BCI) systems are highly dependent on the classification of the EEG signals. These signals are noisy and differ from one subject to another and even for the same subject among different trials, and this is why designing a general classification model is not an easy mission. Convolutional Neural Networks (CNN) approach is dominant in computer vision and image classification, so we followed a trend in EEG signals classification in which these signals are transformed into images, and thus classifying such signals becomes an image classification problem. Surface Laplacian is used to improve the spatial resolution of the raw EEG signals before being transformed into images. The motor imagery EEG activity is mainly in the Mu [8-13 Hz] and Beta [13-30 Hz] bands, so the Surface Laplacian and the raw EEG signals were bandpass filtered accordingly. We used the Physionet dataset for EEG motor movement/imagery tasks which consists of 109 subjects (107 are used). The motor imagery EEG trials were transformed into 2-D images (with 2 channels, one for Mu band and the other for the Beta band) using the azimuthal projection and Clough-Tocher algorithm for interpolation, and these input images are fed to a CNN model to classify 4 different motor imagery classes. The average classification accuracy for the Surface Laplacian based images is 57%, while for the raw EEG images is 56.5%, and both are significantly better than the results of the Support Vector Machine (SVM) over the same dataset which has 54% average accuracy. This suggests that the Surface Laplacian potentials approach is promising when used with the signal to image transformation.

Investigating the effect of female hormone depletion on the progression of Alzheimer's disease

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Introduction: Alzheimer's disease is the most common type of cognitive dementia, affecting elderly women 1.6-3x more than similar aged man, or the younger generations. The advanced progression can be due to decreased hormone synthesis in post-menopause. Estradiol and progesterone both have neuroprotective potentials, and the lack of these hormones possibly aggravates cognitive decline.

Aim: The aim of our experiment is to investigate the relationship between female hormone depletion and the progression of dementia in a triple transgenic mice model of Alzheimer's disorder (3xTg-AD). The pathological hallmark is known to appear in 6-month-old animals; thus, we expect to see cognitive decline in the 4-month-old 3xTg-AD mice only after hormone depletion.

Material and methods: The experiments were performed on 3-month-old genetically modified female 3xTg-AD mice and their control equivalents. As a menopause model ovaries were removed (OVX), control groups received a sham surgery. After 1 month recovery the body composition of the animals were measured by an MRI scan. The cognitive capabilities were investigated with behavioral tests, like Y-Maze and Morris Water Maze (MWM). At the end of the experiment animals were decapitated and uterus was dissected.

Results: The uterus weight decreased, and the body weight increased significantly in the OVX animals. The MRI data showed that the body weight change can be due to fat accumulation. The 3xTg-AD genotype did not influence the somatic changes. In the Y-maze test 3xTg-AD mice moved significantly less, without any effect of OVX. In the MWM a difference between the learning capability of the 3xTg-AD SHAM and 3xTg-AD OVX group could have been detected.

Conclusions: Our experiment show, that the surgery was successful, the animals had menopausal symptoms. The OVX also tended to enhance cognitive decline. Thus, our data confirm that it can be one of the risk factors that aggravate dementia. Further morphological and behavioral tests are needed to understand the pathophysiology and the relationship behind.

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MEDIAN RAPHE REGION SEROTONERGIC NEURONS REGULATE DEPRESSIVE-LIKE BEHAVIOUR RELATED CHANGES IN BODY TEMPERATURE DURING FORCED SWIM TEST

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The midbrain median raphe region (MRR) plays a role in numerous different behaviours, but its effect on vegetative functions is debated. Additionally, it is unknown if there is a connection between the two. The MRR is mostly known for its serotonergic (SERT+) neurons, although they constitute a minor population in the nucleus. On the other hand, the classically stress-related corticotrophin releasing hormone (CRH) positive cells are also present in the MRR, but their role is unknown. Our aim was to investigatethe role of MRR, especially its SERT+ and CRH+ neurons in depressive-like behaviour in parallel with changes in core body temperature (BT) as a vegetative function. Using pharmacogenetics control, excitatory and inhibitory designer receptors (DREADDs) were expressed in Bl/6 mouse MRR. A biotelemetry system was implanted into the abdominal cavity to monitor changes in BT. Following injection of clozapine-N-oxide (CNO), the ligand for DREADDs, behavioural tests were performed. Depressive-like behaviour was measured by forced swim test(FST). The same protocol was repeated in SERT-Cre and CRH-Cre mice, but only with control and excitatory groups. As of the behaviour, excitation of the whole MRR increased floating, while marginally decreasing struggling. The test counts as a cold exposure, and thus, the BT decreased in all animals. However, the drop was smaller in the excitatory group, both during and after the test. The excitation of MRR SERT+ neurons marginally increased floating and significantly decreased struggling. Furthermore, the decrease in BT during and after the FST was reproduced in the SERT-Cre excitatory group. On the other hand, we could not detect any differences between the groups in the CRH-Cre mice. Based on our results, the MRR effectively regulates depressive-like behaviour as well as BT, which is primarily regulated by SERT+ neurons. This finding may have clinical relevance in human depressive disorders, as patients tend to have higher body temperature. CRH positive neurons do not seem to contribute to the regulation of depressive-like behaviour or body temperature.

Contribution of synaptic and intrinsic currents to in vivo-like neuronal activity

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Voltage dependent intrinsic conductances and synaptic currents play complementary roles in shaping the activity of neurons. Intrinsic conductances are essential for action potential generation and endow neurons with their unique electrophysiological properties often used for characterising neuronal subtypes. Conversely, synaptic currents are necessary for signal propagation and synchronization at the network level. However, the contribution of these factors to the neuronal responses, and thus to normal or pathological brain functions, is not known as these currents can not be directly measured experimentally. Our goal is to develop a novel computational framework to measure the contribution of dendritic intrinsic and synaptic currents to the somatic response of biophysically detailed neuron models during in vivo-like input conditions. Here we combine multicompartmental biophysical models of cortical neurons with analysis of their axial and membrane currents based on basic laws of electricity. In a multi-compartmental model the morphologically complex dendritic morphology is modelled as a tree-like graph of isopotential nodes or compartments where each node influences its neighbours through axially flowing electric currents. Hence a dendritically evoked synaptic current can influence the somatic activity by inducing axial currents. Here we partitioned the positive (or negative) axial current flowing from the dendrite into the soma proportionally to the inward (outward, respectively) currents in the dendrite. Applying our analysis to a two-compartmental model of the hippocampal CA_3 pyramidal neuron we show that synaptic excitation targeting the dendritic compartment dominates over somatic voltage dependent Na-currents before burst firing. Similarly, during the burst the dominant source of somatic depolarization are the dendritic voltage dependent Ca currents. Our framework can be recursively extended into multiple compartments with complex geometry and arbitrary spatio-temporally structured synaptic inputs and yields testable experimental predictions. Our work provides a coherent framework to measure the effect of distal currents in models with complex neuronal morphologies. Identifying the dominant conductances responsible for the response of a cell to complex stimuli could guide the refinement of neuronal models and recognising potential therapeutic targets.

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Modelling of Neuronal Responses to Rotating Extracellular Electric Field Gradients

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Modern biophysical simulation environments can model the dynamics of different cell types as well as the responses to extracellular fields in details. Numerous articles characterized the activity of various neuron models in stationary electric fields eliciting subthreshold and suprathreshold responses. In our work, we used the NEURON simulation environment to simulate the effects of extracellular fields of different orientations on geometrically precise models of various neuron types. Our aim was to study the underlying dynamics of the integrative nature of cell membranes exposed to repetitive subthreshold and suprathreshold stimuli. We also tracked the course of charge-accumulation during exposure to high-frequency rotational field leading to the emerge of an action-potentials. The software NEURON was developed to model responses to simple stationary stimuli and thus, it is less suitable to handle stimuli with complex temporospatial layout. In order to model the instantaneous effects of the sequential pulses of high-freqeuncy rotational stimulation, we developed a Python wrapperaround the original C-based environment of NEURON. Neuron cell models were taken from the database of Aberra et al. (2018). These models include five types of morphologies and channel dynamics, from five cortical layers: layer 1 neuroglia cells (L1-NGC), layer 2/3 pyramidal cells (L2/3-PC), layer 4 large basket cells (L4-LBC), layer 5 tick-tufted pyramidal cells (L5-TTPC) and layer 6 tufted pyramidal cells (L6-TPC).One stimulation period was defined by a set of consecutive rotations of the electric field, where the dendroaxonic axis of the modelled neuron and the normal of the field vector was moving from parallel to orthogonal in seven steps. Rotation was advanced at four frequencies (200, 500, 1000, 2000 Hz) and 12 amplitudes (from 0 V/m to 80 V/m in 5 V/m steps) were tested. In conclusion, higher field intensities were needed to generate the first spike when the field was rotated faster around the modelled neurons. Therefore, we conclude that charge accumulation plays an importantrole in action potential generation. Angular variability showed that in general, directions perpendicular to the axons required higher intensities to generate spikes, but the neuronal morphology results in complex spatiotemporal response profiles, which play a more important role in neuronal responsivity than the structural components.

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Effects of intracerebroventricularly injected streptozotocin treatment on the cognitive performance of aged, experienced rats

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Streptozotocin (STZ) injected intracerebroventricularly (icv) into rats produces many symptoms of Alzheimer's disease (AD) such as cognitive decline, increased phospho-tau protein, and appearance of amyloid deposits. As the model has been used in naïve albino rat strains, in our previous experiments (Gáspár et al., 2021) we tried to transfer it to naïve Long-Evans rats, and found that a higher dose of STZ was required to induce subtle AD-like symptoms. In this study we examined the effect of icv STZ treatment on aged, experienced rats. In the study 29 male rats were used which had almost two years learning experience in the following paradigms: five choice serial reaction time task (5CSRTT), Morris water maze (MWM), pot jumping test (PJ) and pairwise discrimination (PWD) in a touchscreen apparatus. At the age of 22 months, they were bilaterally injected with 3x1.5 mg/kg STZ or vehicle into the lateral ventricles on days 1, 3 and 5. Learning and memory capabilities of the rats were then investigating in the above assays supplemented with novel object recognition (NOR), step through passive avoidance (PAL), fear conditioning (FC), open-field (OF) and elevated plus maze (EPM) tests. 15 weeks after STZ treatment animals were sacrificed and the hippocampal phospho-tau/tau protein ratio and β-amyloid level were determined by Western blot technique (WB). We found significant impairing effects of STZ treatment in the NOR and MWM tests while in the FC test, STZ-treated animals showed significantly stronger freezing responses. In the PWD paradigm STZ-treated rats initiated a significantly higher number of trials but with similar percentage of correct responses to the controls. In the 5CSRTT, they made significantly more premature responses than the vehicle-treated group but again with similar correct% and accuracy%. We also found a significant increase in motor activity in the OF together with higher dwelling time in the central zone. In the EPM, STZ-treated animals more frequently visited the open arms. We did not find significant difference between the two groups in the PAL and PJ test, neither in the tau and β-amyloid levels. Our findings suggest, that icv STZ treatment impaired visual recognition and spatial memory but did not affect procedural memory, fear memory and attention in aged, experienced Long-Evans rats. We interpret the overall behavioural changes induced by STZ as increased impulsivity.

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Immunhistochemical characterization of the human and mouse septal area

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Associative learning is essential in adaptation to our continuously changing environment. It is well established that basal forebrain (BF) nuclei play an important role in learning mechanisms. Although malfunction of basal forebrain cholinergic neurons (BFCNs) is closely linked to the patomechanism of Alzheimer's dementia, very little is known about other forebrain cholinergic cell populations. Lateral to the medial septal nucleus lies the lateral septum (LS), which previously have been identified as a mainly GABAergic nucleus. Our colleagues – in coherence with early anatomical studies – identified a significant cholinergic neuronal population in the LS. The area presumably plays an important role in social learning and pathological function of this area is associated with anxiety, mood disorders and schizophrenia. Moreover, dementia and age-related neuronal changes affect this area as well. We hypothetize that cholinergic neurons of the LS play a major role in associative learning as well as BF neuronal populations, therefore we would like to study the neurochemical composition and cellular anatomy of this area more extensively. In our experiments we are examining the coexpression patterns and morphology of lateral septal cholinegic neurons with light- and electron microscopic techniques. We extrapolate our findings by replicating our experiments on human septal complex tissue samples. Our preliminary findings indicate that a major population of LS cholinergic neurons show calbindin-positivity (CB+) as well. By using 3xTg transgenic Alzheimer's disease model mouse line, we show that LS is also affected by ß-amiloid plaque formation. These plaques are surrounding CB+ (putative cholinergic) neurons. We managed to show an extensive CB+ neuronal populations in the human septal complex as well. In the future, we would like to confirm the possible coexpression of CB and cholinergic neuronal marker ChAT (cholin-acetyltransferase) in the human LS and characterize the possible morphological changes of cholinergic neurons associated with dementia.

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Investigation of a pentapeptide carrier on culture models of biological barriers

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Biological barriers block the delivery of large biopharmaceutics at therapeutically relevant concentrations, hence treatment of many diseases is difficult. Targeted delivery of protein drugs to the intracellular space and through biological barriers to achieve a better therapeutic effect is an actively investigated area. We demonstrated that a galectin-1 derived pentapeptide binds to GM1 ganglioside in nanomolar concentration and delivers large proteins into the intracellular space via lipid-raft mediated/caveolar endocytosis (Imre et al 2020, PMID: 32099761). This peptide is small and does not influence the viability of cells, therefore, it is a promising candidate as a carrier for proteins to cross biological barriers. Since no data are available if this complex could be used as a shuttle through biological barriers, our aim was to compare the cellular entry of the peptide complexes into cells and their penetration across culture models of the blood-brain and different epithelial barriers. We observed that the peptide-antibody construct entered the epithelial and endothelial cells and localized in the cytoplasm. Incubation with the complexes did not alter the immunostaining pattern of tight junctional proteins indicating no effect neither on the epithelial nor on the endothelial barrier integrity. In case of the epithelial cells, both the peptide-antibody complex and the antibody complex alone penetrated across the barriers better at the four-hour time point compared to the one-hour time point in all cell lines. Our results showed that there was no difference between the penetration of the peptide-antibody and the antibody complexes across the different epithelial barriers. In contrast, the permeability of peptide-antibody complex was higher across a human co-culture model of the blood-brain barrier. In conclusion, the pentapeptide can increase the entry of large protein complexes into epithelial and human brain-like endothelial cells and promote the transfer of a protein complex, but only across the blood-brain barrier.

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The impact of environmental exposures on the neuronal differentiation of pluripotent stem cells

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Increasing evidence demonstrates that altered conditions during the periconceptional (PC) period of gamete maturation and early embryonic development can have lasting effects on the health of progeny. Such effects can result in the onset of neurological disease and neurodevelopmental disorders ('Developmental Origins of Health and Disease (DOHaD) concept). The present study aims to link mouse models and human clinical observations with the establishment of a human induced pluripotent stem cell (hiPSC)-derived model for brain development. To assess the effect of early-life stress on neurodevelopment, the impact of oxidative stress inducing compounds will be determined experimentally using in vitro hiPSC-derived neuronal tissue with qualitative (Immunocytochemistry) and quantitative (RT-qPCR and Western Blotting) methods. iPSC-derived neuronal cultures have been established and validated with positive expression of neuroectodermal (SOX1, PAX6) and neuron-specific markers (MAP2, TUB3) in 2D, while the neural induction of iPSCs to neural stem cells (NSCs) has been conducted in 3D format. Preliminary toxicity testing including cell viability assays and reactive oxygen species measurements have been undertaken using oxidative stress inducing compounds such as Menadione and Paraquat. This study will establish an in vitro hiPSC-derived model for embryonic brain development. Our model will be utilized to investigate gene expression and phenotypic alterations, as well as epigenetic changes in brain development induced by a chronic oxidative challenge. Conclusively, this study will promote preventative measures and potential therapeutic applications for neurological and neurodevelopmental disorders that can arise due to early-life stress, complementing animal studies and human clinical observations in the DOHaD field.

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Travelling slow waves in the thalamus of anesthetized rodents

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Brain oscillations that play an important role in brain functions, such as slow waves or sleep spindles, often behave like waves. Numerous in vivo and in vitro experiments have shown that travelling waves have a significant impact on the brain's computational performance and synaptic regulation. Nowadays, it is clear that propagating waves are also essential for sleep and also in wakeful functioning. The thalamus we study is essential for the generation of slow waves and sleep spindles. Propagation of sleep spindles has been observed previously in the thalamus of cats in vivo but travelling slow waves with thalamic origin have only been described in in vitro brain slices. The application of multi-shank and high-density silicon-based probes allows us to investigate the firing patterns of neuronal populations at high spatial and temporal resolution. Using this technique, we investigated in vivo whether propagating waves can be recorded in the thalamus of anesthetized mice (n=10) and rats (n=27). In these experiments we used either NeuroNexus multi-shank probes (having 64 or 128 recording sites) or high-density Neuropixels probes with 384 channels (selectable from 960 sites). In a single animal, we recorded thalamic activity from multiple insertions and at multiple depths to map the firing patterns of a high number of thalamic nuclei. Based on our preliminary results, we found that propagating slow waves only appear in some, mainly higher-order thalamic nuclei (e.g., Po or LDVL) and the properties of propagation might also depend on the actual brain state. In addition to slow-wave propagation, we observed that cycles of sleep spindles emerging during the active states of slow waves also propagate (e.g., in the first-order nuclei VPM) and that the strongly intertwined nature of these two types of oscillations results in the appearance of complex spatiotemporal propagation patterns. Besides investigating population activity, we also applied spike sorting on the collected dataset to analyze thalamic single-unit activity. Furthermore, we are also interested in how different anaesthetics (ketamine/xylazine, isoflurane and urethane) can affect the appearance of propagating waves. In summary, the propagating waves observed in the thalamus in vivo are significantly influenced by a number of factors including the depth of anaesthesia, the substance type or the degree of synchronization of the thalamocortical network.

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Post-stress activity of calretinin positive cells in the paraventricular thalamic nucleus is required for long term, stress induced disturbance of sleep behavior

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Sleep disorders caused by stress affect millions of people around the world, but its neurobiological bases are still unclear. The calretinin-positive neurons of the paraventricular thalamus (PVT/CR+) are in a unique position to participate in stress induced sleep disturbances since their activity is significantly affected both by stress and by sleep-wake transitions. In this study we aimed to determine the activity of PVT/CR+ cells before and after the exposure to a natural stressor (fox odor, 2MT, 10 min) and to test causal relationship between post-stress PVT/CR+ activity and post-stress sleep behaviour using optogenetic inhibition (1 hour) of PVT/CR+ cells after the stress situation. Since sleep disturbances not only involve sleep but also the pre-sleep behaviour we separately analysed neuronal activity and behaviour in the nest before sleep. Recordings involved 3 hours sessions for five days before and after stress using movable tetrodes and optogenetic tagging of PVT/CR+ cells.PVT/CR+ cells displayed strongly state dependent activity during pre-stress days. Wake activity in the nest was lower than outside the nest and firing further decreased at the onset of sleep. At the day of the exposure to 2MT both firing rate and synchrony among PVT/CR+ cells increased. Firing rate remained elevated for four days after the stress, with strongest change in the nest. Bursting activity of PVT/CR+ cells decreased during NREM sleep after the stress. These changes were accompanied by altered locomotor activity (EMG, displacement) and nesting behaviour, showing disturbed sleep. Optogenetic inhibition of PVT/CR+ neurons for one hour after 2MT presentation prevented altered locomotion before sleep, the normal nesting behavior were reinstated and the unit activity did not change either in firing rate or in auto and cross correlations on the poststress days. These data together strongly indicate that altered post-stress activity of PVT/CR+ cells is crucial to establish the neuronal network responsible for the emergence of stress induced sleep behaviour.

Information flow between the dentate gyrus and CA3 regions during sharp wave-ripple complexes in rat hippocampal slices

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The hippocampal formation is yet in the focus of attention among the most widely examined parts of the nervous system. It is presumed to have a crucial physiological role in cognitive functions, such as learning, memory formation, and spatial orientation. Nonetheless, our knowledge about how diverse memories are coded and stored has gaps. Sharp wave-ripple complexes (SPW-Rs) are considered to be the neuronal correlates of memory trace formation and transmission. These synchronous population discharges are observed in the mammalian hippocampus on EEG during slow-wave sleep and immobility. An in vitro SPW-R model was investigated on rat hippocampal slices using a 24-channel linear electrode. Instead of assuming a classical trisynaptic circle of information flow from the DG to the CA3 (Type I), our studies showed that SPW-Rs could also be propagated in the opposite direction (Type II), or generated simultaneously in both areas (Type III). Based on these results, it can be stated that bidirectional information flow occurs between the DG and the CA3 region of the rat hippocampus. The role of reverse information flow might be to intensify the information packages transmitted by the SPW-R complexes by adding another network to the trisynaptic circle for more efficient memory consolidation. In the second part of the project, the role of different cell types was investigated in the generation of distinct SPW-Rs. More cells were activated at the initiation site of the SPW-R complexes than at other regions. Pyramidal cells of the CA3 fired more and showed denser connectivity than granule cells of the DG. A metabotropic glutamate receptor agonist, DCG-IV was used to investigate the role of the mossy fibres in the generation of SPW-Rs. In DCG-IV bath, the recurrence frequency of Type I SPW-Rs increased, while that of Type II SPW-Rs showed a slight drop. Furthermore, the propagation of SPW-Rs decelerated, while the LFPg deflections and the superimposed multiunit activities were reduced. Our results emphasise the prominent role of CA3 region on the one hand and the influential but not essential role of mossy fibres, on the other hand, in the generation of SPW-R complexes.

Simultaneous representation of environmental variables in the hippocampus

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Introduction: It is well-established that the hippocampus is critical for successful completion of spatial memory tasks and that hippocampal pyramidal neurons show location dependent activity. However, it is not known how the hippocampal code adapts to changes in the environment to enable flexible behavior. Here we analyzed data from two-photon Ca2+-imaging experiments from head restrained mice running to collect water rewards in different virtual corridors recorded in the Laboratory of Neuronal Signaling (KOKI).

Goal: Our aim was to understand how the hippocampal neuronal population encodes the variables relevant in this task. Specifically, we wanted to test whether the representation of the position is specific to each corridor, or some aspects of the code is shared across different contexts?

Methods: We applied deconvolution and temporal smoothing on the recorded Ca2+ signal and divided the position into 50 discrete bins. For decoding position or corridor identity, we binarized the inferred spike data and used either a static Bayesian decoder assuming Bernoulli likelihood (SBB) or support vector machine (SVM), both with 10-fold cross validation.

Results: We first trained and tested the SBB decoder in just one corridor at a time. We found that the position of the animal could be estimated with high accuracy from the activity of the ~1000 neurons recorded simultaneously. Mean decoding error was typically the lowest near the reward zones and was significantly ($p < 0.001$) lower in the corridor where the reward was near the end, indicating that motivation may have an impact on representational strength. Importantly, the identity of the corridors could be decoded with high accuracy (0.99) irrespective of the location of the animal using an SVM decoder. This demonstrates the high specificity of the representations in the two corridors. To test the generalizability of positional mappings across context we used the decoder trained in one of the corridors to decode the position in the other corridor. We found that the relative distance of the animal from the reward zone could be accurately decoded even in this case.

Conclusion: We conclude that multiple environmental variables are simultaneously represented in the hippocampus: alongside a precise representation of context, both a corridor specific positional mapping and a generalizable, relative encoding of location can be observed.

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Microglia-neuron interactions in an animal model of Alzheimer's disease

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Alzheimer's disease is a neurodegenerative disorder, one of the main causes of dementia, and a serious social and financial burden to our society. Previous studies described that the resident immune cells of the brain – microglia – undergo different changes in morphology and secretory activity in Alzheimer's disease. Our lab identified a direct connection between microglia and neurons termed somatic junction, through which microglia can control neuronal activity and also sense molecules secreted from neurons. The potential significance of this connection is yet to be tested in Alzheimer's. SORLA protein – Sorting receptor with A-type repeats – is a sorting protein found in neurons, which is implicated in neuronal secretory pathway, while playing a role in the elimination of β-amyloid and its mutation is shown to be a factor in the pathogenesis of Alzheimer's. In our research, we investigated the possible changes of microglia-neuron somatic junctions through aging and in the animal model of Alzheimer's disease, in addition to the neuronal and microglial distribution of SORLA in these groups. For our studies, we used post mortem human brain slices and samples from 85-95 days old „young" mice, 488-502 days old „old" mice. The control group consisted of wild-type mice, while triple transgenic (PSEN1//App_swe//tauP301L) animals were used as the model of Alzheimer's. Following immunofluorescent staining, the sections were studied using Confocal Laser Scanning Microscopy (CLSM) and we analyzed our results with the Fiji software. We discovered that microglia-neuron connections, microglial morphology and the microglial coverage of neurons change throughout aging and in the animal model of Alzheimer's disease. We also examined the distribution of SORLA protein in neurons and microglia and found the following: in the old control group and the young Alzheimer's model group there was an elevated level of SORLA compared to the old transgenic animals, where we observed a decreased amount of the protein. We conclude that microglial changes and altered distribution and amount of SORLA protein can play important roles in the pathogenesis of Alzheimer's disease. We hope that our results can bring us closer to understand the precise pathophysiology of Alzheimer's, and help discovering new, targeted therapies to treat the disease.

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Sleep effect of bromocriptine-evoked prolactin release suppression during the reproductive cycle

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Sleep characteristics were addressed in female rats after bromocriptine treatments for 72h applied during the different stages of the estrus cycle and for 24h in the early- and middle postpartum period. Sleep changes after bromocriptine injections showed strong dependency on the estrus cycle phase of the drug application. Strongest wakefulness elevation and slow wave sleep- and rapid eye movements (REM) sleep reduction appeared during diestrus-proestrus and middle postpartum treatments. Stronger sleep-wake effects appeared in the dark phase in case of the estrus cycle treatments, but in the light phase in postpartum treatments. Slow wave sleep and REM sleep loss in case of estrus cycle treatments was not compensated at all and sleep loss seen in the first day post-injection was gained further later. In opposition, slow wave sleep loss in the light phase after bromocriptine injections showed compensation in the postpartum period treatments. These results can be explained by the interplay of dopamine D2 receptor agonism, lack of prolactin release and the spontaneous homeostatic sleep drive being altered in the different stages of the estrus cycle and the postpartum period. The interplay of these factors is present in physiological sleep-wake stages although the significance of D2 agonism may be different due to the systemic bromocriptine injections applied in this study. Our results emphasize the role of D2 agonism and PRL in the homeostatic sleep regulation.

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Unique properties of dendritic Ca2+ spikes in hippocampal CA3 pyramidal neurons

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The hippocampus has an essential role in spatial representations and contextual memory. Its subfields are thought to form a sequential information processing network with special input systems and different roles. The CA3 region, a circuit formed by recurrently connected pyramidal cells (CA3PCs), is often proposed to be involved in pattern separation and completion; however, the cellular and subcellular mechanisms underlying these functions are not well understood. Dendrites of PCs can produce regenerative responses that fundamentally influence input-output transformation. As a prominent dendritic spike type, Ca2+ spikes may have an important role in dendritic integration. Ca2+ spikes are generally considered to produce a slow afterdepolarization (ADP) following action potentials (APs) that evokes complex spike bursts (CSB) at the soma. CA3PCs have high propensity to produce CSBs, but there is pronounced heterogeneity of this property among individual neurons. Putative dendritic Ca2+ spikes have been observed in CA3PCs, but remained poorly characterized, and their role in CSB generation remained incompletely understood. We combined somatic and dendritic patch-clamp recordings with two-photon microscopy in acute adult rat brain slices to elucidate the generation and biophysical properties of dendritic Ca2+ spikes and understand the relationship of these properties with the unusually high propensity and variability of CSBs in CA3PCs. We found a large cell-to-cell variability in the kinetic properties of dendritic Ca2+ spikes, which strongly depended on the proximo-distal position of the cells. Using direct dendritic recordings, we discovered distinct types of dendritic Ca2+ spikes: 1) ADP-type global Ca2+ spikes that promote bursts, and 2) a novel fast Ca2+ spike form that is initiated without backpropagating APs, is compartmentalized to the activated dendritic subtree and promotes strictly single APs at the soma. Our results point to unique properties of Ca2+ spikes in CA3PCs compared to other PC types and suggest a potentially important role for these spikes in input-output transformation of CA3PCs during navigation and associative memory functions of the CA3 network.

Chemogenetic evidence that posterior intralaminar thalamic neurons stimulate maternal behavior in rats

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In a previous study, we established the activation of the posterior intralaminar thalamic (PIL) neurons during social interactions between adult female rats. In this study we focused on the role of PIL in a special type of social interaction, maternal behavior, during which the animals display specific behavioral patterns such as suckling, anogenital licking, pup grooming and nest building which is accompanied with neuronal activation of certain brain areas. For manipulation of PIL neurons, adeno-associated virus was injected into the PIL using stereotaxic apparatus. The virus expressed the fluorescent tag mCherry and a DREADD (Designer Receptors Exclusively Activated by Designer Drugs) in the infected cells. mCherry was used for tract-tracing and the DREADD for chemogenetic stimulation. We used excitatory (hM3D) DREADD, which was activated by clozapine-N-oxide (CNO). Behavioral tests were recorded during the chemogenetic stimulation. After perfusion of the animals, we performed histological analysis. We found mCherry positive fibers in multiple brain areas. The anterogradely labeled fibers were most abundant in the medial preoptic area, the lateral septal nucleus, the paraventricular hypothalamic nucleus and the infralimbic cortex. We also identified the brain areas activated by maternal care using the c-Fos method. We found neuronal activation in the PIL, and also in the medial preoptic area, and the lateral septal nucleus. The behavioral tests were performed on the first week of the postpartum period, 2-7 days after parturition. On the first day of the experiment, vehicle was injected to the animals. Pup preference test, spontaneous maternal behavioral test and pup retrieval tests were recorded. On the second day, the same behavioral tests were repeated starting 1.5 hour after CNO administration. Chemogenetic stimulation significantly increased the pup preference index, the duration on pup related behavioral elements, such as suckling, pup grooming and anogenital licking. The activation of the PIL neurons also increased the duration of nest building and reduced the latency of the pup retrieval. We also performed elevated plus maze test and forced swim test to measure anxiety- and depression-like behaviors, on which the chemogenetic stimulation of the PIL had no effect. Based on these results, PIL neurons may participate in the regulation of maternal behavior conveying sensory inputs from the pups to higher brain areas.

Shape memory polymer based transparent electrode array for long-term multimodal neuroimaging

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Multimodal neuroimaging methods are efficient tools to map the brain functionalities with high spatial and temporal resolution. Combining two-photon microscopy with electrophysiological signal recording is feasible with using transparent electrode materials. The shape memory polymer (SMP) thiol-ene acrylate is an excellent substrate material due to its transparent nature. The stability and biocompatibility of intracortical SMP probes have been shown and the tunable elastic characteristics are beneficial to achieve long-term stability. In our work, we demonstrate a multimodal neuroimaging scheme using a thiol-ene acrylate based cortical implant. The micro-electrocorticography (µECoG) device's feasibility of measuring intracranial EEG and fluorescent GCamP6 signals using two-photon excitation through the device is presented in mice. The stability of electrode yield was presented with in vivo impedance measurement over 75 days. During this period no sign of delamination or material degradation appeared. The recording quality was seen also by the high signal-to-noise ratio (1.04 to 5.74) throughout the course of the experiment and by the identifiable theta oscillations. The chronic immune response was characterized by Glial Fibrillary Acidic Protein (GFAP) staining of astrocytes and fluorescent Nissl (NeuroTrace) staining of neurons. After 80 days of implantation, the histological analysis revealed only a modest foreign body response. The result of cortical thickness measurement confirms the advantage of thiol-ene acrylate as a substrate as no significant difference was shown between implanted and control cortices. To determine the effect of the device on optical distortion and resolution, the sizes of fluorescent beads, neuronal cell bodies, and dendrites were determined without and under the transparent device placed in the light path of the two-photon microscope. The captured sizes of the detected object on the in vitro images showed a small difference between the presence and the absence of the device. In addition, the change in the relative intensity of fluorescent signals was determined in in vivo images under the long-term implanted device. During the 22 weeks in vivo measurements, the fluorescent activity remained and Ca2+ signals were captured. Based on the results we can say our device is suitable for multimodal imaging.

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The dorsal midline thalamus effect over prefrontal cortex by different parallel pathway

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The dorsal midline thalamus (DMT) has a key role in regulation of cognitive processes through frontal thalamo-cortical (FTC) interactions. Our previous data showed that the DMT can be separated into calretinin (CR) expressing (CR+) and non-expressing (CR-) cell groups, which have distinct arousal-related activity (Matyas, Komlosi et al, 2018). This suggests a functional dichotomy in FTC; however, the cellular and network features as well as the effector mechanism(s) of the CR+ and CR- pathways are poorly understood. Here, by integrating in vivo acute and chronic electrophysiological recordings, anatomical and optogenetic approaches in mice we demonstrate that the CR+ and CR- DMT populations have qualitatively and quantitatively different cortical and subcortical input/output organization and possess diverse cortical effect. In general, CR+ DMT neurons (homologue with paraventricular thalamic cells) have global efferent connections and persistent cortical activation. In contrast, CR- DMT (homologue with mediodorsal thalamic (MD) cells) cells have much fewer brain targets and their cortical effects is rather local. In addition, the proportions of the CR+ and CR- DMT activated principal cells and interneuron are different in the prefrontal cortex. Notably, the CR- DMT cells do not form a homogeneous population either; rather, there is a topographic segregation of distinct CR- neuronal groups (which could be analogue with medial, central and lateral MD). Furthermore, our preliminary data also suggest that majority of the CR+ DMT cells show general arousal-mediated activation pattern, while CR- DMT cells only follows the arousal changes. Finally, activation of the CR+ network can be transmitted to the CR- neurons, via indirect antero-medial thalamic reticular nuclear and the prefrontal cortical loops. These findings indicate that, although the CR+ and CR- FTC networks are anatomically and functionally different, they form sequentially activated 'inter-loop' system. Building on each other, they can collectively mediate complex, arousal-dependent brain functions.

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SPATIO-TEMPORAL MEMBRANE POTENTIAL AND RESISTIVE CURRENT RECONSTRUCTION FROM PARALLEL MULTIELECTRODE ARRAY AND INTRACELLULAR MEASUREMENTS IN SINGLE NEURONS

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Here we show, that based on parallel multichannel extracellular and single-channel intracellular potential recordings, it is possible to reconstruct the spatio-temporal distribution of membrane potential with the spatial resolution of the extracellular recordings in a single neuron. Moreover, we show, that reconstruction of intracellular membrane potential made possible the distinction between two components of the current source density (CSD): the resistive and the capacitive currents. This distinction would provide a clue to the proper interpretation of the CSD distribution, as the resistive component corresponds to the active channel currents, both synaptic and voltage-sensitive channel membrane currents, while capacitive current corresponds to the passive counter currents. The importance of this distinction is further emphasized by different features of the resistive membrane current distribution compared to the CSD. As the CSD is a net membrane current, the sum of the CSD along a whole intact cell should be zero at each time moment, according to the charge conservation law. In contrast to this, the sum of the resistive current should not be necessarily zero since it governs the membrane potential dynamics. Thus, estimation of the spatial distribution of the resistive membrane current makes possible the distinction between active and passive sinks and sources of the CSD map and localization of the synaptic input currents, which makes the neuron fire. We validate our reconstruction approach on simulations and demonstrate its application on simultaneous and co-localized extra- and intracellular in vitro recordings in the hippocampal CA1 region using in vitro rat brain slice preparations.

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Morphological and neurochemical characterization of glycinergic neurons in laminae I to IV of the mouse spinal dorsal horn

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It is becoming increasingly evident that glycinergic neurotransmission in the spinal dorsal horn plays an important role in spinal pain processing, especially in the development of mechanical allodynia. However, how glycinergic neurons contribute to the formation of neural circuits underlying spinal pain processing is still waiting for exploration. The lack of this essential knowledge makes the interpretation of the role of glycinergic neurons in spinal pain processing vague. Therefore, we investigated the morphological and neurochemical properties of glycinergic neurons in laminae I-IV of the spinal dorsal horn using a GlyT2::CreERT2-tdTomato transgenic mouse line. By using immunohistochemical and in situ hybridization methods, first in the literature, we provided experimental evidence that there are glycinergic neurons in laminae I-II that do not express GABA and can thus be referred to as glycine-only neurons. We have reconstructed the dendritic morphologies of tdTomato labeled glycinergic neurons from 100 µm thick sagittal sections. According to dendritic morphologies and the shape of cell bodies, we divided the labeled glycinergic neurons into three morphological categories in laminae I-II and classified them into six groups in laminae III-IV. Investigating the co-localization of tdTomato labeling with neuronal markers, which were identified earlier as markers of inhibitory neurons in the spinal dorsal horn, we demonstrated that proportions of the labeled glycinergic neurons co-express neuronal nitric oxide synthase, parvalbumin, the receptor tyrosine kinase RET and the retinoic acid-related orphan nuclear receptor β (RORβ), but they do not express galanin, calretinin, and neuropeptide Y. The present findings may advance our knowledge about glycinergic neurons in the spinal dorsal horn, and thus may contribute to a better understanding of spinal pain processing.

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Mathematical modeling of ATP-evoked Ca2+ signaling in the Deiters' cells along the tonotopic axis of the cochlea

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Deiters' cells are important supporting cells of the sensory receptor cells in the organ of Corti : providing a physical and metabolic support to the outer hair cells, modulating their electromotility and contributing to the cochlear amplification of the sound. Their role and their mechanism of action are not completely understood. Investigating the ATP-evoked intracellular Ca2+ signaling in the Deiters' cells provides information about the regulation of their intracellular processes and their intercellular communication. The purpose of deeper understanding the Deiters' cells' functions and properties might have an interest in pharmacological and therapeutical terms which could be used with the aim of improving hearing and a possible cure for deafness. In this study, we aim to develop computational mathematical models for this ATP-evoked Ca2+ signaling, while comparing it to the data obtained from our Ca2+ imaging experiment in order to simulate the Ca2+ activity of the Deiters' cells. The cochlea, in which the organ of Corti lies, has a characteristic snail shape which creates a tonotopic organization of this organ. The sounds of different frequencies activate different regions of the organ of Corti along the tonotopic axis of the cochlea. This spatial arrangement permits the distinction of different frequencies before they are transmitted to the brain. The brain can then interpret sound information according to their characteristics. This tonotopic organization ensures that the auditory nerve fibers fire in a frequency-coded manner : based on the structural and mechanical organisation of the cochlea (hence the organ of Corti) along its length, the high frequency sounds stimulate the base of the cochlea while the low frequency sounds stimulate the apex. To simplify this arrangement, we divided the cochlea into 3 main regions : apical, middle and basal turns, and developed one model for both the apical and the middle turns from where our data originated. The complex morphology and the size of the cochlea of mice make difficult the investigation of the basal turn, where the cells are more sensitive. While using both mathematical models for the apical turn and the middle turn, we extrapolate to the basal turn in order to create a model for this region of the cochlea as well.

Reliability of functional connectivity estimation and modularity detection in resting-state EEG networks

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A large number of fMRI studies have reported that large-scale brain networks are organized into separated but interacting modules, typically studied in resting-state. Modularity is assumed to reflect functional segregation within the integrated network and is thought to confer robustness and adaptability to brain connectivity networks. However, fMRI data capture functional relations among brain regions on a timescale of seconds, making it difficult to link networks with cognitive functions, while MEG data remains relatively scarce. The present study aims to explore the similarity and modular structure of networks as measured with resting-state EEG rhythms (0.5-80 Hz) on a large sample of young healthy adults (N = 202; mean age = 22.4 +/- 3.1). We tested whether stable resting-state networks could be identified with EEG on the individual and the group-level and if those networks could be characterized with a shared modular structure. Phase synchrony (PLV; iPLV) and amplitude envelope correlation (AEC) was calculated to estimate functional connectivity between reconstructed cortical signals in five frequency bands (delta 0.5-4Hz; theta 4-8 Hz; alpha 8-13 Hz; beta 13-30 Hz; gamma 30-80 Hz). Modularity was defined on the subject-level, with the structural resolution parameter estimated against randomized (null) data. Individual reliability was assessed by halving the resting state data and group-level reliability by randomized subgroups. Similarity of individual average networks was calculated using five metrics (correlation, Euclidean distance, adjacency spectral similarity, Laplacian spectral similarity, DeltaCon). Our results reveal a trade-off between spatial leakage correction and consistency of connectivity measures, with uncorrected measures showing higher consistency. Observed modularity of connectivity might play an important role in state and trait processes of cognitive functioning.

Comparison of popular fluorescent actin markers to measure actin dynamics in dendritic spines

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Excitatory synapses in the central nervous system are mainly localized on dendritic spines, highlighting the importance of these protrusions in learning and memory. Changes in synaptic activity induces rapid remodelling in the actin cytoskeleton leading to morphological changes of the dendritic spines. FRAP (fluorescent recovery after photobleaching) is widely used to study the dynamics of actin cytoskeleton, based on photobleaching fluorescently labelled actin-bound signals within a small area, followed by measuring the return of fluorescent signal intensity within the bleached regions. This technique provides tools to calculate the kinetics of the actin remodelling and determine the proportion of stable and rapidly rearranging microfilaments within certain cellular areas. Within the last decades, numerous actin labelling fluorescent markers have been developed. To select the most suitable for FRAP experiments, murine embryonic hippocampal cell cultures were transfected with three different actin labelling fluorescent markers. The EGFP-Actin fusion protein is covalently labelled with EGFP and incorporates into the F-actin network. Actin-Chromobody-GFP is a monomeric camelid antibody, while LifeAct-GFP has an actin binding domain which can bind to filamentous and monomeric actin. Neurons expressing the freely diffusible EGFP protein only were used as controls. Actin-FRAP experiments were performed in dendritic protrusions under control conditions and after F-actin stabilization by Jasplakinolide. In addition, we compared how the different labelling methods affected the motility of dendritic protrusions and general neuronal morphology. There was no significant difference between the motility of the filopodia expressing Actin-Chromobody-GFP, LifeAct GFP and EGFP, while EGFP-actin expression reduced motility. Sholl analysis of dendritic arborisation revealed that cells expressing Actin-Chromobody-GFP have shorter dendrites and lower number of branches. Fluorescence recovery of the covalently labelled EGFP-Actin was completely blocked by F-actin stabilization. On the other hand, both indirect actin labelling constructs recovered almost completely after bleaching, indicating that free, diffusible fusion proteins mask the detection of actual actin dynamics. Thus, only the covalently labelled EGFP-actin method is suitable for FRAP experiments.

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DEEP PLASMA PROTEOMICS REVEAL AGE-RELATED MOLECULAR PATHWAYS MODULATED BY GRF6019 TREATMENT IN ALZHEIMER'S DISEASE PATIENTS

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Objectives: Blood has been widely investigated to discover biomarkers and gain insights into the biology of aging and age-related diseases. Its protein composition provides information about complex biological processes, as proteins are often direct regulators of cellular pathways. Using recent methodological developments allowing the measurement of thousands of proteins with very high sensitivity and specificity, we sought to understand comprehensive proteomic changes in two AD clinical trials.

Methods: Phase 2 clinical trials (GRF6019-201 n=40 and GRF6019-202 n=26) testing the safety, tolerability, and feasibility of repeated infusions of the plasma fraction GRF6019 in Alzheimer's disease (AD) were used as the source to measure more than 7000 proteins in plasma using the SOMAscan and Olink assays. To evaluate the relevance of the proteomics changes induced by GRF6019, we compared these changes to those observed in a healthy aging cohort (~5000 proteins measured in 370 subjects).

Results: Standard statistical analysis at the protein levels lacked power due to the small sample size in phase 2 clinical trials. By analyzing trajectories of groups of proteins, clinical proteomics revealed multiple clusters of proteins responding to GRF6019. Remarkably, several pathways modulated by GRF6019 were particularly relevant for the biology of aging and AD – including the complement/coagulation cascades and neuronal pathways ($q<0.05$).

Conclusion: Altogether, our results suggest that the treatment of AD patients with a complex plasma fraction modulates biological pathways that are relevant to aging and AD. Our results establish deep proteomics as a powerful tool to study human response to treatment in clinical trials.

Computerized socio-behavioral analysis in color coded rodents

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Behavior consists of different elements conspecifics demonstrate when they are together. Experimental analysis of social behavior revealed its alteration depending on the state including previous history of the animals. In the present study we analyzed the social interaction between 3 rats, of which an experimental animal underwent previous manipulations. The automated analysis of the videorecordings in hindered by the difficulty to distinguish the otherwise similar, equally while animals from each other. To circumvent this problem, we color coded the rats and adapted a previously developed custom-made software, which could recognize the differently color-coded rats using adjustment of Hue Saturation and Value (HSV). The male rats we used, freely interacted in an open-field arena during the 30 min sessions and were videorecorded throughout. One session was performed each day. The videorecordings were analyzed with the software, which detected the trajectories of all 3 animals, and specifically calculated the time spent near and away from the wall as an indicator of the anxiety level of the animals. In addition, 2 other behavioural elements were calculated: approach-avoiding behavior, nosepoking the mates. Each experiment started with 2 days of baseline recordings. Statistical analysis was used to establish if the behavior of the animals changed in these 2 days. Then, on the third days, the experimental rats were manipulated. We addressed the effect of spending the previous night with a conspecific as opposed to being isolated. In this experiment, we found a significant reduction in the number of approach-avoidance behavior of the experimental animals when the animal was grouped together even before the experimental session while the number of node pokes. In the other 2 experiments, the animals were fasted for a day before the experiment, or a cell group in the posterior thalamus was chemogenetically stimulated. In these cases, we observed amount of time spent near the wall changed the behavior of the animals. The data suggest that grouping vs. isolation is the strongest factor modulating the behavior of rats. The observed reduction in approach avoidance with unaltered frequency of nosepoking suggests that the animals touch each other with the same frequency but avoid contact less frequently if being together the preceding day. The neurons in the PIL may be involved in the control of stress or exploration in rats.

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A NEW PATHWAY FROM BASAL FOREBRAIN SOMATOSTATIN NEURONS TO CORTICAL AREAS

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The mammalian basal forebrain (BF) modulates cortical activation and the sleep/wake cycles, and it has important roles in motivation, learning and memory. BF cholinergic, glutamatergic and GABAergic parvalbumin neurons target different cortical regions and play a crucial role in cortical rhythmic activity. Here we discovered a previously unrecognised, long-range GABAergic, somatostatin-expressing cell population in the BF, which innervates interneurons in the dentate gyrus of the hippocampus and in the retrosplenial cortex (RSC). The RSC is thought to play an important role in spatial navigation, contextual memory encoding and retrieval in tandem with the hippocampus, providing similar coding, thus making these systems more robust. Using viral tract tracing in SOM-Cre/vGAT-Flp double transgenic mice, immunohistochemistry and confocal laser scanning microscopy, we found that BF SOM cells establish multiple putative inhibitory synaptic contacts on the somata and dendrites of parvalbumin-, somatostatin- and calretinin-expressing GABAergic interneurons in the RSC. Our results suggest that BF SOM cells may disinhibit selected subpopulations of RSC principal neurons, which may have a crucial role in modulating the involvement of these principal cells in RSC related coding.

Testing modified cyclodextrins on cell culture models of the blood-brain barrier

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Cyclodextrins (CDs) are cyclic oligosaccharides which can be used in therapy both as active agents or carrier molecules. Some CDs are used as therapeutic drugs, while others are currently tested in clinical studies to treat human neurological diseases, but the exact mode of action of cyclodextrins is unclear. CDs can interact with lipid membranes and selectively remove lipids from cell membranes. However, it is still a question whether CDs are able to cross the blood-brain barrier (BBB). BBB is a protective system that separates the central nervous system from the circulation and is anatomically based on the endothelial cells of the brain capillaries. The main roles of the BBB are creation of ionic homeostasis for neuronal functions, the supply of the brain with nutrients and protection from toxic insults. Most of the therapeutic compounds developed for neurological diseases have limited access to their brain targets because of the BBB. Our research hypothesis is, that coupling CDs with peptides that target BBB receptors may help the binding of these complexes to brain endothelial cells and their penetration across the BBB. In our experiments, we used beta-cyclodextrin (BCD) complexed with adamantane labelled with the fluorescent molecule rhodamine B (BCD/Ad-RhB), and BCD/Ad-RhB complexes modified with a peptide targeting the transferrin receptor of the BBB. These complexes were investigated in toxicity measurements and the entry of the complex into cells was assessed by fluorescence spectrophotometry. We investigated the cellular uptake of BCD-peptide complexes in brain endothelial cells and the permeability of these molecules across a cell culture model of the BBB. We demonstrated that none of the complexes affected the viability of brain endothelial cells in the tested concentration range during two-hour incubation time. The uptake of CDs was temperature dependent suggesting an active, energy demand cellular entry process. The permeability studies showed that the penetration of peptide-modified BCD/Ad-RhB complexes across the BBB was significantly higher as compared to unmodified CDs. Our results may contribute to the future application of different modified cyclodextrins in the therapy of neurological diseases.

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The protective effects of PACAP1-38 on the retinal vasculature and hypoxic molecules in rat glaucoma model

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Introduction: Despite the critical impact of glaucoma on blindness, its cause is not fully understood. Animal models are important for better understanding the mechanism behind this disease. Elevated intraocular pressure (IOP) is a risk factor for glaucoma. In our previous study, we described an inducible, microbeads model in Sprague Dawley (SD) rats in which we were able to prove the neuroprotective effects of PACAP1-38 eye drops treatment. Vascular factors have been suggested to play an important role in the development of glaucoma, based on numerous studies.

Aim: In our present study, we aimed to examine the possible protective effects of PACAP1-38 eye drops on the retinal vasculature and the molecular patterns of hypoxia in the hypertensive glaucoma model.

Methods: We induced hypertension through injection of polystyrene microbeads into the anterior chamber of SD rats, PBS receiving rats served as controls. Intraocular pressure was recorded every two weeks. We assessed retinal degeneration, vascular and molecular changes through immunofluorescence. HIF1α protein level was also measured by western-blot.

Results: Significantly increased IOP was observed in the glaucomatous vehicle-treated group (Beads+S) however, in the PACAP1-38 treated group (Beads+P) the IOP remained in a normal range. Optical coherence tomography images suggested severe retinal degeneration in the glaucomatous group, although protective effects were measured after topical administration of PACAP1-38. We also found several vascular parameters changed in the Beads+S group. The examination of molecular patterns suggested hypoxic conditions in the Beads+S group, however after PACAP1-38 administration retinoprotective effects were observed in HIF1α protein level.

Conclusions: Our results show that PACAP1-38, given in form of eye drops, is retinoprotective in glaucoma, providing the basis for potential future therapeutic administration.

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Neurobiological correlates of trait anxiety

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Anxiety disorders are the most prevalent psychiatric illnesses to date. Nevertheless, their pharmacotherapy is only effective in 60% of the treated population. Over the past decades, nearly half of promising anxiolytics in animal models failed in clinical trials. This discrepancy could result from the fact that preclinical models of anxiety do not reflect the clinical disorder. We hypothesize that current preclinical anxiety tests only capture transient emotional states of animals, rather than their anxious traits, the core of clinical diagnosis. To increase the clinical translational value of animal models of anxiety, we developed a repeated behavioral sampling protocol to measure trait anxiety in rats, using the 3 most popular anxiety tests in rodent models, repeating each of them 3 times in a semi-randomised order. Using repeated behavioral data from the same individual, we have created summary measures (SuM, average of 3-time sampling in one test) and compared them to single measures (SiM, behavior in the first test). As opposed to SiMs, SuMs were strongly correlated between different anxiety tests, and were stronger predictors of behavior in a highly aversive environment and of generalised fear response, indicative of trait anxiety. Next, we performed a whole-transcriptome analysis of the medial prefrontal cortex, the highest top-down regulator of affective processes. Following false-discovery-rate (FDR) corrected regression analysis of associations between transcript levels and anxiety-like behavior, we have detected a fivefold increase in differentially expressed genes using SuMs, compared to SiMs. As further functional analysis of differentially expressed genes revealed, SiMs were correlated mostly with expression levels of stress markers, whereas SuMs were strongly associated with neural plasticity-related gene expression. Supporting our results, a high proportion of genes correlating with SuMs had been described previously in association with clinical disorders and their preclinical models. Additionally, we also have discovered new targets previously not associated with anxiety. In conclusion, we have developed a novel behavioral sampling protocol to measure trait anxiety. Using this approach, we have detected a considerably high number of gene correlates of trait anxiety, on which we performed the most detailed genetic profile analysis thus far. We also identified novel genes that may be targets of new, more effective pharmacotherapy.

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Functional ultrasound imaging of deep visual cortex and beyond in awake cats

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Optical imaging has become the imaging method of choice as it is compatible with genetically specified access to brain activity across the meso- to the subcellular scale. Yet, to access brain tissue beyond mm depth, optical methods invoke invasive solutions like implantation of GRIN lenses or prisms. Functional ultrasound imaging (fUSI) is a novel technique, which enables access to brain activity in deep layers of the cortex or even subcortical structures via imaging changes in the haemodynamic signal. We imaged deep cortical and subcortical sensory-evoked responses in Brodmann areas 17, 18 and 19 of awake and anaesthetized cats using fUSI. We achieved signal-to-noise ratio high enough to reliably detect single-trial responses with imaging depth reaching 1.6 cm. Using a custom motorized imaging chamber design, we were able to construct 3D activity maps in a large volume, while limiting the length of imaging sessions to an amount well-tolerated by awake subjects. This method bridges the gap between functional magnetic resonance imaging and optical imaging techniques as a versatile mesoscale activity imaging option that does not rely on head fixation or anaesthesia-based immobilization.

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Examination of the PAC1 receptor colocalization with Ca2+-binding proteins and cochlea-efferent markers in the auditory pathway of pituitary adenylate cyclase-activating polypeptide - knock out (PACAP KO) and wild type (WT) mice

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

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

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

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

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Topographical mapping of the frontal cortex related thalamic circuits

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The main elements of the corticothalamic circuits are the pyramidal cells of the layer 5 and layer 6 of the neocortex, the excitatory relay nuclei and the GABAergic thalamic reticular nucleus (TRN), which is the main source of the intrathalamic inhibition. Our group previously discovered a novel frontal cortex specific corticothalamic pathway originating in the layer 5 of the cortex and targeting the anterior region of the TRN (Hadinger et al, in prep). Anterior TRN cells innervate the relay nuclei, which project back to the frontal cortex, closing the frontal cortex-thalamus-frontal cortex loop. We showed that the fontal L5-TRN pathway had a clear topography, meaning that L5 fibers from neighbouring frontal cortical regions targeted different parts of the anterior TRN. Frontal cortical functions are divergent and linked to different frontal areas. Because of this, behavioral studies combined with optogenetical and in vivo electrophysiological approaches require precise placemet of optic fibers and electrodes both at the cortical and at the corresponding thalamic levels. This requires precise knowledge about the topography of both corticothalamic and intrathalamic connections. Although we previously mapped the topograpy in the frontal cortex- anterior TRN pathway, little is known about the topography of the anterior TRN-relay nuclei bidirectional connection. We had only preliminary data, reagarding how the position of the TRN cell soma within the TRN determines the projection target of the cells. Similarly it was not clear if TRN cells received reciprocal input from the relay territories that they innervated or if they were forming open loops with relay cells outside of their synaptic target zone. We investigated this questions via anterograde and retrograde, classical and viral anatomical tracing methods targeting different parts of the anterior TRN or different frontal cortex related relay nuclei. For viral tracing experiments we used the VGAT-Flp transgenic mouse line in which we could selectively label the inhibitory TRN cells. Our results showed a loose but clear topography at the level of TRN-relay pathway and preliminary data suggest that anterior TRN cells are targeted by the relay cells from their synaptic target zones. In addition to help to design our subsequent behavioral experiments these data can brings us closer to understand the principles of operation in the frontal corticothalamic circuits.

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Simultaneous examination of neuromodulatory systems by fiber photometry and electrophysiology

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The essential role of neuromodulators such as acetylcholine and dopamine in learning is supported by a number of studies. However, the relationship between different neuromodulatory systems and possible synergistic or antagonistic effects are almost completely unknown. Furthermore, the known association of the degeneration of cholinergic and dopaminergic neurons with neurodegenerative diseases such as Alzheimer's and Parkinson's lends special importance to studying these systems. Recent findings suggest that reward prediction error, previously associated exclusively with dopamine, may also be encoded by the cholinergic system. It is hitherto unclear what similarities and differences the information represented by the two systems show. In this study, we investigated the two systems simultaneously in a special auditory operant conditioning task using fiber photometry and electrophysiological methods. In the fiber photometry experiments, acetylcholine and dopamine sensors were used to measure the release of the respective neuromodulators. In electrophysiological experiments, specific light-sensitive ion channels were expressed in cholinergic and dopaminergic cells, providing an opportunity for their optogenetic identification. Examining the temporal relationships of the defining events of learning-the cellular response to the sound predicting reward and punishment, and the neural response to the delivery of reward and punishment-we found groups of neurons following different characteristic firing patterns. Of these, dopaminergic and cholinergic populations were identified among several other groups with unidentified neurochemical identities. We found that dopaminergic neurons responded earlier than cholinergic cells after reward-predicting tones, while neither of them responded to punishment-predicting tones. More activity was observed after the delivery of unexpected than expected reward in both cell types. Cholinergic neurons responded earlier than dopaminergic neurons to both expected and unexpected reward. Dopaminergic neurons were heterogeneous in their responses to punishment, being either suppressed or activated. A group of cholinergic neurons responded to punishment with an extremely fast and precise activation, while some neurons did not respond. Our results show that the information encoded by the two systems correlates but evolves over different time scales. These results hint at a complex relationship between the two systems.

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Excitability changes in prefrontal cortical networks in a rat model of autism

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The experiments focused on brain excitability and plasticity changes in autism spectrum disorder model rats. People diagnosed with ASD exhibit impairment in social interactions and communication skills as well as repetitive behaviours. Underlying this triad of impairments, changes in neural network connectivity and excitability can be observed in several brain areas. Experiments carried out by our group focused on the prefrontal cortex, an area, which is connected to a wide variety of functions, among others, the interpretation of others' emotions and the evaluation of social situations. Valproate was administered to rat dams on the 12th day of pregnancy. Pups were subjected to behavioural tests at postnatal days 3-45, to observe the expected delayed development and autistic traits. Acute brain slices were prepared from 6-week-old and 3-month-old offspring of both sexes. To investigate network functions, evoked field potentials were recorded in the prefrontal cortex. Basic excitability was tested with input-output curves. To test network plasticity, long-term potentiation was induced with two different protocols (1 or 4 stimulation trains of 100Hz). Excitability of individual neurons was tested with patch clamp recordings. Valproate treatment evoked significant delays in postnatal development and impaired social behaviour. According to preliminary electrophysiological results, the amplitude of evoked potentials' early component was lower, while late component amplitude was higher in treated 6-week-old males compared to controls, indicating an altered circuit excitability. The results also pointed out, that there is a significant sex difference between the threshold of excitation of the treated 6-week-old male and females. LTP efficacy did not differ significantly. Intracellular recordings revealed that cells in the treated 6-week-old females were more excitable compared to their control peers, based on the finding that the membrane resistance increased and the rheobase decreased. The excitability difference was observed in the 6-week-old males due to the membrane potential becoming more positive, but the resistance of the membrane decreasing. The difference of the valproate treated and control animals diminished in the 3-month-old groups. Further investigation is needed to increase the sample numbers in each treatment group. Testing network sensitivity by recording epileptiform activity is also planned.

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Saliency-map-based feature selection for electrocorticography-based brain–computer interfaces

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In brain–computer interfacing (BCI), electrocorticography (ECoG) is mainly used for capturing fine motor-imagery-related cortical patterns. In practice, the majority of ECoG electrodes are implanted into epileptic patients scheduled for neurosurgery and serve diagnostic purposes reflected in the electrode placement, as well. Thus, a large number of ECoG channels prove to be redundant during signal processing and is beneficial to be removed—opening a niche for feature selection algorithms. We implemented a two-dimensional convolutional neural network (2D-CNN) and a smaller dense network (DNN). The former was trained on the data of 16 subjects performing simple, repetitive hand, foot and tongue movements (the recordings were acquired at the National Institute of Mental Health, Neurology and Neurosurgery). As training and test samples, the amplitude spectrum of 1-second-long data chunks restricted to the (0, 200] Hz range were applied. After the training, saliency maps for each movement type were produced using the test data: these maps provide a quantitative measure of the importance of a specific feature (i.e. a frequency component on a particular channel) during classification. The most salient features were selected and the small DNN was trained using them (we applied the 1, 10, 100 and 1000 best features for each category). We performed 3 training/test sessions for each subject. The accuracy of the 2D-CNN applied as baseline was above 82.5 % for each subject (with an average of 95.6 %). Using only 1 feature per class, the accuracy of the DNN was insufficient, only 51.5 % on average (53.6 % of the baseline). 10 features per class yielded an average accuracy of 69.1 % (72.1 % of the baseline). 100 features per class gave the greatest accuracy, 81.9 % (85.8 % of the baseline); a slightly smaller value, 81.6 % (85.3 % of the baseline) was obtained using 1000 features per class. The 2D-CNN uses 12288 features in average; our finding support the thesis that satisfactory performance can be obtained applying a much smaller network using the appropriate features that can be more efficient in terms of runtime and hardware resources.

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An alternative cholinergic innervation of the hippocampus

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Cortical functions are highly regulated by ascending subcortical pathways, some of which originate from the basal forebrain. Cholinergic cells of the medial septum (MS) and the horizontal diagonal band (HDB) in the basal forebrain play a vital role in the regulation of attention and memory formation. While the cholinergic innervation of the hippocampus is known to originate from the MS, we discovered an additional cholinergic pathway from the HDB. Using tracing techniques combined with immunohistochemistry and electron microscopy, we found that HDB cholinergic cells mostly target hippocampal layers that are only sparsely targeted by the MS and HDB preferentially target the hilar mossy cells. Our preliminary chemogenetic behavioral experiments suggest that HDB cholinergic cells drive hippocampal novelty detection and memory formation via the mossy cells. Our results provide new insights into the regulation of memory formation and may help better understand cholinergic system-related neurodegenerative diseases.

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Cortical and subcortical neural dynamics during absence epilepsy

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Absence seizures (ASs) are sudden, transient lapses of consciousness associated with lack of voluntary movements and generalized 2.5–4 Hz spike-wave discharges (SWDs) in the EEG. In addition to the primary somatosensory (S1) cortico-thalamo-cortical system, where these pathological oscillations are generated, multiple neuronal circuits have been involved in their modulation and associated comorbidities. Most previous studies exploring the ictal neuronal dynamics in various brain regions have been performed under anesthesia, a regime known to drastically alter neuronal activity. Here we performed intracellular and extracellular single unit recordings from freely moving and immobilized animals to explore the neuronal dynamics in various brain areas. We found that neuronal activity in the cortical initiation network is more heterogeneous than previously thought with only a fraction of neurons being phase locked to SWDs. A prominent rhythmic activity characterizes S1 FS neurons which also undergo ictal hyperpolarization. In higher order thalamic nuclei the activity of individual TC neurons is characterized by ictal rhythmic burst firing. In the lateral hypothalamus neuronal activity is coupled to SWDs exclusively on a long timescale and not to the individual cycles of the SWDs. These results provide novel insights into the neuronal activity in various brain areas and highlight the importance of using drug free preparations when revealing the neuronal activity during (patho)physiological functions.

Examination the role of nesfatin-1 in the supraoptic nucleus of rats

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Nesfatin-1 is an anorexigenic neuropeptide that also inhibits water intake. It is coexpressed with oxytocin (OT) and vasopressin (AVP) in the osmosensitive magnocellular cells of the hypothalamic paraventricular and supraoptic nucleus (SON). In order to investigate the role of nesfatin-1 in the magnocellular cells, we silenced the expression of nesfatin-1 in the SON by AAV-delivered shRNA in rats. A scrambled shRNA (scr)-AAV was used as control, delivered into the SON of a second group of rat. After three weeks, the rats were divided into two additional groups, and received 2% NaCl solution, or tap water to drink for a week. The bodyweight as well as the daily water and food intakes of rats were measured. After 7 days, the animals were perfusion-fixed. Serial coronal sections containing the SON were immunostained for nesfatin-1, OT and AVP, and the cell nuclei were labelled with DAPI. Pictures were taken by a confocal microscope and analyzed using the ImageJ software. Our findings show that the nesfatin-1-shRNA fully inhibited the expression of nesfatin-1 in the infected neurons. Salt loaded animals lost weight during the experiment, which effect was amplified by the lack of nesfatin-1 in the SON. Salt loaded animals consumed higher amount of fluid and their food intake was reduced compared to the tap water group. In the SON, both salt loading and nesfatin-1 deficiency increased the density of AVP immunoreactive fibers, and the lack of nesfatin-1 potentiated this effect. Salt loading reduced the intensity of OT immunostaining, but the absence of nesfatin-1, reduced this effect. Additionally, salt loading increased the size of the SON and the absence of nesfatin-1 in the nucleus enhanced this effect. Based on our results, we suggest that nesfatin-1 plays a role in the development of dehydration-induced anorexia.

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Role of PACAP in age-related systemic amyloidosis

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Introduction: PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide) is a neuropeptide, which can be found in many tissues and organs of the body. Its general cytoprotective, anti-inflammatory, and anti-apoptotic effects have been proven; however, there are just a few data available of its role in aging. [1] The aim of our experiment was to compare the tissues of wild-type (WT) and PACAP (KO) deficient mice of different age groups to explore the role endogenous PACAP in aging.

Methods: Samples were taken from more than 20 organs of two age groups of WT and PACAP KO mice (n=30). We divided the following age groups: 3-12-months, 13-24-months-old animals. 3-µm-thick sections of the samples were stained with hematoxylin-eosin, Congo-red staining and anti-ß-amyloid immunohistochemistry, after we have found signs of amyloid deposits. A semi-quantitative scoring to grade Congo-positive deposits from 0-3 was performed according to pathological criteria. Complete blood count, serum analysis from the animals' blood and cytokine array examinations from kidney samples were performed.

Results: Histopathological analysis showed that in the PACAP KO mice the lesion in all organs seemed more severe and was present at a younger age. Among the WT and PACAP KO mice, significant difference occurred in the esophagus, kidney, liver, spleen, thyroid, and skin. Complete blood count, serum analysis and cytokine array examinations (BLC, IL-1ra, RANTES) have shown differences, due to the lack of PACAP.

Conclusion: Using young and aging PACAP KO mice, here we demonstrated that in mice lacking endogenous PACAP senile amyloidosis appeared accelerated, more generalized, more severe and affected more individuals. In summary, here we describe accelerated systemic senile amyloidosis in PACAP KO mice, which might indicate an early aging phenomenon in this mouse strain. Thus, PACAP KO mice could serve also as a model of accelerated aging, with human relevance.

Abnormal hypothalamic–pituitary–thyroid axis might influence the outcome of food-motivated learning tests in the triple transgenic Alzheimer's disease model mice

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Alzheimer's disease (AD) is an age-related neurodegenerative disease with progressive memory decline, which could be aggravated by other factors such as changes in thyroid hormone levels. Its transgenic mouse models are promising tools in understanding the underlying mechanisms. We investigated male, 6-8-month-old triple transgenic (3xTg-AD) mice, known to show some pathological hallmark of the disorder. First, the cognitive decline as well as disturbances of fine motoric - as first sign of AD - were studied using motivation-based pellet retrieval (PR) and staircase tests. Next, blood glucose and lipid parameters were checked, as well as their thyroid axis was studied using ELISA for measuring free thyroxine (FT_4) level in serum and qPCR for detecting changes in their pituitary thyroid-stimulating hormone β (TSHβ) and thyroid receptor-β2 (TRβ2) mRNA levels. Paradoxically, 3xTg-AD mice provided better learning performance compared to age-matched controls with some disturbances in their fine motoric. In these 3xTg-AD mice, the blood glucose level was increased without any differences in the cholesterol and triglyceride levels. Increased blood FT_4 level and higher pituitary TSHβ mRNA expression was observed in 3xTg-AD animals, while their pituitary TRβ2 mRNA level was reduced. In summary, we confirmed the presence of impaired motor skill as an early symptom in 3xTg-AD mice. The results of food-driven learning tests may be influenced by altered driving force for food intake in 3x-Tg-AD animal. The hyperactive hypothalamic-pituitary-thyroid axis of 3xTg-AD mice might be the background for this „food craving". Although hypothyroidism was more associated with dementia, but hyperthyreoidism observable in 3xTg-AD animals might also contribute to the cognitive decline.

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USE OF GLUCOSE OXIDASE-BASED ELECTRODE (AMPEROMETRIC BIOSENSOR) IN ANIMAL EXPERIMENTS

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The optimal functioning of all the cells in our body requires an adequate supply of energy, the main source of which is glucose. A good indicator of tissue activity is a decrease in their glucose levels. Our main goal is to develop biosensors that can monitor changes in glucose concentration in vivo. As this issue also has clinical implications for the monitoring of diabetics, appropriate methods have been developed in humans to monitor peripheral tissue glucose levels. However, there is currently no microelectrode available to monitor changes in glucose concentration in brain tissue. Furthermore, adapting the electrodes set to human measurements to the mouse dimension is not solved either. The most widely used group of biosensors are amperometric enzyme electrodes based on oxidoreductase enzymes. To measure sugar levels, the oxidoreductive effects of the enzyme glucose oxidase can be exploited. Principle of measurement: the enzyme glucose oxidase is applied to the surface of the platinum electrode, where it forms hydrogen peroxide from glucose and oxygen. It diffuses to the electrode surface through an H_2O_2-selective, size-exclusion layer covering the electrode surface. The electrons formed during the electrocatalytic decomposition on the surface of the electrode give a detectable current signal, the magnitude of which is proportional to the glucose level of the solution to be measured. For biosensors with amperometric detection of H_2O_2, a size exclusion membrane is used on the platinum electrode surface to ensure selectivity. It is also important to consider the optimal layer thickness of the biosensors. As a first step in in vitro measurements, it is necessary to record a calibration curve in the measurement range that would be expected in in vivo experiments. Overall, the developed sensor should be small (microelectrode) but stable enough, sensitive, and selective to collect only specific information about a small area of the mouse brain, informing us of changes in activity in that brain area with good temporal resolution.

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Functional imaging of hippocampal CA1 pyramidal neurons during virtual navigation in mice

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The hippocampus plays a critical role in the formation and storage of spatial and episodic memories, and disruption of its functions leads to impaired memory, navigation and cognitive ability in affected individuals. The cellular mechanisms whereby the hippocampus supports these memory processes remain incompletely understood. During exploration the hippocampus is thought to create and store maps of the visited environments, encoded by ensembles of hippocampal place cells, which typically fire in a specific location of a given environment. However, there is still a lot to learn about the generation, consolidation and use of hippocampal neuronal maps during learning and navigating different environments under variable cognitive demands. To examine the development, refinement and flexible remapping of place coding activity by hippocampal CA1 pyramidal cells (CA1PCs), we aimed to develop a virtual spatial navigation paradigm for head-fixed mice allowing two-photon imaging of Ca2+ activity in large populations of CA1PCs during learning and navigation. Transgenic Thy1-GCaMP6s mice were implanted with an imaging cannula over the CA1 region of the left hippocampus, and a metal head plate was attached to the skull allowing for head fixation during behavioral training and two photon imaging. After recovery, mice were trained to collect small water rewards at specific locations in two different virtual environments. We recorded behavioral parameters (speed, licks) and imaged GCaMP6s-mediated Ca2+ signals in hundreds of CA1PCs over consecutive days, including during 1) initial learning, 2) switches between randomly varied or continuous blocks of presented environments, and 3) exposure to a novel environment. Behavioral parameters demonstrated that mice were able to efficiently differentiate between the two virtual corridors and learn the location of the reward zone in both. In the novel environment the behavior pattern was disrupted, reflecting unfamiliarity of the new corridor. Analysis of the imaging data is currently underway, but our preliminary results indicate reliable place cell activity, selective representation and enrichment of cells active near the reward zone in familiar environments, and global remapping in the new environment. Further analysis will be directed towards elucidating the CA1 coding dynamics during learning and in response to subtle and robust changes in the environment and task structure.

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Optical recording of unitary synaptic connections using Voltron

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A longstanding goal in neuroscience is to understand synaptic connectivity that underlies function-specific neuronal activities. Patch-clamp recording enables precise resolution to map the synaptic properties between individual neurons. However, the number of connections that can be tested is limited. Our goal is to replace patch-clamp recordings of individual postsynaptic neurons with optical imaging that enables simultaneous monitoring of several potential postsynaptic cells. To measure small voltage fluctuations at high spatio-temporal resolution we sparsely expressed a new genetically encoded voltage indicator (GEVI), the Voltron in hippocampal neurons using rAAV vectors. 4-7 weeks after the injections we prepared acute slices that were incubated with a fluorescent dye (Janelia Fluor 549) that bounds to the expressed Voltron protein and enables simultaneous monitoring of membrane voltage in many neurons using epifluorescent illumination and a fast CMOS camera at high speed (0.67-1 kHz) and large field of view (375x235 µm). First, we tested the applicability of Voltron imaging in acute slices. For this, we determined the scattering of fluorescent signals in simultaneously recorded neurons, and tested various illumination equipments and developed analysis algorithms. Our results showed that the Voltron is capable to report small membrane potential changes with high fidelity. We also observed a quasi-linear correlation between voltage changes and fluorescent emission changes, within experiments. Next, we tested whether unitary synaptic connections can be resolved. For this, we stimulated individual dentate gyrus granule cells using patch-clamp recording and imaged putative postsynaptic target neurons in the hilus. The results showed that Voltron signal can resolve individual postsynaptic responses in individual cells and it also captures the cell-to-cell variability of the responses. Subthreshold responses can be readily distinguished from postsynaptic firing due to the sufficient temporal resolution of the imaging system. Furthermore, the persistence of Voltron signal after conventional fixation allowed us to map the identity of imaged neurons (both responding and silent) using posthoc immunolabelling. Together, these results indicate that Voltron-imaging is a powerful tool for detecting synaptic responses in a large pool of neurons and it is suitable for detailed and precise mapping of synaptic connectivity.

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Examination of viral peptide-targeted nanoparticles on a culture model of the blood-brain barrier

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Drug delivery to the central nervous system is challenging because of the blood-brain barrier (BBB), which is composed of endothelial cells of brain capillaries. Nanoparticles (NPs) are promising new tools to increase the transfer of drugs across the BBB. The advantage of vesicular NPs is that they increase the penetration of cargo molecules across biological barriers and by decorating their surfaces with appropriate ligands they are suitable for targeted drug delivery. The aim of this study was to develop an innovative targeted vesicular NP that crosses the BBB and may act as a brain shuttle for therapeutic molecules. We investigated the ability of PepH3, a BBB shuttle peptide isolated from the capsid protein of Dengue virus alone, and as a targeting ligand of NPs to elevate cargo uptake and penetration across our well-characterized rat primary cell-based co-culture model of the BBB. In our experiments, we used Quasar570-labeled PepH3 and prepared PEGylated PepH3-targeted NPs loaded with the fluorescent protein mCherry as cargo. The physico-chemical properties of NPs, such as particle size, polydispersity index and surface charge were measured by dynamic light scattering. The effect of PepH3 alone and PepH3-targeted NPs on the viability of primary rat brain endothelial cells (RBECs) was monitored by impedance measurement. The cellular uptake of PepH3 and PepH3-targeted NPs were visualized by confocal microscope. We investigated the entry of the peptide and peptide-targeted nanovesicles into cells and their penetration across the culture model of the BBB with fluorescence spectrophotometry. The mean diameter of untargeted and PepH3-targeted particles was 114 nm and 164 nm, respectively. The NPs have slightly negative surface charge and relatively narrow size distribution. The encapsulation efficiency of mCherry cargo was around 10% in both cases. PepH3 had no effect on the viability of RBEC and was rapidly taken up by RBEC cells supported by the visualization and cellular uptake studies. PepH3 peptide alone had high penetration across the BBB model compared to marker molecules. The mCherry cargo loaded in PepH3-targeted NPs showed significantly higher entry into the cells compared to non-targeted NPs, but the penetration of PepH3-targeted NPs across the BBB model was lower than the non-targeted group. We are further investigating PepH3, as a shuttle peptide for targeted drug delivery across the BBB using therapeutic biomolecules.

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Lateral septum affects maternal adaptation via a parathyroid hormone 2 neuropeptide-containing pathway arising from the thalamus

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Parental care is a special form of social behaviour, which increases the survival of the offspring. Our research group previously described that neurons in the posterior intralaminar thalamic nucleus (PIL) express an excitatory neuropeptide, parathyroid hormone 2 (PTH2), whose expression is induced in mothers. Here, we demonstrate that PIL neurons expressing PTH2 send projection to the lateral septum (LS). Furthermore, we confirmed that lateral septal neurons show significantly elevated number of c-Fos-activated neurons in mother rats following pup exposure compared to control mothers without pup interaction. Nerve terminals of LS-projecting PTH2+ fibres arising from the PIL show the same distributional pattern within the LS as pup-activated c-Fos+ neurons. Therefore, we made further examinations and found that PTH2+ terminals closely appose c-Fos-activated septal neurons. Furthermore, PIL neurons projecting to the LS show c-Fos-activation in mother rats following interaction with the pups. Beside the LS, PTH2-expressing neurons of the PIL also send dense projection to the medial preoptic area (MPOA), therefore, we further examined this neuronal pathway. We determined that projections to LS and MPOA origin from the calbindin-positive neuron population of the PIL. By injecting different retrograde tracers into the LS and MPOA of the same rats, we demonstrated that PIL neurons projecting to the LS send axon collaterals to the MPOA, too. We conclude that calbindin-positive PIL neurons project both to the lateral septal neurons and MPOA neurons. We investigated the septal projections where the terminals contain PTH2 neuropeptide, and innervate lateral septal neurons. Since pup exposure activates neurons in all 3 brain regions, our data suggest that PIL neurons convey the stimulatory signal of pups to both the lateral septum and the MPOA thereby contributing to their role in the maternal adaptation.

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Investigation of microglia's morphological changes in human post-mortem and surgical removed focal cortical dysplasia type 2 samples

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Neurodevelopmental epilepsies such as epilepsy with focal cortical dysplasia (FCD) are often treated by surgical removal of the epileptogenic brain tissue. Besides the damage of lamination, FCD type 2 is characterized by the appearance of large, irregular dysmorphic neurons. Neuronal and macroglial changes were investigated in several studies, but the role of microglia has been little researched to date. It has been suggested that microglia are activated in these areas, but their detailed description, including quantitative elements, has not yet been established. The aim of our study was to describe the morphological changes of microglia and microglia-neuron contacts in FCD type 2 brain tissue compared to post-mortem control. Samples of 6 subjects of each group were included in the study. FCD samples from a variety of cortical areas were matched with control samples of the same regions. After fixation, 60 µm thick slices were prepared, on which immunofluorescence reactions were performed. Neurons were labelled with NeuN immunostaining, while the P2Y12 antibody was used to specifically mark the microglia. The neuronal membrane became visible with Kv2.1 antibody. For the imaging we used confocal laser microscopy. Within the sections regions of interest were selected in layers 3 and 5 of cortical grey matter, and white matter were sampled as well. We examined the density of microglia and the proportion of different morphologies of microglia (ramified, hypertrophic, dystrophic, amoeboid, rod). Furthermore, we examined microglial coverage on the surface of the neuronal bodies in layer 3 and whether neurons with dysmorphic morphology differed from normal neurons in this aspect. Accordin to our results, the mean density of microglia in FCD type 2 does not change in grey matter, whereas it increases in white matter. All three types of samples show an increase in density of dystrophic microglia, which may indicate functional differences. Based on our preliminary results, the microglial coverage of neurons is higher in epileptic tissue. However, it is relatively decreased on dysmorphic cells. Our results suggest that microglia are functionally altered in FCD type 2. Given that these cells play a major role in apoptosis as well as in the maintenance of homeostatic functions of neurons, their injury may play a role in epileptogenesis. Our plan is to characterize microglial cells and their somatic neuronal connections in the electron microscope.

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Using appetitive motivation to train mice for spatial learning in the Barnes maze

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Barnes-maze, a well-known spatial learning paradigm, is based on the innate fear of rodents from large open spaces and their drive to hide. However, the apparatus itself is often not aversive enough to provoke the hiding response so additional factors (strong light, threatening sounds or odors) are often used for increased aversiveness, but these additional elements may render the method cumbersome. The objective of this study was to establish a Barnes-maze learning paradigm with appetitive motivation in mice. We used 12 C57BL6/J and 12 NMRI male mice in two experiments. The Barnes-maze was a circular metal table (1 m diameter) with twenty holes (5 cm Æ) evenly spaced along the perimeter. Under one of them we placed the escaping box where the mice could hide (target hole). Extra maze cues were placed in the room. We used chocolate cereal as reward. First, we habituated the mice to the chocolate cereal in their home-cage for 2 nights. Then the animals were put in the escaping box with a piece of reward and placed in the middle of the maze for 20 and 10 min on two consecutive days. After the habituation period the maze-learning started. At the beginning of a trial the mouse was placed in the middle of the maze and were allowed to move around and find the target hole for 5 minutes. There were 2 trials a day. The learning criterium was finding the target hole with less than 1 hole error. When the animal reached this criterium we changed the target hole location. Mice needed to re-learn the new position with the same criterium. We measured the latency to find the target hole (LT), number of visited holes until finding the target hole (NH), latency to reach the first visited hole (LF), initial error (distance of the first visited hole from the target hole, IE). NMRI mice reached the criterium in trial 31, C57BL6/J mice in trial 21. LT showed a steep decrease until trial 5 (C57BL6/J) or trial 4 (NMRI) and a slow gradual decrease afterwards. During the trials LF remained less than 6 s. NH and IE decreased proportionally with time. After changing the box location LT, NH signficantly increased while LF remained the same. IE pointed to the original target hole. Mice learned the new target location within 12 trials (NMRI) or 18 trials (C57BL6/J). In summary, appetitive motivation can be used to establish Barnes-maze learning. The contextual part of the task was learnt quickly, while the exact localization of the target hole required more time.

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Perisomatic inhibition and its relation to epilepsy and to synchrony generation in the human neocortex

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Inhibitory neurons innervating the perisomatic region of cortical excitatory principal cells are known to control the emergence of several physiological and pathological synchronous events, including epileptic interictal spikes. In humans, little is known about their role in synchrony generation, although their changes in epilepsy have been thoroughly investigated. Now, we describe how parvalbumin (PV)- and type 1 cannabinoid receptor (CB1R)-positive perisomatic interneurons innervate pyramidal cell bodies, together with their role in synchronous population events spontaneously emerging in the human epileptic and non-epileptic neocortex, in vitro. Quantitative electron microscopy showed that the overall, PV+ and CB1R+ somatic inhibitory inputs remained unchanged in epilepsy. However, the size of PV-stained synapses increased, and their number decreased in epileptic samples, in synchrony generating regions. Pharmacology demonstrated – in conjunction with the electron microscopy – that although both perisomatic cell types participate, PV+ cells have stronger influence on the generation of population activity in epileptic samples. The somatic inhibitory input of neocortical pyramidal cells remained almost intact in epilepsy, but the larger and consequently more efficient somatic synapses might account for a higher synchrony in this neuron population. This, together with the epileptic hyperexcitability might make a cortical region predisposed to generate or participate in hypersynchronous events.

Attempt to transfer a pharmacological neurovascular uncoupling model from mice to rats

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The objective of the present study was to establish a pharmacologically induced neurovascular uncoupling (NVU) method in rats as a translationally valid animal model of human cognitive decline. Diminished neurovascular coupling (NVC) has been shown during aging and in various brain disorders. Pharmacologically induced NVU with subsequent neurological and cognitive defects was described in mice (Tarantini, 2015), however, no similar procedure has been reported so far in rats.In this study, we used 32 male Hannover Wistar rats. NVU was induced by intraperitoneal administration of a pharmacological "cocktail" consisting of N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MSPPOH, a specific inhibitor of epoxyeicosatrienoic acid)-producing epoxidases, 5 mg/kg), L-NG-nitroarginine methyl ester (L-NAME, a nitric oxide synthase inhibitor, 10 mg/kg) and indomethacin (a nonselective inhibitor of cyclooxygenases, 1 mg/kg) and injected twice daily for 8 consecutive days. Animals were tested in Morris water-maze and fear-conditioning assays on days 5-7 and 4 and 8 of the treatment period, respectively. Blood pressure of the animals was monitored on days -1, 2, 5 and 7. NVC was measured in the barrel cortex in a non-recovery operation. A laser Doppler probe was used to detect changes in cerebral blood-flow (CBF), while the contralateral whisker pad was stimulated. Brain and small intestine tissue samples were collected post mortem and processed for prostaglandin E2 (PGE2) level measurements. When contrasted with the control group, the animals treated with the "cocktail" showed no impairment in their performance in any of the cognitive tasks. However, we observed an overall higher blood pressure in these rats. They also showed a greater than 50 % decrease in CBF, while their barrel cortex was under stimulation. Intestinal bleeding and ulcers were found in some of the treated animals and ELISA assays of the tissue samples revealed significantly decreased levels of PGE2 both in the brain and small intestine. Although we could evoke NVU by the applied mixture of pharmacons, it also induced adverse side effects such as hypertension and intestinal alterations. Furthermore, the treatment did not cause cognitive impairment. Thus, further refinements are still required for the development of an applicable model, mostly with regard to finding the appropriate dosages and learning assays.

Investigation of the nasal barrier function, inflammatory processes and brain morphology in healthy and diseased mice

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Objectives: Our goal was to improve understanding of the process of physiological and pathological aging. In this study, we focused on the alteration of the nasal barrier function, brain morphology and cerebral cytokine expression in aged wild-type and genetically modified mice with Alzheimer's disease-like and atherosclerotic pathology.

Methods: In in vivo dual-probe microdialysis experiments, the permeability and functionality of the nasal barrier were studied by intranasal quinidine (P-gp substrate) in APOB-100 and APP-PSEN1 transgenic mice compared to age-matched healthy wild-type animals. The drug levels in dialysate samples were monitored by LC-MS/MS technique. Furthermore, the cerebro-morphological changes were investigated by MRI, and inflammatory processes were studied by cytokine ELISA plate arrays in aging healthy and diseased mice.

Results: For both transgenic mouse strains, similar quinidine concentrations were measured in blood and brain after intranasal treatment due to the absorption of quinidine across the nasal mucosa, by bypassing the blood-brain barrier. Significant cerebroventricular dilatation was observed in APOB-100 mice by MRI, however no changes were seen in APP-PSEN1 animals. The results of the cytokine assay showed a remarkable overexpression of VEGF, PDGF-BB and IL-17A in APOB-100, and a cerebral upregulation of GM-CSF, IL-17A and resistin in APP-PSEN1 mice.

Conclusion: Based on the results of the microdialysis experiments, it can be concluded that the blood-brain barrier does not play a significant role in the brain penetration of the P-gp substrate model drug, quinidine at nasal administration either in the healthy or in diseased animals. The MRI scans confirmed that APOB-100 dependent neurodegeneration affects the brain morphology, and the result of the cytokine assay showed an inflammatory balance shift not only for neurodegenerative model animals but also for aged control mice. The remarkably increased levels of different cerebral inflammatory markers of the transgenic mouse models can be explained by the cerebrovascular pathology for APOB-100 and Alzheimer's–like neurodegeneration for APP-PSEN1 mice.

Can locomotor impairments and anxiety-like behaviour alter the measurable memory-decline in the triple transgenic mouse model of Alzheimer's disease?

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Preclinical studies with animal models play crucial role in revealing the pathomechanism and identifying new treatment options for Alzheimer's disease (AD). In our study, the triple transgenic mouse model of AD (3xTg-AD) was used. During our previous observations decreased locomotor activity had been discovered, which might influence the outcome of other behavioural tests. In the attempt to better understand the 3xTg-AD mouse model different aspects of its motor skills and its anxiety-like behaviour was tested. Several behavioural tests were performed in order to measure the locomotor activity (open field (OF) test, rotarod test, grip test) and anxiety (fox odor (FO) test, elevated plus maze (EPM) test). Cognitive tests that are strongly based on motivation (social discrimination (SD), active avoidance, Morris Water Maze (MWM) test) were also performed. The experiments were carried out on six-month-old male 3xTg-AD animals in comparison with C57Bl/6 controls. In the OF test 3xTg-AD mice moved significantly less, while during the rotarod test, there was no difference between the genotypes. The performance of the 3xTg-AD animals was worse in the grip test. 3xTg-AD animals spent more time in immobile, 'freezing', posture during the FO test. In the EPM test, the transgenic mice stepped fewer times into the closed arm, without any genotype difference in the locomotion-independent anxiety measures. During SD and MWM the memory decline of the 3xTg-AD mice was confirmed. In contrast, during the active avoidance the strong stimulus of the electric foot-shock forced even the 3xTg-AD mice to learn the task as fast as their controls. We were able to conclude that the 3xTg-AD mice show decreased locomotor activity, decreased strength, and enhanced innate anxiety which might contribute to the differences observable during cognitive tests. In memory tests based on strong motivation (such as MWM test), the locomotor difference may disappear as 3xTg-AD mice might be more motivated to learn quickly. This phenomenon might influence the results of the mentioned memory tests. Thus, it is also important to take into account different motivational factors.

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Unusual Perceptual Experiences and Beliefs Are Associated with Amplified Mnemonic Discrimination and Attenuated Generalization

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Introduction. Tendency to experience inaccurate beliefs alongside perceptual anomalies constitutes positive schizotypal traits in the general population and shows continuity with the positive symptoms of schizophrenia. It has been hypothesized that the positive symptomatology of schizophrenia, and by extension, positive schizotypy, are associated with specific alterations in memory functions. Imbalance between memory generalization and episodic memory specificity has been proposed on several counts; however, the direction of the imbalance is currently unclear. Objectives. We aimed to contrast two competing hypotheses regarding the association between positive schizotypy, and memory alterations in a general population sample (N=71) enriched for positive schizotypy from a larger pool of individuals (N=614). Methods. Positive schizotypy was measured with the short-version of the O-LIFE questionnaire, and memory specificity and generalization was captured by the well-established Mnemonic Similarity Task. Results. Distortions in the behavioural memory performance indices were found to correlate with positive schizotypy: individuals prone to unusual experiences demonstrated increased discrimination and reduced generalization (explaining 10% and 17% of variance, respectively). Associations were robust when controlled for the disorganized, negative and impulsive-asocial dimensions of schizotypy and associated psychopathology. Conclusions. Our findings show that people who are prone to irrational beliefs and unusual experiences also show measurable alterations in memory and likely have difficulty grasping the global picture and rather be overpowered by fragments of information.

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Age-related changes in the activity of basal forebrain cholinergic neurons during Pavlovian conditioning

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Acetylcholine is a neuromodulator that has a crucial role in mediating cognitive functions like arousal, attention, sensory processing, reinforcement expectation, reward and addiction. The basal forebrain cholinergic neurons' widespread projections to the cortical mantle play a key role in these modulatory processes. Age-related loss of cholinergic function has been observed across species, characterized by the degeneration of dendrites, synapsis, and axons. However, the link between cholinergic activity during learning and the normal or pathological age-related neurodegeneration is still missing. In order to better understand the age-related changes in the activity of basal forebrain cholinergic neurons, we combined fluorescent in vivo techniques and optogenetic manipulations in headfixed mice during an auditory cued Pavlovian conditioning task. On one hand, we imaged the acetylcholine release in the basolateral nucleus of the amygdala (BLA) using fiber photometry techniques and the recently developed acetylcholine sensor. On the other hand, we optogenetically manipulated learning acquisition by specifically inhibiting the horizontal diagonal band of Broca (HDB) cholinergic neurons during tone presentation (conditioned stimuli, CS). Our results suggest that cholinergic cells respond with an increase in activity and acetylcholine release after US (both punishment and reward) and reward-predicting CS. Our data suggest that acetylcholine release in the BLA occurs during reward-predicting but not punishment-predicting sensory stimuli. Optogenetic inhibition of HDB cholinergic neurons during the auditory CS presentation impaired the learning process of the animals compared to mice injected with control virus lacking the optogenetic actuator. This suggests that acetylcholine release from basal forebrain neurons is required during the acquisition of the CS-US association during Pavlovian conditioning.

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Brainstem can recall fear memory via hippocampal somatostatin interneurons

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Contextual fear memories are encoded by a sparse population of hippocampal principal neurons that are selected based on their inhibitory-excitatory balance during memory formation. Yet the details of this mechanism are still unclear. Disinhibition of principal neurons could facilitate their selection and then also their re-selection during memory recall. Using optogenetic behavioral experiments, we found that if a subpopulation of dendrite targeting somatostatin (SOM) positive interneurons in the dentate gyrus (DG) of the hippocampus are inhibited during fear conditioning, their re-inhibition can recall fear memory even in a novel environment. We discovered that SOM cells are selectively innervated by brainstem nucleus incertus (NI) GABAergic cells. We also found that if NI GABAergic fibers in DG are stimulated optogenetically during fear conditioning, their re-stimulation can recall fear memory via hippocampal SOM cells. Using c-Fos immunostaining, we found that NI neurons showed correlated activity with DG principal neurons during contextual fear memory recall, but not during rest. Furthermore, we observed that inhibition of NI GABAergic cells impaired contextual fear memory recall. Our data suggest a key disinhibition-based memory mechanism in the hippocampus that is supported by local SOM interneurons and their brainstem inputs.

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Analysis of ultrasonic vocalizations (USV) in mice

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Ultrasonic vocalizations (USVs) are fundamental forms of communication between conspecifics to promote social interactions and survival. Still, the underlying neural mechanisms of this communication channel are mostly unknown. Thus, we first recorded ultrasonic vocalizations of the male and female laboratory mice in their home cage and during several diverse stressful situations (separation, shock, restraint, cat fur). The recorded USV signals are acoustically analysed, with the help of the individual call extractions. These individual calls were further processed with noise filtering and principal component analysis for call characterization. In our analysis we could group mice USV calls (n = 459) into 5 distinct clusters. One of the clusters' frequency range reached 120-150 kHz and was only recorded during restrain stress. As this high frequency USV has not been identified in the literature, we have started to investigate its behavioural effects and the related underlying neuronal circuitries. Thus, we used an early-immediate gene(c-Fos) approach to identify those brain regions which are activated by the replay of this distress USVs (dUSV). Especially, we were interested whether the calretinin-expressing lateral thalamic neurons, which plays an important role in learnt auditory fear responses (Barsy, Kocsis et al, 2020), also take part in the process of these calls. Furthermore, we are planning to analyse the dUSV-evoked activation pattern in vivo. Taken together, our date can shed light on a novel communication channel between mice in stressful conditions. Furthermore, our research may reveal a neuronal network mechanism in which evolutionary conserved as well as experience-dependent auditory signals can be processed.

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