NeuroConnect 2026

Organoid Neurophysiology Workshop

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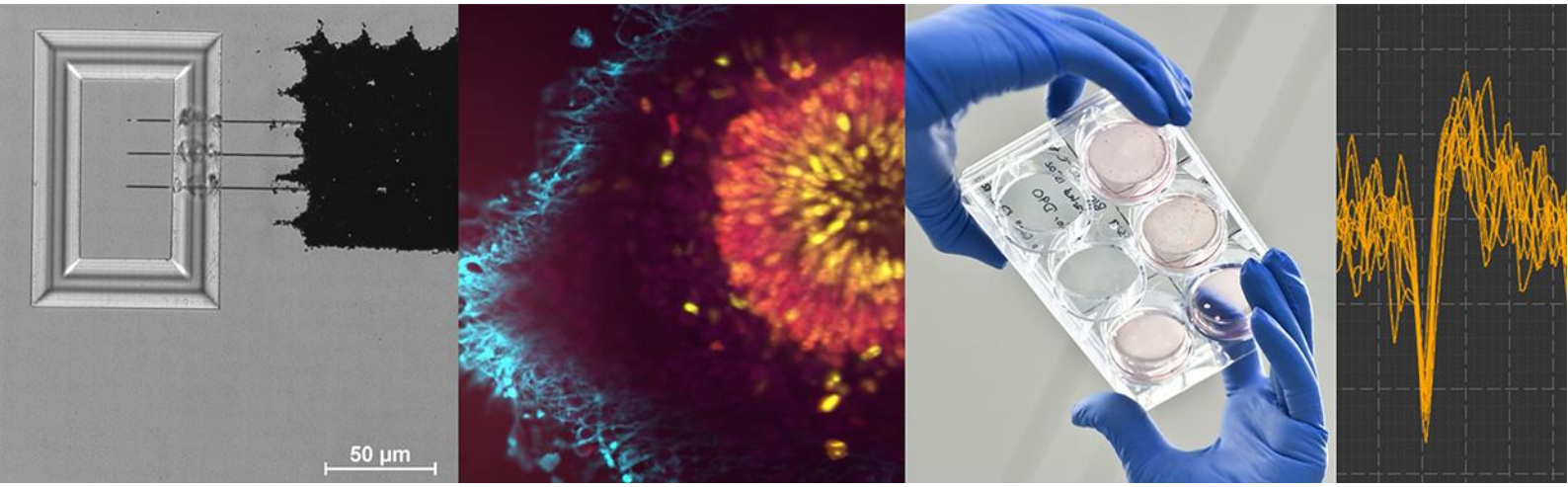

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Poster 1

Early Transcriptomic Dysregulation in PCH2a Cerebellar Organoids Reveals Altered WNT Signaling and Developmental Patterning

Daria Andreeva, Theresa Kagermeier, Simone Mayer

Zoological Institute, Karlsruhe Institute of Technology (KIT), Germany.

Pontocerebellar hypoplasia type 2a (PCH2a) is a rare autosomal recessive neurodevelopmental disorder characterized by cerebellar and pontine hypoplasia, leading to severe neurological impairment. It is caused by a point mutation in a gene involved in tRNA splicing and maturation (TSEN54). Despite the ubiquitous nature of this molecular process, the mechanisms underlying the selective vulnerability of specific brain regions during early human development remain poorly understood. To investigate early pathogenic events, we utilized patient-derived human induced pluripotent stem cells (hiPSCs) differentiated into cerebellar organoids. Previous work demonstrated structural alterations in progenitor zones and reduced organoid size in PCH2a lines during early differentiation (Kagermeier et al., 2022). To define the earliest molecular changes, we performed bulk RNA sequencing at day 0, day 7, and day 12 of differentiation in control and PCH2a organoids. Our analysis revealed early transcriptomic dysregulation in PCH2a organoids. We observed significant downregulation of genes associated with WNT signaling, midbrain specification, regionalization, and developmental patterning. In contrast, genes involved in cell adhesion and cell-to-cell communication were upregulated. Differentially expressed non-coding RNAs were linked to neurodevelopmental processes, and WNT pathway regulation. These findings suggest that impaired developmental signaling and early patterning defects precede overt morphological abnormalities. Ongoing work aims to validate these findings using isogenic lines and functional WNT pathway modulation to determine whether restoring signaling can rescue early developmental phenotypes.

Poster 2

3D X-ray Imaging Across Biological Scales: From a single cell to whole organism

Fariha Mahzabin Annesha[1,3,+], Kristaps Kairiss[2,3,+], Prof. Dr. Venera Weinhardt[3,+]

[1] Heidelberg Graduate School of Mathematics and Computer Science (HGSMathComp), Heidelberg University, Im Neuenheimer Feld 205, 69120 Heidelberg, Germany.
[2] Centre for Organismal Studies (COS), Heidelberg University, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany.
[3] Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.
[+] equal contribution.

Biological form emerges across a vast range of spatial scales, from the nanometer architecture of individual cells to the millimeter structure of whole tissues and organisms. Capturing this hierarchy requires imaging methods that preserve structural context while spanning these scales. Synchrotron-based X-ray imaging provides such a capability, enabling non-destructive three-dimensional visualization of intact biological specimens from nanometer to millimeter resolutions. Techniques including soft X-ray tomography and X-ray computed tomography allow quantitative characterization of cellular ultrastructure, tissue morphology, and organismal anatomy within their native structural environments. In this work, we showcase how X-ray imaging can reveal biological organization across scales through representative examples spanning cellular to organismal structures in 3D. These multiscale observations highlight the power of X-ray methods for quantitative morphometric analysis and demonstrate their growing role as versatile tools for investigating the hierarchical architecture of biological systems.

A defined monolayer culture system for PSC-derived BRG cells facilitates mechanistic insights into Human Neurodevelopment

Annasara Artioli[1,2,3,+], Matteo Gasparotto[1,2,3,+], Andrea Carlo Rossetti[1,2,3], Yannick Hass[1,2,3], Anne Hoffrichter[1,2,3], Ryszard Wimmer[4,5], Fabio Marsoner[1,2,3], Raquel Perez Fernandez[1,2,3], Catello Guida[1,2,3], Philipp Koch[1,2,3,6], Alexandre D. Baffet[4,5], Ammar Jabali[1,2,3,7], Julia Ladewig[1,2,3]

[1] Department of Translational Brain Research, Central Institute of Mental Health (ZI), University of Heidelberg/Medical Faculty Mannheim, Mannheim, Germany.
[2] Hector Institute for Translational Brain Research (HITBR gGmbH), Mannheim, Germany.
[3] German Cancer Research Center (DKFZ), Heidelberg, Germany.
[4] Institut Curie, CNRS UMR144, PSL Research University, Sorbonne University, Paris, France.
[5] Institut national de la santé et de la recherche médicale (Inserm).
[6] German Center for Mental Health (DZPG), partner site Mannheim-Heidelberg-Ulm, Mannheim, Germany.
[7] Present: ISAR Bioscience GmbH, Semmelweisstr. 5, 82152 Planegg, Germany.
[+] equal contribution.

Modeling human corticogenesis has been hindered by limited access to primary fetal tissue. While human induced pluripotent stem cell (hiPSC)-derived cerebral organoids have advanced our understanding of cortical development, they underrepresent certain progenitor populations, including basal (outer) radial glia (bRG). Predominantly localized in the outer subventricular zone (oSVZ), bRG are key neural progenitors that drive the expansion of the human neocortex and whose dysfunction have been implicated in neurodevelopmental disorders (NDD). They are defined by characteristic molecular markers, specific signaling pathways and hallmark behaviors including mitotic and interphasic somal translocation (MST and IST). To date, however, bRG could only be studied in small numbers, either in primary tissue or within cerebral organoids. While acute isolation into 2D cultures is possible, this yields very limited numbers of cells that cannot be expanded, posing major technical barriers to systematic and scalable analyses. Here, we present a defined 2D-culture system enabling generation, expansion and differentiation of bRG-like cells from human iPSCs. These cultures closely resemble primary bRG, retain canonical markers and exhibit niche-like behaviors including cell-cell crosstalk and dispersive movement. Network-based transcriptomic screen identified PAK2 as regulator of MST, a defining feature of bRG dynamics. This simplified and reproducible 2D system provides an accessible experimental platform for dissecting bRG biology, enabling controlled interrogation of human cortical progenitor dynamics and signaling mechanisms. By bridging the gap between primary tissue and complex organoid models, it advances our capacity to model human neocortical development and associated NDD *in vitro*.

Poster 4

Organotchi: an AI-based segmentation tool for the morphological characterization and tracking of brain organoids

Lízia Branco[1], Theresa Kagermeier[1], Pierre Collignon[2], Zeynep Yentür[1], Daria Andreeva[1], Simone Mayer[*1,2]

[1] Zoological Institute, Karlsruhe Institute of Technology (KIT), Germany.
[2] Institute of Biological and Chemical Systems - Functional Molecular Systems, Karlsruhe Institute of Technology (KIT), Germany.

Brain organoids are powerful *in vitro* models for studying early brain development and neurodevelopmental disorders. However, longitudinal assessment of organoid growth and morphology - which is essential for quality control, evaluation of batch-to-batch homogeneity, and the identification of subtle developmental changes associated with genetic or environmental perturbations - typically relies on the manual characterization of brightfield microscopy images, a labor-intensive process susceptible to observer bias and with limited scalability. Moreover, substantial protocol-dependent morphological heterogeneity and dynamic growth-related changes demand segmentation approaches that are both robust and adaptable. To address these challenges, we developed Organotchi, a deep learning-based, high-throughput tool for automated organoid segmentation, tracking, and morphological characterization. Organotchi was trained on a published dataset of 1,400 brightfield images acquired over 30 days from two independent laboratories using an unguided organoid protocol, enabling the model to be resistant against broad morphological variability. Additionally, classical segmentation filters were added to allow for the detection and removal of common imaging artifacts. The model achieved >90% performance across three complementary segmentation metrics (pixel accuracy, intersection over union, and Dice coefficient) and was validated across five distinct organoid protocols, multiple developmental stages, and diverse experimental conditions. Organotchi enables rapid, standardized, and unbiased quantification of organoid morphology across batches, thereby improving reproducibility, throughput, and scalability in organoid-based neuroscience research.

Poster 5

3D-printed connectors for multifunctional fiber-based neural interfaces

Emilija Boštogaitė[1,2*], Anagha Navale[1,2], Kunyang Sui[3,4], Marcello Meneghetti[3,4], Christos Markos[3], Thomas Stieglitz[1,2,5]

[1] Laboratory for Biomedical Microtechnology, Department of Microsystems Engineering – IMTEK, IMBIT//NeuroProbes, University of Freiburg, Germany.
[2] BrainLinks-BrainTools Center, University of Freiburg, Germany.
[3] DTU Electro, Department of Electrical and Photonics Engineering, Technical University of Denmark, Lyngby, Denmark.
[4] Department of Neuroscience, University of Copenhagen, Denmark.
[5] Bernstein Center Freiburg, University of Freiburg, Germany.

In order to enable local recording of neural signals in response to optical stimulation, neural implants combining electrophysiology and optical technologies have become increasingly prevalent. Such multimodal probes can be produced as a fiber by leveraging fiber drawing processes for polymer fibers. Multifunctional polymer-based fibers integrate a waveguide, electrodes, and fluidic channels within a single device. The main challenge of integrating multiple functionalities in a small fiber (<200 µm diameter) is connectorization. From accessing individual modalities to interfacing external equipment, the connector must be reliable and small-scale to bridge the gap, thus enabling optical stimulation, electrical recording, and fluid delivery. This work presents 3D-printed connectors for multifunctional fibers using commercially available resins. These 3D-printed connectors include a ferrule for delivering light, electrode connections, and a fluid inlet. Resin 3D-printing or stereolithography (SLA) is a process for producing high-resolution and high-accuracy parts. The wide availability of 3D printers makes this technology more accessible than standard cleanroom processes used for microfabrication, while 3D printing accelerates the connector design development and optimization. Moreover, it allows for component designs to easily be adapted from *in vivo* applications to better suit studies with *in vitro* models.

Poster 6

Isogenic iPSC-Derived Cerebellar Organoids Reveal Early Developmental Vulnerabilities in PCH2a

Francesco Castagnetti[1*], Theresa Kagermeier[1,2*], Lízia Branco[1*], Zeynep Yentür[1,2], Daria Andreeva[1], Isabel Potthof[1], Pierre Collignon[1,3], Simone Mayer[1,2,3]

[1] Zoological Institute, Karlsruhe Institute of Technology (KIT), Germany.
[2] Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.
[3] Institute of Biological and Chemical Systems - Functional Molecular Systems, Karlsruhe Institute of Technology (KIT), Germany.

Pontocerebellar hypoplasia type 2a (PCH2a) is an ultra-rare, autosomal recessive neurodevelopmental disorder characterized by cerebellar and pontine hypoplasia and progressive microcephaly, for which no effective therapies currently exist. The disease is caused by a homozygous founder mutation (A307S) in TSEN54, encoding a core subunit of the tRNA splicing endonuclease (TSEN) complex. Despite its monogenic origin, the pathogenic mechanisms linking defective tRNA processing to selective hindbrain vulnerability remain unresolved, largely due to the absence of faithful human model systems. Building on recent advances in human region-specific neural organoids (Kagermeier et al., 2024), we established a system based on complementary isogenic induced pluripotent stem cell (iPSC) lines through genome editing. In control iPSCs, we introduced the A307S mutation, while in patient-derived iPSCs we corrected the pathogenic variant (S307A), generating isogenic pairs. These lines were differentiated into cerebellar organoids following established protocols (Silva et al., 2020; Atamian et al., 2024) and profiled by single-cell RNA sequencing at days 30 and 50 of differentiation. PCH2a cerebellar organoids exhibited disrupted regionalization and impaired developmental patterning. Across multiple cellular populations, we observed consistent downregulation of RNA splicing, translation, and ribosome biogenesis pathways. Notably, mTORC1 signaling, unfolded protein response (UPR), and ER-associated protein biogenesis were negatively enriched, suggesting a compromised capacity to sustain proteostatic and metabolic stress responses during early cerebellar development. Collectively, these data position defective translational control and stress adaptation, beyond defective tRNA splicing, at the core of PCH2a pathogenesis, thereby providing a conceptual framework to investigate whether modulation of proteostatic and metabolic resilience can modify disease progression.

Field-Effect Transistors for Biological Applications

Merve Nur Ekmekci, Gabriel Cadilha Marques, Michael Hirtz and Jasmin Aghassi Hagmann

Institute of Nanotechnology, Karlsruhe Institute of Technology (KIT), Germany.

Cancer is a disease with one of the highest mortality rates. Despite significant advancements in modern science and medicine, cancer is still challenging to treat. To preserve tissue homeostasis, cells go through strictly controlled processes of growth, division, and programmed death. Therefore, it is crucial to detect cancerous cells at an early stage. The BioFETs consist of ITO drain and source electrodes as well as inkjet-printed In_2O_3 as the channel. Subsequently, biotinylated lipid membranes are patterned via capillary printing on the channel. For electrical characterization, phosphate-buffered saline and a composite solid polymer electrolyte are constrained in the channel area of the BioFET. Finally, bovine serum albumin and streptavidin layers are sequentially applied via pipetting. In the In_2O_3 liquid-gated device, PBS deposition caused a negative shift in threshold voltage, due to electric double layer formation and strong ionic gating. In the absence of lipids, the threshold voltage shifted to more negative values. This shift is indicative of enhanced gate coupling and a more stabilized electrolyte/semiconductor interface. However, when a lipid layer was present, PBS produced a strong degradation in subthreshold behavior together with a pronounced negative shift of the threshold voltage, suggesting reduced effective gate capacitance and increased interfacial polarization/trapping. After BSA blocking, the threshold voltage only showed a minor shift toward more positive values in both lipid and no-lipid cases. Finally, streptavidin adsorption resulted in a small additional positive shift of the threshold voltage for both conditions, indicating biomolecular-induced interfacial charge/dipole effects and enhanced trapping or polarization dynamics. In future works, the sensing ability of the BioFET/EGFET device will be further characterized by immobilizing certain biorecognition probe molecules, such as antibodies, and then exposing the sensors to cancer-related protein biomarkers. The variations in the transfer curve parameters, such as Vth changes, subthreshold slope, and hysteresis, will be monitored and analyzed to confirm the sensing and binding effects.

Poster 8

HD-MEA Functional Profiling of Patient-Specific iPSC-Derived Neuronal Networks for Precision Drug Discovery

Jay English[1,2], Jinghui Geng[3,4], Tal Sharf[3], ChangHui Pak[1]

[1] Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst MA 01003.
[2] Graduate Program in Molecular and Cellular Biology, University of Massachusetts Amherst, Amherst MA 01003.
[3] Department of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, CA 95064.
[4] Department of Electrical and Computer Engineering, University of California Santa Cruz, Santa Cruz, CA.

Patient derived induced pluripotent stem cells (iPSCs) provide a powerful platform for modeling disorder specific mechanisms in neurodevelopmental and neurological conditions and for accelerating individualized therapeutic discovery. Using mixed glutamatergic/GABAergic induced cortical neurons (E–I iNs), we previously identified disorder specific synaptic phenotypes in autism spectrum disorder (ASD) cultures via whole cell patch clamp electrophysiology. ASD derived E–I iNs exhibit elevated spontaneous excitatory transmission and impaired homeostatic synaptic upscaling following chronic silencing, while control cultures maintain robust compensatory plasticity. During these studies, we also observed qualitative alterations in synchronous burst dynamics, suggesting that homeostatic plasticity deficits in ASD extend beyond single synapse physiology to the level of network organization. To systematically quantify these network level alterations and evaluate their therapeutic tractability, we propose to translate this platform to high density microelectrode arrays (HD MEAs). HD MEAs enable longitudinal, non-invasive monitoring of large-scale neuronal network activity, providing scalable readouts of firing patterns, synchrony, and adaptive responses to chronic perturbations. We will characterize how ASD and control derived networks respond to chronic silencing or activation, focusing on burst structure, firing regularity, and network level signatures of homeostatic plasticity. We will then perform targeted drug screening using compounds with known or emerging roles in synaptic scaling, including retinoic acid and lithium, to determine whether any restore adaptive network responses. Finally, we aim to extend this framework to additional monogenic neurological disorders – such as genetically defined epilepsies – to determine whether HD MEA-based homeostatic plasticity signatures can serve as a generalizable functional biomarker for personalized therapeutic selection.

TBR2 Coordinates ATP-Dependent Chromatin Remodeling to Drive Neuronal Differentiation in Human Cortical Organoids

Catello Guida[1,2,3], Matteo Gasparotto[1,2,3], Anne Hoffrichter[1,2,3], Fabio Marsoner[1,2,3], Annasara Artioli[1,2,3], Julia Ladewig[1,2,3]

[1] Central Institute of Mental Health (ZI), University of Heidelberg/Medical Faculty Mannheim, Mannheim, Germany.
[2] Hector institute for Translational Brain Research (gGmbH), Mannheim, Germany.
[3] German Cancer Research Center (DKFZ), Heidelberg, Germany.

Hearing loss. The T-box transcription factor TBR2 (EOMES) plays a central role in intermediate progenitor (IP) cell identity during cortical development, yet its mechanistic contribution to human neuronal differentiation remains incompletely understood due to early embryonic lethality associated with total TBR2 loss *in vivo*. To address this limitation, we generated CRISPR/Cas9-engineered human induced pluripotent stem cell (hiPSC) lines lacking TBR2 and differentiated them into forebrain organoids to model early stages of human corticogenesis. Comprehensive characterization of TBR2 knockout organoids using immunofluorescence, metabolic assays, and single-nucleus transcriptomic and chromatin accessibility profiling revealed marked accumulation of neural progenitors accompanied by a profound failure of neuronal differentiation. All neuronal identities were severely compromised, indicating that TBR2 loss primarily disrupts neuronal maturation rather than exerting a direct effect on progenitor specification. Multi-omics analyses further demonstrated widespread downregulation of neuronal gene programs and pronounced alterations in chromatin organization within neuronal populations. Because chromatin must adopt a permissive state to enable neuronal gene expression, and because ATP-dependent chromatin remodeling complexes undergo defined developmental subunit switches during neurogenesis, we examined the regulation of the NuRD and neuronal BAF (nBAF) complexes as potential downstream direct targets of the transcription factor. TBR2-deficient organoids failed to execute normal subunit transitions, retaining progenitor-associated components while failing to upregulate neural-specific subunits essential for neuronal identity. Given the established involvement of chromatin remodeling complexes in a wide spectrum of neurodevelopmental disorders, these results position TBR2 as a potential regulatory hub whose dysregulation may contribute to disease pathogenesis through disrupted chromatin remodeling during human cortical development.

A Human Microglia-Containing Cortical Organoid Model to Investigate Neuroinflammation and rTMS-Induced Network Modulation

Marsela Hakani[1], Anna Pancho[1], Ramya Rama[1], Andreas Vlachos[1,2,3]

[1] Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany.
[2] Center for Basics in Neuromodulation (NeuroModulBasics), Faculty of Medicine, University of Freiburg, Freiburg, Germany.
[3] Center BrainLinks-BrainTools, University of Freiburg, Freiburg, Germany.

Human brain organoids, three-dimensional (3D) structures derived from human induced pluripotent stem cells (hIPSCs), represent a powerful model system that recapitulates key aspects of human brain development, thereby providing a suitable platform to study the development and progression of neurological diseases. However, despite their promising features, the value and applicability of organoids are often hindered by several unresolved limitation. These include substantial inter-organoid heterogeneity, as well as the development of a necrotic core during long-term culture, which can limit cellular maturation and circuit formation. The lack of microglia, the central nervous system (CNS) immunocompetent cells, within developed organoids further contributes to these limitations. In this study, we aimed to develop microglia-containing human cortical organoids that resemble the cellular and structural human neocortex. To improve tissue viability and maturation, organoids were additionally cultured as air-liquid-interface cortical organoids (ALI-COs) following vibratome slicing. This approach reduces necrotic core formation by improving nutrient and oxygen diffusion. We further evaluated the integration, survival, and maturation of microglial progenitors within the organoid tissue. The development of such an advanced platform provides a physiologically relevant model to investigate mechanisms of neurodegeneration. Specifically, we aim to employ this system to study amyloid-β plaque formation and associated neuroinflammatory responses in Alzheimer's disease (AD). Furthermore, we will use repetitive transcranial magnetic stimulation (rTMS) as a non-invasive neuromodulatory approach to investigate its impact on neuronal maturation, microglial activation states, and emergent network activity within human cortical organoids.

Human otic Bioengineered Neuronal Organoids (oBENOs) mimic early inner ear development and physiology (oBENOs)

Angeliki Koufali[1,3,4], Guobin Bao[1], Emre Taylan Duman[2], Maria-Patapia Zafeiriou[1,3,4]

[1] University Medical Center, Georg-August-University Göttingen, Germany, Pharmacology and Toxicology.
[2] University Medical Center, Georg-August-University Göttingen, Germany, NGS Integrative Genomics Core Unit (NIG).
[3] Georg-August-University Göttingen, Germany, Else Kröner Fresenius Center for Optogenetic Therapies (EKFZ L2T).
[4] Georg-August-University Göttingen, Germany, Collaborative Research Center 1690 (CRC1690).

Hearing impairment is the most common sensory disorder. Unraveling the underlying disease mechanisms and developing effective therapies for the most prevalent form, sensorineural hearing loss, are essential to address the large unmet medical need. Human otic organoid models provide a promising platform for disease modeling, drug screening, and regenerative medicine. Here, we report the generation of a human induced pluripotent stem cell-derived organoid model, the otic bioengineered neuronal organoid (oBENO), which reproducibly develops functional hair cell (HC)-like cells and spiral ganglion neuron (SGN)-like cells along supporting cell populations, and an intricate vascular network. Temporal bulk RNA sequencing analysis of oBENOs, between days 0 and 60, revealed that the oBENO development closely mimics *the in vivo* stages of early inner ear formation. By day 60, transcripts related to the otic epithelium development, including HC differentiation, as well as neuronal maturation, are abundant. Notably, the latter were significantly enhanced upon retinoic acid addition. To genetically trace the SGN-like cells, we generated a transgenic reporter line (trSGN). Calcium imaging of the labeled cells demonstrated that endogenous glutamate neurotransmission drives their activation. In the future, a double tracing line labelling both HC and SGN-like cells could allow for the investigation of their potential synaptic connectivity. Altogether, the defined multicellularity, high reproducibility, and functional characteristics of oBENOs render them a promising model for investigating inner ear development and assessing potential therapies for restoring sensory hearing loss.

Poster 12

Functional and molecular profiling of iPSC-derived cell types from Bipolar Disorder patients in Spanish multiplex families

Ankita Kumar Bhamidipati[1,2], Dr. Anne Hoffrichter[1,2], Dr. Malin Schmidt[1,2], Dr. med. Philipp Koch[1,2]

[1] Central Institute of Mental Health (ZI), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.
[2] Hector Institute for Translational Brain Research gGmbH (HITBR), CIMH, Mannheim, Germany.

Bipolar Disorder (BD) is a highly heritable neuropsychiatric condition, yet the molecular and cellular mechanisms linking genetic risk to neural function remain poorly understood. This study utilises induced pluripotent stem cells (iPSC)-derived cell types to investigate cell type-specific disease relevant phenotypes associated with BD type 1. Our central hypothesis is that patient-derived iPSCs exhibit disruptions in neurodevelopmental trajectories and calcium signalling across distinct cellular lineages. We differentiated iPSC lines from four BD type 1 patients (from multiplex families) and four age- and sex-matched unrelated controls into cortical progenitors, neurons, and astrocytes. To characterise molecular and cellular differences, we conducted transcriptomic profiling and high throughput immunocytochemistry using the Opera Phenix (PerkinElmer/Revvity) platform. In cortical progenitors, differential expression analysis revealed dysregulation of neurodevelopmental genes, notable members of the ephrin signalling family. BD iPSC-derived neurons exhibited dysregulated expression of protocadherin family genes, along with key neuropsychiatric risk genes including DISC1 and NRXN1. BD iPSC-derived astrocytes displayed differential expression of astrocytic glutamate transporter genes, potentially indicating impaired maturation and cellular stress responses. Integrative transcriptomic analysis revealed consistent dysregulation of calcium signalling across the examined cell types. We are currently validating these findings and correlating them with morphological and electrophysiological alterations. Through this cell type specific approach, we aim to uncover key pathways underlying BD pathophysiology for the development of pathway-based therapeutic strategies.

Poster 13

Functional Characterization of L-Dopa responses in Midbrain Organoids using 3D HD-MEA Technology

Elisa Monz[1], Xiaobo Han[2,3], Remi Yokoi[2,3], Bumpei Noda[2,3], and Ikuro Suzuki[2,3]

[1] 3Brain AG, Pfäffikon, Switzerland.
[2] Tohoku Institute of Technology, Sendai, Miyagi, Japan.
[3] VitroVo, Inc, Sendai, Miyagi, Japan.

The development of brain organoids has opened a powerful avenue for modelling the richness and complexity of human neural circuitry *in vitro*. Neural network dysfunction can be a hallmark of many prevalent neurological disorders, therefore, the ability to investigate the functional network activity of brain organoids is becoming essential for therapeutic development. Yet, dissecting the functional organization of these complex 3D structures remains technically challenging. Here, we present an approach built on 3Brain's CorePlate™ 3D, a next-generation high-density multi-electrode array (HD-MEA) platform engineered to capture and analyse the electrical dynamics of three-dimensional neural tissues such as brain organoids. CorePlate™ 3D enables the simultaneous acquisition of signals from thousands of electrodes at a high signal-to-noise ratio, offering unprecedented access to the spatial and temporal features of 3D neuronal networks. Using this platform, we functionally characterized a four-month-old midbrain organoid and investigated its response to varying concentrations of L-3,4-dihydroxyphenylalanine (L-Dopa). From these recordings, we extracted single-unit spikes and isolated the activity of individual putative neurons embedded within the organoid's architecture. L-Dopa application induced pronounced changes across multiple functional metrics, including firing rate, burst structure, and network connectivity, clearly demonstrating the sensitivity of CorePlate™ 3D to modulatory effects ranging from single-cell physiology to large-scale network dynamics. Overall, our findings establish 3Brain's CorePlate™ 3D as a robust and versatile technology for unlocking the full functional potential of midbrain organoids, positioning it as a powerful tool for mechanistic research, disease modelling, and drug development.

Poster 14

A Surface-Attached Protein-repellent Hydrogel Coating to Enhance the Biocompatibility of Neural Implants

Fernanda Narváez[1,3], Shubham Tiwari[2,3], Jürgen Rühe[2,3], Simon Binder[1,2,3]

[1] Center BrainLinks-BrainTools, University of Freiburg, Germany.
[2] Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), Cluster of Excellence livMatS, University of Freiburg, Germany.
[3] Department of Microsystems Engineering - IMTEK, University of Freiburg, Germany.

Implantable neural interfaces are engineered to interact with the nervous system to restore, replace, or modulate sensory and motor functions. However, their long-term performance remains limited by foreign body reactions at the implant–tissue interface. Surface coatings that prevent nonspecific protein adsorption and subsequent cellular adhesion represent a promising strategy to mitigate this response while preserving device functionality. In a previous study, polyimide spinal cord implants were coated with a surface-attached hydrogel copolymer containing N,N-dimethylacrylamide (DMAA) and 4-methacryloyloxybenzophenone (MABP). A P(DMAA-co-2.5%-MABP) multilayer was achieved via spray coating with tunable thickness between 100nm to 350nm resulting in slightly increased impedance by 19%, and reduced NIH-3T3 fibroblast adhesion to 9.4%. In this work, we extend this strategy to the novel ultra-high channel-count, highly flexible brain implant NeuralaceTM recently presented by Blackrock Neurotech. The implant's silicon-based, mechanically compliant design necessitates substantial adaptations regarding the coating process and surface chemistry. We thus investigated the surface-attachment of prepolymer gels, containing 5% of 2-acryloyloxyanthraquinone (AOAQ) as the photoreactive comonomer, along with 95% of DMAA. To achieve uniform coating on the complex NeuralaceTM geometries, a recently developed foil transfer printing was successfully implemented. Hydrogel layer thicknesses of test substrates ranged from 2 to 10 µm and highly depended on precursor concentration and copolymer molecular weight. Further studies will evaluate foreign body responses using organotypic brain slices and *in vivo* experiments to assess tissue reactions under physiologically relevant conditions, thus establishing a platform for systematical investigation and modulation of tissue responses in brain tissue environments with high density neural interfaces.

Brain–Surface Interfaces: Optical Voltage Readout and Peptide-Engineered Microenvironments for Neural Organoids

Alexander Nesterov-Müller[1], Stefan Bräse[2], Véronique Orian-Rousseau[3]

[1] Institute of Microstructure Technology, Karlsruhe Institute of Technology (KIT), Germany.
[2] Institute of Organic Chemistry, Karlsruhe Institute of Technology (KIT), Germany.
[3] Institute of Biological and Chemical Systems, Karlsruhe Institute of Technology (KIT), Germany.

The development of functional *in vitro* models of the human brain requires both precise readout of neuronal activity and controlled modulation of cell–surface interactions. Here, we present a two-part technological platform that integrates optical voltage sensing with peptide-engineered interfaces for neural organoids and cell networks. In the first part, we introduce transparent voltage-to-fluorescence conversion surfaces that enable optical readout of electrical activity in neural cells and organoids with high spatial and temporal resolution. This approach allows non-invasive monitoring of surface voltage dynamics and supports the study of emergent network behavior in engineered neural systems. In the second part, we develop high-density peptide microarray-based surfaces to screen and identify peptide sequences that modulate cell–surface interactions, including adhesion, repulsion, and functional organization of neural assemblies. These peptide-functionalized interfaces provide a tunable microenvironment to influence organoid maturation and network formation. Together, these technologies establish a framework for integrating electrical stimulation, optical readout, and molecular surface engineering. This combined platform opens new opportunities for studying brain physiology *in vitro* and advancing brain–computer interface concepts at the organoid level (Sonnentag et al., Commun Biol 7, 870, 2024)..

Functional Modeling of the Human Blood–Brain Barrier Using Neurovascular Assembloids

Anna Pancho[1], Marsela Hakani[1], Ramya Rama[1], and Andreas Vlachos[1,2,3]

[1] Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany.
[2] Center for Basics in Neuromodulation (NeuroModulBasics), Faculty of Medicine, University of Freiburg, Freiburg, Germany.
[3] Center BrainLinks-BrainTools, University of Freiburg, Freiburg, Germany.

The blood–brain barrier (BBB) is essential for brain function, preserving microenvironmental homeostasis by restricting pathogens and toxins while regulating ion, metabolite, and signaling exchange. Neuronal activity dynamically modulates BBB transporter expression and permeability, reflecting bidirectional neurovascular cross-talk. BBB dysfunction contributes to stroke, multiple sclerosis, Parkinson's disease, and Alzheimer's disease. Age-related hippocampal BBB breakdown is an early event in cognitive decline and represents a translational gap between animal models and human biology, emphasizing the need for advanced human *in vitro* BBB models. To address this, we have successfully differentiated human induced pluripotent stem cells (hiPSCs) into vascular organoids exhibiting robust vessel formation, as well as hippocampal organoids. In parallel, we are differentiating hiPSCs into microglia, with the goal of assembling a multicellular model that recapitulates the principal cellular components of the human neurovascular unit. To generate a neurovascular model, we have assembled hippocampal and vascular organoids into assembloids. Due to necrotic core formation from limited diffusion during long-term maturation, we are evaluating hippocampal organoids in an air–liquid interface (ALI) system at two time points. To better mimic BBB development, we are generating ALI-grown assembloids that integrate vascular and brain tissue components. This platform enables study of activity-dependent BBB regulation properties in a fully human, multicellular context. We are currently assessing neuronal activity using patch-clamp electrophysiology and plan to integrate multi-electrode array (MEA) recordings. By linking functional network activity to neurovascular organization, this approach allows us to study how neuronal activity shapes barrier maturation and stability in health and disease.

Poster 17

Using isogenic control lines for an in-depth characterization of a patient derived neocortical organoid model of PCH2a

Isabel Potthof[1*], Theresa Kagermeier[1,2*], Lízia Branco[1*], Francesco Castagnetti[1], Daria Andreeva[1], Zeynep Yentür[1,2], Pierre Collignon[1,3], Simone Mayer[1,2,3]

[1] Zoological Institute, Karlsruhe Institute of Technology (KIT), Germany.
[2] Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.
[3] Graduate Training Centre of Neuroscience, University of Tübingen, Tübingen, Germany.
[4] Institute of Biological and Chemical Systems - Functional Molecular System Karlsruhe Institute of Technology (KIT), Germany.

Pontocerebellar hypoplasia type 2 (PCH2) is a rare neurogenetic disorder characterized by a severe disruption of brain development, primarily affecting the cerebellum and pons, accompanied by neocortical atrophy and microcephaly. The subtype PCH2a is caused by the homozygous founder mutation p.A307S in TSEN54 which is part of the tRNA splicing endonuclease complex. To model this pathology, a neocortical organoid system was previously established using patient-derived induced pluripotent stem cells (Kagermeier et al., Dis Model Mech, 2024). To further characterize this model, isogenic cell lines were generated by introducing the p.TSEN54(A307S) mutation using CRISPR-Cas9 into control cell lines. These lines were then differentiated into neocortical organoids. We assessed changes in overall growth of the organoids using brightfield microscopy. With immunohistochemistry, we investigated the expression of layer-specific neuronal markers, apoptotic cell death, and neural progenitor maintenance. Furthermore, single-cell RNA sequencing enabled high resolution characterization of mutation-associated alterations in cellular composition and gene expression. Using this approach, we reproduced aspects of our previous findings and identified differential regulation of RNA processing, mTORC1 signalling, and cellular stress responses. These findings further strengthen the mutation-associated effects on early neurodevelopment and highlight the utility of the isogenic organoid model system.

High-Frequency Oscillations and Network Hyperexcitability in DEPDC5 Knockout Cerebral Organoids

Brian P. Rummell[1], Denise Haslinger[1,2], Sophie v. Brauchitsch[2], Andreas G. Chiocchetti[2], Julio C. Hechavarría[1,3]

[1] Ernst Strüngmann Institute (ESI) of the Max Planck Society, Frankfurt, Germany.
[2] Goethe University Frankfurt, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Therapy and Research Center of Excellence, Frankfurt am Main, Germany.
[3] Freie Universität Berlin, AG Brain and Behavior, Berlin, Germany.

Focal epilepsies have been increasingly linked to pathogenic variants in DEPDC5 (DEP-domain containing-5), a component of the GATOR1 complex that inhibits the mechanistic target of rapamycin complex-1 (mTORC1) signaling pathway. Mutations in DEPDC5 are hypothesized to drive epileptogenesis through unchecked mTORC1 activity, yet the underlying mechanisms remain poorly understood. Here, we generated DEPDC5 homozygous and heterozygous knockout cerebral organoids to model DEPDC5-related epileptogenesis *in vitro*. Using high-density microelectrode arrays, we recorded spontaneous extracellular activity and identified functional alterations in knockout organoids compared to isogenic wild-type controls. These included elevated spiking rates and increased high-frequency oscillations (HFOs), a promising electrophysiological biomarker associated with epileptogenic networks. Pharmacological characterization revealed that the hyperexcitable activity in DEPDC5 knockout organoids was dependent on glutamatergic neurotransmission but insensitive to GABAergic perturbation, suggesting a primary excitatory mechanism. These findings demonstrate that DEPDC5 deficiency disrupts excitatory/inhibitory balance in developing neural circuits and establish cerebral organoids as a tractable platform for investigating mTORopathy-associated epileptogenesis.

HELMHOLTZ Acceleration Alliance (HELMA)

Ute Schepers, www.helma.kit.edu

Institute for Functional Interfaces, Karlsruhe Institute of Technology, Karlsruhe, Germany.

The HELMHOLTZ Acceleration Alliance (HELMA) will become Germany's leading consortium for autonomous AI/ML-based research based on Self-Driving Labs (SDLs) with the greatest activity in the autonomous innovation development of health technologies through biomedical engineering and the digital acceleration of materials system discovery addressing communication technology, electronics, energy storage/conversion, and catalysis. Together with academic, clinical and industrial partners, HELMA will accelerate the path from ideas to data and innovation by redefining labs in smart data centers. Redefining the labs of the future with seamless automation and innovative AI/ML solutions and enabling remote infrastructure access via a cloud lab will unlock transformative increase of discoveries, scaling scientific impact to unprecedented levels without escalating resource use. This framework is set to drive significant change, transition research into the digital age, moving away from traditional, manual approaches to innovation development, while minimizing the environmental impact of large-scale research activities.

Methodological Influences on the Evaluation of Chronic Neural Implant Stability: Implications for Post-Implantation Interpretation and 3R-Aligned *In Vitro* Models

Ioana-Georgiana Vasilaș[1,2], Paul Čvančara[1,2], Jennifer Schulte[1,2], Larissa Graner[1,2,3], Anagha Navale[1,2], Thomas Stieglitz[1,2,4]

[1] Laboratory for Biomedical Microtechnology, Department of Microsystems Engineering –IMTEK, IMBIT // NeuroProbes, BrainLinks-BrainTools Center, University of Freiburg, Freiburg, Germany.
[2] BrainLinks-BrainTools Center, University of Freiburg, Freiburg, Germany.
[3] Analytical Magnetic Systems-Microstructures and Process Sensors, Institute of Microstructure Technology, Karlsruhe Institute of Technology, Karlsruhe, Germany.
[4] Bernstein Center Freiburg, University of Freiburg, Freiburg, Germany.

Chronic stability of neural implants is essential for interpreting long term performance and guiding continuous device improvement. It is commonly assessed through impedance measurements and material inspection at different stages of implantation: pre-implantation, *in vivo*, and after implantation. However, these assessments are highly sensitive to how they are carried out. Tissue handling, post explant processing, site-related differences, and variations in *in vitro* testing protocols can alter both material and electrode-tissue interface. As a result, apparent chronic degradation may partly reflect the measurement setup rather than true *in vivo* related changes. Access to animals with long term implants is often limited, restricting systematic comparisons across device configurations. From polyimide foils to fully assembled implants, methodological details are still not fully understood. Here, we used impedance measurements of chronically implanted ECoG arrays across multiple stages of implantation to examine how strongly outcomes depend on the chosen method. Both post-explant measurements and minor protocol changes affected impedance, material or interface stability, suggesting that common evaluation approaches may not directly represent the functional state of chronically implanted devices. These findings highlight the need for carefully defined and standardized strategies to assess chronic neural implant stability. *In vitro* biological models, such as neural spheroids or organoids, enable longitudinal monitoring of electrode-tissue interfaces and allow key variables to be examined in a controlled manner. Combining such models with *in vivo* observations can improve interpretation of implant stability and contribute to careful and efficient use of experimental resources.

The effects of cerebrospinal fluid treatment on the development of forebrain organoids

Zeynep Yentür[1,2,3,4,7,+], Peter Müller-Wöhrstein[2,6,+], Kseniia Sarieva[2,3,4], Shokoufeh Khakipoor[2], Theresa Kagermeier[2,4,7], Katharina Becker[2], Niklas Schwarz[2,6], Thomas Wuttke[*2,6], Simone Mayer[*1,2,7,8]

[1] The Heidelberg Academy of Sciences and Humanities, Heidelberg, Germany.
[2] Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.
[3] International Max Planck Research School, Graduate Training Centre of Neuroscience, University of Tübingen, Tübingen, Germany.
[4] Graduate Training Centre of Neuroscience, University of Tübingen, Tübingen, Germany.
[5] University of Tübingen, Tübingen, Germany.
[6] University Clinics Tübingen, Tübingen, Germany.
[7] Systemic Cellular Neurobiology, Zoological Institute, Karlsruhe Institute of Technology (KIT), Germany.
[8] Institute of Biological and Chemical Systems - Functional Molecular Systems, Karlsruhe Institute of Technology (KIT), Germany.
[+] equal contribution.

Cerebrospinal fluid (CSF), produced by the choroid plexus, surrounds the brain and spinal cord, and provides essential signaling cues. Although CSF is the primary physiological environment of the developing brain, its role in neural organoid development has not been investigated. To investigate this, we generated forebrain organoids from human iPSC lines and exposed them to human CSF collected from normal pressure hydrocephalus patients. Organoids were cultured with CSF for 30 days, from day (D)60 to D90, followed by transcriptomic analysis and single-cell transcriptomic analysis. CSF exposure altered cell type proportions, delayed maturation and favored progenitor cell populations. These findings demonstrate that physiologically relevant environmental signals shape developmental trajectories in forebrain organoids. Overall, our work highlights the importance of incorporating environmental factors into *in vitro* neural models to better recapitulate human brain development.