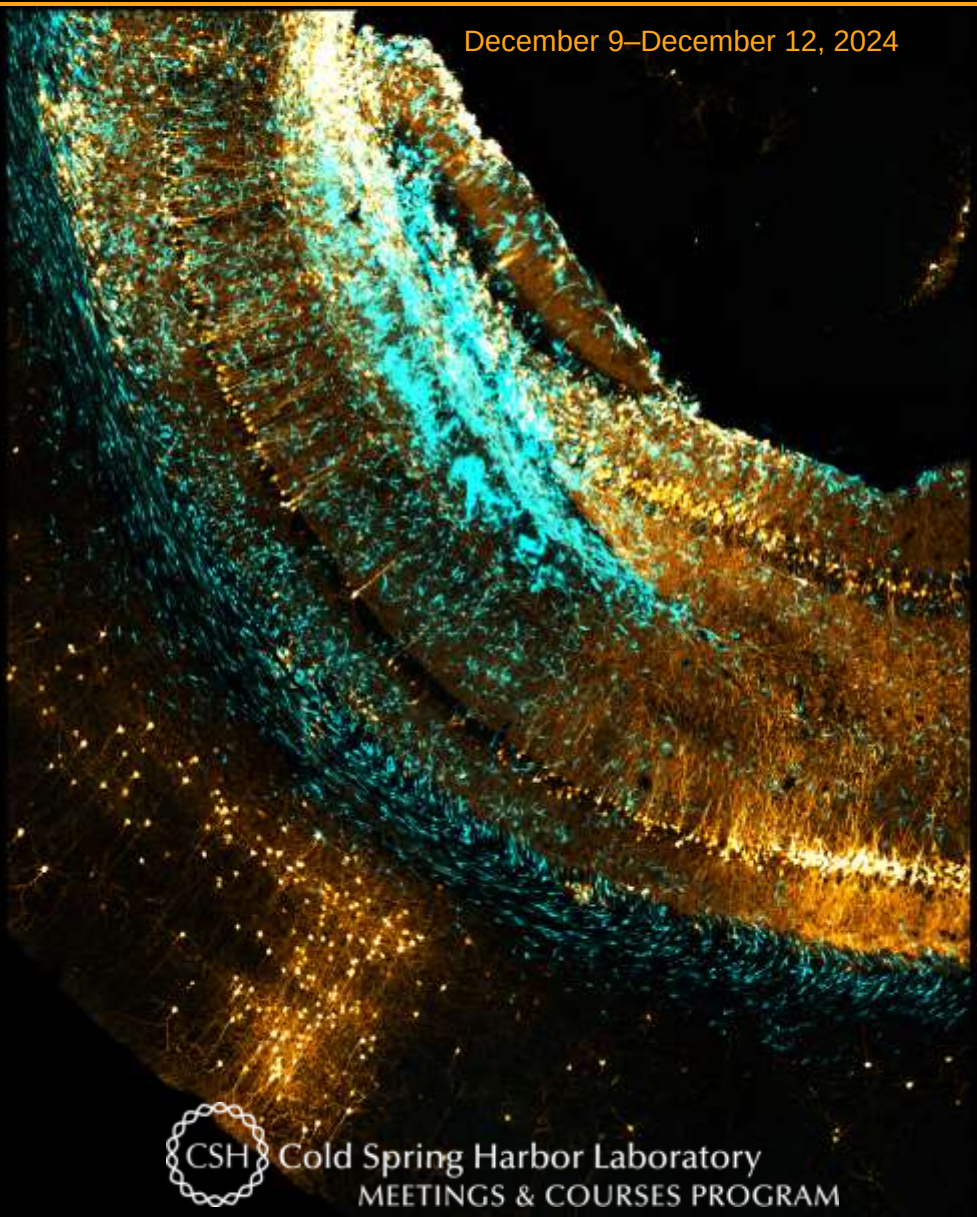
Abstracts of papers presented
at the 2024 meeting on

DEVELOPMENT & 3-D MODELING OF THE HUMAN BRAIN

December 9–December 12, 2024



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2024 meeting on

DEVELOPMENT & 3-D MODELING OF THE HUMAN BRAIN

December 9–December 12, 2024

Arranged by

Guo-li Ming, *University of Pennsylvania Perelman School of Medicine*
Sergiu Pasca, *Stanford University*

CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

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Back cover: Sergiu Pasca, Stanford University.

DEVELOPMENT AND 3D MODELING OF THE HUMAN BRAIN
Monday, December 9 – Thursday, December 12, 2024

Monday	7:30 pm – 10:00 pm	**1** Keynote Session
Tuesday	9:00 am – 12:00 pm	**2** Neural Development and Evolution
Tuesday	2:00 pm – 5:00 pm	**3** Neural Models of Development and Disease I
Tuesday	5:00 pm	*Wine and Cheese Party*
Tuesday	7:30 pm – 10:30 pm	**Poster Session I**
Wednesday	9:00 am – 12:00 pm	**4** Neural Models of Development and Disease II
Wednesday	1:30 pm – 2:30 pm	**5** Keynote Speaker
Wednesday	2:45 pm – 4:30 pm	**6** Neural Models of Development and Disease III
Wednesday	4:30 pm – 6:30 pm	**Poster Session II**
Wednesday	6:30 pm	*Cocktails and Banquet*
Thursday	9:00 am – 12:00 pm	**7** Neural Models of Development and Disease IV

All times shown are US Eastern: Time Zone Converter

Workshops
StemCell Technologies, December 10 (Lunchtime), ***p. T-1***
3Brain AG, December 10 (Dinner), ***p. T-2***
Maxwell Bio, December 11 (Lunchtime), ***p. T-3***

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

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PROGRAM

MONDAY, December 9—7:30 PM

SESSION 1 KEYNOTE SESSION

Chairperson: **Sergiu Pasca,** Stanford University, California

KEYNOTE SPEAKER

TUESDAY, December 10—9:00 AM

SESSION 2 NEURAL DEVELOPMENT AND EVOLUTION

Chairperson: **Helen Bateup,** University of California, Berkeley

SESSION 3 NEURAL MODELS OF DEVELOPMENT AND DISEASE I

Chairperson: **Orly Reiner,** Weizmann Institute of Science, Rehovot, Israel

TUESDAY, December 10—5:00 PM

Wine and Cheese Party

TUESDAY, December 10—7:30 PM

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Chairperson: **Flora Vaccarino,** Yale University, New Haven, Connecticut

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Guo-li Ming.
Presenter affiliation: University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania.

Giuseppe Testa.
Presenter affiliation: Human Technopole, Milan, Italy.

Cellular crosstalk during brain development
Silvia Cappello.
Presenter affiliation: Ludwig Maximilian University of Munich, Munich, Germany. 18

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Presenter affiliation: UCLA, Los Angeles, California. 25

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Lorenz Studer.
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Presenter affiliation: University of Michigan, Ann Arbor, Michigan. 193

DOES NEURAL DEVELOPMENT HAVE LESSONS FOR ALZHEIMER'S DISEASE?

Carla J Shatz[1,2]

[1]Stanford University, Bio-X, Stanford, CA, [2]Stanford University, Biology and Neurobiology, Stanford, CA

During brain development, circuits form sequentially, initially by creating a basic scaffold of connectivity according to strict molecular guidance cues. Subsequently the final details of each circuit emerge by pruning and sculpting synapses. This synapse selection process is also genetically specified but in this case the program requires neural activity. Prenatally, the brain generates its own internal neural activity patterns to jump-start the sculpting process. Postnatally, experience of the world takes over to sharpen brain wiring during critical periods. Neural activity and sensory experience regulate expression of sets of genes including several previously thought to act only in the immune system. These genes, including Major Histocompatibility Class I family members and a cognate receptor Paired immunoglobulin-like receptor B (PirB), are expressed in cortical and hippocampal neurons and are required for activity-dependent synapse pruning and plasticity in mouse brain. Memories are stored at synapses and circuits, which are lost and destroyed in Alzheimer's disease (AD). Unexpectedly, PirB and its human homolog LilrB2 are also high affinity receptors for soluble oligomers of beta amyloid. In human cerebral cortex, LilrB2 is expressed in neurons and at excitatory synapses, and is also present in neurons in human cortical organoids. It could be that well before amyloid plaque formation, excessive levels of soluble beta amyloid put normal synapse pruning mediated by PirB/LilrB2 into overdrive. PirB knockout protects AD model mice from cognitive loss and alterations in Hebbian synaptic plasticity. Thus, changes in expression and/or function of these molecules may contribute to synapse pruning disorders in neurodegenerative disease, as well as in development. Supported by NIH EY02858, NIA AG065206, Mathers Charitable Foundation, Sapp Family Foundation, Phil and Penny Knight Stanford Initiative for Brain Resilience.

A MOUSE ORGANOID PLATFORM FOR MODELING CEREBRAL CORTEX DEVELOPMENT AND CIS-REGULATORY EVOLUTION IN VITRO

Daniel Medina-Cano*[1,2], Mohammed T Islam*[1,2], Veronika Petrova*[3,4], Sanjana Dixit[1,2], Zerina Balic[1,2,7], Marty G Yang[5], Matthias Stadtfeld[6], Emily S Wong#[3,4], Thomas Vierbuchen#[1,2]

[1]Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, Developmental Biology Program, New York, NY, [2]Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, Center for Stem Cell Biology, New York, NY, [3]Victor Chang Cardiac Research Institute, Sydney, Australia, [4]School of Biotechnology and Biomolecular Sciences, Sydney, Australia, [5]Gladstone Institute for Data Science & Biotechnology, San Francisco, CA, [6]Weill Cornell Medicine, Sanford I Weill Department of Medicine, New York, NY, [7]Weill Cornell Graduate School of Medical Sciences, Cornell University, Cell and Developmental Biology Program, New York, NY

Over evolutionary timescales, natural selection has shaped the gene regulatory networks that control neuronal cell fate specification and terminal differentiation, leading to changes in neural circuit formation, function, and ultimately behavior across mammals. Neural organoids generated from pluripotent stem cells have emerged as a powerful model system for mechanistic dissection of brain development. However, although most *in vivo* studies of brain development and function use mouse models, current *in vitro* studies overwhelmingly use human organoids, making it difficult to distinguish between species-specific and *in vitro*-specific phenomena. To address this issue, we developed a rapid and reproducible protocol to generate cerebral cortical organoids from mouse epiblast stem cells. Mouse cortical organoids develop with kinetics that mirror the embryonic cortex *in vivo*, generating cortical neurons (pre-plate, deep-layer, and upper-layer) and glial cells (oligodendrocyte precursor cells, myelinating oligodendrocytes, astrocytes, ependymal cells). As a proof of concept, we generated organoids from F1 hybrid crosses between standard laboratory mice (C57Bl/6J) and four wild-derived inbred mouse strains (PWK/PhJ, MOLF/Ei, CAST/Ei, SPRET/Ei) from distinct sub-species spanning >1.5 million years of evolutionary divergence. F1 hybrid-derived cortical organoids allowed us to comprehensively map cis-acting transcriptional regulatory variation across developing cortical cell types using scRNA-seq. We identify hundreds of genes that exhibit dynamic allelic imbalances during cortical neurogenesis, providing the first insight into the developmental mechanisms underpinning changes in cortical structure and function between mouse strains. These experimental methods and cellular resources represent a powerful new platform for investigating mechanisms of gene regulation in the developing cerebral cortex across evolutionary timescales.
*authors contributed equally

FROM ORGANOIDS TO INSIGHTS: NOVEL MICROCEPHALY PATHWAYS IN HUMAN BRAIN DEVELOPMENT

Orly Reiner[1,2], Rami Tshuva[1,2], Jeyoon Bok[3], Mio Nonaka[1,2], Tamar Sapir[1,2], Xufeng Xue[3], Jianping Fu[3]

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[2]Weizmann Institute of Science, Molecular Neuroscience, Rehovot, Israel,
[3]University of Michigan, Bioengineering, Ann Arbor, MI

The intricate processes governing human brain development are highly susceptible to genetic mutations, often resulting in conditions such as microcephaly—a disorder characterized by reduced brain size. Traditional models have struggled to fully capture the complexity of human cortical development. To overcome these limitations, we introduce the NeuroMorphoChip, an innovative organoid-based platform that replicates key aspects of brain development. By applying controlled morphogen gradients, this platform enables the spatial organization of major neural regions, including the forebrain, midbrain, hindbrain, and spinal cord, within a single organoid, providing a more accurate model of neural tube patterning.

Using this system, we generated brain organoids to model microcephaly and observed significant abnormalities in neural progenitor cell cycle dynamics. While microcephaly is typically associated with centrosome dysfunction and disrupted mitotic progression, as we observed, our NeuroMorphoChip model revealed a novel mechanism tied to abnormal brain patterning. To further investigate this, we analyzed the underlying molecular mechanisms using both the human NeuroMorphoChip model and mutant mouse embryos.

Our study demonstrates the power of the NeuroMorphoChip platform in uncovering a previously unrecognized microcephaly mechanism driven by disrupted brain patterning and signaling pathways and provides new insights into the molecular pathways contributing to this neurodevelopmental disorder.

CELL-EXTRINSIC CONTROLS OVER NEOCORTICAL NEURON FATE AND DIVERSITY

Denis Jabaudon

University of Geneva, Basic Neuroscience, Geneva, Switzerland

Cellular diversity in the neocortex emerges gradually during prenatal and postnatal development. While environmental interactions occur during this extended maturation period, the impact of extrinsic cues on determining the fate of distinct neuron types remains unknown. To address this question, we exposed developing neocortical cells to various environmental conditions and examined how this affects cell fate and diversity. Our developmental analyses reveal a hierarchical molecular program where cell class-distinguishing features emerge first, followed by subclass- and type-related characteristics, with distinct developmental paces among cell populations. Environmental contribution was assessed in vivo, using genetically modified mice models in which position or innervation are altered, and in vitro using two-dimensional cultures. Acquisition of cellular identity and diversity remained stable across in vivo models. In contrast, in vitro glutamatergic neurons showed decreased expression of identity-defining genes, reduced diversity and alterations in canonical cortical connectivity. Cellular identity and diversity were restored towards in vivo values in organotypic slice cultures. These findings reveal cell population-specific responses to environmental conditions and highlight the role of extracellular context in shaping cell diversity in the maturing neocortex.

PRINCIPLES OF NEURAL STEM CELL LINEAGE PROGRESSION

Simon Hippenmeyer

Institute of Science and Technology Austria (ISTA), Developmental Neuroscience, Klosterneuburg, Austria

The concerted production of the correct number and diversity of neurons and glia by neural stem cells is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. Clonal analysis by exploiting the single cell resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology revealed an inaugural quantitative framework of RGP behavior in the developing neocortex. However, the cellular and molecular mechanisms controlling RGP lineage progression through proliferation, neurogenesis and gliogenesis remain largely unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated neuron and glia genesis. Ultimately, our results shall translate into a deeper understanding of brain function and why human brain development is so sensitive to the disruption of particular signaling pathways in pathological neurodevelopmental and psychiatric disorders.

GENETIC UNDERPINNINGS OF CORTICAL EVOLUTION

Debra Silver

Duke University Medical Center, Durham, NC

The cerebral cortex controls our higher cognitive capacities and helps define us as humans. Cortical development is orchestrated by neural progenitors which give rise to neurons and glia. Aberrant cortical development can result in devastating neurodevelopmental diseases. Our lab aims to elucidate genetic and cellular mechanisms controlling cortical development and contributing to neurodevelopmental pathologies and brain evolution. This talk will highlight some of our recent discoveries using mouse as well as primate 2D and 3D models to understand mechanisms of cortical evolution. Our work demonstrates how small changes in regulatory DNA directly impact species differences in brain development.

MOLECULAR AND CELLULAR DYNAMICS OF THE DEVELOPING HUMAN NEOCORTEX AT SINGLE-CELL RESOLUTION

Arnold R Kriegstein, Li Wang, Cheng Wang, Jingjing Li

University of California, San Francisco, Neurology, San Francisco, CA

The human cerebral cortex is more than three times expanded compared to our closest non-human primate relatives. The cortex emerges from an initially pseudostratified neuroepithelium that gives rise to radial glia, the neural stem cells of the cortex. A number of subtypes of radial glia have been identified, and single cell RNA sequencing (scRNAseq) has contributed to a novel model of primate corticogenesis, highlighted human-specific features of cortical development, suggested a relationship between oRG cells and brain tumors, and provided a benchmark for in vitro organoid models of brain development and disease. Recently, we conducted paired RNA sequencing and ATAC-seq on single nuclei derived from multiple regions and age groups of the developing human neocortex. In addition, spatial transcriptomic analysis was utilized to reveal cellular niches and cell-cell communication. These datasets have enabled the construction of a multi-omic atlas of the human neocortex across different developmental stages at single-cell resolution. The results illuminate molecular and cellular dynamics of the developing human neocortex, including cellular composition, spatial organization, intercellular signaling, gene regulatory networks, lineage potential, and disease susceptibility, highlighting novel progenitor cell lineages and shedding light on mechanisms of glioblastoma and neuropsychiatric disorders.

NEURAL ORGANOID SINGLE-CELL TECHNOLOGIES: MULTIMODAL SPATIOTEMPORAL RECONSTRUCTIONS AND COMPARATIVE CELL ATLASES

J. Gray Camp[1,2]

[1]Institute of Human Biology (IHB), Roche Pharma Research and Early Development, Roche Innovation Center, Basel, Switzerland, [2]Biozentrum, University of Basel, Basel, Switzerland

Neural organoids generated from pluripotent stem cells provide insight into development, disease, and evolution, but quantitative measurements across spatial scales and molecular modalities are lacking. Here I will give an overview of recent work implementing single-cell technologies to explore these complex biomimetic systems.

First, we have generated multiplexed protein maps over retinal and cerebral organoid time courses. We developed a toolkit to visualize progenitor and neuron location, the spatial arrangements of extracellular and subcellular components and global patterning in each organoid tissue. We integrated genomic data with spatially segmented nuclei into a multimodal atlas to explore organoid patterning and reconstruct spatiotemporal representations of multimodal features.

Second, we have integrated a human neural organoid cell atlas (HNOCA) totaling over 1.7 million cells. Mapping to developing human brain references reveals primary cell types and states that have been generated in vitro, and estimates transcriptomic similarity between primary and organoid counterparts across protocols. We provide a programmatic interface to browse the atlas and query new data sets, and showcase the power of the atlas to annotate organoid cell types and evaluate new organoid protocols. The atlas can be used as a diverse control cohort to annotate and compare organoid models of neural disease, identifying genes and pathways that may underlie pathological mechanisms with the neural models.

RESOLVING THE THREE-DIMENSIONAL INTERACTOME OF HUMAN ACCELERATED REGIONS DURING HUMAN AND CHIMPANZEE NEURODEVELOPMENT

Atreyo Pal[1], James P Noonan[1,2]

[1]Yale School of Medicine, Genetics, New Haven, CT, [2]Yale School of Medicine, Neuroscience, New Haven, CT

Human Accelerated Regions (HARs) are highly conserved across species but exhibit a significant excess of human-specific sequence changes, suggesting they may have gained novel functions in human evolution. HARs include transcriptional enhancers with human-specific activity and have been implicated in the evolution of the human brain. However, our understanding of how HARs contributed to uniquely human features of the brain is hindered by a lack of insight into the genes and pathways that HARs regulate. It is unclear whether HARs acted by altering the expression of gene targets conserved between HARs and their chimpanzee orthologs or by gaining new gene targets in human, a mechanism termed enhancer hijacking. We generated a high-resolution map of chromatin interactions for 1,590 HARs and their orthologs in iPSC-derived human and chimpanzee neural stem cells (NSCs) and excitatory neurons to comprehensively identify gene targets in both species. HARs and their chimpanzee orthologs targeted a conserved set of 2,963 genes enriched for neurodevelopmental processes including neurogenesis and synaptic transmission. Changes in HAR enhancer activity were correlated with changes in conserved gene target expression. Conserved targets were enriched among genes differentially expressed between human and chimpanzee NSCs or between human and non-human primate developing and adult brain. Species-specific HAR gene targets did not converge on known biological functions and were not significantly enriched among differentially expressed genes, suggesting that HARs did not alter gene expression via enhancer hijacking. HAR gene targets, including differentially expressed targets, also showed cell type-specific expression patterns in the developing human brain, including outer radial glia, which are hypothesized to contribute to human cortical expansion. Our findings support that HARs influenced human brain evolution by altering the expression of conserved gene targets and provide the means to functionally link HARs with novel human brain features.

MECHANOMORPHOGENESIS GOVERNED BY GUIDANCE RECEPTOR PLEXIN-B2 IS CRITICAL FOR GATING NEURONAL DIFFERENTIATION

Daniel Halperin[1], Chrystian Junqueira Alves [1], Roland H Friedel [1,2], Hongyan Zou [1,2]

[1]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Nash Family Department of Neuroscience, New York, NY, [2]Icahn School of Medicine at Mount Sinai, Department of Neurosurgery, New York, NY

Neuronal differentiation involves cell fate specification by instructive cues and also major physical transformation to extend neurites. The signaling pathways orchestrating the nuclear program and cytoskeletal reorganization are unclear. During development, neural tube closure is also a critical step; whether cell biomechanics of neuroprogenitors (NPCs) and differentiating neurons affects the mechanomorphogenesis of the neuroepithelium is poorly understood. Recent human genetics studies revealed rare pathogenic variants of axon guidance receptor *PLXNB2* in families presenting with intellectual disability, overlapping with phenotypes seen in *Plxnb2* mutant mice that displayed neural tube closure defect. Here, we reveal high expression of Plexin-B2 in the germinal matrix of the human fetal brains relative to cortical regions. In hESC-derived brain organoids, Plexin-B2 is also robustly expressed in ventricular zones and cortical plates. We show that Plexin-B2 ablation severely disrupted structural integrity of ventricle-like structures, leading to smaller cerebral organoids and spatial disarray of NPCs and cortical neurons. EdU pulse-chase study also revealed premature cell cycle exit and shrinkage of the $Sox2^+$ NPC pool in the ventricular zone of Plexin-B2 deficient cerebral organoids.
For mechanistic understanding, we studied neuronal differentiation from hESCs with or without *PLXNB2* knockout using an established neural induction protocol. Strikingly, Plexin-B2 deficient cells exhibited accelerated neuronal differentiation, forming exuberant $TUJ1^+$ neural networks. This phenomenon was linked to a reduced actomyosin cortical layer, favoring neurite outgrowth, as well as gene expression changes featuring cytoskeleton regulation and neurogenesis. These observations are in line with the role of Plexin-B2 in controlling actomyosin contractility and cell stiffness of hNPCs as shown in our recent study, which in turn impacts neurogenesis gene program.
Structure-function analysis further demonstrated that Plexin-B2 signals through its Ras-GAP domain to regulate mechanomorphogenesis, while the bendable extracellular ring domain was also indispensable, as locked-ring mutants of Plexin-B2 failed to rescue the KO phenotype. Ongoing single-nuclei RNA sequencing analysis of Plexin-B2 KO brain organoids and of prematurely differentiated Plexin-B2 KO neurons will uncover mechano-regulatory elements underlying the role of Plexin-B2 in neurogenesis. Altogether, our data support a primary role of Plexin-B2 in regulating the cell mechanics of physical transformation during neuronal differentiation. Unraveling the mechanomorphogenetic function of Plexin-B2 during early stage of neuronal cell fate specification will help advance understanding of neurodevelopmental disorders associated with Plexin mutations and facilitate generation of induced neurons for disease modeling.

ANALYZING FATE SPECIFICATION AND NEURAL NETWORK ARCHITECTURE BY TRANS-SYNAPTIC LABELING USING BARCODED RABIES VIRUS

Ramsey Najm*[1,2], Abel Vertesy*[1,2], Balint Doleschall*[1,2], Chong Li[1,2], Thomas Burkard[1,2], Maria Novatchkova[1,2], Yoav Ben Simon[1], Jürgen A Knoblich[1]

[1]Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria, [2]Medical University of Vienna, Department of Neurology, Vienna, Austria

Cerebral organoids derived from pluripotent human stem cells can recapitulate morphogenesis and cell fate specification in the fetal human brain. Our group uses stem cell derived 3D cultures to explore the mechanistic basis for neurodevelopmental disorders. We have modeled Tuberous sclerosis (TSC), a neurodevelopmental disorder caused by overactivation of the mTOR pathway and have identified a progenitor cell type responsible for tumor formation and brain pathologies in TSC patients. Our data suggest that these progenitor cells located in the caudal ganglionic eminence might be responsible for the generation of inhibitory interneurons that migrate into the cerebral cortex after birth in humans but not in mice. By analyzing neural network activity using extracellular recordings, we have identified characteristic phenotypes also seen in TSC patients during the interictal phase. To understand the modifications in neural network architecture underlying those changes, we developed “Connectomics by sequencing” a bardoded trans-synaptic labeling methodology that allows identification of synaptic connections in single-cell transcriptome experiments. Together, our results offer new insights into human brain development and the mechanistic defects that underlie neurodevelopmental disorders including Epilepsy or Autism.

ELUCIDATING THE ROLE OF CCP1 IN HUMAN CORTICAL INTERNEURON MIGRATION

Antonela Bonafina[1], Anaïs Boutsen[1], Bernard Coumans[1], Bernard Lakaye[1], Miriam Javier-Torrent[1], Sylvia Tielens[1], Romann Close[1], Christopher Kune[2], Gabriel Mazzucchelli[2], Anne Firquet[3], Carsten Janke[4], Silvia Cappello[5], Ira Espuny Camacho[1], Laurent Nguyen[1]

[1]University of Liège, Laboratory of Molecular Regulation of Neurogenesis, GIGA Institute, Liège, Belgium, [2]University of Liège, Laboratory of Mass Spectrometry, Liège, Belgium, [3]C.H.U Citadelle, Liège, Belgium, [4]Institut Curie, Paris, France, [5]Ludwig Maximilians Universität, Munich, Germany

The cerebral cortex comprises a wide variety of excitatory projection neurons (PNs) and inhibitory interneurons (cINs). The majority of cINs are generated in the ventral forebrain and undergo tangential migration to cortical regions, where they integrate into local circuits. Previous studies conducted in mice by our laboratory have demonstrated that CCP1 (cytosolic carboxypeptidase 1) regulates the mode of cIN migration by facilitating the deglutamylation of myosin light chain kinase. The loss of CCP1 in cINs impairs their migration and indirectly increases the generation of PNs, thereby influencing cortical morphogenesis. However, the role of CCP1 in regulating the migration of human cortical interneurons remains unknown. In the present study, we demonstrate that disruption of CCP1 expression in human cINs, derived from both primary human forebrain cultures and human cerebral assembloids, impairs their migratory behavior. The absence of CCP1 causes a shift from saltatory migration to a gliding motion. These results suggest that CCP1, as in mouse cINs, governs nucleokinesis in human-derived cINs. However, the dynamic branching of the leading process during migration is only disrupted in human cINs in the absence of CCP1 activity, suggesting that CCP1 may have acquired a distinct function in cIN migration through evolution.

MODEL OF TUBEROUS SCLEROSIS COMPLEX

Helen Bateup

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the *TSC1* or *TSC2* genes, which presents with early onset epilepsy, variable intellectual disability, and psychiatric conditions including autism spectrum disorder. A hallmark pathology of TSC is the presence of cortical tubers, which are focal brain malformations that arise during embryonic development. Tubers contain dysplastic and abnormal neurons and glia and can often become seizure foci. How cortical tuber cells drive seizures and epileptogenesis is not well understood. Analysis of resected patient tissue has revealed marked signatures of astrocyte reactivity and neuroinflammation within tubers. Based on this, we hypothesize that altered glial cell function, in particular astrocytes, may be a key contributor to seizure pathophysiology in TSC.

To test this idea, we established genetically-engineered human cortical organoid models of TSC to investigate how loss of *TSC2* affects neural development. We generated a conditional knock-out model consisting of stem cells with one non-functional *TSC2* allele and one conditional allele that can be disrupted through the addition of Cre. Exposure to a low dose of Cre during neural differentiation generates a subpopulation of *TSC2*-/- cells within a background of *TSC2*+/- cells in the same organoid. With this genetically mosaic strategy we can mimic a somatic "second-hit" mutation, which is proposed to drive the localized formation of tuber cells.

We used this model to delete *TSC2* from a subset of progenitor cells early during cortical organoid development. We profiled these organoids at different developmental time points using single cell RNA sequencing to uncover changes in gene expression programs. We find that *TSC2*-/- cells preferentially generate glial cell lineages, including astrocytes, compared to *TSC2*+/- cells in the same organoid. *TSC2*-/- cells have numerous gene expression changes associated with altered proteostasis, autophagy, and reactive astrogliosis. Notably, *TSC2*-/- astrocytes have the most pronounced changes, which include upregulation of a gene expression module associated with disease-induced reactivity. Immunopanning of TSC2-/- astrocytes from brain organoids reveals dramatic changes in cell morphology and properties. Many of these changes in astrocyte morphology and expression are also observable in primary resected TSC patient tuber samples, lending validity to the organoid model. Together, this work reveals that loss of TSC2 during neural development biases developmental trajectories toward the generation of astroglial cells, which show pathophysiological changes at early developmental stages.

NON-CANONICAL FUNCTION OF VOLTAGE-GATED SODIUM CHANNEL GENE *SCN3A* IN EARLY BRAIN DEVELOPMENT

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Voltage-gated sodium channels (VGSCs) have well-established roles in neuronal physiology, including action potential initiation and propagation. Accordingly, mutations in VGSCs are associated with severe neurological impairments. Certain variants in the predominant developmental VGSC gene *SCN3A* are associated with severe cortical malformations and Developmental and Epileptic Encephalopathy (DEE). Surprisingly, these phenotypes do not align with the classical role of VGSCs in cellular excitability. Using human-induced pluripotent stem cell (hiPSC) derived human cortical organoids (hCOs), we found that clinically identified *SCN3A* mutations cause irregular morphology consistent with cortical malformation phenotypes observed in patients. To explore the molecular mechanisms underlying this defect, RNA sequencing of *SCN3A* hCOs identified the premature upregulation of genes involved in later stages of development, particularly those regulating cell adhesion, assembly, and migration. We used a high throughput platform to generate neural rosettes and observed defects in cellular organization as early as four days in vitro (DIV). To measure the functional consequence of *SCN3A* mutations on neural networks, we performed calcium imaging of hCOs and observed increased activity in mutant cultures as early as DIV 30. Together, these data highlight the importance of non-canonical functions of *SCN3A* in early brain development and point to a mechanism of *SCN3A* disorder involving neuronal hyperexcitability, impaired neuronal migration, and cellular disorganization.

EVOLUTIONARY MECHANISMS ENABLING INCREASED COMPLEXITY OF THE HUMAN BRAIN

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The human brain is greatly enlarged and complex, even when compared with our closest living relatives, the other great apes. How this evolutionary elaboration has come about is still unclear, but neural organoids are enabling comparative studies to uncover the underlying mechanisms. It is now becoming clear that a large part of this increased size and complexity relates to a delay in brain development. In particular, we have uncovered a delay in neuroepithelial transition to neurogenesis involving a process similar to a partial epithelial-to-mesenchymal transition, a process that can be accelerated or delayed by perturbing EMT factors. Turning to later stages of development, namely the development of long-range projections, we have discovered a similar human delay in a transition, in this case the transition from long range axon growth to local synapse formation. These studies are revealing mechanisms that could explain the slowed human tempo, thus enabling an impressive number of neurons and connectivity.

HUMAN-SPECIFIC MECHANISMS LINKING SYNAPTIC DEVELOPMENT, EVOLUTION AND DISEASES OF THE HUMAN BRAIN

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A salient feature of human brain development is the considerably prolonged, or neotenic, tempo of cortical neuronal maturation, taking months to years in humans, instead of days to weeks observed in other mammals. Cortical neuron neoteny affects in particular synaptic development and maturation, which is thought to lead to prolonged and/or enhanced synaptic plasticity, thereby contributing to advanced cognitive and social skills characterizing Homo Sapiens.

The species-specific developmental timing of cortical synaptogenesis is partly intrinsic to cortical neurons, as it is retained in human cortical neurons when xenotransplanted in the fast-developing mouse cortex. The underlying mechanisms involve several human-specific genetic modifiers of cortical neuron development and function, which act by regulating well-conserved genes that are mutated in intellectual deficiency and autism spectrum disorders. The mechanisms underlying human neuronal and synaptic neoteny shed new light on human brain evolution and neurodevelopmental diseases.

CHRONIC CHANGES IN *IN VIVO* VASCULARIZED, GLIAL ENRICHED ORGANOIDS WITH INJURY AND DISEASE

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It has long been recognized that glial cells are critically involved in various neurodegenerative disorders such as Alzheimer's disease (AD). Microglia and Astrocytes are highly dynamic cells that can take on several reactive phenotypes. Despite the critical role of Glial cells in various brain pathologies, the cellular and molecular mechanisms that govern the diverse and context-dependent set of phenotypes are poorly understood.

To understand and study human Glia phenotypes in health and disease, novel platforms are needed that feature functionally mature cells operating within a physiologically relevant human brain environment. To develop these platforms, it is crucial to complement the existing *in vitro* approaches with platforms that capture the physiological environment present *in vivo*. Therefore, we have developed an *in vivo* approach that allows the investigation of human iPSC-derived microglia and astroglia within a vascularized, human brain-like environment. This approach mimics the colonization of human brain organoids through iPSC-derived erythromyeloid progenitors (EMPs), as well as early generation of astrocytes and capitalizes on our ability to graft these units into a immunocompromised rodent host for vascularization. Transplantation into the brain of an animal host allows infiltration of the host vasculature within a few days and grafted human brain organoids do not exhibit the cell death observed *in vitro* even when maintained long-term. The colonizing human microglia and astroglia show morphological features indicative of a resting and reactive states, and express microglia and astroglia-specific markers. In this presentation we reveal systematic changes in glia and human neurons derived spheroids and organoids in chronic models of injury and disease.

CELLULAR CROSSTALK DURING BRAIN DEVELOPMENT

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Cellular crosstalk is an essential process during brain development and is influenced by numerous factors, including cell morphology, adhesion molecules, the local extracellular matrix, and secreted vesicles. Inspired by mutations associated with neurodevelopmental disorders, we focus on understanding the role of extracellular mechanisms essential for proper human brain development. Therefore, we combine the in vivo mouse model and the in vitro human-derived progenitors, neurons, astrocytes, cerebral organoids, and dorso-ventral assembloids to better understand the molecular and cellular mechanisms involved in progenitor proliferation and fate, migration, and maturation of excitatory and inhibitory neurons during human brain development and to address the causes of neurodevelopmental disorders. In particular, we focus on mutations in genes that affect cell-cell contacts, extracellular matrix, and vesicle secretion to study intrinsic and extrinsic mechanisms that contribute to brain formation. Our data reveal an important contribution of non-autonomous cell mechanisms in the pathogenesis of neurodevelopmental disorders.

MODELING JUVENILE BATTEN DISEASE USING BRAIN REGION-SPECIFIC ORGANOIDS: INSIGHTS INTO PATHOLOGY AND THERAPEUTIC STRATEGIES

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Batten disease is a devastating genetic lysosomal storage disorder that affects children globally, with a prevalence of approximately 1 in 100,000 births and a higher incidence in Scandinavia. Juvenile Batten disease, or juvenile neuronal ceroid lipofuscinosis, is the most common form and is associated with loss-of-function mutations in the CLN3 gene, which encodes a lysosomal transmembrane protein of unknown function. Juvenile Batten disease is characterized by progressive vision loss starting between ages 4 and 12, neurodegeneration resulting in cognitive decline and motor impairment, and premature death typically occurring between ages 15 and 30. Currently, there are no available cures for this fatal condition. Pathological hallmarks of the disease include the accumulation of intracellular autofluorescent storage material, considerable neuronal loss, glial reactivity, and retinal degeneration.

To systematically model CLN3 disease, we generated induced pluripotent stem cells from both CLN3 patients and controls, with most affected individuals carrying at least one allele with a 966 bp deletion, and established various region-specific organoids, including dorsal cortex, hippocampus, midbrain, hypothalamus, retinal and neuromuscular junction. Notably, CLN3 organoids were able to recapitulate key features of the disease. Our preliminary data indicate that CLN3 deficiency significantly impacts brain developmental processes, such as impaired proliferation and structural organization of progenitors and neurogenesis. Moreover, the abnormal accumulation of the lysosomal protein LAMP1 in CLN3 organoids suggests compromised lysosomal function.

We have explored the therapeutic potential by treating these organoids with selected candidate drugs and rAAV-mediated gene therapy strategies by evaluating their efficacy and safety in the CLN3 patient-derived organoid models. Additionally, we are conducting single-cell transcriptomics and proteomics to further characterize phenotypes, underlying mechanisms, and treatment responses in these models.

This study underscores the potential of brain organoids as powerful tools for modeling juvenile Batten disease, providing insights into pathophysiology and avenues for developing targeted treatments.

A GENETIC SCREEN IN CEREBELLAR ORGANOIDS TO GENERATE NOVEL PEDIATRIC BRAIN CANCER MODELS OF MEDULLOBLASTOMA

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Brain neoplasms are the most frequent type of cancer during childhood, with Medulloblastoma accounting for the majority of embryonal cases. Medulloblastoma is subdivided into four main subgroups, WNT, SHH, Group 3 (G3), and Group 4 (G4). Despite being thoroughly molecularly characterised, only a modest selection of the most prominent Medulloblastoma driver genes have been studied for their role in tumorigenesis. This is especially true for G3/G4, which collectively account for >65% of paediatric Medulloblastoma diagnoses and have the highest rates of metastases, relapse, and mortality. Although a variety of MYC/MYCN-driven models have been reported in the literature, these account for restricted G3 subgroups, and none of have confidently replicated the diverse biology of these tumors. Each tumor type has a specific developmental origin, and the transcriptomic and epigenomic landscapes are heavily biased towards specific neuronal signatures. SHH originates from granule neuron progenitors of the cerebellum, G3 likey originates from progenitors of the sub-ventricular zone of the rhombic lip, while G4 from unipolar brush cell progenitors. Rhombic lip progenitors in developing human embryos differ from mouse development, providing a likely explanation as to why attempts to faithfully model G3 and G4 in the mouse have been largely unsuccessful. Therefore, we generated novel Medulloblastoma cancer models from human iPSC-cerebellar organoids. We designed a genetic screen in which oncogenic insults are activated in the putative cell-of-origin of SHH/G3/G4. We generated three transgenic human-iPSC lines expressing a lineage-specific dox-inducible CRE. In a separate locus, we inserted a dox-inducible promoter driving the expression of CRE. These lines allow temporal and cell type-specific activation of genetic lesions, controlling the onset of tumor initiation. Using these models, we are testing proof-of-prinicple oncogene combinations before performing a novel genetic screen to reveal new combaintions of oncogenes that drive medulloblastoma. These novels models allow us to study human-specific features of the disease and provide reliable models to develop new treatments.

YOU CONTAIN MULTITUDES: SOMATIC MUTATION IN HUMAN CEREBRAL CORTEX

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Recent work shows that every cell division creates somatic mutations even during normal development, and that postmitotic neurons continue to accumulate mutations throughout life even in the absence of cell division. Clonal somatic mutations that activate the mTOR pathway have long been implicated in intractable focal epilepsy of childhood, associated with focal cortical dysplasia, while more recently, mosaic mutations that activate the PTPN11/RAS/RAF/MAPK pathway have been found in mesial temporal lobe epilepsy, where seizures start most often in adulthood. Recent work suggests that a portion of schizophrenia brains (10-12%) show somatic mutations at transcription factor binding sites (TFBS) in recurrent patterns. While many of these somatic mutations disrupt normal gene regulation, establishing their definitive relevance to SCZ risk will require larger sample sizes. Nonetheless, these recurrent patterns of mutation suggest the existence of fetal exposures that may influence somatic mutation in brain.

We recently developed a new method to sequence single cell genomes at high throughput based on the Tn5 transposase, and applied it to studying somatic CNV in human cerebral cortical neurons before and after birth. Neurons from the prenatal cortical plate are frequently aneuploid, with large-scale CN gains and losses occurring in most (>60%) of cortical neurons, likely carried over from chromosome mis-segregation known to occur frequently during early fetal development. Remarkably, donor brains analyzed after birth show far fewer somatic CNV (<20%), and this low rate is stable, or slightly decreases, through postnatal life. The dramatic perinatal loss of neurons with large-scale CNV suggests a selective removal of neurons with damaged genomes, acting as a process of genomic quality control among dispersed, nondividing neurons. Genomic regions affected by CNV in postnatal neurons are depleted for dosage-sensitive genes and genes implicated in synaptic development, and removal of CNV-bearing neurons occurs contemporaneously with the previously described “pruning” of synapses and neurons that occurs perinatally. This perinatal removal of neurons with damaged genomes, potentially by processes relating to neuronal competition, may be essential to normal brain development and function.

PASSING OF TIME IN BRAIN ORGANOIDS: THE JOURNEY TO UNDERSTAND HUMAN BRAIN DEVELOPMENT AND MATURATION

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Much remains unknown regarding the mechanisms governing human brain maturation and aging. Human brain organoids offer a unique platform for these studies. Here, we investigated human cortical organoids cultured for periods ranging from 6 months to over 4 years in vitro. Module scores of maturations trained on the endogenous tissue show that organoids continue to develop and mature while in culture for these extended time frames. In agreement, methylation profiling revealed a strong correlation between predicted age of the organoids and time in culture. Using extracellular single-unit recordings with multielectrode arrays (MEA), we detected network bursts and action potentials with features that changed over developmental and maturation trajectories in culture. Notably, we find that cortical progenitors age in culture as indicated by a progressively more restricted ability to generate early-born progeny when exposed to inductive developmental signals. The work suggests that human brain organoids are capable of recording and recalling developmental time.

BRAIN-WIDE NEURONAL CIRCUIT CONNECTOME OF HUMAN GLIOBLASTOMA

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Glioblastoma (GBM), a deadly adult brain cancer, infiltrates the brain and can be synaptically innervated by neurons, which drives tumor progression. Synaptic inputs onto GBM cells identified so far are largely short-range and glutamatergic. The extent of integration of GBM cells into the brain-wide neuronal circuitry is not well understood. We applied monosynaptically-restricted rabies virus-mediated retrograde transsynaptic tracing and herpes simplex virus-mediated anterograde transsynaptic tracing approaches to systematically investigate circuit integration of human GBM organoids transplanted into adult mice. We found that GBM cells from multiple patients rapidly integrate into brain-wide neuronal circuits and exhibited diverse local and long-range connectivity. Beyond glutamatergic inputs, we identified a variety of neuromodulatory inputs across the brain, including functional interaction between long-range cholinergic projections from the basal forebrain and GBM cells. Acute acetylcholine stimulation induces long-lasting elevation of calcium oscillations and transcriptional reprogramming of GBM cells into a more motile state via the metabotropic CHRM3 receptor. CHRM3 activation promotes GBM cell motility, whereas CHRM3 downregulation suppresses GBM cell proliferation and migration. Together, these results reveal the capacity of human GBM cells to rapidly and robustly integrate into anatomically diverse neuronal networks of different neurotransmitter systems in the adult brain, highlighting GBM as a brain-wide disease. Our findings further support a model wherein rapid connectivity and transient activation of upstream neurons may lead to a long-lasting increase in tumor fitness.

HUMAN OLIGODENDROCYTE PROGENITOR CELLS MEDIATE SYNAPSE ELIMINATION THROUGH TAM RECEPTOR SIGNALING: INSIGHTS FROM 2D AND 3D BRAIN MODELS

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Synaptic pruning is a crucial neurodevelopmental process, traditionally associated with microglia. Recent animal studies suggest that oligodendrocyte progenitor cells (OPCs) may also contribute to synapse remodelling, but a mechanistic understanding as well as the extent to which this feature occurs in humans remains unclear.
To address these questions, we established a human multi-lineage organoid model harbouring OL-lineage cells that transition through various developmental stages in proximity to neurons, astroglia, and microglia. We then leverage this 3D model, utilizing high-resolution confocal imaging and 3D volumetric reconstruction, to demonstrate that human OPCs spontaneously internalize synaptic structures, notably at levels comparable to microglia. Computational analysis of cellular crosstalk patterns using single cell RNA-seq data highlighted intricate interactions between OPCs and neurons as well as microglia, involving synaptic adhesion molecules and growth factors.
Given their involvement in microglia- and astrocyte-mediated phagocytosis, we then focused on the TAM receptors and observed that the amount of engulfed synaptic material in OPCs largely depended on the protein levels of the TAM receptor ligand GAS6 and the TAM receptors AXL and TYRO3, while the corresponding co-expression networks strongly enriched for synaptic genes implicated in the bidirectional communication between neurons and OPCs. To confirm a mechanistic role of TAM receptors in human OPC-mediated synapse engulfment, we employed a live-imaging assay in which hiPSC-derived OPCs were exposed to pHrodo-labelled synaptosomes. Utilizing a small molecule to inhibit phosphorylation and activation of TAM receptors in a dose-dependent manner, we observed a reduction in synaptosome engulfment foremost implicating AXL. Thus, we proceeded with a specific siRNA-mediated inhibition of AXL and confirmed a clear decrease of synaptosome uptake by OPCs.
Our findings suggest that human OPCs can remodel neuronal circuits by internalizing synaptic structures, a process partially dependent on TAM receptor signaling. This work provides novel insights into human neurodevelopment and lays the groundwork for future investigations into OPC-mediated synaptic pruning in both physiological and pathological contexts, while offering a valuable 3D organoid platform for studying complex cellular interactions in human brain development.

MODELING CORTICAL VERSUS HIPPOCAMPAL NETWORK DYSFUNCTION IN A HUMAN BRAIN ASSEMBLOID MODEL OF EPILEPSY AND INTELLECTUAL DISABILITY

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Developmental and Epileptic Encephalopathy 13 (DEE-13) is a severe neurodevelopmental disorder caused by gain-of-function mutations in the SCN8A gene, which encodes the Nav1.6 sodium channel. DEE-13 is characterized by early-onset epilepsy and intellectual disability, with the underlying mechanisms affecting these different clinical outcomes remaining poorly understood. Here, we leverage human brain organoid models to explore how SCN8A mutations differentially affect cortical and hippocampal networks.

Using assembloids derived from induced pluripotent stem cells (iPSCs) of DEE-13 patients with SCN8A variants, we modeled both cortical (Cx+GE) and hippocampal (Hc+GE) brain regions. Cortical assembloids exhibited overt hyperexcitability, manifesting as increased spikes and long-duration discharges in local field potential (LFP) recordings, mirroring the epileptic activity seen in patients. By contrast, hippocampal assembloids did not display overt hyperexcitability but instead revealed network dysregulation, including disrupted theta-gamma phase-amplitude coupling (PAC) and reduced high-gamma/ripple frequency oscillations, features critical for hippocampal function and memory processes.

To further investigate these aberrant dynamics, we developed a computational model of hippocampal circuitry, which predicted that increased persistent sodium currents and selective loss of Oriens-lacunosum/moleculare (O-LM) interneurons drive the observed theta-gamma coupling disruptions in DEE-13 hippocampal assembloids. Immunohistochemical and single-nucleus RNA sequencing analyses confirmed these predictions, showing a significant reduction in O-LM interneurons alongside an increase in excitatory neurons in mutant hippocampal assembloids. These findings were corroborated by in vivo analyses of human temporal lobe epilepsy patients, which revealed similar disruptions in theta-gamma coupling in epileptic versus non-epileptic hippocampi.

Our results highlight distinct mechanisms of network dysfunction in cortical versus hippocampal circuits in SCN8A-related DEE-13. While cortical hyperexcitability underlies seizure phenotypes, hippocampal-specific PAC deficits and interneuron loss may contribute to the cognitive impairments observed in patients. These findings underscore the utility of region-specific human brain organoid models in elucidating the complex pathophysiology of neurodevelopmental disorders like DEE-13 and pave the way for targeted therapeutic strategies.

APOE4 VASCULAR PHENOTYPES IN THE BRAINS OF COGNITIVELY NORMAL INDIVIDUALS ARE REPLICATED IN IPSC VASCULAR MODELS AND HIGHLIGHT BARRIER IMPAIRMENT AS AN EARLY CONTRIBUTOR TO ALZHEIMER'S DISEASE

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Breakdown of the blood-brain barrier (BBB) occurs early in Alzheimer's Disease (AD) and AD-related dementias (ADRD). APOE4 is a risk factor for vascular complications associated with AD, vascular dementia, and Cerebral Amyloid Angiopathy (CAA). Moreover, newly approved anti-amyloid immune therapies are more likely to cause brain hemorrhage and edema in APOE4 carriers. Recent studies have demonstrated that cognitively normal aging APOE4 carriers have greater vascular leakage compared to non-APOE4 carriers and that this is exacerbated with the onset of AD symptoms. These observations implicate APOE4 in BBB impairment, but the underlying mechanisms remain unclear. We used 2D and 3D self-assembling vascular models derived from isogenic APOE4/4 and APOE3/3 induced pluripotent stem cells (iPSCs) to reveal vascular phenotypes that may contribute to barrier function impairment in APOE4 carriers.

Combining several single-nucleus RNA-seq brain datasets enabled us to define gene expression differences in APOE4 carriers and non-carriers in endothelial cells (ECs), pericytes, smooth muscle cells, and fibroblasts in postmortem human brains. In cognitively normal (control) brains, APOE4 ECs were enriched for interferon-gamma pathway-related genes and reduced oxidative phosphorylation metabolic pathways. Using a mesoderm patterning process, we then generated iPSC-derived ECs and mural cells from 5 isogenic pairs of APOE4/4 and APOE3/3 lines. Several interferon-gamma and related metabolic pathway gene expression changes were also captured in the iPSC-derived vascular models. We established functional differences in barrier strength and response to inflammation in APOE4/4 iPSC-EC monolayers, including reduced barrier recovery after a TNFα + IL1β inflammatory challenge. APOE4/4 vascular cells showed increased expression of fibronectin (FN1), consistent with previous reports of increased FN1 deposition around blood vessels in the brains of APOE4 carriers with AD. This is notable given recent findings that loss of function variants in FN1 can protect against the increased risk of AD due to APOE4. Further analysis is ongoing to define APOE4-associated vascular changes and their impact on vascular amyloid deposition and neuronal health in neurovascular 3D models.

SOMATIC MOSAICISM AND GENETIC AND PHENOTYPIC VARIATION BETWEEN iPSC LINES

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Embryonic pattern formation drives regional and cellular specification in the vertebrate neural tube. Distinct neuronal subtypes are generated in a precise spatial order according to their location along the anterior-posterior and dorsal-ventral axes. To understand the sufficiency of this dual patterning in human brain development, we exposed human induced pluripotent stem cells (iPSC) to concomitant orthogonal gradients of a posteriorizing and a ventralizing morphogen, activating WNT and sonic hedgehog signaling, respectively, in a custom-made chamber. We demonstrate that morphogen's crosstalk triggered by this dual gradient activates in just 5 days specific genetic and molecular programs in precursor cells, specifying organoids into the major neuronal lineages of forebrain, midbrain, hindbrain and cerebellum. Human iPSC lines from different individuals demonstrated biased sensitivities to the same morphogen gradient, resulting in different regional and cellular specification of the organoids. Using clonal replicates of each iPSC line we established that differential responses to morphogens among lines are not substantially contributed by technical (batch-to-batch) variation, reprogramming technique or age of the donor, but rather to interindividual variations in genetic background (person-to person variation) and additional factors specific to each line (line-to-line variation). Line-to-line variations can be attributed to somatic mutations occurring in the iPSC founder cell *in vivo* and inherited by all cells of the line. Most somatic mutations accumulate during prenatal development at frequencies of 1-5 single nucleotide variations (SNVs) per cell division, and in this fashion represent natural barcodes that can be used to trace ancestral relationships between cells. Cells differ from each other at birth by ~1,000 SNVs and 1-5 CNVs/SVs and accumulate more variations during each person's lifetime; however, about 6% individuals harbor a 3 to 20-fold higher than average number of SNVs in their brain. While early somatic mutations are typically shared by all tissues in an individual, later mutations can be private to a tissue or organ, and all can confer variability to reprogrammed cells. These data suggest that both germline and somatic variations should be considered major factors influencing the epigenetic state and developmental genetic programs of iPSC lines.

DEVELOPMENT OF NOVEL ALZHEIMER'S DISEASE MODELS THROUGH IPSC-DERIVED BRAIN ORGANOIDS AND GLIAL CELLS.

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Advancements in iPSC technology have revolutionized regenerative medicine, disease modeling, and drug discovery, particularly in neuroscience. Our research focuses on novel differentiation protocols that derive a range of neuronal and glial cells from iPSCs, contributing to over 40 neurodegenerative disease models. While significant progress has been made, there remain substantial hurdles in translating iPSC-based findings into clinical applications, especially due to the limitations of current brain organoid models.

We have developed protocols for differentiating iPSCs into astrocytes, oligodendrocytes, and microglia, which we integrated into co-culture systems and brain organoids. This integration enhances the study of glial roles in neurodegenerative diseases like Alzheimer's disease (AD). For example, our study of astrocytes expressing the APOE4 gene, which is a major AD risk factor, revealed their inhibitory effect on dendritic spine formation, mediated by an overexpression of EDIL3, an extracellular matrix factor found to co-localize with amyloid plaques in AD brains. This suggests EDIL3 may play a critical role in AD pathology. Additionally, the APOE Christchurch mutation, associated with resistance to familial AD, was shown to reduce tau propagation and inhibit the reactive transformation of astrocytes.

We also developed forebrain organoids (FBOs) from AD patient-derived iPSCs, observing amyloid-β accumulation and phosphorylated tau, though tau aggregation was not detected. To model tauopathies, we introduced P301L mutant tau into FBOs via adeno-associated virus (AAV), successfully producing tau aggregates similar to those found in frontotemporal lobar degeneration (FTLD) patients.

Current organoid models lack blood vessels and require long culture times to induce mature glial cells. To address this, we are creating astrocyte-enriched brain organoids through transcription factor overexpression, which promotes synapse formation and could reveal more distinct phenotypes in APOE4-enriched organoids. Furthermore, we have integrated iPSC-derived microglia into neuroimmune organoids and are developing vascularized organoids through transplantation into mouse brains.

By advancing brain organoid technology, we aim to generate more mature astrocytes and microglia, providing novel insights into the molecular mechanisms of dementia and facilitating new avenues for drug discovery.

CONSTRUCTION OF HUMAN BRAIN REGION SPECIFIC ORGANOIDS AND ASSEMBLOIDS REVEAL ABNORMAL NEURODEVELOPMENT IN DOWN SYNDROME

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Down syndrome is a genetic disorder that results from an extra copy or part of chromosome 21 in the human genome. Individuals with Down syndrome have significant developmental delays, intellectual disabilities, and MRI results suggest that patients with Down syndrome have volumetric atrophy in multiple brain regions, including the cortex, cerebellum, and basal forebrain. The cognitive impairments in DS may result from early developmental defects in various brain regions, and the underlying molecular mechanism remains unknown.Here we differentiated human pluripotent stem cells into basal forebrain cholinergic organoids (hBFCOs) and cerebellar organoids by treating differentiation morphogens, respectively. Then we fused hBFCOs with cortical organoids (hCOs) to form assembloids and transplanted hBFCOs into immunodeficiency mice to construct chimeric brains. We validated the structural and functional connectivity of basal forebrain cholinergic neurons to the cortex in assembloids and chimeric brains. In addition, we identified neural morphological and projective defects in cholinergic neurons in Down syndrome patient iPSC-derived assembloids by histological assays. Cognitive impairment was further detected in chimeric mice that transplanted with Down syndrome patient iPSC-derived organoids. After performing single-cell/single-nucleus RNA-Seq analysis on organoids in the normal and Down syndrome groups, we found that axonogenesis was significantly affected in Down syndrome group, given the decreased projected fibers in the cortex of assembloids or chimeric brains; this may have been responsible for the cognitive impairment due to projection defects. Furthermore, suppression of the DSCAM pathway, which showed enhanced activity in DS, using CRISPR/Cas9 and RNA interference, reversed morphological defects in cholinergic neurons, thereby increasing the projection fiber in the cortex. Meanwhile, cerebellar organoids derived from individuals with Down syndrome recapitulated disease phenotypes, including decreased levels of cell proliferation and differentiation. More importantly, our data showed that Down syndrome cerebellar organoids have longer primary cilia, which may ultimately contribute to abnormal cerebellar development.Our work models the abnormality of basal forebrain-cortical cholinergic circuit and the developmental disorder of cerebellar cell proliferation in DS by using organoids and chimeric brains, which presents the direct influence of the DSCAM pathway and length of primary cilia on developmental brain defects in DS.

HUMAN DORSAL ROOT GANGLION ORGANOID MODEL: INSIGHTS INTO SENSORY NEURON DEVELOPMENT AND FUNCTION

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Dorsal root ganglia (DRG) house cell bodies of sensory neuron and play crucial role in transmitting sensory signals from the periphery to the central nervous system. While the development of DRG has been extensively studied in mouse models, the specifics of human DRG development and the mechanisms governing cell fate determination remain less understood. Here, we present a functional human dorsal root ganglion organoid (hDRGO) model, which incorporates both external signaling pathways and internal transcription factors identified through the analysis of developmental human sensory neurons. This model provides a reliable platform for studying the development and physiological characteristics of human sensory neurons. By simulating the in vivo environment through sequentially adding signal factors, we recapitulate the differentiation process of pluripotent stem cells into sensory neurons in vitro, generating proprioceptors, mechanoreceptors, and nociceptors. Long-term culture of hDRGOs also led to the formation of Schwann cells that surround nerve fibers in a manner similar to native DRG tissue structure, highlighting the potential of this model for studying sensory neuron-glial cell interactions. In particular, we point out the presence of a human-enriched sensory neuron subtype in hDRGOs characterized by the expression of DCC/NTRK3/NTRK1. Using calcium imaging, we demonstrate that this sensory neuron subtype is specifically activated by capsaicin, confirming its identity as a nociceptor and highlight the ability of hDRGOs to mimic human-specific cell characteristics. Additionally, hDRGOs also allowed us to verify the roles of transcription factors in regulating the development of sensory neuron lineages, emphasizing its significant value for study of human sensory neuron development. In summary, this study establishes a robust human DRG organoid model that integrates external stimuli and internal signals, simulates the process of establishing sensory neuron diversity in vitro, and emphasizes its potential in exploring the developmental regulation and physiological functions of human sensory neurons.

Keywords: human dorsal root ganglia, sensory neuron, neurogenesis wave, transcription factors, single-cell spatial transcriptome, DRG organoid

MAST1 IS AN IMPORTANT REGULATOR OF NEURONAL DEVELOPMENT AND MITOCHONDRIAL FUNCTION IN HUMAN STEM CELL-DERIVED TELENCEPHALIC NEURONS AND ORGANOIDS

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Neurodevelopmental disorders associated with autism, intellectual disability, and cortical malformations are frequently caused by rare genetic abnormalities. Rare de-novo mutations in the microtubule-associated serine-threonine kinase 1 (MAST1) have been detected in multiple patients with severe intellectual disability and cortical malformations. However, the cellular and molecular mechanisms that are disrupted by MAST1 mutations remain largely unknown. We characterized neurodevelopmental deficits in induced pluripotent stem cell (iPSC) derived telencephalic neurons and organoids from patients, parents, and engineered stem cells with MAST1 mutations. We observed that organoids with MAST1 mutations were smaller as compared to control organoids due to reduced number of neurons and impaired neuronal processes. To gain insights into the cellular mechanisms underlying these phenotypes, we investigated the timing, cell-specificity, and subcellular localization of MAST1 expression. Interestingly, we discovered that MAST1 was associated with neuronal mitochondria. We also found that MAST1-mutated neurons demonstrated reduced mitochondria mass and increased proportions of hyperactive mitochondria with elevated levels of reactive oxygen species. We demonstrated that expression of WT-MAST1 in patient MAST1-deficient neurons rescued the neurite outgrowth deficits. Collectively, these results suggest that MAST1 is an important mitochondria-associated protein that has a nonredundant role in regulating neuronal properties and that genetic restoration of MAST1 expression is a promising approach for future therapy development for patients.

DEVELOPMENT OF MULTICELLULAR hPSC-DERIVED 3D MYELIN SPHERES TO EXPLORE BRAIN DEVELOPMENT AND IN VITRO DISEASE MODELING

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The combination of stem cell biology, genome engineering and bioengineering has revolutionized the field of disease modelling by providing novel human in vitro systems to neuroscientists to understand neurodevelopment and disease pathogenesis. However, creating a multicellular 3D brain/cortex model that mimics human brain in vitro remains a challenge. Brain organoids, contain neural progenitor cells (NPCs) that self-organize and evolve over time into mature neuronal subtypes; allowing study of neurological diseases. However, problems like long culture duration, organoid heterogeneity, absent neuron-glia interactions like myelination, interferes with drug discovery for human neurological diseases. In current study, we aim to develop 3D Myelin Spheres from pre-differentiated human pluripotent stem cell (hPSC)-derived neural cells, to model *in vitro* neuron-glia interactions.

We combined growth-factor and transcription factor overexpression-based protocols to differentiate hPSC to NPCs and glial cells (astrocytes and oligodendrocytes). To define the optimal medium and cell ratio, we initially optimized 2D neuron-glia cocultures. We next converted the 2D model to a complex 3D Myelin Spheres using the hanging-drop method. We demonstrated the maturation of neurons, astrocytes and oligodendrocytes over time by immunostaining, single nuclei RNA sequencing and lipidomics in the developed 3D Myelin Spheres. Moreover, transmission electron microscopy (TEM) established presence of loose myelin sheaths as early as after 6 weeks of culture, which compacted and became more complex by week 15. Finally, CMOS-multi-electrode arrays indicated the beneficial effect of glial cells on neuronal electrophysiological maturation.

We used Rabies infection as a prototype for *in vitro* disease modeling using developed Myelin Spheres. We demonstrated successful rabies infection in 3D Myelin Spheres by secretion of rabies virus in culture supernatants, and by imaging (IF and TEM). We also detected presence of viral particles around degenerated myelin sheaths depicting the role of myelin biology in human rabies infection for the first time. Currently, we are using both the 2D and 3D models to assess the effect of anti-viral drugs on rabies infection.

3D Myelin Spheres serves as a novel hPSC-derived neuronal/glial culture system for disease modelling and drug development, and towards the fulfilment of the 3Rs principle in animal research.

FOLIC ACID IMBALANCE DISRUPTS NEURODEVELOPMENT AND CONNECTIVITY IN HUMAN CEREBRAL ORGANOIDS: RELEVANCE TO AUTISM

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Background: Folate is essential for cell growth and gene regulation through nucleotide synthesis and methylation, making it important during pregnancy for the rapid development of fetal tissues. Folic acid (FA), an oxidized synthetic form of folate, is commonly included in prenatal supplements and fortified foods to prevent neural tube defects. However, some studies have reported that elevated maternal plasma folate levels beyond WHO recommendations are linked to a higher risk of autism spectrum disorders in children. Although the benefits of FA are well-known, the impact of excessive FA intake on human fetal brain development, cortical organization, neurogenesis, and neuronal connectivity, is not fully understood.

Objectives: To investigate the neurodevelopmental, neurophysiological, and transcriptional consequences of imbalanced maternal FA supply during pregnancy in cultured human cerebral organoids.

Methods & Results: Human cerebral brain organoids (HCBOs) were generated from induced pluripotent stem cell (hIPSC) lines derived from healthy donors. These organoids were cultured for 40 days in media containing varying levels of FA or folinic acid (5-FTHF, a reduced natural form of folate), followed by histological, flowcytometric, and RNA sequencing analyses to assess changes in developmental outcomes. Neurophysiological measurements were conducted on 100-day-old organoids using high-density microelectrode arrays to record network-level electrical activity. Remarkably, we observed changes in neuronal differentiation in HCBOs cultured under conditions of excess FA supply compared with control and 5-FTHF groups. Neurophysiological recordings revealed reduced synchronous electrical activity, suggesting that neurons exposed to excess FA formed less functional synaptic connections. Gene ontology analysis of differentially expressed genes further highlighted significant dysregulations in processes related to axonogenesis, neuron projection development, and forebrain development.

Conclusions: Optimal prenatal FA intake is crucial as excessive FA exposure during pregnancy could potentially impair brain development and function, raising concerns about its neurodevelopmental effects.

HUMAN BRAIN ORGANOID MODEL FOR STUDYING RADIATION-INDUCED EFFECTS ON NEURAL STEM CELLS AND NEUROGENESIS

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Neural stem cells play a pivotal role in neurogenesis, making them essential for cognitive function. However, these cells are highly sensitive to chronic stressors and environmental insults, which can significantly reduce neurogenesis and lead to depression and cognitive decline. For long-term space travel, astronauts experience more hazardous environmental insults than on Earth, including heightened risks from proton radiation in galactic cosmic rays (GCRs) and solar particle events (SPEs). Radiation induces neural stem cell apoptosis, elevates oxidative stress, and reduces neural stem cell proliferation impairing neurogenesis. This impairment can manifest as memory decline, anxiety, and depression. Therefore, preserving neural stem cells is critical for preventing neurogenesis-associated pathologies during extended space missions. To investigate the effects of proton radiation on neurogenesis, we used a human brain organoids abundant in neurogenic niches during early developmental stages. We exposed 25-35 day-old organoids to a single dose of 0.5 Gy, replicating the accumulated dose of space radiation experienced on the Moon over the course of a year. We found an increase in apoptosis and decrease in cell proliferation that resulted in reduced organoid size. We also observed a significant increase in the expression of genes linked to the P53 signaling and apoptosis pathways. Trajectory inference and RNA velocity analysis strongly suggested that proton irradiation disrupted the normal progression of neurogenesis. In addition, metabolomics analysis revealed significant dysregulation in key energy pathways. Further, we found reduced expression of multiple genes linked to lipid synthesis, suggesting that proton radiation may compromise membrane integrity, cellular signaling, and energy storage – processes that are essential for the survival and function on neural stem cells and their progeny. By uncovering the early and significant effects of proton radiation on lipid metabolism, this research not only provides a deeper understanding of how radiation impairs neurogenesis at a cellular level but also opens the door to novel therapeutic approaches. Targeting the lipid metabolic pathways could lead to the development of neuroprotective strategies, offering a way to rescue neurogenesis and maintain cognitive function in astronauts exposed to the harsh environment of space.

IDENTIFYING TRANSCRIPTIONAL REGULATORS OF HUMAN FOREBRAIN NEURULATION

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The brain and spinal cord form from an epithelial sheet of neural progenitor cells through a process called neurulation, in which the neural plate folds into a neural tube. The closure of the neural tube occurs in a stereotypical sequence along the anterior-posterior axis of the embryo and is a prerequisite for proper morphogenesis and function of the nervous system. However, its molecular regulation at different axial positions is poorly understood, especially in humans, necessitating the application of reverse genetic screens to in vitro human stem cell-based models. Current screening approaches are typically conducted in a pooled manner, which potentially masks non-cell-autonomous aspects of morphogenetic processes such as intercellular signaling. Here we develop a cost-effective method based on CRISPR interference to rapidly perturb individual genes in an arrayed format homogeneously and scalably in a reproducible human embryonic organoid model of anterior neural tube closure. Using this method, we identify a network of key transcriptional regulators and downstream molecular pathways involved in forebrain neurulation, with implications for mechanisms underlying anterior neural tube defects such as exencephaly.

T CELLS REGULATE HUMAN CORTICAL DEVELOPMENT

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While entry to the central nervous system is tightly regulated, there is a growing body of evidence that immune cell types are present within and impact the function of neural tissue across the lifespan. However, the contribution of immune regulation on brain development remains unclear. Given the known association of immunodeficiency with neurodevelopmental delay, the dynamic neuro-immune interactions during neurogenesis may be particularly important for longitudinal brain function. Within developing human neocortex tissue, we identified CD4+ naive and memory T lymphocytes that are present within the angiogenic, progenitor niche, located adjacent to CNS-resident microglia. Flow cytometry-isolated brain T cells were molecularly characterized using single cell transcriptomics. Gene expression profiles indicate similar composition of T cell populations across paired organs isolated from the same biological sample. However, discrete expression profiles of organ-specific memory T cells suggest specific molecular identity, and perhaps function, of T cells in the brain. We then functionally assessed the interactions between CD4+ naïve and memory T cells and relevant neural populations through co-culture with regionalized forebrain organoids, during neurogenic periods. We observed T cell migration to the progenitor zone of the organoid, similar to their location in vivo. After T cell co-culture, or exposure to cytokines secreted from specific T cell populations, we observed decreased cell death and increased neural stem cell composition. Together, these results suggest the presence and active contribution of T cells to neural tissue health and growth during development of the brain.

DE NOVO START-LOSS VARIANTS IN GLUL LEAD TO GLUTAMINE SYNTHETASE DYSREGULATION AND NEURODEVELOPMENTAL DEFECTS

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Glutamine synthetase (GS), encoded by GLUL, catalyzes the conversion of glutamate to glutamine, and is crucial for neurotransmitter production and ammonia detoxification in the brain. GS levels are tightly regulated by glutamine availability. High glutamine concentration induces acetylation of the degron in the N-terminus of GS, and thereby a ubiquitin-mediated degradation of GS. Although GS deficiency is linked to severe neurological defects and early death, the impact of disrupted degron-mediated regulation on neurodevelopment is unclear. We reported nine individuals with severe developmental delay, seizures, and white matter abnormalities, all carrying de novo GLUL variants (Jones 2024). Seven had start-loss variants, and two had splicing disruptions leading to translation initiation from Met18, bypassing the degron. This truncation produces a stable, yet enzymatically active GS that is insensitive to glutamine feedback. GLUL was found to be widely expressed in neuro- and glial-progenitor cells, mature astrocytes, and microglia, but not in post-mitotic neurons. These findings highlight the critical role of GS regulation in human neurodevelopment. To further investigate the effect of this variant, we took advantage of CRISPR/Cas9-mediated genome editing to generate iPSC lines possessing the pathogenic variant. These edited lines will be used to generate iPSC-derived astrocytes and microglia, which will help us elucidate the role of GS regulation in human neurodevelopment.

EVALUATION OF HUMAN NGN2-INDUCED EXCITATORY NEURONS GENERATED VIA AAVS1-INSERTION, LENTIVIRUS TRANSDUCTION, OR PIGGYBAC TRANSPOSITION OF INDUCIBLE NGN2-CASSETTE

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Transcription factor-based differentiation strategies offer a more cost-effective method to generate human iPSC-derived cells at large-scale compared to traditional small molecule patterning approaches, which often involves longer timelines and expensive growth factors. The differentiation of neurons via the overexpression of the pro-neuronal transcription factor NGN2, for example, has been one of the most widely published methods to generate excitatory neurons efficiently and rapidly. Compared to small molecule differentiation, which take approximately 2-3 months to differentiate hiPSCs into functional neurons, NGN2-overexpression produces functional neurons in 2-3 weeks. Multiple methods exist to overexpress NGN2 in iPSCs, each differs significantly in scalability, reproducibility, and cost. Towards establishing and standardizing internal capabilities to generate NGN2-induced excitatory neurons (NGN2-iNs; iNs), we directly compared iNs generated from iPSC lines engineered with a DOX-inducible NGN2-overexpression system via three distinct methods, AAVS1-safe harbor locus insertion, lentiviral transduction, and PiggyBac transposon system. Specifically, we highlight the timeline, cost, scalability, reproducibility, and efficiency in producing NGN2-iPSCs and -iNs. To accomplish this, we evaluated cell morphology (microscopy, qPCR gene expression, immunocytochemistry), differentiation efficiency (FACS, qPCR), and functionality (MEA) of iNs derived from the three methods, and with multiple donors. These human iPSC-derived NGN2-iNs will serve as a powerful cellular system for screening and translational studies including target identification, target validation, and mechanistic studies.

UTILIZING 3D HUMAN CORTICAL AND GANGLIONIC EMINENCE ORGANOIDS TO INVESTIGATE THE INTERACTION BETWEEN *APOE4* AND SARS-COV-2 INFECTION

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COVID-19 is caused by the SARS-CoV-2 virus, which has infected over 750 million people and resulted in more than 7 million deaths worldwide. Despite initially being identified as a respiratory disease, over 30% of hospitalized COVID-19 patients experience acute and chronic neurological symptoms, including headaches, anosmia, and brain fog. Several studies have associated *APOE4*, the leading genetic risk factor for developing late-onset Alzheimer's Disease (AD), with an increased risk for severe COVID-19 infection. Our lab and others have shown that *APOE4* differentially affects excitatory and inhibitory neurons and increases astrocyte markers. Previous findings have also shown that there is an increase in SARS-CoV-2 infection in glial cells. However, whether *APOE4* exacerbates SARS-CoV-2 infection response in a cell type-specific manner has not been investigated. To address this question in a human-relevant model system, we generated human cortical organoids (COs) enriched in excitatory neurons and human ganglionic eminence organoids (GEOs) enriched in inhibitory neurons, using induced pluripotent stem cells (iPSCs) from *APOE3/3* control and *APOE4/4* patients. We also used isogenic lines to control for patient background. After 220 days in vitro (DIV), when organoids express mature neuronal and glial markers, we infected the organoids with SARS-CoV-2 for 1 hour and harvested them 7 days post-infection for RT-qPCR and immunohistochemistry to determine the severity of infection and affected cell types. Our results indicate that the *APOE* genotype does not affect overall SARS-CoV-2 infection in COs and GEOs. In infected COs, both *APOE* genotypes exhibit increased markers of mature neurons, outer radial glia, and immature astrocytes. In infected GEOs, both genotypes show a reduction in mature neuron markers. This suggests that at low viral titers, *APOE4* may not affect CO and GEO susceptibility to SARS-CoV-2 7 days post-infection. Investigating *APOE4*'s role in COVID-19 severity or susceptibility is important for understanding the underlying cell-type specific mechanisms and identifying potential therapeutic interventions for at-risk populations.

SUPERNUMERARY X CHROMOSOMES SHAPE BRAIN ORGANOID ARCHITECTURE AND FUNCTIONS IN A DOSE-DEPENDENT FASHION

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Klinefelter syndrome (KS) is the most prevalent aneuploidy in males and is characterized by a 47,XXY karyotype, with an incidence of approximately 1:600 live male births. Higher-grade sex chromosome aneuploidies (HGAs), such as 48,XXXY and 49,XXXXY are rarer conditions occurring in 1:40.000-1:80.000 males. Both KS and HGA patients exhibit a broad spectrum of neuronal impairment, including cognitive deficits, seizures, autistic traits, and motor, speech, and language delays. The severity of these clinical features correlates directly with the number of additional X complements. While KS patients typically display a mild phenotype, HGAs are associated with profound cognitive defects and reduced viability. Despite the prevalence of X chromosome aneuploidies, there is a critical need for cellular models that elucidate the transcriptional and epigenetic consequences of X chromosome overdosage and its implications for neurodevelopmental disorders. To solve this gap, we generated a paradigmatic cohort of induced pluripotent stem cells (iPSCs) derived from KS and HGA patients (47,XXY, 48,XXXY, 49,XXXXY) with preserved X chromosome inactivation status. We used these iPSCs to model the impact of sex chromosome aneuploidies on early neurodevelopment through cortical brain organoids. Our findings revealed that X aneuploid brain organoids retain the epigenetic inactivation status of supernumerary X chromosomes during extended differentiation periods. Through a multi-layered analysis integrating morphological, bulk, and single-cell transcriptomic data and functional assessments, we demonstrated that the presence of additional X chromosomes impairs neural patterning, disrupts cortical architecture, and alters the electrophysiological properties of cortical organoids in a dose-dependent manner. Relevantly, patch-clamp analysis revealed X chromosome dosage-sensitive deficits in long-term potentiation (LTP), likely linked to impaired synaptic plasticity. Additionally, severe astrocyte differentiation defects were observed in HGA-derived organoids, potentially contributing to synaptic dysfunction. Single-cell RNA profiling further uncovered altered neuronal and non-neuronal cell population proportions and dysregulated progenitor proliferation in organoids carrying supernumerary X chromosomes. Our results highlight the role of genes escaping X inactivation in driving the neurodevelopmental defects associated with X chromosome overdosage. Overall, our study underscores the use of brain organoids as a valuable platform for elucidating the molecular and cellular consequences of X chromosome aneuploidy during early human brain development.

SYSTEMATIC EVALUATION OF MORPHOGEN PATTERNING REPRODUCIBILITY IN HUMAN NEURAL ORGANOIDS USING A MULTIPLEXED SINGLE-CELL TRANSCRIPTOMICS SCREEN

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The mesencephalon or midbrain arises from the second vesicle of the neural tube forming the rostral part of the brainstem, while the rhombencephalon or hindbrain emerges from the third vesicle, forming the cerebellum and the caudal part of the brainstem. Mid- and hindbrain function to coordinate motor movements and process sensory inputs and their impairment is associated with diverse disorders including Multiple Sclerosis and Parkinson's disease. In vitro organoid models promise new inroads to understand human brain development, model disease, and develop therapeutics. However, existing human neural organoid atlases are enriched in cell types with forebrain identity, whereas cell type diversity of the posterior brain, such as mesencephalon and rhombencephalon, is under-represented. To derive organoids with novel brain regional identities, focused on mid- and hindbrain, we designed a multiplexed morphogen patterning screen taking existing midbrain organoid protocol as a basis. We tested 48 conditions, varying concentrations and combinations of 10 morphogens involved in brain patterning. Each condition was tested minimum in duplicate with single-cell transcriptomic readout of single organoids, enabling us to assess the reproducibility on the organoid level. We identified conditions allowing for the emergence of under-represented cell types, such as medulla glycinergic neurons and cerebellum glutamatergic neurons, thereby expanding existing mid- and hindbrain organoid models.
In order to systematically investigate, to which extent cell lines and experimental batches affect the consistency of organoid patterning, we conducted an additional multiplexed morphogen patterning experiment to study the reproducibility of the effects induced by retinoic acid, FGF-8, SHH and CHIR. We have tested influence of 2 neural induction methods, 2 experimental batches and 4 cell lines: H1 (XY), H9 (XX), WTC (XY) and WIBJ (XX) on cell type composition consistency after morphogen patterning using single-cell transcriptomic readout and pooling 3 organoids for each condition. Altogether, we present the first comprehensive resource, systematically evaluating the reproducibility of morphogen patterning in human neural organoids, and extending current morphogen atlases with cell types within the midbrain and hindbrain.

ARMCX2 REGULATES MITOCHONDRIAL CLUSTERING AND HUMAN NEURAL PROGENITOR CELLS PROLIFERATION

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Mitochondria are highly dynamic organelles that undergo frequent fusion and fission. Mitochondrial fusion is essential for neural stem cell self-renew and maintaining neural stem cell stemness. However, little is known on how mitochondrial dynamics is regulated during early stages of human neural differentiation. Therefore, we use a rather simple in vitro two-dimensional neural differentiation system to study how mitochondrial dynamics may affect neural stem cell function. We have found that mitochondria formed big clusters close to the nucleus in the majority of day 9 differentiated cells in the two-dimensional differentiation system. This mitochondrial clustering phenotype is caused by the high expression of Armcx2 during differentiation,as Armcx2 knock out led to mitochondrial declustering,neural progenitor cells overproliferation and neuronal differentiation defects. Furthermore, we found that Armcx2 can interact with the nuclear import protein Transportin1 and recruit Transportin1 to the mitochondria. Mechanistically, we are testing whether the interaction between the PY_NLS motif of Armcx2 and Transportin1 is essential for neural progenitor cells proliferation and thus neuronal differentiation.

EMERGING NEURAL NETWORK DYNAMICS IN HUMAN BRAIN ORGANOIDS FROM MECP2 DUPLICATION AND MECP2 DELETION (RETT) SYNDROMES

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Methyl-CpG-binding protein 2 (MECP2) gene, located in the Xq28 region of the X-chromosome, is a dosage-dependent gene that causes devastating neurodevelopmental disorders if its expression deviates from normal. MECP2 gain-of-function leads to MECP2 Duplication Syndrome (MDS) mostly occurring in boys, while loss-of-function causes Rett syndrome mostly seen in girls and Male Rett-Like (MRL) syndrome in boys. Despite their opposing genetic origins, these disorders have similar clinical features, including autistic behaviors, learning deficits, epilepsy, and impaired motor function. To investigate the MeCP2-dosage-dependent mechanisms underlying these disorders, we generated human cortical organoids from molecularly confirmed MDS and MRL patients, as well as typically developing sex-matched controls. We employed multi-electrode arrays to study neuronal network activity patterns in 3-month-old organoids, conducting recordings over four consecutive weeks. This approach allowed us to capture the evolution of network activity during a critical period of organoid development. Analysis of spiking activity from continuous voltage measurements revealed distinct electrophysiological profiles in MDS and MRL organoids compared to healthy controls. MDS organoids exhibited an increased weighted mean firing rate, while this was reduced in MRL organoids. Interestingly, the control organoids demonstrated an intermediate firing rate, suggesting that MeCP2 expression could be contributing to the difference in the firing rate. Examination of network burst dynamics over 18 recording sessions revealed further differences between the organoid types. Network burst synchrony, burst frequency, burst percentage, and the number of electrodes participating in bursts showed distinct patterns for MDS, MRL, and control organoids. These findings highlight the complex relationship between MECP2 dosage and neuronal network function. Our study provides novel insights into the emerging neural network dynamics in human brain organoids derived from patients with MECP2-related disorders. These findings contribute to our understanding of how MECP2 dosage affects neuronal network development and function, potentially leading to the identification of therapeutic targets or prognostic markers for disease progression and treatment efficacy.

INTEGRATION OF HUMAN iPSC- DERIVED MICROGLIA IN 2D NEURAL CULTURES AND ADHERENT CORTICAL ORGANOIDS

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Microglia are the resident immune cells of the brain, playing a crucial role in neural development, maintaining brain homeostasis, neuroinflammation and disorders. Recent developments in human induced pluripotent stem cell (hiPSC) field permit the integration of human derived microglia with cortical cell culture models for research and drug development. The current study focuses on supplementation of hiPSC- derived neural models with microglia and the impact of such an integration on the different cells involved. The 2D neural cultures are generated by over-expressing *NGN2* in hiPSCs, which gives rise to neurons, and then supplementing with human iPSC- derived astrocytes. To obtain the 3D adherent cortical organoids, hiPSC-derived neural progenitor cells (NPCs) are differentiated in standard 384-wells to develop radially organised, functionally mature synaptic networks, with neuronal and glial cell types. The cortical organoids, additionally, provide a platform to study neural development over longer periods of time in a reproducible small scale format, that is suitable for high-throughput studies. The hiPSC- derived microglia, following characterisation, are sequentially seeded at different time points to observe their survival and integration in these models. Microglia were detected in the adherent cortical organoids up to 6 months post seeding without supplementation with additional growth factors. Highly motile microglia integrate efficiently in the neural cultures, preferentially positioned in the densely populated regions. We observed that microglia exhibit dynamic and mature morphologies in co-culture with neural networks compared to pure microglial cultures. To further elucidate the effects of microglia on the neural cultures, we examined the development and functionality of the models, including cell proliferation, organoid structure formation, cytokine analysis, single cell RNA sequencing, and electrophysiological activity using calcium imaging. Supplementation of the neural cultures with microglia, contributes to our understanding of neuronal- microglial interactions and enables further investigations of neuroinflammation and early cortical development in health and disease.

HIGH-THROUGHPUT MULTI-SCALE TOOLS FOR AUTOMATED BRIGHTFIELD BRAIN ORGANOID SCREENING USING A MULTI-CAMERA ARRAY MICROSCOPE.

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We describe novel tools and techniques developed to enable automated high-throughput screens of 3D iPSC-derived cortical organoids grown in multi-well plates. The developed method leverages a multi-camera array microscope (MCAM™) acquire high resolution brightfield images (1um / pixel to 9 um/pixel) in a few seconds to fully digitize a well plate for subsequent analysis. In the time immediately following the imaging period, the images are analyzed using a machine learning based segmentation algorithm (Segment Anything-2 - SAM-2) to create precise masks of the organoids. In one case, we demonstrate how the tool can be used to capture and process organoids cultivated in a conical 96 well plate (day 1-35) when their diameter is no larger than several hundred micrometers. In this mode of operation, the MCAM™ is operated at a resolution of 1 um per pixel (similar to that of a 10x optical objective). 24 objectives, and 24 image sensors, each capturing 9 megapixels of image data, are used to digitize the well plate in ~15 seconds. The conical shape of the wells ensures that the organoids stay near the vicinity of the center of each image. The algorithm uses this knowledge and biases the initial prompt used by SAM-2 as the indicator of the organoid away from the exact center of the image towards the darker region. Each of the 96 9MP acquisitions are resampled to 1024x1024 and segmented by SAM-2. The masks are then used to compute the area and circularity of each organoid creating a pipeline that can be completed in under 1 minute using a workstation with a powerful graphical processing unit (RTX 4090).At the later time points (35 days and above), we demonstrate how multiple organoids can be studied when they are transferred to 24 well plates. At these ages, each organoid can be as large as several millimeters in diameter necessitating larger volumes of media for healthy growth making imaging at 10x equivalent magnification challenging when considering the 3D size. Instead, we utilize an MCAM™ configured to have a pixel size of 9 um in the object plane (2x equivalent magnification) to capture an entire 24 well plate in a single instant. In this configuration, each well is synchronously imaged with a unique objective and image sensor avoiding image tiling artifacts. The flexibility of the SAM-2 enables the software to provide multiple point prompts to individually segment each organoid. This enables the quantification of the area and circularity of multiple organoids in a 24 well plate in as little as 40 seconds.

By reducing the entire capture to analysis pipeline for a full 24 or 96-well plate to under 1 minute, we offer a robust solution for non-destructive, high-throughput optical imaging while enhancing the precision of surface area and circularity measurements ensuring high-quality data collection.

CEREBROSPINAL FLUID AS A KEY EXTRACELLULAR SIGNAL DURING HUMAN NEOCORTICAL DEVELOPMENT

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Cerebrospinal fluid (**CSF**), secreted by the choroid plexus (**ChP**), is increasingly recognized as an emerging underexplored signal in neocortical development. CSF is enriched with extracellular cues, and it directly contacts the apical side of neuronal progenitors (**NPCs**) in the brain's ventricles. This suggests that CSF could influence cortical development by modulating NPC signaling.
To explore this, we have generated choroid plexus organoids and compared the proteomic profiles of *in vitro*-derived CSF with fetal human CSF, revealing key molecular similarities.
Moreover, we have identified potential candidate molecules in the developing CSF, which are known for guiding neuronal migration and axonal pathfinding, potentially impacting brain architecture and signaling.
To investigate how CSF affects developing cortical signaling, we have treated NPCs with *in vitro* CSF and performed proteomic and transcriptomic analyses, which showed significant changes in protein and gene expression, underlining the potential regulatory role of CSF in NPC behavior.
Further, by treating cortical organoid slices with potential candidates, we will explore whether they may influence cortical development by modulating proliferation, neuronal differentiation, or migration.
The CSF also contains Galectins, including Galectin-3-binding protein (**LGALS3BP**) which has been previously demonstrated to be a crucial extracellular signal during cortical development. Specifically, a *de novo* E370K mutation has been shown to lead to pathological conditions, such as autism and epilepsy.
We found that LGALS3BP mutation in the ChP organoids alters CSF composition during development, possibly affecting NPC signaling. Therefore, we are investigating whether physiological CSF can reverse LGALS3BP mutation phenotypes, providing crucial insights into linking extracellular signals to disease mechanisms and potential treatments.
This study is advancing our understanding of how developing CSF, and its components are crucial factors in human neocortical development, and it is also uncovering potential implications for understanding neurodevelopmental disorders, opening new paths for neurodevelopmental disorders research.

DEVELOPMENT OF A PATIENT-REPRESENTATIVE ORGANOID MODEL FOR DIFFUSE MIDLINE GLIOMA (DMG) TO INVESTIGATE EARLY TUMORIGENESIS AND THERAPEUTIC RESPONSE.

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Diffuse **M**idline **G**lioma (*DMG*) is a highly aggressive and rare pediatric cancer primarily found in the hindbrain, particularly the pons, with no curative treatment. Disease-representative models can aid the search for effective treatments, but human preclinical models that reflect the unique developmental background, environment, and anatomical complexity of DMG are so far lacking. Here, we present a novel guided brain organoid model with pontine identity, genetically engineered to model H3.3K27M-altered DMG, termed *DMGO's* (**D**iffuse **M**idline **G**lioma **O**rganoids). The resulting tumors, grown over months in vitro, faithfully recapitulate the infiltrative, diffuse nature and human-specific transcriptomic heterogeneity observed in patients. DMGO's reflect a similar methylation profile as primary tumors and allow for the genetic tracing of early tumorigenesis, demonstrating that aggressive clonal expansion is fundamentally linked to hindbrain-specific gliogenesis, not neurogenesis. Furthermore, we evaluated promising GD2 CAR T cell treatments, revealing a patient-representative level of functional T cell heterogeneity, enabling us to investigate and recapitulate treatment schemes similar to those used in patients. To further enhance the biological accuracy and predictive power of our preclinical model, we incorporated non-neural cells (primitive macrophage progenitors and brain endothelial cells) within a bioprinted microfluidic chip system, successfully recreating a complex multicellular environment. Together, we have engineered a physiologically relevant and life-like model for studying healthy hindbrain development as well as DMG onset and progression. The unmatched similarity of this setup offers a promising tool to accelerate the understanding and development of targeted treatments, such as GD2 CAR T cells, for this devastating pediatric cancer.

GLIOBLASTOMA CELLS CONVERGE ONTO AN INVASIVE NEURONAL/GLIAL PROGENITOR-LIKE CELL STATE AFTER ENGRAFTMENT INTO HUMAN BRAIN ORGANOIDS.

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Glioblastoma (GBM) tumors are highly diverse and contain cell populations that closely mirror neurodevelopmental cell types. However, unlike normal brain development, the potential for plasticity is retained by all stem-like and differentiated cells within GBM tumors. GBM cells can exploit these state-shifting capacities to resist therapeutics, invade into the normal brain, and propagate new tumors. Thus, there is a need to identify and target the infiltrative cell states within GBMs and the molecular regulators within the microenvironment that underlie their emergence. To address this, we use human brain organoids as a recipient environment in which we engraft tumor cells isolated directly from surgically resected GBMs or patient-derived cell lines. The use of human brain organoids allows us to study the impact of human-specific spatial and maturational milieu on the biological behaviors of GBM tumors, including their cell state plasticity, proliferation, and invasion. We performed paired single-cell RNA sequencing on tumor cells pre-engraftment and 14 days post-engraftment and found that GBM cells are uniformly overrepresented by a neural progenitor-like (NPC-like) transcriptomic state within organoids. The dominance of this NPC-like GBM cell state was consistent across organoids mimicking forebrain, midbrain, and hindbrain identities, and within immature vs. more mature forebrain organoids. To determine the functional significance of this NPC-like cell state bias, we applied an established invasivity gene signature onto our datasets and identified that NPC-like GBM cells have a higher score for invasivity genes post-engraftment than cells belonging to other transcriptomic states. Collectively, our data highlight the existence of intrinsic tumorigenic factors (e.g.: invasivity) or shared extrinsic factors across multiple microenvironmental milieu that may bias GBM cells toward a NPC-like transcriptomic state. We are now using this glioblastoma/organoid coculture platform to test if neural progenitor-like GBM cells may propagate new tumors in a model of serial transplantation and to determine the extrinsic factors responsible for infiltration using computational pipelines of cell-cell interactions.

MITOCHONDRIAL DYSFUNCTION AND ROS IMBALANCE IN 16P12.1 DELETION SYNDROME: IMPLICATIONS FOR NEURODEVELOPMENTAL DISORDERS

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The 16p12.1 deletion is linked to various neurodevelopmental disorders, including autism spectrum disorder (ASD). This study explores the molecular mechanisms underlying 16p12.1 deletion syndrome, focusing on mitochondrial dysfunction and reactive oxygen species (ROS) imbalance. Mitochondria regulate cellular energy metabolism and redox homeostasis, and their dysfunction can impair ATP production, increase ROS levels, and trigger apoptosis pathways. Emerging evidence connects mitochondrial abnormalities to the pathophysiology of 16p12.1 deletion syndrome, with recent findings showing that this deletion increases ROS production and affects mitochondrial complex III.

We employed a multidisciplinary approach, integrating biochemical assays and cellular models, to investigate the effects of 16p12.1 deletion on mitochondrial function and ROS regulation. Our hypothesis is that genes within the 16p12.1 region are critical for mitochondrial biogenesis, dynamics, and redox balance, and their haploinsufficiency disrupts these processes, contributing to the neurological symptoms seen in 16p12.1 deletion syndrome.

Our analysis examines mitochondrial DNA content, respiratory complex activity, and ROS production in 16p12.1-deficient cells, alongside expression levels of key mitochondrial genes and proteins. Additionally, CRISPR activation (CRISPRa) technology will be used to upregulate 16p12.1 genes in human induced pluripotent stem cells (hiPSCs) and neuronal lineages (NPCs and Mature Neurons), testing for potential rescue of mitochondrial function.

This research provides novel insights into the molecular mechanisms driving 16p12.1 deletion syndrome, highlighting mitochondrial dysfunction and ROS imbalance as central factors. Our findings may reveal new therapeutic targets and enhance our understanding of neurodevelopmental disorders such as autism.

MULTI-OMIC ANALYSIS OF GUIDED AND UNGUIDED FOREBRAIN ORGANOIDS REVEAL DIFFERENCES IN CELLULAR COMPOSITION AND METABOLIC PROFILES

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Neural organoids serve as invaluable models for studying neurodevelopment and neurological diseases. Two primary methods are currently employed for generating forebrain organoids: the guided approach, where differentiation toward neuroectoderm and specific CNS regions is directed by external signaling molecules, and the unguided approach, which leverages the intrinsic potential of pluripotent stem cells to form neuroectoderm without external cues, predominantly resulting in telencephalic specification. Despite their importance in the field, the distinct outcomes of these models have not been directly compared.
To address this gap, we conducted a comprehensive multi-omic analysis of forebrain organoids generated via guided and unguided methods, focusing on proteomic, lipidomic, and metabolomic variations. Additionally, we examined key neurodevelopmental post-translational modifications (PTMs) by characterizing differences in protein phosphorylation and sialylation, and performed single-cell transcriptomics (scRNAseq).
Our multi-omic analysis revealed significant differences in the neuronal, synaptic, and glial composition between the two approaches. Guided organoids exhibited a higher proportion of neurons, including GABAergic interneurons, and synapses, whereas unguided organoids showed increased numbers of GFAP+ cells and choroid plexus. Metabolically, unguided organoids demonstrated elevated oxidative phosphorylation and fatty acid β-oxidation, while guided organoids showed a greater reliance on glycolysis. Extending this analysis to forebrain organoids from patients with schizophrenia and healthy controls, we confirmed the identified differences and demonstrated that distinct disease phenotypes may emerge based on the organoid generation method.
Overall, our study comprises a thorough description of the multi-omic differences arising when generating guided and unguided forebrain organoids and provide an important resource for the neural organoid field studying neurodevelopment and -disease.

INTEGRATIVE FRAMEWORK FOR SINGLE-CELL TRANSCRIPTOMIC ANALYSIS OF HUMAN CORTICAL DEVELOPMENT

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The cerebral cortex is a critical region of the brain responsible for higher-order cognitive functions. Its development is a particularly complex process that follows precise temporally and spatially defined steps that are not yet completely understood. The advent of single-cell omics technologies provides scientists with a tool to investigate biological systems at unprecedented resolution, thus leading to an exponential increase of datasets investigating human brain cell diversity.
However, an integrative resource to effectively analyze cortical development is still missing, as no dataset completely covers the time span of this process. The inherent sparsity and noisiness of single-cell transcriptomics data represent a non-trivial challenge in the biological interpretation, de novo discovery and integration of multiple datasets, which further complicates integrative single-cell analysis of cortical development.
We present a computational framework that addresses these challenges, allowing efficient integration, analysis, and interpretation of single-cell transcriptomics datasets in the context of human cortical development. We analyzed 11 datasets ranging from fetal to adult developmental stages, performing per-batch analyses and defined prototypical signatures that capture relative intra-batch differences, thus removing sample specific variability. To then merge the 1756 prototypes that we obtained into one consistent reference, we built a network that reflects the similarity of each prototype signature with a set of biologically interpretable cell-type signatures, which come from 6 well-annotated datasets that span the whole cortical development. The similarity to the reference cell-type prototypes is used to interpret each prototype, while retaining all the additional metadata of the datasets in which the prototypes were obtained. Graph-layout algorithms are used to visualize the integrated, interpretable network.
To enable easy interpretation of independent data, we additionally developed an approach to easily map single-cell or bulk transcriptomic data onto the reference network. Mapping allows intuitive data visualization and interpretation by transferring knowledge from our integrative reference. Finally, we introduce mapping quality metrics to estimate the accuracy and precision of the knowledge transferred to the new data .
Our computational method thus provides a new approach to easily integrate multiple heterogeneous datasets, while also providing a simple framework to analyze the cellular complexity of human cortical development.

MODELING MAJOR DEPRESSIVE DISORDER RISK VARIANT NEGR1 IN HUMAN BRAIN ORGANOIDS

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Major depressive disorder (MDD) is one of the most significant global health challenges affecting over 185 million people globally. Large-scale genome-wide association studies (GWAS) demonstrate a highly polygenic architecture, in which many variants of small effect size influence the likelihood of developing the disorder. NEGR1 has been consistently identified as one of the most significant risk genes for MDD, wherein its upregulation in brain tissue has been positively associated with the disease. NEGR1 encodes for a cell adhesion molecule that promotes neuronal spine plasticity. The biological effects of increased NEGR1 levels on gene expression and cellular function in human brain development remain unclear. To investigate these effects, CRISPR/Cas9 technology will be used to introduce MDD risk variant rs3101339, shown to increase NEGR1 expression, into induced pluripotent stem cells (iPSCs) to generate cortico-striatal assembloids and hippocampal organoids. Single cell RNA-seq analysis and calcium imaging will be performed to determine transcriptomic and functional changes between conditions.

ASSESSING OLIGODENDROCYTE DEVELOPMENT AND MYELINATION IN DOWN SYNDROME USING HUMAN FOREBRAIN ASSEMBLOIDS

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Down Syndrome (DS) is a genetic condition caused by the triplication of chromosome 21 and is associated with global disturbances in gene expression and abnormal phenotypic and functional properties of brain cells, ultimately resulting in intellectual disability. While the mechanism responsible for intellectual disability is unknown, it has been linked to aberrant oligodendrocyte and myelin biology. We aim to define the molecular and developmental features in oligodendrocytes underlying deficits in myelin in the DS brain.

We used three pairs of isogenic DS patient-derived induced pluripotent stem cell (iPSC) lines to generate cortical organoids (COs). The oligodendrocyte populations were expanded, and COs were regionally patterned to mimic the fate of ventral and dorsal forebrain (vCOs and dCOs, respectively). The COs were fused to generate forebrain assembloids (FAs). We assessed markers of oligodendrocyte development using immunohistochemistry (IHC) and performed single-cell RNA sequencing to determine the changes in gene expression. Myelin structure was detected using electron microscopy (EM) and quantitative birefringence microscopy (qBRM).

We detected significant changes in the early cell fate specification and lineage commitment in 2 month old trisomic vCOs, which exhibited increased number of Sox10+ cells, as well as cells co-expressing Sox10 and NKX2.2. At 3 months, increased CC1 expression (a pan-oligodendrocyte marker) and its colocalization with Sox10 and PDGFRα suggests increased generation of OPCs in trisomic vCOs, though these changes were absent in our FAs. By 5 months, the total number of oligodendrocytes expressing CC1 did not differ between euploid and trisomic FAs, but the number of oligodendrocytes co-expressing CC1 and MBP (myelin basic protein) was decreased in trisomic FAs and trisomic vCOs. Interestingly, trisomic FAs showed increased levels of co-expression of CC1 and BCAS1, a marker of actively myelinating oligodendrocytes, suggesting neuronal activity-dependent dysregulation. ScRNA-seq of FAs identified changes in transcriptomic signature in trisomic oligodendrocytes. We have demonstrated the feasibility of EM and qBRM to assess myelin integrity in trisomy in our FAs.

Using our CO and FA model, we identified stage-specific dysregulation in oligodendrocyte development in trisomy 21. This platform can be used in the future for the assessment of new targeted therapeutic approaches.

DISRUPTION OF MITOCHONDRIAL AND PURINE METABOLISM IN *MECP2* DUPLICATION SYNDROME: INSIGHTS FROM PATIENT DATA AND ORGANOID MODELS

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The brain is exquisitely sensitive to gene dosage, as maintaining the correct dosage is critical for neuronal homeostasis. MeCP2 is a dosage-sensitive gene: too much of the produced protein leads to *MeCP2* Duplication Syndrome (MDS) seen mostly in boys, and too little of it causes Rett syndrome seen mostly in girls. Altered dosage of MeCP2 results in clinically similar neurodevelopmental disorders, characterized by Intellectual disabilities, epilepsy, and impaired motor function. Although the genetic basis of the *MeCP2*-related disorders is well established, the cellular mechanisms underlying these conditions remain poorly understood. Emerging evidence suggests that metabolic imbalances may play a role in Rett syndrome, leading to the hypothesis that MDS could similarly be linked to metabolic dysfunction. To test this hypothesis, we analyzed plasma and cerebrospinal fluid (CSF) from affected patients and modeled MDS using patient-specific cortical organoids. Utilizing a multi-omics approach, we conducted untargeted metabolomics on plasma samples and examined gene-metabolome coupling in CSF and cortical organoids derived from the same patients. Notably, our analyses identified significant disruptions in mitochondrial and purine-related pathways across various datasets from the MDS patients. Mitochondria/bioenergetic assays revealed reduced size and branching of mitochondria, and lower ATP and reduced-glutathione (GSH) levels in MDS organoids. In contrast, markers of oxidative stress (oxidized-GSH and 8-hydroxyguanosine) were elevated. The purinosome, an enzymatic complex involved in purine metabolism, has already been associated with mitochondrial dysfunction, and proteomic analyses showed decreased expression of purine-related proteins in both CSF and organoids. Given that MeCP2 functions as a transcription factor we interrogated if genes involved in mitochondria-purine pathways were transcriptionally regulated by MeCP2. *MeCP2* binding motifs were identified in promoters of 434 mitochondrial-purine metabolism genes. In conclusion, our study provides compelling evidence that metabolic dysfunction, particularly mitochondria/purinosome coupling, plays an important role in the pathogenesis of MDS. By integrating patient-derived samples with advanced in vitro models, such as cortical organoids, we have identified critical molecular pathways that may serve as key drivers of disease progression. The parallels between MDS and Rett syndrome, both driven by altered MeCP2 dosage, suggest that these disorders may share common underlying mechanisms, especially in terms of disrupted mitochondrial function.

NEUROCHEMICAL AND METABOLIC ASSESSMENT OF HUMAN PLURIPOTENT STEM CELL–DERIVED CEREBRAL ORGANOIDS BY HIGH-RESOLUTION MAGIC-ANGLE SPINNING NMR SPECTROSCOPY

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Human-derived cerebral organoids (COs) are three-dimensional cell culture systems derived from human pluripotent stem cells that resemble human brain tissue. COs can be analysed using various 'omics' approaches to study the transcriptome and proteome, providing valuable information that is typically inaccessible in human brain tissues *in vivo*. However, these methods are destructive, costly, and indirect. An alternative for metabolic measurement is nuclear magnetic resonance (NMR) spectroscopy, a non-destructive technique that directly quantifies metabolites involved in various cellular pathways. Proton (^{1}H) NMR offers a key advantage in that it is translational and can readily be performed in the human brain *in vivo* using clinical MRI systems. However, to our knowledge, ^{1}H-NMR spectroscopy has yet to be performed in human-derived COs. Here, we report the use of high-resolution magic-angle spinning (HR-MAS) NMR spectroscopy for metabolic analysis in intact human embryonic stem cell (hESC)-derived COs at different stages of maturity. With this approach, we were able to detect and quantify up to 17 metabolites in hESC-derived COs, greatly improving the spectral quality and metabolic information that can be obtained from these intact human brain models. While the CO neurochemical profile showed similarities to that of *in vivo* human brain, we also observed some notable differences, including high levels of lactate, high levels of hypotaurine, a balanced glutamine/glutamate ratio, and low levels of N-acetyl aspartate.

To better understand metabolic differences between CO and *in vivo* human brain, we compared CO expression of selected metabolic genes against publicly available spatial transcriptomics datasets from human brain (both adult and foetal), and rat brain. The low observed NAA concentrations appear to be explained by the pattern of gene expression in COs, whereas some of the metabolic changes in glutamine, glutamate and hypotaurine did not appear to be explained by the pattern of gene expression. Our results suggest HR-MAS NMR spectroscopy as a useful methodology to assess neurochemistry and metabolism in human brain models, such as hESC-derived COs.

Key words: human pluripotent stem cells; cerebral organoids; HR-MAS NMR spectroscopy.

PAX6 LOSS IN HUMAN CEREBRAL ORGANOIDS RESULTS IN ALTERED EXCITATORY/INHIBITORY NEURONAL RATIO.

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The transcription factor PAX6 is a crucial regulator of multiple aspects of embryonic forebrain development. Its well-known roles in mice include regulating differentiation of excitatory and inhibitory neurons in the embryonic cortex, in which we recently showed that in mice, Pax6 acts to limit the competence of developing cortical cells to respond to inductive intercellular signals. PAX6's roles during human forebrain development, however, are less well understood. Therefore, we investigated PAX6's roles in human neurodevelopment using human cerebral organoids. We found homozygous *PAX6* mutant ($PAX6^{-/-}$) organoids were larger than controls and contained inhibitory cell types not found in $PAX6^{+/+}$ controls. These inhibitory cells exhibited transcriptomic similarities and comparable distribution to analogous inhibitory cells previously described in $Pax6^{-/-}$ mice.

To uncover what is driving the changes in excitatory/inhibitory cell fate decisions in *PAX6* null organoids, we furthered explored the differentiation trajectory of excitatory and inhibitory cells to understand what are the genetic programmes driving these decisions. Further, we also examined cell-cell communication differences caused by the loss of PAX6 in the organoids using CellChat. Our findings suggests that while PAX6's role in controlling excitatory versus inhibitory neural differentiation is conserved, there might be alterations in the activities of intercellular signalling pathways in human $PAX6^{-/-}$ cortical progenitors that have not been described in mice, indicating potential species-specific mechanistic differences.

ENHANCER-TARGETED CRISPR-ACTIVATION OF ASD RISK GENES RESCUES MUTANT GENE EXPRESSION AND PHENOTYPES IN PATIENT-DERIVED CORTICAL ORGANOIDS.

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Autism Spectrum Disorders are highly heritable neurodevelopmental disorders that affect millions of people globally and represent a significant public health burden. Over the past two decades, hundreds of rare de novo mutations of major effect have been identified, most known to or predicted to lead to truncated mRNA products, strongly implying haploinsufficiency as the mechanism of action, in which a single copy of a functional gene is insufficient for normal function. Given this, one potential therapeutic avenue would be to upregulate the expression of the wildtype allele to restore gene dosage levels to wildtype.
We previously demonstrated that *CHD8* and *SCN2A*, two well-characterized ASD genes, could be successfully rescued with enhancer-targeted CRISPR-A. Using CRISPR-edited stem cell lines, we established cortical forebrain organoids to characterize altered neurodevelopment in *CHD8*$^{+/-}$ and *SCN2A*$^{+/-}$ organoids. By applying enhancer-targeted CRISPR-A, we were able to rescue both mutant gene expression levels and mutant phenotypes. This approach takes advantage of endogenous gene regulatory mechanisms to modulate gene expression, which is necessary for haploinsufficient genes - in which both over and under-expression lead to loss of fitness. From this successful result, we hypothesized that this approach could be more broadly applied to other monogenic forms of ASD.
We thus targeted two additional high confidence genes, *ARID1B* and *KMT2C*, which lead to Coffin-Siris and Kleefstra syndromes, respectively. We established *ARID1B*$^{+/-}$ organoids using CRISPR-editing and observed that CRISPR-A + guideRNA treatment of organoids led to an increase in *ARID1B* expression and a correction of Wnt signaling levels, suggesting both gene expression and function were rescued. For *KMT2C*, we utilized a cohort of four patient iPSC lines, each with different truncating mutations, from different genders and ethnicities. This cohort gave us the opportunity to test the extent of CRISPR-A rescue on different backgrounds and mutations. Characterization of the organoids showed microcephaly-associated gene expression patterns, in line with clinical observations, as well as premature neuron differentiation. We found that there was no significant difference in the level of *KMT2C* activation resulting from CRISPR-A treatment across the different patient lines, suggesting that targeted therapies may be able to be used across a spectrum of mutations for a gene of interest. Ongoing work will further characterize *KMT2C*$^{+/-}$ organoids, measure phenotypic rescue, and determine whether we can develop improved rules for developing guides for enhancer targeting with CRISPR. Our results, now in multiple patient lines and several different mutations, provide a proof of principle for the broader application of gene activation as a therapeutic intervention in ASD.

PATIENT DERIVED MODEL OF UBA5-ASSOCIATED ENCEPHALOPATHY IDENTIFIES DEFECTS IN NEURODEVELOPMENT AND HIGHLIGHTS POTENTIAL THERAPIES

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UBA5 encodes for the E1 enzyme of the UFMylation cascade, which plays an essential role in ER homeostasis. The clinical phenotypes of UBA5-associated encephalopathy include developmental delays, epilepsy and intellectual disability. To date, there is no humanized neuronal model to study the cellular and molecular consequences of UBA5 pathogenic variants. We developed and characterized patient-derived cortical organoid cultures and identified defects in GABAergic interneuron development. We demonstrated aberrant neuronal firing and reduction in size of patient-derived organoids. Mechanistically, we show that ER homeostasis is perturbed along with exacerbated unfolded protein response pathway in cells and organoids expressing UBA5 pathogenic variants. We also assessed two gene expression modalities that augmented UBA5 expression to rescue aberrant molecular and cellular phenotypes. Our study provides a novel humanized model that allows further investigations of UBA5 variants in the brain and highlights novel approaches to alleviate cellular aberrations for this rare, developmental disorder.

MEASURING ACTIVITY IN HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL ORGANOIDS FOR DISEASE MODELING AND DRUG DISCOVERY

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Neural organoids have emerged as a powerful technology for modeling developmental processes, cell-cell interactions, cytoarchitecture, and disease mechanisms. However, the structural heterogeneity observed between organoids, as well as from a lack of protocol standardization, can complicate functional analysis. To address this challenge, we present standardized workflows for the generation and long-term culture of cerebral and spinal cord organoids. Additionally, we show how to induce disease-related phenotypes in these organoids and measure functional outputs using multielectrode arrays (MEAs). Cervical spinal cord organoids (SCOs) were generated using STEMdiff™ Spinal Cord Organoid Differentiation Kit from 8 different human pluripotent stem cell (hPSC) lines. In a single experiment, three 30-day-old SCOs were adhered to 48-well Axion™ MEA plates within 100% Matrigel® domes and cultured for 40 days in Organoid Maturation Medium or BrainPhys™ Neuronal Medium. These spinal cord organoids were cultured for an additional 40 days and treated with 10 µM MG-132, a compound known to induce aggregation of TAR DNA-binding protein 43 (TDP-43) and used for modeling amyotrophic lateral sclerosis (ALS). In a separate experiment, three 60-day-old hPSC-derived unregionalized cerebral organoids generated using STEMdiff™ Cerebral Organoid Differentiation Kit were plated onto 3D HD-MEA (4096 µpillars, ~90 µm high, size ~12x12 μm^2 – 3Brain), according to the manufacturer's instructions in BrainPhys™ Neuronal Medium + 10 mM glucose. Cerebral organoids were then treated with 100 µM 4-aminopyridine (4-AP) to induce an epileptic phenotype, with reversal of this phenotype modeled by subsequent treatment with 1 mM valproic acid, an anti-epileptic. SCOs cultured in BrainPhys™ Neuronal Medium activated twice as many electrodes and displayed networking bursting activity compared to SCOs cultured in Organoid Maturation Medium. Acute treatment of SCOs with MG-132 reduced the number of active electrodes and spikes to 0 and the weighted mean firing rate to 0 Hz. For cerebral organoids, acute treatment with 4-AP resulted in a 10 - 15-fold increase in the mean firing rate compared to the baseline (untreated) rate, resulting in an epileptic-like phenotype. Subsequent treatment with valproic acid resulted in a 3 - 5-fold reduction in the firing rate compared to the untreated baseline rate. These data suggest that neural organoids generated using STEMdiff™ organoid differentiation kits display robust organoid to organoid neural activity after long-term culture in either STEMdiff™ Neural Organoid Maintenance Kit or BrainPhys™ Neuronal Medium, providing a reliable platform for neuronal disease modeling and drug discovery.

INVESTIGATION OF REGION-SPECIFIC INTERACTIONS BETWEEN NEURAL PROGENITORS AND MICROGLIA DURING DEVELOPMENT

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Microglia are a unique cell type that has been extensively studied in the adult brain, due to their implication in neurodegenerative diseases and physiological brain homeostasis. Deriving from the yolk sac, microglia are among the first glial cell types to colonize the developing neuroepithelia, starting already after GW4.5 in humans, and preceding the peak of neurogenesis and neuronal migration in the developing telencephalon (Menassa & Gomez-Nicola, 2018). Previous studies have shown that during early telencephalic development, microglia heavily colonize the proliferative zones of the primate cortex (Penna et al., 2021).

Microglia exhibit one of the highest levels of regional heterogeneity in the brain, at the morphological, transcriptional and proteomic levels. However, very little is known about the communication between microglia and neural progenitors during development (Lilienberg et al., 2022).Thus, our study seeks to investigate whether microglia show similar heterogeneity during development, specifically during telencephalic development and explore the effects of developing microglia on neural progenitor pools in different regions of the developing neuroepithelia.

To achieve this, we utilize a model system where hematopoietic progenitor cells (HPCs), derived from induced pluripotent stem cells (iPSCs) are incorporated into patterned cerebral organoids of two different identities: dorsally patterned and ventrally patterned cerebral organoids. Subsequently, these HPCs are differentiated into microglia-like cells within the organoids, allowing us to investigate both direct and indirect interactions between microglia-like cells and the neuronal population in environments with different cellular composition. We will accomplish this by combining multiOMICS and imaging approaches. Through comparative analysis we aim to underline how the profile of microglia may change when situated in different neuronal environments. Furthermore, we aim to decipher the effects that microglia-like cells can mediate in these environments with different cellular identities, particularly in influencing neuronal differentiation and identity acquisition during early developmental stages.

SCALABLE AND GENETIC EDITABLE hPSC-DERIVED ASTROCYTES

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Astrocyte malfunction is found in many neurodegenerative and neuroinflammation diseases, including Alzheimer disease, Parkinson disease, Huntington disease and multiple sclerosis. Since mouse and human astrocytes are transcriptionally and functionally different, human pluripotent stem cell (hPSC)-derived astrocytes serve as a valuable tool for studying human neurological disorders and for target discovery and validation. CRISPR/Cas9 system is a powerful tool for genetic editing. We aim to combine these two tools to generate a scalable hPSC-derived astrocyte model equipped with CRISPR/Cas9 system, facilitating target identification and drug development. We explored three recently published astrocyte differentiation methods. We found that the cells express astrocyte markers and Cas9 and respond to neuroinflammatory stimuli. Interestingly, we observed some differences in the marker expression and functional properties of the astrocytes generated with these three methods.

RESCUING NEURONAL MATURATION IN OLIGODENDROCYTE-ENRICHED CORTICAL ORGANOIDS

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Introduction: Cortical organoids (COs) containing mature neurons and astrocytes exhibit spontaneous neuronal activity and provide an invaluable platform for mimicking cell-to-cell interactions in the human brain. Oligodendrocyte containing organoids (OCOs) have mature, myelinating oligodendrocytes (OLs), offering a valuable approach for understanding OL biology in a 3D human neural system. However, preliminary observations suggest that expansion of the oligodendrocyte progenitor cell (OPC) population in OCOs results in diminished neuronal maturation and activity. The aim of this study is to establish a reproducible protocol for the generation of OCOs populated with functionally mature neurons and OLs.
Methods: BrainPhys is a medium optimized for electrophysiological activity and neuronal maturation; long term culture in this "maturation-optimized" environment is a promising avenue for rescuing neuronal maturation in OCOs. COs and OCOs were generated from three induced pluripotent stem cell (iPSC) lines and divided into three conditions: one receiving BrainPhys prior to OL expansion, the second receiving BrainPhys after expansion, and the final remaining in standard basal medium. Using these three groups, our goal was to determine the conditions that will generate an OL population while preserving neuronal activity, and assess the impact of this enhanced neuronal activity on OL maturation.
Results: Immunohistochemical staining prior to OL expansion was performed to confirm the presence of ventricular zones and quantify the baseline OL population. Staining of OL lineage markers Sox10 and CC-1 at day 70 following OPC expansion confirms a preserved OL population in all groups. Functional neuronal assessment was performed with calcium imaging of GCaMP8s-labeled neurons. Calcium activity at day 110 suggests transitioning to BrainPhys prior to OL expansion generates OCOs with functional neurons. In contrast, exposure of OCOs to BrainPhys at day 70 following the expansion of OPCs resulted in diminished neuronal activity. Interestingly, OCOs exposed to BrainPhys prior to OPC expansion exhibit increased area relative to OCOs receiving BrainPhys post expansion or no BrainPhys.
Conclusion: These results demonstrate the importance of enhancing neuronal maturation through stage-specific exposure to BrainPhys to generate OCOs with functional neurons and mature OLs. Furthermore, we are validating the ability of this model to recapitulate known network abnormalities and cellular pathologies in neurodevelopmental disorders. Taken together, this study presents an increasingly comprehensive organoid capable of modeling neuronal activity, OL lineage, and neuron-OL crosstalk.

EXTRACELLULAR VESICLES IN ASD

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Exosomes are small extracellular vesicles that mediate intercellular signaling in the brain without requiring direct contact between cells. These vesicles are enriched with miRNAs and proteins. Current evidence for exosome signaling in the brain points toward their role in translational regulation, neurogenesis, synaptic plasticity, and neuroinflammation, all of which have been implicated in several neurodevelopmental diseases. However, changes in exosome signaling in mental disorders have yet to be explored. Exosomes have been isolated from nearly all mammalian cell types, including cells in the central nervous system such as neurons, astrocytes, oligodendrocytes, and microglia. Neurons and glia release exosomes in vitro and in vivo. Exosome-mediated intercellular signaling has been implicated in neurodegenerative diseases and neurodevelopmental disorders. More specifically, altered neuronal exosome signaling has been implicated in Rett syndrome, a form of Autism Spectrum Disorder (ASD). However, the role of exosome signaling in ASD and other mental disorders remains to be fully understood. We have recently explored whether exosome content and numbers are altered in ASD. We have isolated exosomes from healthy control and patient brain organoid cultures by differential ultracentrifugation and evaluated purified exosomes using transmission electron microscopy, western blotting for biochemical markers, and nanoparticle characterization system. In purified exosome fractions, we defined exosome proteomes, using Tandem mass tag [TMT] liquid chromatography [LC] mass spectrometry, and small RNA content, using next-generation sequencing. Comparison between control (8 distinct lines) and patient (8 distinct lines) groups indicated that hundreds of proteins and small RNAs, majority comprising miRNAs, were differentially expressed in ASD exosomes compared to control exosomes. The top function categories among the differentially expressed genes are linked to ribosome and translation regulation. Our studies have the potential to provide novel insights into the etiology of ASD. Identifying alterations in content of brain exosomes in ASD reveals underappreciated modifications in cellular communication during mental disease states.

A CURATED COMPENDIUM OF TRANSCRIPTOMIC DATA FOR THE EXPLORATION OF NEOCORTICAL DEVELOPMENT

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Vast quantities of multi-omic data have been produced to characterize the development and diversity of cell types in the cerebral cortex of humans and other mammals. To more fully harness the collective discovery potential of these data, we have assembled gene-level transcriptomic data from 188 published studies of neocortical development, including the transcriptomes of >33 million single-cells, extensive spatial transcriptomic experiments and RNA sequencing of sorted cells and bulk tissues: nemoanalytics.org/neocortex. Applying joint matrix decomposition to mouse, macaque and human data in this collection, we defined transcriptome dynamics that are conserved across mammalian neurogenesis and which elucidate the evolution of outer, or basal, radial glial cells. Decomposition of adult human neocortical data identified layer-specific signatures in mature neurons and, in combination with transfer learning methods in NeMO Analytics, enabled the charting of their early developmental emergence and protracted maturation across years of postnatal life. Interrogation of data from cerebral organoids demonstrated that while many molecular elements of in vivo development are recapitulated in vitro, specific transcriptomic programs in neuronal maturation are absent. We invite computational biologists and cell biologists without coding expertise to use NeMO Analytics and to fuel it with emerging data.

MODELLING ADA-SCID NEUROPATHOLOGY WITH HUMAN FOREBRAIN ORGANOIDS

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Adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID) is a rare autosomal recessive disorder caused by the loss of ADA, an enzyme which catalyses the hydrolytic deamination of adenosine and deoxyadenosine. ADA-SCID has been widely studied for the impairment in both function and differentiation of immune cells, leading to a fatal outcome in early childhood due to the incapability of facing infections. In addition to a severe immunodeficiency, ADA-SCID patients develop neurological deficits, including reduced verbal expression, learning disability, attention deficits, structural abnormalities and hearing loss. Despite the immunological correction of hematopoietic stem and progenitor cells by targeted *ex vivo* gene therapy or the use of enzyme replacement therapy, children affected by ADA-SCID continue to present these neurological and behavioural impairments throughout their lifetime after treatment.
Here, we aim to understand how ADA-deficiency leads to the manifestation of neuropsychiatric phenotypes by leveraging an *in vitro* organoid platform combined with the chemical perturbations of ADA expression and its downstream metabolites, the generation of ADA knock-out (KO) hPSCs, and the reprogramming of ADA-SCID patient iPSCs. We found that the chemical inhibition of ADA enzymatic activity, the supplementation of exogenous adenosine to the culturing media, and the genetic KO of ADA all lead to a growth impairment during forebrain organoid formation, suggesting that both ADA enzymatic dysfunction as well as a dysregulation of purinergic signalling impact key neurodevelopmental processes. During early neural precursor cell (NPC) development, mitochondrial metabolism is perturbed in ADA-KO forebrain organoids, with the downregulation of key genes responsible for aerobic glycolysis, suggesting a possible switch to mitochondrial oxidative phosphorylation. Transcriptional profiling over time of ADA-KO and ADA-SCID patient-derived forebrain organoids reveal a divergent patterning signature, with an upregulation of WNT and TGF-ß pathways. Further, analyses of organoid development at a cellular level by immunofluorescence staining demonstrate cell fate misspecification during organoid differentiation. Overall, this study represents a step forward to understand how ADA modulates forebrain neurogenesis and specifically NPC fate dynamics, which once affected in the context of ADA-SCID ultimately converge in neurological manifestations.

LATE BORN CGE DERIVED INTERNEURON PROGENITORS IN HUMAN BRAIN DEVELOPMENT AND DISEASE

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The human brain has undergone a tremendous expansion enabled by an amplified and diversified repertoire of progenitor cell types. Human brains have a higher proportion of interneurons versus excitatory neurons compared to mice. In addition, human interneurons generate specific interneuron-interneuron networks (Loomba et al., 2022). Interneuron progenitors are also involved in the characteristic protracted development of the human brain, as streams of newly born interneurons migrate into the human cortex in the perinatal and postnatal period (Paredes et al., 2016).

We have previously demonstrated that interneuron progenitors play an instructive role in the development of Tuberous sclerosis (TSC), a neurodevelopmental disorder (Eichmüller et al., 2022). TSC is characterized by morphological alterations in the developing brain including subependymal giant cell astrocytomas (SEGAs), a type of benign tumor as well as cortical tubers, disorganized areas in the cortex that include dysmorphic and enlarged cells. TSC is caused by heterozygous mutations in TSC1 or TSC2, inhibitors of the mTOR signaling pathway.
We found that heterozygous TSC2 mutations in a specific set of interneuron progenitors resulted in amplification of these cells and development of TSC brain pathology including tumors and tubers. We termed these cells caudal late interneuron prognitors (CLIP) as they are derived from the caudal ganglionic eminence (CGE) and proliferate during mid gestation.

Following up on these results we are now analyzing the role of late born interneurons in the neurophysiological changes that cause epileptic phenotypes in TSC patients. In addition, we are exploring the function of CLIP cells more broadly in health and disease focusing on their role during normal brain development and their potential contribution to pediatric brain tumors.

A PLATFORM FOR REPRODUCIBLE AND SCALABLE BRAIN ORGANOIDS TO MODEL NEURODEVELOPMENTAL DISORDERS

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The development of the human cerebral cortex and the generation of distinct neuronal cell populations is a highly intricate process. In the last decade, in vitro models such as cerebral organoids have become a standard tool for modelling human neurodevelopment and associated diseases. However, they also have limitations, including limited reproducibility in terms of size and morphology, as well as low scalability, which makes them challenging to use in high-throughput settings.

To address these limitations, we here use micropatterned organoids-on-a-chip as an alternative approach. Human embryonic stem cells are confined to defined shapes, allowing cells to attach and grow in a 3D-like manner in a highly controllable way, such that they generate organoids with a more uniform morphology characterized by one single rosette in a highly scalable setting. Using immunofluorescence and single-cell RNA sequencing, we have observed the presence of distinct cellular populations at different development stages, demonstrating the ability of this model to recapitulate important aspects of early human brain development.

We have applied this system to investigate a severe neurodevelopmental disorder caused by mutations in DYRK1A, a critical gene involved in neuronal proliferation and differentiation. DYRK1A syndrome is characterized by microcephaly and is associated with autism spectrum disorder (ASD). To recapitulate the disease condition, we have generated an isogenic cell line carrying a heterozygous nonsense mutation observed in patients. The DYRK1A-mutated organoids showed an increased number of intermediate progenitors and a reduction in deeper- and upper-layer neurons, recapitulating important aspects of the disease phenotype observed in patients.

Overall, our micropatterned organoids represent a scalable tool to study human brain development and disorders such as ASD. Their high reproducibility and scalability allow for the precise analysis of early pathogenic behaviors of cells with high statistical significance, which can help to unravel disease mechanisms and could lead to fast development of therapeutic avenues.

A THREE-DIMENSIONAL SPINAL CORD MODEL FROM PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS TO EXPLORE THE THERAPEUTIC AND MOLECULAR MECHANISMS OF RISDIPLAM-LIKE COMPOUND IN SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a severe neurological disorder characterized by the premature degeneration of lower motor neurons and resultant denervation, muscle atrophy and weakness. Establishing human models is crucial for enhancing our knowledge about this disorder and identifying novel therapeutic approaches. In our research, we generated and phenotypically assessed human spinal cord organoids from induced pluripotent stem cells (iPSCs) of SMA type I subjects (n=3) and of one healthy control (n=1). Our study aimed at better understanding the molecular features of the disease in 3D model and the consequences of treatment with Risdiplam-like compound (RIS-L) whose main action is restoring SMN protein level. We observed that SMA presents a pervasive cellular and molecular developmental alteration in multiple cell populations, beyond motor neurons. This was ascertained using bulk transcriptomics, single cells RNA sequencing and multi-electrodes array analysis, along with immunophenotypic characterization. Our preliminary results on treatment demonstrated that RIS-L modulates at least 15% of disease affected genes, long-term in vitro treatment is well-tolerated, the ratio between full length SMN2 and Δ7 is robustly restored, pathological hallmarks are reverted. Interestingly, the molecular analysis revealed alterations in the expression levels of genes associated with the primary cilium organelle network, known to play a crucial role in nervous system development. Some of these genes were found to be downregulated in SMA and modulated upon RIS-L treatment, suggesting the compound may impact cilia-related processes in SMA organoids. At the functional level, we also evaluated the neurophysiological profile of CTRL. SMA and RIS-L treated spinal cord organoids with high density multielectrode array (HD-MEA) technology. We assessed their basal electrophysiological properties and their response to chemical modulation.
Optimizing RIS-L for all SMA patients is a key aspect in clinical perspective and can be achieved by understanding its molecular and functional effects. Our investigation contributes to the identification of possible targets for complementary treatment intervention.

TRACING THE ORIGINS OF INTERNEURON DEFECTS IN FCDII: DIFFERENTIAL EFFECTS OF *DEPDC5* MUTATIONS IN DORSAL AND VENTRAL FOREBRAIN LINEAGES

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Focal cortical dysplasia type II (FCDII) is a major cause of drug-resistant epilepsy and the most common brain malformation detected in children undergoing epilepsy surgery. Characterized by focal cortical dyslamination and cytomegalic cells, FCDII has recently been linked to somatic mutations in mTOR pathway-related genes. These postzygotic somatic mutations are present in only a small percentage of cells. While traditionally thought to arise in dorsal telencephalic progenitors, recent findings reveal that mutated cells are found in different lineages in the brain, including interneurons.

This project investigates the cellular origins of interneuron defects in FCDII, specifically examining whether these abnormalities stem from mutant dorsal telencephalic progenitors through non-cell autonomous effects or require mutations within the ventral interneuron lineage. Using cortical and subpallial organoids along with forebrain assembloids carrying mutations in *DEPDC5* (a gene frequently mutated in FCDII patients), we examine mTOR pathway activity through its downstream target, phosphorylated S6 ribosomal protein (pS6). Our findings reveal elevated pS6 levels in both cortical and subpallial organoids in $DEPDC5^{-/-}$ cells compared to $DEPDC5^{+/-}$ and $DEPDC5^{+/+}$ cells. Tracking interneuron migration in DEPDC5 forebrain assembloids shows $DEPDC5^{-/-}$ interneurons moving at increased velocities in the dorsal region compared to $DEPDC5^{+/-}$ interneurons. Ongoing studies reveal altered migration and calcium transient patterns in DEPDC5 forebrain assembloids with $DEPDC5^{-/-}$ ventral telencephalic progenitors. This comprehensive approach aims to elucidate the cellular mechanisms underlying FCDII pathogenesis.

ROLE OF NR2F1 IN DOPAMINERGIC DIFFERENTIATION USING MIDBRAIN ORGANOIDS

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Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases affecting the elderly. While the number of patients is expected to grow in the next years due to the increasing number of aging individuals, we still do not understand the etiology of the disease enough to develop an effective cure. Most cases are idiopathic, but certain mutations are implicated in the development of the familial form of the pathology, such as the LRRK2-G2019S mutation. Previous research has tried to elucidate the role of the LRRK2-G2019S mutation in PD. However, it is still unknown how the mutated protein causes PD-relevant phenotypes. We recently showed that in midbrain dopaminergic neurons (mDA) derived from induced pluripotent stem cells (iPSCs) reprogrammed from PD patients carrying the mutation, the transcriptional regulator NR2F1 is downregulated. NR2F1 is involved in several aspects of neuronal differentiation during development including fate determination, migration, and neurogenesis. We observed that neuroepithelial stem cells (NESC) carrying the LRRK2-G2019S mutation showed accelerated differentiation into mDA followed by increased apoptosis. We identified NR2F1 as a main member of the core regulatory circuit of the NESC state. Furthermore, we showed that this LRRK2-G2019S-associated mDA phenotype and NR2F1 downregulation was also observed in midbrain organoids generated from LRRK2-G2019S patients. This suggests NR2F1 dysregulation might be a contributor to the observed PD phenotypes. To better understand the role of NR2F1 in midbrain development and PD pathology we used iPSC NR2F1 knock-out lines. We identified altered NESC differentiation potential and increased cell death suggesting a peculiar role of NR2F1 in dopaminergic neurons' function in different stages of development. This gives us a better understanding of the role of NR2F1 in midbrain development and offers a potential therapeutic candidate for restoring dopaminergic degeneration in PD.

DEVELOPING AN IMMUNOCOMPETENT *IN VITRO* 3D BRAIN MODEL OF ALZHEIMER'S DISEASE TO STUDY THE NEUROPROTECTIVE EFFECTS OF ESTROGEN

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Alzheimer's disease (AD) is a neurodegenerative disease, characterised by extracellular amyloid (Aβ) depositions and intracellular neurofibrillary Tau tangles (NFTs) that lead to progressive neuronal cell death and cognitive decline. Despite extensive research, the exact mechanisms behind AD remain elusive, owing partially to the fact that mouse models are almost exclusively used in AD research despite obvious species differences. Recently, three-dimensional (3D) cerebral organoid models derived from human induced transgenic pluripotent stem cells (iPSCs) have shown promise to recapitulate hallmarks of AD, and may be novel tools to better understand AD.

We have developed a cerebral organoid model of familial AD from iPSCs carrying knock-in of three mutations in amyloid precursor protein (APP) that elevate the total Aβ levels similarly to the established App^{NLGF} mouse model of AD. This new *in vitro* model of AD can be used to study the molecular underpinnings behind the development of amyloidosis and Tau pathology in a more human-relevant setting.

In this study, we are characterising the pathology and the neurodevelopmental differences between the human APP^{NLGF} and APP^{WT} organoids. Our preliminary observations suggest that the APP^{NLGF} organoids show an accelerated neuronal differentiation compared with their isogenic control. Importantly, the APP^{NLGF} forebrains exhibit altered APP processing, and successful development of the amyloid pathology, as it is reflected in the elevated levels of Aβ42 already from Day 45 of culture and phosphorylated tau from day 60. Noteworthy, these APP^{NLGF} models show prominent astrogliosis indicating AD-like pathology as it was previously mentioned.

To validate our findings, we will compare the transcriptional and cellular composition of the APP^{NLGF} brain organoids with brain samples of AD patients and the App^{NLGF} mouse model. Lastly, since estrogen and estrogen receptors are highly involved in brain development and glial regulation and have been proposed to contribute to the sex differences in AD, we will study the estrogenic neuroprotection in this model. Overall, this project can provide a more human-relevant experimental AD model and serve as a tool for studying the driving mechanisms behind familial AD pathology, offering advances in drug discovery and personalised modelling.

CSTB-DEPENDENT ALTERATIONS IN EXTRACELLULAR SIGNALING AND INTERNEURON SPECIFICATION IN PROGRESSIVE MYOCLONUS EPILEPSY TYPE I

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The extracellular space, encompassing the extracellular matrix, soluble molecules and extracellular vesicles (EVs), is critical for brain development. A clear example of its importance is observed during cortical development, where both excitatory projection neurons and inhibitory interneurons rely on cues from the extracellular environment to mature and reach their proper locations within the cerebral cortex. Disruptions within this extracellular space can lead to significant neuronal dysfunction, which may contribute to neurodevelopmental disorders. Progressive Myoclonus Epilepsy Type I (EPM1) is a significant and rare form of epilepsy, caused primarily by mutations in the CSTB gene.
Our previous studies using cerebral organoids (EPM1-CO) derived from somatic cells of EPM1 patients, have shown that CSTB is implicated in human cortical development and plays a role in extracellular signaling, cell proliferation, interneuron recruitment and synapse physiology.
Here we focused on ventrally patterned COs (vCO), which give rise to interneurons and hypothesized that altered signaling, due to low pathological CSTB protein levels, may lead to defects in cell fate, interneuron maturation, and function, as indicated by the altered electrophysiological properties. We demonstrated that EPM1-vCO exhibit altered patterning, leading to an impaired excitatory/inhibitory balance. Importantly, we propose that CSTB alters the extracellular environment by affecting the biogenesis and composition of EVs, particularly by modulating SHH levels within these vesicles. This altered SHH signaling via EVs is likely to be a critical factor in influencing neural differentiation and fate, thereby playing a significant role in the pathogenesis of EPM1.

INTERSPECIES ORGANOIDS REVEAL HUMAN-SPECIFIC MOLECULAR FEATURES OF DOPAMINERGIC NEURON DEVELOPMENT AND VULNERABILITY

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Midbrain dopaminergic (DA) neurons coordinate multiple aspects of cortical- and subcortical functions and regulate ancestral motor control, as well as recently evolved cognitive and social behaviors. Dysregulation and degeneration of these neurons have been implicated in various disorders that are unique or enriched in humans, including schizophrenia and Parkinson's disease (PD), but the origins of human-specific developmental trajectories and vulnerabilities remain poorly understood.
In this study, we established a phylogeny-in-a-dish approach, generating interspecies ventral midbrain cultures of human and chimpanzee cell lines, and performing combined single cell RNA and ATAC sequencing during midbrain progenitor maturation. By comparing homologous cell types across primate species, we identified conserved and human-specific gene regulatory networks influencing developmental trajectories of DA neurons and related floorplate cell types. To unmask genetic variation in regulatory elements and gene expression related to DA neuron vulnerability, we subjected midbrain organoids to rotenone-induced oxidative stress revealing conserved gene regulatory networks driving stress response and recovery as well as human-specific responses. Together, these results provide a comparative multiomic atlas of primate DA neuron differentiation and implicate candidate molecular pathways supporting DA neurons specializations in the enlarged human brain.

HIGH-RESOLUTION TEMPORAL MULTI-OMIC ATLAS OF CEREBRAL ORGANOID DEVELOPMENT

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Cerebral organoids (CBOs) are generated from pluripotent stem cells that undergo neuroectoderm specification and neuronal differentiation in three dimensions. The developing neurons in CBOs migrate and self-organize into cerebral cortex-like layers, mimicking human brain development. Developmental processes in CBOs occur according to intrinsic signaling mechanisms and gene regulatory networks, which require spatiotemporal regulation of protein expression and function. The latter can be achieved through post-translational modifications (PTMs), which can be added or removed from proteins, allowing cells to quickly adapt to their surroundings. Recent studies have elucidated regulatory mechanisms underlying cell fate specification in developing CBOs. However, to our knowledge, the understanding of protein dynamics, including the involvement of PTMs, in CBO development remains limited. Thus, we aim to provide a high-resolution temporal overview of protein and PTM dynamics in CBOs up to day 200. We have analyzed CBOs across 28 time points using a quantitative proteomic, PTMomic and metabolomic approach. This allowed us to identify and quantify more than 9,300 proteins and several PTMs important for cellular signaling and neuronal development (including phosphorylation, lysine acetylation, sialylated N-glycosylation, and cysteine modifications). We observed significant temporal changes in the abundance of proteins and dynamic PTMs, reflecting transitions from progenitor functions to neuronal maturation and glial development. Pathways related to neuronal differentiation, including axon guidance, showed temporal changes in both protein abundance and dynamic PTMs during organoid development. Conversely, proteins involved in calcium signaling were predominantly regulated by dynamic PTMs. We further show that proteins that localize to synapses have the highest relative expression around day 65-100 and correlate with the presence of neurotransmitters. We investigated the presence of glial cell markers and found these to be associated with metabolic changes at late time points. We compare the proteome of CBOs to that of human fetal brain tissue as well as to transcriptomic data from CBOs, and furthermore demonstrate that disease-related risk genes show dynamic temporal profiles on both the protein and PTM level.

QUANTITATIVE PROTEIN PHENOTYPING OF CORTICAL DEVELOPMENT IN TUBEROUS SCLEROSIS COMPLEX

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Tuberous Sclerosis Complex (TSC) is a genetic neurodevelopmental disorder caused by mutations in the *TSC1/TSC2* genes, resulting in upregulation of mammalian target of rapamycin (mTOR) signaling and unregulated cell growth. Within the brain, dysplastic cortical lesions called tubers emerge during fetal development. Although tuber growth appears to stop postnatally, repercussions do not. TSC patients suffer debilitating neurological sequelae from these tubers, including epilepsy, autism, and neuropsychiatric disorders. The molecular mechanisms underlying tuber development remain unclear.

In this study, we sought to understand changes in cellular identity during cortical development of *TSC2* mutant cells and their association with tuber formation. Using CRISPR-Cas9 technology, three isogenic lines of TSC patient-derived induced pluripotent stem cells (iPSCs) were generated with pathogenic mutations in the *TSC2* gene. iPSCs were differentiated into 2-D and 3-D neural lineages and collected at several timepoints for mass cytometry analysis. Quantitative assessment of differentiation reproducibility revealed minimal batch differences. Across isogenic lines, *TSC2* mutant neural progenitor cells presented a distinct protein phenotype as early as day 10 post-differentiation, including elevated SOX2 and YAP1 expression coinciding with mTORC1-specific phosphorylation events. Mass cytometry analysis of resected patient tubers showed similar abnormal cell signatures. These results indicate a broader level of dysregulation than previously reported in transcriptomic analyses. Functional neuronal data using multi-electrode arrays revealed elevated firing rates in both heterozygous and homozygous *TSC2* mutants, with highest rates in ventrally patterned organoids. Taken together, these results indicate that cell-specific, protein-level changes during cortical development may play a role in tuber formation and can be adequately modeled using human iPSCs.

YAP IS REQUIRED FOR PROPER NEUROEPITHELIA AND CORTICAL LAYER DEVELOPMENT

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Yes-associated Protein (YAP), a key effector of the Hippo signaling pathway, is vital in executing cell programs pertaining to proliferation, survival, maintaining stemness, and cellular morphogenesis. During early neural development, Yap is tightly involved in the development of neuroepithelium, particularly through control of cell cycle progression; expression levels of Yap are tied to advancement in the developmental program, and loss of Yap has been demonstrated to impair neural tube development, leading to birth defects like anencephaly and hydrocephaly. Examining the role of Yap in mouse models of corticogenesis is challenged by embryonic fatality prior to the initiation of neurogenesis; moreover, distinctions between developing murine and human cortices limit recapitulation. Previous studies have used fetal tissue and induced pluripotent stem cells to model early gastrulation and late neurogenesis; however, the specific role of Yap in early neurogenesis has yet to be divulged. Further, previous studies have relied on 2D cell culture of unicellular lines and have not recapitulated the heterogenous cell environment found in the developing embryo. Using isogenic human embryonic stem cells (hESCs) and an unguided cerebral development protocol (STEMCELL Technologies), we successfully generated control and Yap-/- Human Cerebral Organoids (hCOs). Yap-/- hCOs did not undergo increased apoptosis or cell death, but loss of Yap was associated with abolished neural progenitor cell (NPC) development and failure of neural rosette formation. Neural stem cell (SOX2+) and post-mitotic neurons (TUJ1+, MAP2+) were also significantly reduced in Yap-/- hCOs. Additionally, we observed distinct morphological changes in developing cortical regions of hCOs. Single-cell RNA-sequencing analysis revealed that Yap-/- hESCs organized into 3D cultures consisting of heterogenous mesodermal and endodermal cell types; consistent with literature, Yap-/- hESCs failed to develop into ectoderm-containing hCOs, instead presenting a diverse array of mesodermal and endodermal cells. Together, our data suggest that Yap is required for potentiating neuroectodermal development; our model reveals the downstream consequences of Yap-/- deletion and uncovers the non-neuronal fate of ectoderm-null embryoids. Future studies will utilize this model to drive ectodermal specification (via inhibition of SMAD family proteins) to produce guided neural organoids to examine the function of Yap specifically within ectoderm-committed progenitor cell populations.

DECIPHERING THE ROLE OF PDE4DIP IN NEURAL DEVELOPMENT AND 1Q21-ASSOCIATED DISORDERS

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During Central Nervous System (CNS) development in vertebrates, the neural tube, comprising neural progenitor cells (NPCs), serves as the primordium of the brain and spinal cord. Precise regulation of NPCs proliferation within the neural tube is crucial for proper CNS growth. Genetic mutations impacting NPCs proliferation often manifest as microcephaly, characterized by a diminished brain size. Despite extensive efforts to elucidate genes involved in CNS development, most instances of microcephaly remain genetically uncharacterized. The 1q21 neurodevelopmental disorder (NDD) presents a distinctive genetic signature- a 1q21 copy number alteration- associated strongly with both micro- and macrocephaly phenotypes and developmental delay. The 1q21 chromosomal region is extremely repetitive and enriched in human-specific genes. Some of these genes have been linked to the evolutionary expansion of the brain size. However, the list of affected genes remains elusive, and the function of most genes still unclear. Our study focuses on unravelling the pathogenic mechanisms underlying 1q21 copy number variations in NDDs and elucidating the role of one associated gene, PDE4DIP in CNS development. Preliminary findings suggest that PDE4DIP could be affected in patient-derived induced pluripotent stem cells (iPSCs) with 1q21 alterations. Furthermore, PDE4DIP encoding proteins localise to the centrosome in NPCs of the chick neural tube, suggesting potential isoform-specific functions in NPCs proliferation. In summary, our study sheds light on the role of PDE4DIP in CNS development and provides insights into the pathogenic mechanisms underlying 1q21 NDDs.

SELF-ASSEMBLING SYNUCLEIN INDUCES PARKINSONIAN PATHOLOGY *IN VITRO* AND *IN VIVO*

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It is challenging to model neurodegenerative diseases with organoids due to the difficulties in accurately recapitulating pathology in disease-relevant cells. For instance, one of the key pathological features of Parkinson's disease (PD) is the abnormal aggregation of alpha-synuclein (αSyn). It remains unclear when, where, and in which cell types αSyn aggregation starts and how it is transmitted between neurons. Inoculation of animal models with αSyn pre-formed fibrils (PFFs) induces aggregation of endogenous αSyn and recapitulates critical aspects of PD pathology. However, this method involves invasive surgeries and lacks cell-type specificity. The PFF model is further hampered in iPSC-derived neurons and organoids systems by limited cellular uptake of PFFs and penetration into organoids. In this study, we therefore aimed to develop a new method to induce temporally controlled, tunable, and cell-type-specific PD pathology that can easily be applied to animal models and cultured cells and organoids. First, we designed a library of self-assembling synucleins (SAS) consisting of different types of self-assembling peptides fused to αSyn. The constructs were designed with a Tet response element to allow precise doxycycline-inducible control of the level and duration of expression. *In vitro* screening identified several constructs that not only form αSyn aggregates but also induce substantial Ser129 phosphorylation (pS129), a hallmark of αSyn pathology. We further validated top hits in several modalities of neuronal culture, including murine primary neurons, and human iPSC-derived neurons and organoids. Our top three SAS constructs robustly induced αSyn aggregation and pS129-αSyn pathology in over 90% of neurons. Of these, one construct (SAS3) also induced secondary nucleation of endogenous αSyn, neuronal axon retraction and neuronal death. Finally, we tested if SAS3 could induce PD-like pathology *in vivo*. By packaging SAS3 into PHP.eB, an adeno-associated viral vector (AAV) engineered by our lab that efficiently transduces the central nervous system in mice, we achieved brain-wide delivery of our construct in C57BL/6J mice. After three weeks of doxycycline treatment, we observed αSyn aggregation and pS129-αSyn pathology across different brain regions, as well as decreased movement in an open field test. In summary, we have developed a genetically-encoded, doxycycline-regulated self-assembling synuclein system to efficiently induce temporal and tunable PD-relevant pathology both *in vitro* and *in vivo*. We expect this to be a valuable tool to study the initiation and spread of pathology in diverse models of PD.

BIOLOGIC POTENTIAL AND PLASTICITY OF VERY LONG-TERM BRAIN CORTICAL ORGANOIDS

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Human brain organoids offer a unique opportunity to explore developmental milestones, though our understanding of the complex molecular mechanisms involved in the long-term maintenance of human neural tissue remains limited. In this study, we profiled brain cortical organoids cultured for periods ranging from 180 to over 1,000 days using single-cell RNA sequencing, electron microscopy, and functional assays. Our analysis revealed various neuronal populations with distinct subclusters that gradually decreased in complexity over time, while astrocyte populations became increasingly prominent and refined.

We hypothesized that spontaneous activity and the establishment of functional synapses might play a crucial role in maintaining neuronal identity over time. To test this, we transferred younger organoids to culture conditions that promote synaptic maturation and evaluated morphology and synapse formation using electron microscopy. We observed an increase in synaptic markers and a rescue of specific neuronal populations, such as callosal projection neurons and corticofugal projection neurons. Additionally, we performed functional assays to confirm that the organoids were electrically active. Using extracellular single-unit recordings with a multielectrode array (MEA), we detected network bursts and action potentials that evolved throughout the developmental trajectory.

In parallel, we explored the potential of cortical progenitors after extended periods of culturing. We discovered populations of committed cortical progenitors that remained responsive to specific environmental triggers.

Our findings provide insight into the developmental capabilities of long-term organoid cultures exploring fundamental processes that might contribute to the maintenance of neuronal identity and progenitor plasticity.

MOLECULAR PREDICTION OF SINGLE PROGENITOR FATE IN THE DEVELOPING NEOCORTEX

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Neocortical apical progenitors (APs) generate a variety of cell types in a temporally regulated manner during development. Self-renewing divisions maintain the progenitor pool by generating new APs, while consumptive divisions generate intermediate progenitors (IPs) and neurons. As corticogenesis proceeds, APs progressively shift from self-renewing to consumptive divisions, with neurons being increasingly generated indirectly through IPs. However, whether past division types are predictive of future division types in single APs remains unknown. Addressing the natural history of AP divisions at the single-cell level is critical as several brain disorders can arise from a somatic mutation in single progenitors during development.

In this study, we used serial TrackerSeq barcode indexing to assess the sequence of division types in single APs during mouse corticogenesis. This revealed that IP-generating divisions bias single APs towards further IP generating divisions. Combining TrackerSeq with single cell RNA+ATAC Multiomic analysis, we identified two transcription factors that predict the occurrence of IP-generating divisions. Functional validation using CRISPR loss- and gain-of-function approaches revealed not only that AP division types can be molecularly modulated using these factors, but that this also determines postmitotic neuron fate, hence uncoupling the normal temporal sequence of neuron type generation during corticogenesis. Together, these findings reveal that past AP divisions types predicts future division types and the fate of their clonal neuronal progeny.

MOLECULAR AND CELLULAR FUNCTION OF GAI1 VARIANTS IN PEDIATRIC NEURODEVELOPMENTAL DISORDER

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Gαi1 is a heterotrimeric G protein involved in a variety of cellular processes. Pathogenic variants in Gαi1 have been implicated in neurodevelopmental disorders (NDD) and epilepsy. When bound to GDP, Gαi1 forms an inactive heterotrimer with Gβγ subunits. Upon activation, GDP is exchanged for GTP, the Gαi1 subunit dissociates from the Gβγ complex, and each G protein subunits (α and βγ) are now active and can act on downstream signaling pathways including cAMP, mTOR, and MAPK/ERK. To date, there is not specific humanized neuronal model or effective treatment available for Gαi1-related NDD. Understanding the impact of pathogenic variants on protein function (loss, gain, novel) as well as the downstream cellular consequences using humanized neuronal model is critical for designing targeted and precise therapies. We hypothesized that pathogenic variants in Gαi1 impair GTP binding and hydrolysis and disrupt downstream signaling pathways including cAMP levels. In this study, we investigated the effects of four pathogenic variants on Gαi1 function.
To study the effect of disease-related Gαi1 mutations on GTP binding, we produced purified wild-type Gαi1 and selected mutant proteins to measure GTPase activity using a fluorescent GTP analog, BODIPY-GTP. We found that four variants impaired GTP binding and hydrolysis. To investigate biochemical behavior of Gαi1 mutations with Gβγ interacting proteins, we performed TRUPATH BRET assay. Outstandingly, while wild-type Gαi1 dissociates from Gβγ subunits upon activation, the mutants remain bound, potentially disrupting cAMP and other signaling pathways. To assess the impact on downstream signaling, we examined adenylyl cyclase activity and cAMP levels using FRET assay (Epac-S-H187). Our results revealed that the Gαi1 mutants fail to inhibit adenylyl cyclase activity resulting in elevated cAMP levels. This suggests a potential mechanism for the pathogenesis of Gαi1-related NDD.

To further validate our findings and explore potential therapeutic strategies, we are developing and characterizing cortical brain organoids derived from patient-derived iPSC carrying Gαi1 mutations. These 3D models will provide a valuable platform for studying the cellular and molecular consequences of Gαi1 dysfunction in a human brain context. Our findings highlight the critical role of Gαi1 function in neurodevelopment and suggest that targeting the pathogenic variant of Gαi1 may hold promise for therapeutic intervention in Gαi1-related NDD.

CELL TYPE SPECIFIC EFFECTS OF *PHOSPHOLIPASE D3* (*PLD3*) ON VASCULAR SIGNALING NETWORKS IN THE CONTEXT OF ALZHEIMER'S DISEASE

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Late Onset Alzheimer's Disease (LOAD) is a complex neurodegenerative disorder stemming from interactions between genes and the environment. Single-nucleotide polymorphisms (SNPs) have been identified through genome-wide association studies (GWAS) that modify the development and progression of LOAD, including low frequency polymorphisms, which contribute to risk. Phospholipase D3 (PLD3), an atypical phospholipase, has a rare variant, p.A442A, found in a previous study to double the risk of LOAD. Studies have also demonstrated that PLD3 plays a role in Amyloid Precursor Protein (APP) processing and Aβ development and yet the mechanism in which PLD3 contributes to disease remains unknown. Interestingly, we have found that the *APP/PSEN1*x*Pld3*^{KO} mouse model demonstrated strong changes in brain vasculature. This data motivated us to test the *PLD3* p.A442A loss-of-function variant along with *PLD3* knockdown (KD) in our human induced pluripotent stem cell (iPSC) 2D and 3D vascular and neurovascular models. Using an isogenic pair (*PLD3* p.A442A vs its CRISPR corrected control) or *PLD3* shRNA knockdown compared to a scrambled control, we have identified transcriptomic changes in both iPSC-endothelial cells (ECs) and -mural cells (mixture of pericytes and smooth muscle cells). We have found each vascular population shows evidence of a heightened inflammatory state at rest and following inflammatory challenge. We also identified *PLD3* p.A442A ECs have altered metabolism and proliferation whereas *PLD3* p.A442A variant mural cells showed changes in the extracellular matrix and several pathways related to vessel structure and development. Additionally, preliminary evidence shows *PLD3* KD has reduced recovery following inflammatory challenge when analyzed by the real-time resistance readout electrical cell-substrate impedance sensing (ECIS). Ongoing work is employing our 3D vascular organoid to capture these critical EC-mural cell interactions, mix-and-match WT vs variant cell types to understand cell-type specific contributions and assess changes in amyloid vascular deposition. This 3D modeling is key to be able to unravel *PLD3*'s role in the vasculature and help understand its impact on the development of LOAD.

DECONSTRUCTING TUMOR-IMMUNE LANDSCAPE OF GBM USING ENGINEERED 3D ASSEMBLOIDS

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Glioblastomas are highly aggressive, treatment-resistant malignancies that lead to debilitating neurological sequelae and have a grave prognosis, with a median survival of less than 15 months. Glioma cells hijack the brain's native immune surveillance, primarily led by resident myeloid cells or microglia, to create an immunosuppressive tumor microenvironment (TME), making GBM extremely difficult to treat. These glioma cells subvert the anti-tumorigenic functions of microglia and reprogram them to create a TME that facilitates diffuse infiltration and disease progression. However, the mechanisms by which glioma cells reprogram microglia from anti-tumorigenic to pro-tumorigenic remain unclear, primarily due to (i) our inability to isolate human tumor-associated microglia; (ii) our inability to model the tumor-microglia interactions within the TME; and (iii) the lack of models that include or allow manipulation of microglia while reliably recapitulating the human disease. To overcome these critical challenges, we have developed a novel human induced pluripotent stem cell (hiPSC)-derived 3D assembloid model. This engineered model allows us to study the complex interactions between human glioma and microglia, as well as their crosstalk with other CNS cell types (e.g., neurons and astrocytes) within the TME. In this model, hiPSC-derived microglia (iMG), astrocytes (iAstro), and neurons (iN) are combined to generate "mini-brains," which are co-cultured with patient-derived GBM tumor spheroids to create 3D assembloids. Using multi-omic RNA sequencing and proteomic approaches, we demonstrate that 3D assembloids can reliably recapitulate the tumor-immune interactions within GBM and create an immunosuppressive TME. Furthermore, glioma cells recruit microglia from mini brain to the TME via secreted cytokines. Additionally, the presence of GBM in 3D assembloids reprograms microglia from a "resting" state to a "reactive" state, as marked by morphological changes in microglia and alterations in the secreted cytokine profile. In conclusion, our novel 3D assembloid model provides a powerful tool to study the tumor-immune and tumor-neuro-immune axes in GBM and can be used to test effective therapeutic strategies targeting these interactions. The transformative potential of this work is not limited to brain tumors but can be broadly applied to investigate neuro-immune crosstalk in neurodevelopmental and neurodegenerative disorders.

EARLY DEVELOPMENTAL ORIGINS OF CORTICAL DISORDERS MODELED IN HUMAN NEURAL STEM CELLS

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The implications of the early phases of human telencephalic development, involving neural stem cells (NSCs), in the etiology of cortical disorders remain elusive. Here, we explored the expression dynamics of cortical and neuropsychiatric disorder-associated genes in datasets generated from human NSCs across telencephalic fate transitions in vitro and in vivo. We identified risk genes expressed in brain organizers and sequential gene regulatory networks across corticogenesis revealing disease-specific critical phases, when NSCs are more vulnerable to gene dysfunctions, and converging signaling across multiple diseases. Moreover, we simulated the impact of risk transcription factor (TF) depletions on different neural cell types spanning the developing human neocortex and observed a spatiotemporal-dependent effect for each perturbation. Finally, single-cell transcriptomics of newly generated autism-affected patient-derived NSCs in vitro revealed recurrent alterations of TFs orchestrating brain patterning and NSC lineage commitment. This work opens new perspectives to explore human brain dysfunctions at the early phases of development.

CORTICAL GRAFTS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS INTEGRATE SPECIFICALLY INTO THE MOTOR CORTEX OF MICE AND IMPROVE FORELIMB MOTOR MOVEMENT.

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Traumatic brain injury often results in a permanent loss of neuronal tissue that causes long-term defects in cognitive and motor abilities. A study done by the Center for Disease Control looking at 5-year outcomes of patients who received inpatient treatment found that 74% of patients had not seen any improvements likely due to the permanent loss of neuronal tissues, specifically the loss of neurons. This project aims to develop an in-vitro cortical graft, which can be transplanted into the brain to establish a de novo neural network, potentially compensating for neuronal loss associated with TBI. Cortical grafts will be engineered from induced pluripotent stem cells using a guided 3D cerebral organoid protocol. These grafts replicate cortical development with glutamatergic and GABAergic neurons, neural progenitor cells, and glial cells. The grafts were grown for at least 2 months and then infected with an adeno-associated virus (AAV) that would express green fluorescent protein (GFP) under the human synapsin (hSYN) promotor. One week following the infection, the grafts were transplanted into NOD-SCID mice. All animal work was carried in accordance with Texas Woman's University Institutional Animal Care and Use Committee's standards. Male mice were randomly assigned to one of three groups: Sham, TBI, and TBI + transplant. The TBI + transplant group received a controlled cortical impact injury in the left motor cortex, followed by excision of the necrotic tissue and transplantation of the graft into the injury site. The TBI group underwent the same procedure but without graft transplantation, while the Sham group only received a craniotomy without injury or graft placement. Following surgery, all mice underwent a modified neuro severity score test for 7 days then once weekly for the remainder of the study. To test specific forelimb motor ability, a water drop test was performed at the same time points previously described. 28 days after surgery the mice were perfused and the brains were collected for histological analysis. We observed significant right forelimb motor ability recovery fourteen days following surgery when comparing TBI + transplant and TBI groups. We observed no statistical difference in left forelimb motor ability among all groups. Using serial histological analysis, we identified efferent projections from the graft to local and distant brain regions. Migratory cells from our graft were also found. This research lays the groundwork for understanding synaptic integration from cortical grafts and specific cell types that promote recovery following injury. Furthermore, it introduces a transformative approach to treating traumatic brain injury by restoring neural connectivity and function.

iPSC-DERIVED CORTICAL BRAIN ORGANOIDS OF PACS1 SYNDROME REVEAL A DYSREGULATION AT THE PROTEOMIC LEVEL IN SYNAPSE ORGANIZATION AND NEURON PROJECTION DEVELOPMENT DURING MATURATION

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PACS1 syndrome is characterized by intellectual disability and distinct craniofacial abnormalities resulting from a de novo p.R203W variant in Phosphofurin Acidic Cluster Sorting protein 1 (PACS1). The role of PACS1 during human neurodevelopment and the impact of the p.R203W variant is still poorly understood yet few therapeutic options exist for patients. Previous transcriptomic analysis in brain organoids revealed a dysregulation in neurogenesis and cell projection organization in immature PACS1 p.R203W neurons, while mature glutamatergic neurons exhibited an upregulation in synaptic signaling processes when compared to controls. In this study, we determined how the regulatory landscape at the proteomic level is dysregulated over brain organoid maturation using tandem mass tag mass-spectrometry. Organoids were collected after 21 days of differentiation, when mostly neural precursors and proliferating cells are present, at day 40, when a population of intermediate progenitors and newly born neurons appear, and at day 90 when the organoids are mainly composed by post-mitotic neurons. Time series analysis showed that brain organoids displayed a proteome which better represented the dynamics observed in earlier fetal brain development. Five major protein abundance trends were detected with temporal differences between genotypes concentrated in the transition from maturation day 40 to day 90. Organoids carrying the PACS1 p.R203W pathogenic variant presented with dysregulated processes when compared to control organoids; upregulated processes were associated with neuron differentiation, neurite projection development and axo-dendritic transport while downregulated include RNA splicing and translation at the synapse. Moreover, proteins involved in synaptic vesicle recycling, transport/localization, ER stress, protein maturation/stability, and dysregulation of kinase activity showed a loss of temporal regulation, remaining stable during maturation only in PACS1 p.R203W but not control organoids. Despite the low overlap between gene identities when comparing proteomic and transcriptomic data, the dysregulated processes were shared, indicating that multiple regulation mechanisms occur at the protein and RNA level. Our data highlights the need of applying both transcriptomic and proteomic approaches to better capture the multiplicity of regulation levels at which NDD-risk genes might be affecting brain development.

THE INTERACTION BETWEEN GENETICS AND HORMONES IN AUTISM USING BRAIN ORGANOIDS

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Genetic risk for Autism Spectrum Disorder (ASD) is associated with hundreds of genes related to a wide variety of biological functions, including the epigenetic control of gene expression and the regulation of synaptic activity. The phenotypic manifestation of ASD varies among individuals, and the alterations caused by the partial loss of function of ASD-risk genes during neurodevelopment remains still obscure. Strikingly, a variable penetrance is observed, with more XY than XX individuals affected in a 4 to 1 ratio. The reasons for these sex-biased differences in the phenotypic manifestation of ASD are still unknown.

Work from our laboratory reveals that sex steroids, namely androgens, increase the proliferation of cortical neural progenitors. At the mechanistic level, transcriptomic analysis shows an effect of androgens on chromatin remodelling and epigenetics, altogether leading to an increase in excitatory neurogenesis during development.

In this work, we made use of gene editing in human cerebral organoids as an experimental platform to interrogate the interaction of ASD-risk genes with androgens. We show mechanistic data in brain organoids during incipient stages concomitant with the prenatal testosterone surge, and the resulting phenotypes in more mature stages modelled here by cortical organoids cultured in the air-liquid interface (ALI-COs) for several months of neuronal maturation.

EFFECTS OF PRENATAL NICOTINE EXPOSURE ON THE MOUSE BRAIN

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Nicotine consumption has many detrimental health effects and is a leading cause of avoidable deaths worldwide. While the incidence for tobacco use has been steadily declining over the last decades, other products containing nicotine, such as vaping liquids or chewable pouches, have become increasingly popular. Additionally, vaping has been advertised as less harmful than smoking and as a potential smoking cessation tool, despite the actual nicotine dose is similar or even increased. Nicotine binds with high affinity to and activates the ionotropic pentameric nicotinic acetylcholine receptors (nAChRs), which are present in the body from an early point in development. Dysregulated transmission of acetylcholine has been described in several models of obsessive-compulsive behaviors (OCD) like Tourette's syndrome, attention-deficit hyperactivity syndrome, or autism spectrum disorder (ASD). Moreover, prenatal nicotine exposure seems to be a risk factor for OCD, ASD, and intellectual disability.
To assess the effects of prenatal nicotine exposure on the brain, we exposed pregnant dams to either vehicle (2% saccharin in drinking water) or nicotine (200 µg/ml and 2% saccharin in drinking water) during gestation. Afterwards, we collected brains from the pups and parents at different postnatal time points: postnatal day (P) 6, P12, P21, adult. and extracted RNA from either an entire hemisphere or the cortical and subcortical areas (hemisphere minus hindbrain, cerebellum, and midbrain). The RNA levels in these samples were quantified using the Neuropathology panel of the Nanostring platform. During post-hoc analysis, we found that prenatal nicotine effects were transient and independent of sex. At P6, we found the largest number of differentially expressed genes. At later timepoints, effects sizes decreased, and adult brains were indistinguishable as to their RNA expression patterns.
We conclude that the brain rapidly adjusts to prenatal nicotine exposure during postnatal development on the brain level.

UPTAKE, PROPAGATION, AND CLEARANCE OF INFECTIOUS PRION AGGREGATES IN BRAIN ORGANOIDS.

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Prion diseases, such as bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in cervids, and Creutzfeldt–Jakob Disease (CJD) in humans, are aggressive, fatal, incurable neurodegenerative diseases. Misfolding of the cellular prion protein (PrPc), results in the formation of self-replicating pathological protease resistant protein aggregates (prions or PrPD). The accumulation of these PrPD aggregates is followed by neuronal loss, spongiform degeneration, and astrogliosis ultimately leading to death.

Until the recent development of a cerebral organoid (CO) model for human prion disease, in vitro models for studying human prion diseases have been limited. COs are three-dimensional self-organizing cultures of cerebral brain tissue generated from induced pluripotent stem cells. COs exposed infectious CJD prions become infected and display subtype specific hallmarks of disease.

COs also provide a platform for investigating zoonotic potential of prions. Consumption of BSE contaminated beef resulted in the emergence of human variant Creutzfeldt-Jakob disease. This raises the question of whether CWD could similarly infect humans. Our initial studies using COs suggest that cross-species transmission of CWD is unlikely. However, we discovered that the exposed COs were still able to take up and retain the PrPD for at least 180 days post infection. The finding that the inocula can persist for long periods in the COs provides us the opportunity to investigate how brain tissue might take up and spread the infectious protein throughout the tissue.

Here we used wild-type or PrPc-knockout COs, the latter of which are not susceptible to prion infection, to investigate these questions. We found that CJD aggregates get internalized independent of PrPc expression, and that this internalization was closely associated with astrocytes. We further looked at the influence of exposure doses and times on the level of inocula retained in the COs compared to the amount of PrPD replication.

These findings will help to optimize our CO model by allowing us to minimize the confounding influence of residual inocula while still allowing for a robust infection. More importantly, our study will also shed light on the mechanisms by which PrPD aggregates are taken into the tissue and distributed by cells to propagate prion infection throughout the brain.

MODELING BLOOD-BRAIN BARRIER FORMATION IN HUMAN PSC-DERIVED ORGANOIDS

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The human blood-brain barrier (hBBB) serves as a highly specialized structure regulating passage between the blood and central nervous system (CNS) compartments. Despite its pivotal physiological role, the lack of reliable in vitro models capable of mimicking hBBB development and function has been a significant challenge. In this study, we addressed this gap by constructing hBBB assembloids using brain and blood vessel organoids derived from human pluripotent stem cells. Through rigorous validation, we confirmed the acquisition of BBB-specific molecular, cellular, transcriptomic, and functional characteristics within these assembloids. Intriguingly, our investigation revealed an intricate neuro-vascular crosstalk with a discernible spatial pattern within hBBB assembloids. Utilizing patient-derived hBBB assembloids to model cerebral cavernous malformations (CCMs), we successfully recapitulated the anatomical features of cavernomas and the associated BBB breakdown observed in patients. Comparative analysis of phenotypes and transcriptomes between patient-derived hBBB assembloids and primary human cavernoma tissues unveiled CCM-related molecular and cellular alterations, offering insights into the underlying mechanisms of this disorder. Furthermore, we developed BBB assembloids derived from fragile X syndrome (FXS) patients, revealing disease-related brain vascular and BBB dysfunction induced by abnormal mTOR and Wnt signaling pathways. Finally, we validated BBB assembloids as a novel model for evaluating AAV virus transduction across the human BBB. In summary, our study presents hBBB assembloids that faithfully mimic core properties of the human BBB, providing a platform for investigating disease mechanisms such as CCMs and ASDs, and offering a new paradigm for testing therapeutic delivery across the human BBB.

IMPROVED MULTIMODAL MONITORING OF HUMAN CORTICAL ORGANOIDS TRANSPLANTED INTO RODENT CORTEX

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Patient-specific tissue models have the potential to dramatically improve our understanding of neurodevelopmental diseases, providing new insights into biomolecular mechanisms, and giving rise to better therapies. Human pluripotent stem cell (PSC) derived cortical organoids (hCOs) are three-dimensional neural cell aggregates that resemble the developing human cortex. They have emerged as an avenue to study pathological processes in a human-centric experimental context. Previous work by us and others demonstrated that implanting hCOs into rodent brain leads to xenograft vascularization and facilitates maturation, synaptic connectivity, and integration of human neurons into host neuronal networks. However, lack of standardized monitoring strategies remains a key challenge of this approach. Here, we demonstrate an improved strategy for multimodal monitoring of hCO xenografts. To probe xenograft function, we labeled human induced PSC-derived neurons with GCaMP6s or GCaMP8s via transduction with AAV before implantation. In combination with optically transparent graphene surface microelectrode arrays, we performed simultaneous electrophysiological recordings of local field potentials (LFPs) and 2-photon calcium imaging to probe both single cell and neuronal network dynamics in xenograft and surrounding host cortex. Additionally, we accessed connectivity between host and graft through LFP recordings during visual stimulation of the rodent contralateral eye. We also demonstrate the use of immunostaining to quantify cell composition and structural features within hCO xenografts using cell type-specific markers (NeuN, Sox9, and CC-1) in combination with human nuclear antigen (HNA) as well as markers of cortical lamination, including CTIP2 and SATB2. We implanted dorsally or ventrally derived hCOs and assessed phenotypic differences in function, structure, and composition. Our results demonstrate the usefulness of this multimodal monitoring system and confirm previous findings claiming high graft-to-graft variability, emphasizing the need for new methods to guide hCO integration and maturation in vivo. The data also highlights the shortcomings of AAV-based strategies to reliably label human neurons in hCO xenografts after transplantation.

DISSECTING THE ROLE OF HUMAN ELAV/HU PROTEINS IN NEUROGENESIS

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Accurate execution of transcription programs is essential to maintain cellular identity in all organisms. In animal neurons, the highly conserved ELAV/Hu protein family regulates alternative splicing and alternative polyadenylation, generating specific mRNA isoforms and defining the unique complexity of the neuronal transcriptome. In this study, we investigate the global impact of the ELAV/Hu proteins in alternative 3′end usage in human neuronal development. We identify mis-regulated transcripts in human iPSC-derived knock-out neurons and investigate molecular and cell state changes at the single cell level. In cultured HuC knock-out neurons, we observe defects in timely differentiation and increased cell death. These phenotypes were accompanied by changes in mRNA isoform expression; in particular, 3′end site usage was strongly affected in knockout 2D and 3D neuronal cultures. Our results show that the ELAV/Hu proteins are essential in the organization and proper regulation of the human neuronal transcriptome, which impacts the development, cell identity and function of the nervous system.

UNDERSTANDING THE DEVELOPMENTAL AND CELLULAR PHENOTYPES OF PATIENT ASSOCIATED NRXN1 DELETIONS USING HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED BRAIN ORGANOIDS.

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Copy number deletions in the neurexin-1 gene (NRXN1; 2p16.3del) pose a significant genetic risk for a wide range of neurodevelopmental disorders such as autism spectrum disorders (ASD), schizophrenia (SCZ), and learning disabilities. NRXN1 deletions in the population show variable penetrance and expressivity, thus resulting in vast phenotypic heterogeneity among NRXN1 deletion carriers. How these NRXN1 deletions from various genetic backgrounds impact developmental trajectories, cellular differentiation and maturation, and synaptic function, thus resulting in neurodevelopmental disorder phenotypes, remains unclear. Here, we investigated the molecular, cellular, and functional consequences of NRXN1 deletions on neurodevelopment using cortical brain organoids differentiated from human iPSC lines reprogrammed from ASD and SCZ patients who carry various NRXN1 deletions. Using single-cell transcriptomic analysis at 3.5 months, we elucidate differences in cell type-specific gene expression and developmental trajectories across disorders. Calcium imaging analysis shows differences in network connectivity in ASD versus SCZ-NRXN1 deletions, suggesting disorder-specific circuit outcomes. In conclusion, we predict that the cell type-specific differential gene expression profiles of these variable NRXN1 deletions that impact the maturation of neurons and astrocytes as well as the trajectory of neural progenitor cells will provide valuable insights into understanding the pathogenic mechanisms underlying NRXN1 deletion-mediated neurodevelopmental phenotypes in patients.

FUNCTIONAL ANALYSIS OF ACTIVE INGREDIENTS FOUND BY SCREENING IN ER STRESS-INDUCED BRAIN ORGANOIDS

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Stress is an inevitable after-effect of both intrinsic and extrinsic life processes. If left uncontrolled, it connects to the poor quality of life, specially towards later years of human lifespan when there is a decrease in efficiency of damage-repair processes. Stress management early-on is expected to decrease the disease burden and benefit the health care system. We generated an in vitro cell-based screening system to search compounds possessing protein de-aggregation potential. Genetically engineered amyloid β (Aβ; an established causative factor of Alzheimer's disease)-expressing plasmids encoding Aβ with strong aggregating characteristics were recruited. By three rounds of screening of ~80 compounds, we selected Fucoxanthin as a strong candidate possessing protein-de-aggregation potential. It is a popular carotenoid found in marine organisms and known for its antioxidant properties. We validated the effects of Fucoxanthin on various stress inducers such as heat shock, tunicamycin (ER stress), sodium arsenite (NaAsO2, heavy metal) and cellular senescence per se. It was seen to protect cells against heat, oxidative and protein aggregation stresses as supported by level of several molecular markers in treated Vs control cells. Cells treated with Fucoxanthin showed delay in cellular senescence as evidenced by decrease in the expression of p16, and accumulation of reactive oxygen species (ROS). Of note, Fucoxanthin-treated cerebral organoids representing in vitro brain model showed reduction in TM-induced ER stress. Taken together, the study suggested usefulness of Fucoxanthin for management of aging, stress and related brain pathologies.

BARCODED LINEAGE TRACING TO INVESTIGATE CEREBELLAR CELL FATE TRAJECTORIES IN HUMAN BRAIN ORGANOIDS

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The cerebellum has often been associated with motor control, but emerging evidence over the years points toward additional roles in other higher-order cognitive functions and emotions in humans. Understanding how the human cerebellum develops its unique cellular diversity is essential for understanding its broader functions beyond motor control and in the pathogenesis of neurodevelopmental disorders. Studies reveal differences in brain development between humans and mice, particularly in the expansion of progenitor zones. In humans, specifically, ventricular and rhombic lip progenitor regions grow more extensively, forming sub-ventricular zones populated by basal progenitors. These differences are crucial for understanding brain structure and function differences between species. Due to the difficulty of accessing the human brain during key developmental stages, alternative models are essential for study. While current rodent models do not fully represent human neuronal development, and 2D cultures lack in vivo cytoarchitecture and physiological relevance, 3D human brain organoids offer cellular complexity and biological relevance. Single-cell transcriptomics has allowed for highly resolved identifications of cell states within this system; however, uncovering the fate choices these cells take as they mature within this 3D culture system remains elusive. We have recently established a reproducible human induced pluripotent stem cell-derived (hiPSC) cerebellar organoid protocol to model cerebellar development, which generates all the major cell types of the developing cerebellar, including excitatory and inhibitory neurons as well as several glial subtypes. To study how lineage and cell fate choices are determined in cerebellar organoids, we engineered hiPSCs with a novel recording system termed “hypercascade”, in which a genomically integrated barcode array is progressively and stochastically mutated over time at a set of designed target sites by adenine base editors. Reconstructing lineage trees from these data will provide insights into the history of individual cerebellar cell progenitors by revealing their transcriptomic profiles. This approach will allow the elucidation of lineage alterations in both healthy and disrupted cerebellar development.

GENERATING MULTI-LINEAGE PERIPHERAL SOMATOSENSORY ORGANOIDS TO MODEL SENSORY DYSFUNCTION

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The peripheral nervous system (PNS) consists of complex neuronal networks that mediate normal sensation, movement, and organ function by relaying information between the body and the central nervous system. PNS disorders often result in debilitating somatosensory pathology such as peripheral neuropathies, chronic pain, and sensory ataxia. Recent studies have generated dorsal root ganglion (DRG)-like organoids, offering a potential to model more sophisticated aspects of human somatosensory development and pathogenesis *in vitro*, such as Friedrich's Ataxia and pain insensitivity. However, these protocols can be highly variable in yield, structure, and produce limited cellular diversity and maturation. Furthermore, they do not account for immune and other supportive cell interactions that are largely uncharacterized in early DRG neurodevelopment, yet increasingly associated with sensory pathogenesis. To more reliably model DRG development and disease, we established a new hPSC-derived peripheral somatoSENSORy organoid (PNS SENSOR) model that efficiently generates high somatosensory cell yields, including peripheral somatosensory neuron and glial subtypes as well as hPSC-derived macrophages, and uniform structural features reminiscent of human DRG, such as elaborate neurite projections. Preliminary electrophysiology results reveal that PNS SENSORs are functionally active and respond to evoked stimuli, and work is currently underway to further characterize their cellular and molecular features using established pipelines performed on other neural organoids in our lab, such as single-cell RNA sequencing, calcium imaging, tissue clearing, and immunological assays. Further, we have performed preliminary studies xenografting PNS SENSORs into endogenous rodent DRG, offering an unprecedented opportunity to examine more complex humanized somatosensory cell innervation, function, and DRG-spinal circuitry *in vivo*. Given limited donor tissue accessibility, there is a critical need to generate a more comprehensive human *in vitro* somatosensory model to examine the biological mechanisms underlying impaired sensory processing and signalling.

ABERRANT HIPPO SIGNALING DUE TO TAOK1 MUTATION LEADS TO BRAIN ORGANOID OVERGROWTH

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Mutations in the kinase encoding gene TAOK1 (Thousand and one amino acid kinase 1) cause a syndromic neurodevelopmental disorder (NDD) with developmental delay, macrocephaly and autism. Despite its association with brain development deficit, the precise function of TAOK1 in brain development and how its dysfunction leads to neuropathology remains unknown. To model TAOK1 dysfunction during early brain development, we generated human iPSC lines harboring a TAOK1 NDD and macrocephaly associated mutation, S111F that abolishes a key residue required for catalytic activity. Using iPSC derived 2D and 3D neuronal models in combination with discovery proteomic approaches, we sought to determine the biological bases of TAOK1 associated NDD. Dorsal forebrain progenitors derived from TAOK1 mutant stem cell exhibited significantly increased proliferation compared to isogenic controls. Cortical organoids generated from TAOK1 mutant iPS lines showed a dramatically expanded proliferative zone (Pax6+ive) with higher numbers of actively dividing progenitors (CyclinA2 and Ki67 +ive) compared to isogenic control organoids at both DIV70 and 90. To elucidate mechanisms that underlie increased proliferation in TAOK1 mutant organoids and macrocephaly in TAOK1 syndrome patients, we performed comparative phosphoproteomic analysis of control and TAOK1 mutants NPCs. Pathway analyses and phosphopeptide analyses revealed differential phosphorylation of YAP/TAZ proteins in TAOK1 mutant NPCs indicating aberrant Hippo signaling. Deficits in Hippo signaling were confirmed in TAOK1 S111F mutant neuronal progenitors by immunocytochemistry and western blot analyses. In summary, our findings reveal aberrant Hippo signaling as a likely and druggable driver of the TAOK1 related neurodevelopmental disorders.

NEURAL ACTIVITY READOUTS AND MATURATION OF TRANSPLANTED HUMAN FOREBRAIN ASSEMBLOIDS

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Stem cell-based models such as organoids and assembloids hold promise for investigating human neural development and disease. In vitro organoids grow in the absence of coordinated neural activity, limiting their development. While transplantation of cortical organoids into newborn rats leads to advanced human neuronal maturation and engagement of host circuitry, these grafted organoids generally lack GABAergic neurons. Leveraging transplantation and assembloid approaches, we integrated organoids containing human cortical glutamatergic and GABAergic neurons in the developing rat somatosensory cortex. These transplanted human forebrain assembloids (t-hFA) grow an order of magnitude over several months. Compared to stage-matched in vitro human GABAergic neurons, t-hFA GABAergic neurons are more mature at the transcriptomic, structural, and electrophysiological levels. This allows for advanced tissue-level electrophysiological readouts and a functional investigation into PCDH19 gene loss, which is associated with early-onset genetic epilepsy. Notably, mosaic loss of PCDH19 in t-hFA, but not t-hCO, revealed sensitivity to hyperthermia-induced seizures, a key symptom of PCDH19-related epilepsy. Taken together, transplantation of assembloids to create human microcircuits in living animals has the potential to facilitate pre-clinical studies of neurological and psychiatric disorders, including epilepsy-related conditions.

NEURON-ASTROCYTE-MICROGLIA TRIPLE-CULTURES: A FLEXIBLE AND SCALABLE MODEL TO STUDY MICROGLIA FUNCTIONS IN HEALTH AND DISEASE

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Microglia play a key role in neurodegenerative disease. Recently, large scale genetic association studies have identified many Alzheimer's disease genes highly expressed in microglia in the CNS, but tools for studying human microglia function in vitro have been limited. Historically, microglia have been studied in monocultures in vitro. This has been achieved through primary mouse cultures, and more recently, the directed differentiation of iPSCs. Unfortunately, microglia lose their identity very quickly in the absence of the brain environment. Therefore, in many in vitro assays, microglia reflect generic myeloid cell responses rather than unique microglia responses critical for understanding microglia function in the brain.

Here, we present a human in vitro model containing iPSC-derived neurons and microglia and fetal derived astrocytes to study microglia functions in a CNS-like environment. Neurons:microglia:astrocytes seeded in a 3:1:1 ratio with microglia added at the precursor state resulted in high microglial survival and integration into the neuron-astrocyte network. Flow cytometric analysis of key microglia surface markers showed a more homeostatic profile when compared to our standard monocultures, and image analysis of GFP-labeled microglia showed ramified morphology, similar to microglia observed in vivo. Using live cell imaging, we compared microglia motility in response to different CNS substrates. Like microglia in the brain, microglia in triple cultures show increased motility of their processes in the presence of CNS substrates. Like microglia monocultures, triple culture microglia take up pHrodo-labeled CNS substrates such as amyloid beta, myelin and synaptosomes, but show less cell body movements compared to microglia monocultures, mimicking the behavior observed in mouse brain.

Together, we present here a reproducible and scalable in vitro model to study diverse microglia functions in a more brain-like environment that allows for genetic manipulation using CRISPR or the use of patient derived iPSC lines and is easily accessible for evaluating drugs and antibody therapeutics.

DOMINANT MALIGNANT CLONES LEVERAGE LINEAGE RESTRICTED EPIGENOMIC PROGRAMS TO DRIVE EPENDYMOMA DEVELOPMENT

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ZFTA-RELA is the most recurrent genetic alteration seen in pediatric supratentorial ependymoma (EPN) and is sufficient to initiate tumors in mice. Despite ZFTA-RELA's potent oncogenic potential, ZFTA-RELA gene fusions are observed exclusively in childhood EPN, with tumors located distinctly in the supratentorial region of the central nervous system (CNS). We hypothesized that specific chromatin modules accessible during brain development would render distinct cell lineage programs at direct risk of transformation by ZFTA-RELA. To this end, we performed combined single cell ATAC and RNA-seq analysis (scMultiome) of the developing mouse forebrain as compared to ZR-driven mouse and human EPN. We demonstrate that specific developmental lineage programs present in radial glial cells and regulated by Plagl family transcription factors are at risk of neoplastic transformation. Binding of this chromatin network by ZFTA-RELA or other PLAGL family motif targeting fusion proteins leads to persistent chromatin accessibility at oncogenic loci and oncogene expression. Cross-species analysis of mouse and human EPN reveals significant cell type heterogeneity mirroring incomplete neurogenic and gliogenic differentiation, with a small percentage of cycling intermediate progenitor-like cells that establish a putative tumor cell hierarchy. In vivo lineage tracing studies reveal single neoplastic clones that aggressively dominate tumor growth and establish the entire EPN cellular hierarchy. These findings unravel developmental epigenomic states critical for fusion oncoprotein driven transformation and elucidate how these states continue to shape tumor progression.

NETWORK BIOLOGY AND HIGH-CONTENT CRISPR SCREENING IDENTIFIES REGULATORS OF NEURONAL MATURATION

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Neurodevelopmental disorders are a heterogeneous group of conditions characterized by early onset of developmental abnormalities in cognitive, motor, and social functions. Limited understanding of the underlying neurobiological mechanisms hampers the development of drugs that address the molecular root causes. While neurodevelopmental disorders have complex genetic basis and diverse clinical manifestations, emerging evidence suggests that shared molecular and cellular mechanisms contribute to their pathogenesis. We constructed a biological network of essentially all genes that are known or suspected to contribute to five groups of neurodevelopmental disorders (~3000 genes in total): autism spectrum disorder (ASD), intellectual disabilities (ID), attention-deficit hyperactivity disorder (ADHD), epilepsy and schizophrenia (SCZ). Using pooled CRISPR interference (CRISPRi) screens with FACS-based reporters of cell differentiation in human iPSC-derived neurons, we identified genes and pathways that perturb neuronal maturation and migration. The uncovered network of regulators consists of three major clusters: regulation of DNA damage response, chromatin remodeling, and transcription elongation. Further experiments are ongoing to validate and characterize the biological contribution of the identified regulators and their functional importance in neuronal maturation programs.

Keywords: neurodevelopmental disorders, NGN2 iPSCs, CRISPRi screens, FACS reporter screens, functional genomics, neuron, high-throughput biology, systems neurobiology

GENERATION OF NEURAL ORGANOIDS BY DIRECT CELL REPROGRAMMING TO MODEL HUNTINGTON'S DISEASE.

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Background: Neural organoids are valuable tools for modelling neurological diseases. However, current protocols utilising pluripotent stem cells are lengthy and have limited applicability to neurological diseases of aging due to cells undergoing DNA rejuvenation during reprogramming. Direct-to-induced neural precursor (iNP) cell reprogramming converts adult human fibroblasts into region-specific neural precursor cells and allows the maintenance of epigenetic and aging factors. Objectives: To demonstrate the ability for striatal organoids, created using direct-to-iNP cell reprogramming, to recapitulate Huntington's disease (HD) phenotypes. Methods: Induced neural precursors were directly reprogrammed from neurologically normal or HD patient-derived adult human fibroblasts by transient expression of *SOX2* and *PAX6* using chemically modified mRNA transfection. Organoids were generated by suspending iNPs in ultra-low attachment plates with gentle rotation and timed growth factor exposure. Striatal lineage markers were identified using qPCR and immunocytochemistry. The level of soluble and aggregated mutant huntingtin protein was quantified using the Mesoscale Discovery (MSD) and Single Molecule Counting (SMC) ELISA assays (Evotec SE), respectively. Results: Organoids progressively increased in size over 21 days, reaching a uniform size of approximately 1mm diameter. Direct-to-iNP striatal organoids were positive for the striatal lineage markers *CTIP2*, TUJ1 and DARPP32 at 7 and 14 days of differentiation, as confirmed by qPCR and ICC. Preliminary data demonstrated that mature HD-derived organoids expressed an average of 22.6 pmol/g of soluble mutant huntingtin protein and an average of 15 pmol/g aggregated mutant huntingtin by 14 days of differentiation. Presence of mutant huntingtin aggregates was also confirmed by immunocytochemistry. Discussion: This study demonstrates the ability to generate striatal organoids from neurologically normal and HD patients using direct-to-iNP cell reprogramming. Most importantly, we demonstrate for the first time the ability to generate HD-derived striatal organoids that exhibit soluble and aggregated mutant huntingtin protein within 14 days of differentiation. The reduced time to generate striatal organoids from iNPs, and the ability for direct-to-iNP reprogramming to maintain epigenetic and aging signatures advances current organoid technology and will facilitate future investigation into the pathology and potential treatment of HD.

ABERRANT NEURONAL CONNECTIVITY AND SYNAPTIC PROTEIN EXPRESSION IN A CORTICAL ORGANOID MODEL OF DOWN SYNDROME

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Introduction: Down syndrome (DS) is characterized by intellectual disability, developmental deficits, increased prevalence of seizures, and early-onset Alzheimer's disease (AD). These phenotypes have been, in part, attributed to aberrant neuronal network connectivity in DS individuals. Despite early findings from animal models of DS— which offer limited translational potential— and functional neuroimaging in human patients— which offer limited resolution—the precise mechanisms of this altered neuronal activity are still unknown. Here we assess the cellular, molecular, and functional components of this irregular connectivity using cortical organoids as a 3-D in-vitro DS model.
Methods: We performed an in-vitro neuronal network analysis of human euploid and trisomic iPSC-derived cortical organoids from three isogenic lines. Three-month-old organoids were transduced using an AAV encoding for the calcium sensor GCaMP8s under a synapsin promoter and, at day 120, imaged using confocal microscopy. Subsequent analysis assessed the frequency and synchrony of neurons in trisomic versus euploid organoids. Post-fixation histology assessing synaptic markers PSD-95 and synaptophysin, was then used to assess structure-function correlation.
Results: Calcium-imaging analysis in trisomic and euploid organoids revealed distinct firing profiles for each condition. We identified a significant increase in cell-to-cell correlation of calcium transients suggesting an increased synchrony within circuits. Immunohistochemical assessment demonstrated a higher PSD-95 puncta count in trisomic organoids compared to their euploid counterparts. No significant difference however, was detected when assessing synaptophysin puncta count. Additionally, trisomic neurons demonstrated a trend towards increased PSD-95 and synaptophysin colocalization.
Conclusions: Trisomic neurons exhibit distinct physiological profiles with altered functional connectivity and firing patterns, as well as increased synaptic marker counts. Taken together, this may suggest precocious synaptic maturation in trisomy. These results are in line with the increased neuronal synchrony associated with seizures in DS and early onset AD and recapitulate previous findings in human brains and mouse models. Altogether, we have demonstrated that our 3-D in-vitro DS model recapitulates DS-related neuropathology and can be utilized in future studies to identify mechanisms of early neuronal dysfunction.

HUMAN ASSEMBLOID MODEL OF THE ASCENDING NEURAL SENSORY PATHWAY

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The ascending somatosensory pathways convey crucial information about pain, touch, itch, and body part movement from peripheral organs to the central nervous system. Despite a significant need for effective therapeutics modulating pain and other somatosensory modalities, clinical translation remains challenging, which is likely related to species-specific features and the lack of in vitro models to directly probe and manipulate this polysynaptic pathway. Here, we established human ascending somatosensory assembloids (hASA)– a four-part assembloid completely generated from human pluripotent stem cells that integrates somatosensory, spinal, diencephalic, and cortical organoids to model the human ascending spinothalamic pathway. Transcriptomic profiling confirmed the presence of key cell types in this circuit. Rabies tracing and calcium imaging showed that sensory neurons connected with dorsal spinal cord projection neurons, which ascending axons further connected to thalamic neurons. Following noxious chemical stimulation, single neuron calcium imaging of intact hASA demonstrated coordinated response, while four-part concomitant extracellular recordings and calcium imaging revealed synchronized activity across the assembloid. Loss of the sodium channel SCN9A, which causes pain insensitivity in humans, disrupted synchrony across the four-part hASA. Taken together, these experiments demonstrate the ability to functionally assemble the essential components of the human sensory pathway. These findings could both accelerate our understanding of human sensory circuits and facilitate therapeutic development.
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GENERATION OF MELATONIN-PRODUCING HUMAN PINEAL GLAND ORGANOIDS

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The pineal gland plays a crucial role in regulating circadian rhythms through melatonin production by its resident cell type, pinealocytes. However, the study of human pineal gland biology has been limited by the inaccessibility of this tissue. To address this, we developed melatonin-producing pineal gland organoids (hPGOs) from human pluripotent stem cells. Single-cell RNA sequencing identified both mature and developing pinealocytes, with a transcriptomic profile closely resembling that of the in vivo pineal gland. These organoids exhibit key functional characteristics of pinealocytes, including the expression of adrenergic receptors. Upon receptor activation, we observed significant increases in melatonin production and calcium activity, replicating physiological responses observed in vivo. Additionally, we generated hPGOs from Angelman syndrome (AS) patient-derived induced pluripotent stem cells (iPSCs). Compared to control organoids, AS-derived hPGOs exhibited a marked reduction in pinealocyte numbers, alongside a drastic decrease in melatonin production. These findings suggest impaired developmental processes in AS-derived hPGOs, offering novel insights into potential disruptions in circadian regulation and pineal gland neurodevelopment in Angelman syndrome. Together, this study presents a physiologically relevant, all-human model system for the pineal gland, providing new opportunities to elucidate its development, function, and pathophysiology.

HUMAN ASTROCYTES ACCELERATE AND MAINTAIN SYNCHRONIZED NETWORK ACTIVITY ACROSS NEURAL ORGANOID ENSEMBLES

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It is well established that rodent astrocytes promote neural network maturation in the nervous system through a combination of pro-synaptogenic components and neuroprotective mechanisms. Do human astrocytes have similar capabilities in promoting human neural network activity? To address this question, we optimized and tested a human-specific model with bioengineered neural organoids composed of rapidly matured astrocytes and glutamatergic neurons from human pluripotent stem cells (hPSCs) (i.e., Asteroids, Cvetkovic et. al.). We found that Asteroids more rapidly exhibit organoid-wide synchronous burst activity compared to neuron-only organoids as measured by GCaMP-based calcium imaging and multi-electrode array (MEA) analysis. To determine which astrocyte-derived components underlie this effect, we tested candidates identified in RNAsequencing and proteomic screens of Asteroid-conditioned media. A subset of these candidates was sufficient to accelerate neural network activity of neuron-only organoids. Next, we tested whether astrocyte-mediated neuroprotection maintains network activity via improved viability. We found that Asteroids have improved viability in suboptimal media and are protective against neurotoxic treatments when compared to neuron-only organoids. Given improved network formation and maintenance, we tested if astrocytes enable generation of functionally interconnected ensembles of organoids. Ensembles of multiple interconnected Asteroids (i.e., Asteroid Belts) revealed synchronous burst activity within 24 hours with physical projections extending into neighboring tissue. Taken together, our work highlights the importance of astrocytic mechanisms in rapidly generating and maintaining human neural networks. The robust and rapid functional interconnectivity of neural networks delivers a new human in vitro experimental approach that is expected to extend to models of various distinct regional networks and disease context.

REFERENCES: Cvetkovic C, Patel R, Shetty A, Hogan MK, Anderson M, Basu N, Aghlara-Fotovat S, Ramesh S, Sardar D, Veiseh O, Ward ME, Deneen B, Horner PJ, Krencik R. Assessing Gq-GPCR-induced human astrocyte reactivity using bioengineered neural organoids.
J Cell Biol. 2022 Feb 9;221(4):e202107135. doi: 10.1083/jcb.202107135. PMID: 35139144
FUNDING: Research is supported by National Institute of Neurological Disorders and Stroke (R01NS129788).

OBSERVATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN EARLY NEURAL ORGANOID GENERATION

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Human-induced pluripotent stem cells (hiPSC) have been a game changer in studying the human brain. By providing physiological growth factors in a timely orchestrated manner, the hiPS have successfully been differentiated into numerous important neuron and supporting cell types that functionally and anatomically faithfully recapitulate their in vivo counterparts, including many neurons of the hypothalamus(1). Early cultures were limited to 2D models, which lacked tissue organization. Recently, an arcuate nucleus organoid (ARCO) model, derived from hiPSCs by other researchers, resulted in the development of all major neural progenitor cells of the ARC, closely resembling the cell composition found in human embryonic post-mortem tissue(2). We culture hiPSC to generate early neural organoids for neuropharmacology applications. I present here observations of hiPSC behaviour during the early stages of these cultures.

1. Merkle FT, Maroof A, Wataya T, Sasai Y, Studer L, Eggan K, et al. Generation of neuropeptidergic hypothalamic neurons from human pluripotent stem cells. Development. 2015 Feb 15;142(4):633–43.
2. Huang WK, Wong SZH, Pather SR, Nguyen PTT, Zhang F, Zhang DY, et al. Generation of hypothalamic arcuate organoids from human induced pluripotent stem cells. Cell Stem Cell. 2021 Sep;28(9):1657-1670.e10.

PATIENT DERIVED CORTICO-STRIATAL ASSEMBLOIDS FOR MODELING MITOCHONDRIAL DISEASE

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Defects in the mitochondrial respiratory chain (RC) underlie a spectrum of human conditions typically affecting the central nervous systems (CNS). One of the most severe manifestations of mitochondrial disease in children is Leigh syndrome (LS) that can affect 1/36,000 newborns. LS causes symmetric lesions in CNS and specifically in the striatum, leading to psychomotor regression. Among RC components, complex I (CI) is the most frequently affected in LS. Here, we focus on two mutations in the CI nuclear gene NDUFS4. We generated iPSCs from two LS patients carrying nonsense NDUFS4 mutations (c.316C>T and c.20C>G). Using CRISPR-Cas9, we introduced these two mutations into a control iPSC line. In all mutant iPSCs (patient- and CRISPR-derived), we observed a decrease in expression of the NDUFS4 protein and lower CI activity. We previously found that LS impairs the development of unguided cerebral organoids by disrupting neuro-morphogenesis. We now aim to address the region-specific CNS defects of LS by generating cortical (COR) and striatal (STR) brain organoids.

We confirmed that COR and STR organoids express mature cortical and striatal markers by day 70. Bulk RNA sequencing revealed an up-regulation of inflammatory markers in mutant COR and STR organoids compared to isogenic control organoids, with particular accumulation within STR organoids.

To investigate the connectivity between these different brain regions, we established COR-STRI assembloids. We added a synapsin reporter adeno-associated virus to COR from day 65-70 before co-culturing with STR. After 2 weeks of culture, we observed neural projections from COR to STR, and we are currently addressing potential differences in mutant organoids. We next plan to use high-density multi-electrode array (HD-MEA) to investigate the functionality of cortico-striatal assembloids, and to add iPSC-derived microglia to address the impact of neuroinflammation. Combining these various techniques, we hope to gain a deeper understanding of the striatal-specific defects and the overall neuronal pathology of LS to possibly identify innovative targets of interventions.

EFFECTS OF THE NR2E1 MUTATION ON CELLULAR AND MOLECULAR DETERMINANTS OF NEUROGENESIS

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Nuclear receptor subfamily 2, group E, member 1 (NR2E1) is a master regulator of neurogenesis, the process by which neural stem cells (NSCs) differentiate into mature neurons in the brain. Loss of Nr2e1 in mouse models leads to hippocampal hypoplasia and deficits in learning and memory. However, the role of NR2E1 in humans, particularly the impact of genetic variants, remains unclear, as do potential pathological implications. Recently, we identified several patients with undiagnosed neurological disorders carrying unique missense mutations in the NR2E1 coding sequence. These patients exhibit mild-to-moderate intellectual disability and behavioral issues, including aggression. To investigate causality, we focused on one mutation, Ile385Thr (I385T), located in NR2E1's ligand-binding domain. We hypothesized that the NR2E1 I385T mutation disrupts neurogenesis by impairing NR2E1 function, leading to cognitive deficits. Our in vitro biophysical assays showed that this mutation significantly reduced NR2E1's binding to its co-regulators, compromising its transcriptional activity. To assess if the mutation affects embryonic and adult neurogenesis, we generated NR2E1 I385T knock-in mouse model and human iPSC line using CRISPR/Cas9 gene-editing. Initial gene expression analysis revealed upregulation of the NR2E1 target genes Glial fibrillary acidic protein (Gfap) and Aldh1a1 in the hippocampus of homozygous mutant mice, suggesting increased astrogenesis in this model. We are now evaluating astrogenesis and neurogenesis in the mutant iPSC-derived neural progenitor cells and cortical organoids. This work may provide critical insights into the role of NR2E1 in human neurological diseases and could pave the way for therapeutic strategies targeting NR2E1 dysregulation in neurodegenerative disorders.

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS CARRYING SCHIZOPHRENIA-ASSOCIATED MUTATIONS IN *SETD1A* DISPLAY INCREASED EXCITABILITY *IN VITRO* AND *IN VIVO*

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Schizophrenia is a debilitating psychiatric disorder, affecting approximately 1% of the global population. Estimates of the heritability rate of schizophrenia range around 80%, suggesting a strong genetic component. Loss-of-function mutations in the *SETD1A* gene have been linked to both schizophrenia risk and other neurodevelopmental phenotypes. SETD1A is involved in chromatin remodeling through its function as a H3K4 methyltransferase, but how *SETD1A* loss-of-function mutations lead to altered brain function remains unclear. In order to gain insight into the functional consequences of loss of SETD1A, this work utilized isogenic human induced pluripotent stem cell (hiPSC) clones that carry heterozygous schizophrenia-associated mutations in *SETD1A* or the reference allele. Neural precursor cells were established from hiPSCs and differentiated *in vitro* towards a mixed neuron-astrocyte culture in 8 weeks. Whole-cell patch-clamp recordings of mature hiPSC-derived *SETD1A*$^{+/-}$ neurons *in vitro* revealed a decreased action potential (AP) threshold and half width, and an increased AP amplitude compared to the isogenic control. For *in vivo* differentiation, hiPSCs were fluorescently labelled and directed towards a neuronal fate through forced overexpression of *Ngn2*. Differentially labelled *SETD1A*$^{+/-}$ and *SETD1A*$^{+/+}$ *Ngn2*-neurons were mixed in a single cell suspension in a 1:1 ratio and subsequently xenografted into the brains of immunodeficient *Rag2*$^{-/-}$ pups (P1-3). Whole-cell patch-clamp slice recordings of labelled hiPSC-derived neurons in the mouse brain revealed a similar phenotype as was observed *in vitro*, a decreased AP threshold and half width combined with an increased AP amplitude in *SETD1A*$^{+/-}$ *Ngn2*-neurons. These preliminary results suggest that *SETD1A* loss-of-function mutations could induce increased excitability in hiPSC-derived neurons. Future work will attempt to elucidate and manipulate potential molecular mechanisms underlying this cellular phenotype.

MODELLING OF CHD2 DOSAGE DEPENDENT EFFECTS ON CORTICAL DEVELOPMENT UNCOVERS DYSREGULATION OF AUTOLYSOSOMAL FLUX AS A MECHANISM OF NEOTENY

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CHD2 is a chromatin remodeler critical for the regulation of gene expression. *De novo* mutations in its coding region cause a haploinsufficient neurodevelopmental condition (OMIM #602119) featuring epileptic encephalopathy, cognitive impairment and autism spectrum disorder. Recently, three unrelated patients were reported to carry different *de novo* deletions of CHASERR, a long noncoding RNA known to negatively regulate CHD2 expression, which result in upregulated CHD2 dosage and a severe neurodevelopmental disorder (NDD). Through a comparative paleogenomic analysis, we identified a noncoding single nucleotide variant (SNV) derived in the Sapiens lineage compared to Neandertals and Denisovans that falls in a regulatory region upstream of CHD2 and CHASERR. Computational analyses revealed that the SNV falls in an enhancer region and alters the affinity of an estrogen responsive element for transcription factor binding, likely rewiring CHD2 regulation. To recapitulate the dosage spectrum of CHD2, we generated through CRISPR/Cas9 a set of isogenic induced pluripotent stem cell (iPSC) lines harboring the ancestral SNV or a missense mutation in the coding sequence of CHD2. By comparing the expression levels of CHD2 across iPSCs of chimpanzees and bonobos alongside our informative human cohort, we show that it linearly decreases from chimpanzees to present-day humans down to patients, aligning with a gradual reduction in autophagy flux rate. In NDD and ancestralised induced neurons, we found an accumulation of autolyosomes displaying alterations of their pH and proteolytic activity, as well as mirroring differences in somatodendritic complexity and spontaneous neuronal activity. Then, to characterize the role of CHD2 dosage-dependent effects on human cortical development, we profiled cortical brain organoids at 25, 50 and 90 days of differentiation at bulk and single cell RNA-seq resolution. NDD organoids exhibit a broad transcriptional dysregulation across all timepoints that converge on neurodevelopmental programs and autism-related genes, while ancestralised organoids exhibit differential expression of genes related to somatodendritic complexity and cell cycle progression. Overall, our findings reveal alterations in the pace of neuronal development and maturation.

DEFINING THE FUNCTIONAL IMPACT OF GENOMIC VARIANTS ON GENE REGULATORY NETWORKS USING ANCESTRALLY DIVERSE HUMAN PLURIPOTENT STEM CELL VILLAGES FOR 3D BRAIN ORGANOID DIFFERENTIATION

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A fundamental question in biology is to understand how genetic variation affects genome function to influence phenotypes. Most genetic variants associated with human diseases are located within non-coding genomic regions and may affect genome function through modulating the activity of cis-regulatory elements that are selectively targeted by either transcriptional or post-transcriptional regulators. However, our knowledge about the impact of genomic variants on cell-type-specific gene expression programs and regulatory networks associated with human developmental dynamics is rather limited. Understanding the intricate molecular and cellular mechanisms underlying genetic variability demands unbiased and scalable genomics approaches. Here, we leveraged a panel of multi-ethnic (i.e. European/African Americans, and African hunter gatherers, ~80 lines) and gender-balanced human induced pluripotent stem cell (hiPSC) lines to study the impact of genomic variations on transcriptional and post-transcriptional regulatory networks during 3D brain organoid differentiation at single-cell levels. Specifically, we utilized metabolic labelling-based, time-resolved single-cell RNA sequencing (scNT-seq2) to characterize dynamic transition from pluripotent stem cells to differentiated cell states. Using genetic de-multiplexing based on donor-specific variants, we can assign individual cells to their corresponding donors. In a benchmarking experiment involving 4 African and 4 European lines, we conducted a comprehensive comparative analysis of pooled 8-line versus individual-line differentiation toward forebrain cell fates using scNT-seq2. This analysis revealed significant donor-specific variability during differentiation into 3D brain organoids. While pooled-line differentiation produced more consistent outcomes, it limited the ability to study non-cell-autonomous effects, such as cell-cell interactions, compared to individual-line differentiation. Additionally, we demonstrated efficient labelling of newly transcribed RNAs in day 45 forebrain organoids. By reconstructing gene regulatory networks, we plan to identify genes and pathways with cell-type or state-specific effects, which offers insights into the molecular networks governing 3D brain organoids development.

CONCORDANCE BETWEEN BRAIN ORGANOID MODELS AND PATIENT TISSUE IN TUBEROUS SCLEROSIS COMPLEX

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the TSC1 or TSC2 genes, which presents with early onset epilepsy, variable intellectual disability, and psychiatric conditions including autism spectrum disorder. A hallmark pathology of TSC is the presence of cortical tubers, which are focal brain malformations that arise during embryonic development. Tubers contain dysplastic and abnormal cells and can often become seizure foci. The origins of these tuber cells is not well understood, with one hypothesis being a somatic “second-hit” mutation, where cells with a heterozygous loss-of-function mutation lose their functional allele during development.

To test this idea, we established genetically-engineered human brain organoid models of TSC. We generated a conditional knock-out model consisting of stem cells with one non-functional TSC2 allele and one conditional allele that can be disrupted through the addition of Cre. Exposure to a low dose of Cre during neural differentiation generates a subpopulation of TSC2-/- cells within a background of TSC2+/- cells in the same organoid, mimicking the second-hit mutation.

We differentiated these stem cells into brain organoids and deleted TSC2 from a subset of progenitor cells early during development. We used single cell RNA sequencing to profile gene expression changes in these cells over time. We find that TSC2-/- cells preferentially generate glial cell lineages, including astrocytes, compared to TSC2+/- cells in the same organoid. TSC2-/- cells have numerous gene expression changes associated with proteostasis, autophagy, and reactive astrogliosis. Notably, TSC2-/- astrocytes have the most pronounced changes, which include upregulation of a gene expression module associated with disease-induced reactivity.

To validate these insights, we performed cyclic immunofluorescence staining in primary resected TSC patient tuber samples, a technique that enables the probing of many targets with the same set of cells. Using a novel image processing and clustering approach, we identify a subpopulation of tuber cells that express many of the same genes that we identified in the organoid sequencing data. In particular, these cells do not typically express classic neuronal markers, such as NeuN or HuC/D; however, they do express astrocyte markers such as GFAP. This work reveals that complete loss of TSC2 during neural development biases developmental trajectories toward the generation of astroglial cells, and more broadly demonstrates that in vitro organoid models can recapitulate key features of TSC.

INTERACTION OF ETHANOL AND POLYGENETIC BACKGROUND IN ALCOHOL USE DISORDER IN HUMAN iPSC DERIVED NEURAL CO-CULTURE AND 3D ORGANOID SYSTEM

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Alcohol exerts a range of effects on the human brain, including alterations in neurotransmitter release and neuroinflammation, which are implicated in the development and progression of alcohol use disorders (AUD). Genome-wide association studies (GWAS) have identified numerous gene variants associated with AUD. Integrating these variants by creating polygenic scores (PGS) offers insights into genetic susceptibility to AUD. However, the molecular implications of these PGS remain underexplored. Neuroimmune interactions, especially between microglia and neurons, are increasingly recognized as significant contributors to AUD pathophysiology. We investigated the interaction between AUD PGS and ethanol in a human microglia-neuron coculture and a 3D organoid system derived from iPSCs of individuals with high- or low-PGS of AUD. Transcriptomic analysis of microglia revealed differential expression of genes related to the MHCII complex and phagocytosis following ethanol exposure. Unlike low-PGS microglia, high-PGS microglia exhibited increased phagocytosis in both fluorescent zymosan bioparticles and synaptosomes after exposure to ethanol. Additionally, in microglia-neuron cocultures, we observed a reduction in synapse numbers and decreased frequencies of miniature excitatory postsynaptic currents in co-cultures with HPRS microglia, indicating potential excessive synapse pruning by high PGS microglia upon alcohol induction. Currently, we are investigating microglia-neuron interaction in a 3D organoid model. We hope to provide insights into the intricate relationship between AUD PGS, ethanol, and microglial function, potentially influencing neuronal functions in developing AUD.

MECHANISMS OF INTERNEURON EXPANSION IN THE HUMAN CAUDAL GANGLIONIC EMINENCE

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The mammalian developing ventral telencephalon is comprised of three major germinal zones: the lateral (LGE), medial (MGE), and caudal ganglionic eminences (CGE). The MGE and the CGE are highly proliferative regions that generate nearly all cortical interneurons. In humans, the CGE generates ~50% of cortical interneurons, significantly more than the ~30% produced by the mouse CGE (mCGE). This increase in CGE-derived interneurons is considered key to the greatly expanded cerebral cortex and higher brain function in humans. Yet, how this ventral forebrain germinal center can satisfy the demand of interneurons for a greatly expanded cerebral cortex in the human brain, remains unresolved. Recent work from the host lab has shown that proliferating progenitors in the human CGE (hCGE) persist in the early postnatal human brain and that hCGE-derived interneurons continue to be recruited into the entorhinal cortex and other temporal lobe regions for 1-3 years after birth. Other studies have recently shown that mutations in the *TSC2* gene can lead to selective overproliferation of a subpopulation of CGE progenitors and tumor formation. These findings highlight the importance of the human CGE (hCGE) as a critical source of essential cortical interneurons and suggest that its high proliferation may also make it a potential source of malignancies. However, a comprehensive understanding of how the CGE is organized and sustains this high proliferative capacity is still lacking. My overall goal is to understand how the hCGE is organized to produce enormous numbers of cortical interneurons: what its cellular composition is and how these cells are organized; and determine which cells are the most proliferative. I aim to identify transcriptomic and epigenomic properties associated with these different cell types. My preliminary data suggests that Epidermal Growth Factor Receptor (EGFR) signaling is preferentially active in the hCGE compared to mCGE, and that EGFR signaling is correlated with cell amplification in the developing hCGE. I will use mouse models and human CGE organoids to investigate what role EGFR signaling plays in CGE interneuron production. My overall hypothesis is that the hCGE has expanded intermediate progenitor and neuroblast amplification to increase the output of interneurons born in this germinal zone.

HUMAN CORTICAL ORGANOIDS WITH SIGNIFICANTLY IMPROVED CYTOARCHITECTURE DEVELOPMENT UPON HYPOTHERMAL CULTURE

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3D-cultured human induced pluripotent stem cell-derived cortical organoids can mimic broad features of developing human cortex. However, even with the best protocol developed so far, many fundamental aspects of human corticogenesis have not been fully recapitulated in organoid culture yet. This has been partially due to aberrantly accelerated radial glia differentiation and excessive inner necrosis in an overall non-ideal *in vitro* environment. Here, we found that after being cultured in hypothermal condition, the cortical organoids can retain intact ventricular structures for more than 4 months, therefore an enlarged proliferating progenitor pool and a prolonged neurogenic time window, which led to significantly enlarged organoid size, remarkably increased neuronal production, particularly the upper-layer cortical neurons, as well as improved cortical lamination. Our study also suggested that substantially reduced necrotic core buildup and boosted mitochondrial remodeling at early stage which helped maintain the neuroepithelial cell/early radial glial cells state are two potential underlying mechanisms. Our research revealed an intimate relationship between cellular metabolism and corticogenesis, and strongly suggested that metabolic manipulation may hold great promise as a generalizable strategy to modulate *in vitro* model of neurodevelopment.

CALCIUM DYNAMICS TUNE SPECIES-SPECIFIC DEVELOPMENTAL TEMPO TO CONTROL AXON TRACT LENGTH

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The pace of human brain development and especially its neurons is considerably slow, which correlates with increased brain size, neuron number and structural complexity of neurons. In human evolution, axon trajectories have undergone an even further expansion compared to other neuronal domains resulting in axons with lengths an order of magnitude longer than commonly used model organisms. Using human and mouse brain organoids, we observed that axons extend further and grow for an extended time in humans independent of their tissue environment, pointing to tempo as the main determinant of species-specific differences in axon tract length. Single cell RNA sequencing analysis revealed a subset of “synaptic” genes, particularly calcium-permeable ion channels, with an early expression aligning with the beginning of neuron development. During earlier and later stages of neuron development, human and mouse organoids exhibited respectively spontaneous and synchronous calcium activity. At different stage-matched timepoints, calcium amplitude, but not frequency, was lower in humans compared to mice. Stimulating calcium influx and increasing cAMP levels resulted in premature halting of axon outgrowth and shorter axon tracts, with a bidirectional effect observed upon perturbing calcium influx. Thus, we identified species-specific differences in intracellular calcium regulation as a mechanism to tune developmental tempo and evolutionary neuronal complexity.

DISSECTING STEM CELL HIERARCHIES IN HUMAN BRAIN DEVELOPMENT AND HOMEOSTASIS

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The human cortex undergoes rapid development during the second trimester from a pool of neural stem and progenitor cells (NSPCs), which give rise to the neurons, oligodendrocytes, and astrocytes of the mature brain. The functional study of stem cells is reliant on methods for prospectively isolating pure populations of distinct cell types. This is especially the case in primary human tissues where classical genetic tools are not available. The main challenge in prospective isolation lies in identifying combinations of cell surface markers that can discriminate functionally distinct stem and progenitor populations.

Here, we describe a method for prospectively isolating ten distinct NSPC types from the developing human cortex using fluorescence-activated cell sorting (FACS). The developmental potency of each purified NSPC type was interrogated using differentiation assays and xenotransplantation into neonatal mice. Broadly, these purified cell types include a $CD24^{-}THY1^{-/lo}$ compartment enriched in ventricular and outer radial glia as well as astrocytes, a $THY1^{hi}$ compartment encompassing all oligodendrocyte lineages, and a $CD24^{+}THY1^{-/lo}$ compartment consisting of excitatory and inhibitory neuronal lineages. Notably, we identify and prospectively isolate a transcriptomically- and functionally-distinct bipotent glial progenitor cell (GPC), which we found to be lineage-restricted to astrocytes and oligodendrocytes, but not neurons. These GPCs are enriched in the outer subventricular zone, and we hypothesize that this cell type may play a role in the dramatic white matter expansion and glial diversity characteristic of the human brain.

Our paradigm for developing purification strategies combines index sorting with single cell RNA sequencing, allowing for direct mapping between individual cells' surface marker profile and their transcriptome. This framework is highly generalizable across tissues and developmental time. To demonstrate its versatility, we applied this method to postnatal human brain tissue from neurosurgical resections and rapid autopsy. We identify and isolate a rare population of Nestin-expressing neural progenitors. These cells are enriched in periventricular regions, and we demonstrate through lentiviral barcoding that they are multipotent both in vitro and in vivo following xenotransplantation. Further functional interrogation of these postnatal human neural progenitors may prove fruitful in our understanding of brain homeostasis and pathology.

ALCOHOL USE DISORDER ASSOCIATED GENE *FNDC4* AFFECTS GLUTAMATERGIC AND GABAERGIC NEUROGENESIS IN HUMAN iPSC-DERIVED NEURAL ORGANOIDS

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Alcohol use disorder (AUD) is a chronic brain disorder characterized by continued alcohol use despite harmful health consequences. The genetic etiology of AUD is not fully understood. Large-cohort genome-wide association studies (GWAS) for AUD risk and drug treatment outcomes have identified significant genetic loci that are splicing quantitative trait loci (sQTLs) for the fibronectin III domain containing 4 (*FNDC4*) gene in multiple human brain regions, including cortex, nucleus accumbens and hippocampus. However, in spite of the fact that *FNDC4* is highly expressed in the brain, its function and how it might contribute to AUD pathophysiology remain unknown. In the precent study, we characterized *FNDC4* function using CRISPR/cas9 gene editing, the creation of human induced pluripotent stem cell (iPSC)-derived neural organoids and with single-nucleus RNA sequencing (snRNA-seq). Specifically, we generated *FNDC4* homozygous knock-out (KO) iPSC lines and differentiated two single-colony KO iPSCs, together with wildtype (WT) iPSCs, to generate forebrain organoids. Those neural organoids were harvested at three time points of differentiation (45, 90, and 150 days) for snRNA-seq using a "split-pool" combinatorial barcoding technology which allows sample multiplexing. We found that KO of *FNDC4* results in a significant shift in the relative proportions of glutamatergic and GABAergic neurons in those iPSC-derived neural organoids, suggesting a crucial role of FNDC4 in neurogenesis. We further analyzed those snRNA-seq data for pseudo time of neurodevelopment, which identified a cluster of cells that represented a cell status leaning toward glutamatergic neurogenesis in those *FNDC4* KO organoids. Differentially expressed genes (DEGs) when comparing that cluster of cells from WT to *FNDC4* KO neural organoids, displayed enriched pathways related to neurogenesis, synapse development and neurotransmission. "Top" DEGs including *THSD7A*, *OPCML*, *NFIA*, and *NFIB*, have previously been implicated in addiction and other neuropsychiatric disorders. This series of observations suggests that *FNDC4* may play a role in maintaining balance among neuronal cell types, which may contribute to the effects of alcohol on the brain as well as response to alcohol and AUD drug treatment.

A HUMAN-SPECIFIC ENHANCER FINE-TUNES RADIAL GLIA POTENCY AND CORTICOGENESIS

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Humans evolved an extraordinarily expanded and complex cerebral cortex, associated with developmental and gene regulatory modifications. Human accelerated regions (HARs) are highly conserved genomic sequences with human-specific nucleotide substitutions. Although there are thousands of annotated HARs, their functional contribution to human-specific cortical development is largely unknown. HARE5 is a HAR transcriptional enhancer of the WNT signaling receptor Frizzled8 (FZD8) active during brain development. Here, using genome-edited mouse and primate models, we demonstrate that human (Hs) HARE5 fine-tunes cortical development and connectivity by controlling the proliferative and neurogenic capacity of neural progenitor cells (NPCs). Hs-HARE5 knock-in mice have significantly enlarged neocortices containing more neurons. By measuring neural dynamics in vivo we show these anatomical features correlate with increased functional independence between cortical regions. To understand the underlying developmental mechanisms, we assess progenitor fate using live imaging, lineage analysis, and single-cell RNA sequencing. This reveals Hs-HARE5 modifies radial glial progenitor behavior, with increased self-renewal at early developmental stages followed by expanded neurogenic potential. We use genome-edited human and chimpanzee (Pt) NPCs and cortical organoids to assess the relative enhancer activity and function of Hs-HARE5 and Pt-HARE5. Using these orthogonal strategies we show four human-specific variants in HARE5 drive increased enhancer activity which promotes progenitor proliferation. These findings illustrate how small changes in regulatory DNA can directly impact critical signaling pathways and brain development. Our study uncovers new functions for HARs as key regulatory elements crucial for the expansion and complexity of the human cerebral cortex.

SPATIAL-TEMPORAL DUAL-COMMUNICATION ORGANOID NEURAL INTERFACE WITH 3D CURVED MESOSURFACES

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Neural organoids from self-organized 3D cell cultures hold promise for cutting-edge research in neuroscience and biomedicine. To explore the huge potential of organoid systems requires precise monitoring of the electrophysiological signals, especially when high-resolution spatial temporal information is involved. The three-dimensional geometry of organoids, however, raises critical challenges for spatial temporal electrophysiology. Given that commercial planar multielectrode arrays (MEA) suffer from limited interfacing area with spherical shaped organoids, tremendous efforts have been made to go beyond the 2D plane, including bilayer electrode arrays, foldable ultrathin mesh electrodes, self-folding electrodes, buckled MEAs, and Kirigami electrodes. Nevertheless, these methods only sample activity of a selective portion of organoids with relatively small number of individual channels. Therefore, a platform to probe the systematic electrophysiological behavior of organoids is still elusive.

The principal challenge of achieving high-resolution spatial temporal electrophysiology lies in electrode array patterns that can map across the surface of neural organoids. Distinct from normal 3D MEAs, these spatial electrode arrays require the realization of precisely controlled complex 3D surfaces. In this work, we develop a high-resolution 3D surface neural interface for neural organoids. By employing inverse design strategy and mechanically guided 3D assembly, we realize 3D MEA based on curved mesosurfaces that can access over 75 % of the surface area of organoids with 240 individual channels enabling dual-way communication of recording and stimulating with a spatial resolution below 150 μm. These features make possible the spatial monitoring and chronic tracking of organoids, correlation analysis in 3D space, and pharmacological and disease studies with a systematic level that cannot be achieved before.

In this presentation, we will discuss our inverse design strategy for 3D neural interface and the high-resolution spatial temporal mapping of activities from multiple types of neural organoids. We will discuss the compatibility of our platform with variety of widely used biological tests including pharmacological studies and disease models. We will also cover the feasibility of our methods to enable higher channel numbers, resolution, surface coverage, and to realize free geometry designs for 3D morphing of organoids.

HUMAN CLOCK REGULATES NEOTENY TO EXTEND NEURO- AND GLIOGENESIS DURING EARLY NEOCORTICAL DEVELOPMENT

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The human neocortex experienced a remarkable expansion during evolution. This expansion is characterized by increased both neurons and glia. Due to the human-specific expression of CLOCK in the frontal pole, we previously studied CLOCK in human neural progenitor and CLOCK humanized mouse models. We discovered that human CLOCK increased neuron and oligodendrocyte density in the frontal cortex and regulated neurogenesis genes. These results suggest the potential function of human CLOCK during the embryonic development of neocortex. Herein, we studied the proliferation, differentiation, and cytoarchitecture of developing neocortex on BrdU administrated embryos of CLOCK humanized mouse model (HU). We discovered that CLOCK is only expressed in radial glia rather than intermediate progenitor as early as embryonic day 14.5 (E14.5). Consistent with the cell type-specific expression of CLOCK, human CLOCK appeared to promote the maintenance of radial glia in progenitor fate and enhance their proliferation rate to increase the progenitor pool at both ventricular and subventricular zone. We also observed that human CLOCK enhanced differentiation of postmitotic cells at subplate. These results suggest that CLOCK has distinct functions at different developmental zones. Additionally, human CLOCK also altered the dorsal-ventral organization of developing cortex. At E16.5, HU mice increased relative thickness of ventricular zone and subplate, and this thickening persisted in the germinal zones at postnatal day 0 (P0), suggesting an enhanced neurogenesis in HU mice. To further elucidate the developmental process leading to the increased cell density observed in adult HU mice, we quantified postmitotic neurons and oligodendrocytes across developmental stages. Deep-layer neurons of HU mice showed a consistent higher density at both P0 and P7, whereas the elevated density of upper-layer neurons was only detected in P7 but not P0. Along with the findings in neurons, oligodendrocyte density of HU mice was reduced at P0, while it reached a similar level as wild-type mouse at P7. Given the chronological order to generate deep-layer neurons, upper-layer neurons, oligodendrocytes during neocortical development, the observed alterations of cell density during development suggest a protracted windows of neurogenesis and gliogenesis. Therefore, this study demonstrates that CLOCK may regulate the neoteny of human neocortical development for an extended timeline to expand neuronal and glial populations. Future work will leverage single-nuclei omics and iPSCs-derived organoids to understand the molecular mechanisms that are regulated by CLOCK in human brain development.

MODELING A NEURODEVELOPMENTAL DISORDER CAUSED BY A DE NOVO *NACC1* MUTATION IN BRAIN ORGANOIDS

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Brain organoids developed from human stem cells are powerful models for understanding human neurodevelopmental disorders. A rare developmental disorder involving neurodevelopmental delay, epilepsy, feeding difficulties, congenital cataracts, and delayed myelination, was recently discovered to be caused by a rare mutation in *NACC1*, R298W. Organoids were generated with the mutation genetically edited into healthy human pluripotent stem cells have developmental and functional differences compared to organoids generated from the control line. Mutant organoids also have a phenotype seen on calcium imaging with neural hyperactivity that suggests this model can be used to study developmentally derived epilepsy syndromes. This demonstrates the potential of brain organoids to model neurodevelopmental disorders and a platform for finding new therapeutics for difficult to treat conditions.

Arl2 GTPase ASSOCIATES WITH THE CENTROSOMAL PROTEIN CDK5RAP2 TO REGULATE CORTICAL DEVELOPMENT VIA MICROTUBULE ORGANIZATION

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ADP ribosylation factor-like GTPase 2 (Arl2) is crucial for controlling mitochondrial fusion and microtubule assembly in various organisms. Arl2 regulates the asymmetric division of neural stem cells in Drosophila via microtubule growth. However, the function of mammalian Arl2 during cortical development was unknown. Here, we demonstrate that mouse Arl2 plays a new role in corticogenesis via regulating microtubule growth, but not mitochondria functions. Arl2 knockdown leads to impaired proliferation of neural progenitor cells (NPCs) and neuronal migration. Arl2 knockdown in mouse NPCs significantly diminishes centrosomal microtubule growth and delocalization of centrosomal proteins Cdk5rap2 and γ-tubulin. Moreover, Arl2 physically associates with Cdk5rap2 by in silico prediction using AlphaFold Multimer, which was validated by co-immunoprecipitation and proximity ligation assay. Remarkably, Cdk5rap2 overexpression significantly rescues the neurogenesis defects caused by Arl2 knockdown. Therefore, Arl2 plays an important role in mouse cortical development through microtubule growth via the centrosomal protein Cdk5rap2.

INVESTIGATING THE IMPACT OF COMMON GENETIC RISK VARIANTS FOR SCHIZOPHRENIA ON NEURONAL FUNCTION ELUCIDATES RELATIONSHIPS BETWEEN CELLULAR PHENOTYPES, CLINICAL STATUS AND COGNITIVE PERFORMANCE

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Schizophrenia (SCZ) is a complex, polygenic disorder with marked clinical heterogeneity and no clear pathological mechanism or cellular pathology. Previous human induced pluripotent stem cell (hiPSC) studies have identified cellular phenotypes associated with SCZ diagnosis, yet their relevance to the clinical and cognitive features remains uncertain. Here, we describe results of our investigation into the relationships between cellular measures obtained from hiPSC-derived neurons and the individual's clinical status, severity of symptoms, and cognitive performance. We further identify within-case patterns of association that could serve to illuminate dimensions of illness and subgroups of patients that may be suitable for targeted treatments.

In hiPSC-derived neurons from 13 high polygenic risk score (PRS) SCZ patients and 15 low PRS neurotypical controls (CON), we observed several electrophysiological measures related to Na+ channel function that were associated with diagnosis. Lines derived from SCZ donors showed an increased membrane resistance, increased number of Na+ current peaks in response to a voltage ramp protocol, shifted the activation threshold of the second Na+ peak, significantly hyperpolarized voltage dependence of activation and inactivation, reduced long term inactivation and decreased action potential interspike interval. In SCZ, the number of Na+ peaks showed a positive association with the severity of hallucinations, and the sEPSC amplitude showed a negative correlation with performance on the Wisconsin Card Sort Test. Additionally, we are generating human forebrain organoids (FBOs) from these hiPSC lines and performing extensive cellular characterization using immunofluorescence staining, transcriptomic profiling, and electrophysiology to find novel association with clinical phenotypes. Remarkably, day 150 SCZ FBOs showed similar hyperpolarized shifts in the voltage dependence of activation and inactivation. These consistent findings across two distinct differentiation protocols suggest that the kinetic properties of Na+ channels may represent a convergent biophysical mechanism downstream of common variant risk for SCZ and underscore the potential of this approach for biomarker identification and downstream drug development.

ADIPOSE TISSUE-DERIVED MICROVASCULAR FRAGMENTS SHAPE THE DEVELOPMENT OF HUMAN CORTICAL ORGANOIDS

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Cortical organoids grown from human pluripotent cells are an attractive and efficient method for modeling the human cerebral cortex. However, while these organoids recapitulate much of the structure and cellular diversity of the cortex, they remain relatively immature, small, and morphologically undefined in comparison to the postnatal cortex. Since these organoids are composed only of neural tissue and do not acquire the network of blood vessels critical to the development of the human brain, constructing a vascular network within cortical organoids is a widely proposed method to increase the size, cellular maturity, and cortical morphology of these organoids.

Microvascular fragments (MVFs) derived from adipose tissue provide a robust platform for the construction of vessel networks. MVFs retain the cellular structure of functional blood vessels and spontaneously form vascular networks when grown in a fibrin matrix. We have incorporated MVFs into cortical organoids, reliably resulting in accelerated growth and the development of an expanded neuroepithelial layer as early as 30 days *in vitro*. These MVF-cocultured cortical organoids also show markers of accelerated cortical layering and development relative to controls. Significantly, a similar but more compact organoid morphology is observed when cortical organoids are exposed only to the metabolites generated by MVFs grown in fibrin cultures. These findings suggest that MVFs may produce signaling cues similar to those secreted by cortical blood vessels *in vivo*, driving cortical organoids toward a more developed and physiologically relevant phenotype even in the absence of functional blood vessels.

EXPLORING EVOLUTIONARY VARIATIONS IN FOREBRAIN DEVELOPMENT USING PATTERNED ORGANOIDS

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Vertebrate adult forebrains show a large variation in size and complexity across species despite the conserved nature of the regulatory molecules responsible for patterning. In the developing telencephalon, SHH (ventral) and WNT/BMP (dorsal) signals induce the expression of region-specific transcription factors thereby dividing it into discrete zones of progenitor cells. The relative size and cell type composition of the progenitor zones influence the organization and complexity of the adult brain. The mechanism of how the differences in signalling activities of morphogens and their downstream regulation by gene regulatory networks translate into cell fate decisions of the early progenitor populations and therefore drive inter-species variation remains poorly understood.
To tackle this gap in the field, I am establishing a human organoid system suitable for *in vitro* patterning. I have devised a protocol that generates single lumen telencephalic organoids from human embryonic stem cells. Under the culture conditions, the self-organised human organoids resemble the dorsal region of the nascent telencephalon marked by the expression of WNT. The organoids respond to a global SHH signal and self-organise into a pattern recapitulating a subset of the in vivo dorsoventral pattern. Next, I will be utilising engineered mouse cells carrying optogenetically inducible SHH construct to impose a gradient of the morphogen on the human organoids by making human-mouse chimeras. The protocol for making chimeras and spatially restricted induction of SHH will be optimized to recapitulate the entire dorsoventral organisation of the developing telencephalon. I will quantify cellular responses to morphogen input in the chimeric organoids and analyse how spatiotemporal patterns change in response to perturbations in morphogen dynamics. Using mathematical modelling I will be generating a rigorous framework to understand early telencephalon patterning in humans. We aim to probe how cells and tissues respond to morphogen signals and subsequently determine the evolutionary differences in the developing telencephalon by systematically comparing our results with measurements done in *in vivo* systems.

Keywords: Organoids, morphogen, telencephalon dorsoventral patterning, evolution.

NEXT-GENERATION ELECTROPHYSIOLOGY FOR FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS AND ASSEMBLOIDS

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Organoid and assembloid models derived from human-induced pluripotent stem cells (hiPSCs) have emerged as invaluable tools for investigating neurological disorders such as epilepsy, Alzheimer's, and Parkinson's disease.
Real-time, label-free measurement of electrical activity in such self-organizing 3D *in-vitro* cellular models offers critical insight into the complexity of their neuronal networks. High-density microelectrode arrays (HD-MEAs) offer a non-invasive method for capturing electrophysiological signals from a range of electrogenic samples, including hiPSC-derived neurons, neural organoids, assembloids, retinal tissues, and brain slices.

In this study, we employed the MaxOne and MaxTwo HD-MEA platforms (MaxWell Biosystems AG, Switzerland), which feature 26,400 electrodes per well and high signal-to-noise ratio, to record extracellular action potentials in neural organoids and assembloids. The platforms enabled recordings at various scales, from neuronal networks to single-cell and sub-cellular levels, within disease models and drug testing applications. The ability to flexibly selective electrodes for recording neural activity, significantly enhanced the reproducibility and statistical robustness of the collected data. Key electrophysiological metrics such as firing rates, spike amplitudes, and network burst profiles were analyzed longitudinally.

Furthermore, the AxonTracking Assay was used to characterize axonal function, measuring action potential conduction velocity, latency, axonal length, and branching. This unique assay offers sub-cellular functional recordings and facilitates high-throughput characterization of disease models targeting axon initial segments, axonal branching, development, and conduction.

MaxWell Biosystems' HD-MEA platforms, coupled with automatically generated plots and extracted metrics, offer a unique, user-friendly approach to identifying and isolating functionally active regions in 3D cultures. These powerful platforms enable long-term *in-vitro* disease modeling and compound testing in both acute recordings and/or longitudinal studies.

DEVELOPMENTAL NETWORK TOPOLOGY AND ELECTROPHYSIOLOGICAL DISRUPTIONS INDUCED BY NOS1AP OVEREXPRESSION IN IN-VITRO NEURONAL NETWORKS

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During brain development, neuronal network maturation is critical for cognitive and behavioral functions. Network topology, which dictates connectivity patterns, is key for efficient information transfer. NOS1AP (Nitric oxide synthase 1 adapter protein), involved in synaptic development, disrupts dendritic spine growth when overexpressed, which may impact network development. Although linked to neurodevelopmental disorders like schizophrenia, its effects on network topology and electrophysiology remain unclear. This study examines how NOS1AP overexpression alters neuronal network development, focusing on electrophysiological and topological changes. Using high-density microelectrode arrays (HD-MEAs), we recorded data from rat hippocampal cultures from day 14 in vitro (DIV14). Spike sorting algorithms identified neuronal units, and network maturation was analyzed through firing rate, burst behavior, and topological metrics like centrality and path length. Functional connectivity was inferred using Dynamic Differential Covariance analysis. A total of 19 networks was analyzed (n_control = 8, n_NOS1AP = 11), with statistical comparisons made using Mann-Whitney U-tests. NOS1AP-overexpressing networks exhibited a higher mean firing rate ($p = 0.012$), fewer bursts ($p = 0.023$), and longer burst durations ($p = 0.035$), indicating altered network dynamics. NOS1AP overexpression led to lower centrality ($p = 0.004$), reduced clustering ($p = 0.03$), and longer path lengths ($p = 0.015$), suggesting the development of a sparser, less efficient network topology with fewer connections and reduced local clustering. This study highlights how NOS1AP overexpression impairs neuronal network development by disrupting key topological metrics. Such changes may underlie cognitive deficits seen in disorders like schizophrenia, emphasizing the importance of studying network topology to understand the impact of protein dysregulation in mental health disorders.

Declaration:
This work was supported by the Swiss National Science Foundation project 205320_188910/1

GENERATION OF A HUMAN HIV-1-NEUROCOGNITIVE IMPAIRMENT (HIV-1-NCI) MODEL USING CEREBRAL BRAIN ORGANOIDS INFILTRATED WITH HIV-1 INFECTED MICROGLIA.

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Chronic HIV-1 infection within the central nervous system (CNS) contributes to the development of HIV-1-neurocognitive impairment (HIV-1-NCI). Multiple inflammatory mediators and neurotoxic factors are released by various cell types in the CNS in response to HIV-1 infection. This neuroinflammation generates neuronal damage and cognitive deficits characteristic of HIV-1-NCI. Additionally, although combination antiretroviral drug therapy (ART) suppresses HIV-1 RNA by decreasing inflammation in the periphery, neuroinflammation remains in the CNS of people with HIV-1-NCI. We built a model to recreate neuroinflammatory processes in the CNS during HIV-1-NCI. Here, we used human cerebral brain organoids (CBOs) infiltrated with human Microglia (M-CBOs) as a model to investigate inflammatory mechanisms driven by HIV-1 in the CNS in the presence of ART. Microglia were obtained by differentiating Hematopoietic progenitor cells (HPCs), infected with HIV-1 or left uninfected, then cocultured with CBOs. Microglia infiltration into CBOs, forming M-CBOs, was confirmed via microscopy and flow cytometry. HIV-1-infected M-CBOs were treated with ART or left untreated for 25 days to assess pro-inflammatory measurements with cytokines and p-NF-κB levels. HIV-1 infection was validated using ddPCR and P24Elisa. Our findings show elevated levels of phosphorylated NF-κB (p-NF-κB) in microglia and neurons from HIV-1-infected M-CBOs compared to uninfected M-CBOs. These differences were consistent with higher levels of pro-inflammatory cytokines found in the CSF of people with HIV-1-NCI, such as CCL2 or Osteopontin, detected in the supernatant of HIV-1-infected M-CBOs via ELISA. Importantly, both the levels of p-NF-κB and the pro-inflammatory cytokines remained elevated even in the presence of ART, mirroring observations in people with HIV-1-NCI. In summary, this study provides crucial insights into the intricate interplay between HIV-1 and neuroinflammation using a CBO model. Funding: NIH DA052027

UTILIZING GEOMETRIC ARRANGEMENT OF ORGANOIDS TO GENERATE SIGNALING GRADIENTS

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The use of three-dimensional human neural tube models has been limited by the absence of morphogenesis and patterning. In conventional in vitro models, neuroepithelial cells organize into multiple rosettes, failing to mimic the tube morphology and intricate organization seen in the mammalian neural tube. Creating a model that accurately replicates neurodevelopmental features, alongside scalable perturbation of genetic networks, is critical to understanding the mechanisms that drive human brain development and disease.

Our previous research demonstrated that a WNT signaling gradient could be achieved through the geometric arrangement of micropatterned organoids. This resulted in organoids that contained hindbrain and spinal cord populations, successfully recapitulating the anterior-posterior patterning of the neural tube.

Building on this approach, we show that a BMP signaling gradient can be created in a similar fashion. We used this BMP gradient to pattern single lumen ectoderm organoids. The inner cells of the organoids experienced lower BMP, driving differentiation into neural ectoderm, while the outer cells sensed higher BMP levels, differentiating into surface ectoderm. The interaction between these tissues induced the neural ectoderm to fold, effectively recapitulating the process of neural tube closure. At the end of this morphogenesis, we have a single-lumen forebrain organoid, patterned dorsally by non-neural ectoderm. As these organoids mature, distinct regions along the dorsal-ventral axis emerge.

Concurrently, we developed methods to knock-down 24 genes per experiment, while guaranteeing that a given organoid would only have one gene knocked-down at a time. This meant that we could observe the effects of disrupting a single gene during neural tube closure at scale. We tested the effect of knocking-down one hundred genes to find key genes that are necessary for proper neural tube morphogenesis.

By using the geometric arrangement of our organoids, we aim to develop more accurate and complex models of the human anterior neural tube. This, in turn, can provide deeper insights into brain development, morphogenesis and disease mechanisms, offering novel pathways for addressing neurodevelopmental disorders.

LEVERAGING ORGAN-ON-A-CHIP TECHNOLOGIES TO MODEL THE BRAIN-BONE MARROW AXIS IN HEALTH AND DISEASE

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Organ-on-a-chip (OOC) technologies, including 3D bioengineered human tissues, have emerged as unique human- and patient-specific model systems that can capture how different organs interact under both homeostatic and neuropathological conditions. Our model (i) maintains 3D tissues in their optimized microenvironments, (ii) connects tissues by vascular perfusion, and (iii) separates vascular and tissue compartments by a selectively permeable endothelial barrier. In this study, we adapt this platform to examine the interactions between brain organoids and a constantly repopulating immune system generated by engineered bone marrow tissues (eBM). Our goal is to enable cellular crosstalk along the brain-BM axis via a selectively permeable vascular barrier. Individual 3D bioengineered tissues of the brain and BM were matured for 2 months prior to integration into the OOC platform. Cortical organoids were generated using a modified Pasca protocol and are enriched with neurogenic niches containing neural stem cells, newborn neurons, and mature neurons that dynamically respond to environmental changes. We generated eBMs by infusing hematopoietic progenitors into a stromal BM niche formed by osteoblasts, mesenchymal stem/stromal cells, and endothelial cells, seeded within a mineralized bone matrix. eBMs can release immune cells into the surrounding environment and demonstrate physiological responses to stress and tissue injury. After isolated tissue maturation, brain and eBM tissues were integrated into the OOC platform and cultured in tissue-specific medium, linked by vascular perfusion, for periods of up to 4 months under homeostatic conditions. To examine the functional viability of these tissues, we conducted studies of the transcriptome, metabolome, cytokinome, and calcium transients. Our evidence to date demonstrates our ability to establish, mature, and maintain viable and functional 3D human tissues of the brain and BM for up to 6 months in an OOC platform, enabling long-term studies of multi-organ, neuro-immune responses in injury and disease.

APOE4 IMPACTS CORTICAL NEURODEVELOPMENT AND ALTERS NETWORK FORMATION IN HUMAN BRAIN ORGANOIDS

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Apolipoprotein E4 (APOE4) is the leading genetic risk factor for Alzheimer's disease. While most studies examine the role of APOE4 in aging, human APOE4 carriers demonstrate altered brain structure and cognition as early as infancy. Imaging studies in infants have shown that APOE4 reduces gray matter volume (GMV) and regional myelin water fractionation (MWF) in regions preferentially affected by AD while increasing GMV and MWF in the parietal lobe and frontal cortex. Cognitively, APOE4 infants and toddlers score higher on early mental development tests, but APOE4 children score lower on IQ, attention, and working memory tests. These studies suggest that APOE4 influences brain structure and cognition well before the development of AD, potentially extending to neurodevelopment. Here, we examined human-relevant cellular phenotypes across neurodevelopment using induced pluripotent stem cell (iPSC) derived cortical and ganglionic eminence organoids (COs and GEOs). In COs, we showed that APOE4 decreased BRN2+ and SATB2+ cortical neurons, increased astrocytes and outer radial glia, and was associated with increased cell death and dysregulated GABA-related gene expression. In GEOs, APOE4 accelerated maturation of neural progenitors and neurons. Multi-electrode array recordings in assembloids revealed that APOE4 disrupted network formation and altered response to GABA, resulting in heightened excitability and synchronicity. Together, our data provides new insights into how APOE4 may influence cortical neurodevelopmental processes and network formation in the human brain.

CHRONIC INFLAMMATION INDUCES INNATE IMMUNE MEMORY IN HUMAN MICROGLIA: INSIGHTS FROM A NOVEL ORGANOID-BASED PLATFORM

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Chronic low-grade inflammation, or inflammaging, is a hallmark of aging and is implicated in various age-related brain disorders. Microglia, the long-lived immune cells of the brain, are especially susceptible to immune imprinting, where repeated inflammatory stimuli induce innate immune memory, altering their responses over time. However, the precise cellular and molecular drivers of these state transitions in human cells remain unclear. Using a human organoid-based in vivo platform, we modeled chronic inflammation via systemic administration of lipopolysaccharide (LPS), allowing us to study human microglial phenotypes within a vascularized brain-like environment. This platform enabled us to investigate key aspects of microglial reactivity and immune memory during chronic inflammation. Our data show that chronically activated microglia adopt altered transcriptomic profiles, shifting from homeostatic to reactive states. Through two-photon microscopy, we observed a significantly diminished response to subsequent injuries in chronically stimulated microglia, indicating a state of immune tolerance. These findings align with emerging concepts of immune imprinting, where repeated inflammatory exposures lead to epigenetic reprogramming that impacts microglial behavior. This model provides new insights into the long-term involvement of microglia in brain inflammation and highlights the potential of our organoid-based system for investigating the broader implications of chronic inflammation in brain health and disease.

MECHANICAL CONTRIBUTIONS OF ENCAPSULATING MATRIGEL TOWARDS MIDBRAIN ORGANOID DEVELOPMENT

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Overview. Standard organoid growth protocols require the organoids to be encapsulated in a supportive hydrogel matrix, such as Matrigel or Geltrex as an essential step in their maturation and growth. While these hydrogel materials provide requisite chemical stimuli for tissue maturation, they also provide a mechanical resistance to the growing tissue. Mechanical forces are generally well-established to play a critical role in tissue development, but their role in midbrain organoid (MBO) development remains undefined. Understanding how mechanical forces might affect organoid differentiation is particularly challenging, as the mechanical conditions at the organoid-matrix interface themselves are unknown; and it is currently not possible to precisely control the stresses present at this interface during large-scale morphogenetic growth.
Approach. To measure the stresses present during normal MBO maturation, we developed cell-sized hydrogel stress sensors with well-defined mechanical properties that can be placed at the organoid-Matrigel interface. Monitoring deformation of these sensors provides direct measurements of local stresses during development. To control the stresses present during MBO growth, we developed a granule-based hydrogel matrix that yields and reorganizes itself at tunable and well-defined stresses. In this way, as an encapsulated organoid grows and pushes into the surrounding matrix, stress at the organoid-matrix interface is maintained below the specified yield stress, independently of conventional matrix degradation and MBO proteinase activity.
Results. Induced pluripotent stem cells were formed into embryoid bodies by aggregation, successfully coated with sensors, and differentiated towards MBOs in Matrigel, where they exhibited characteristic neuroepithelial bud formation by week 1, and well-defined internal rosettes by week 2. Preliminary analysis of stress sensors indicates that stresses increase during growth, but are quite small (10s of Pascals) early bud formation. We then tuned the yield stress of our granular hydrogels from 2 to 200 Pa, and found that bud formation only occurred at yield stresses of between 10 and 50 Pa. The number of rosettes increased at greater yield stresses, but only formed large structures at 20-50 Pa. This suggests that a small but appreciable stress is essential for MBO development.
Significance. The exquisite sensitivity of MBOs to matrix yield stress suggests that mechanics of the encapsulating hydrogel plays a critical but overlooked role in organoid growth. Variations in yield stress properties may therefore explain why MBO growth seems so sensitive to batch-to-batch variations in Matrigel. Furthermore, this study suggests a generalized approach towards designing replacements for Matrigel based on tuning the mechanical properties of synthetic substitutes.

UNCOVERING TRANSCRIPTIONAL SIGNATURES OF UNIQUELY HUMAN THALAMIC NEURODEVELOPMENT BY ALIGNING HUMAN AND CHIMPANZEE ORGANOID MODELS TO THE DEVELOPING HUMAN BRAIN

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The human brain exhibits unique features compared to other primates, including an expanded number of neurons, a greater complexity of neuronal connections, and prolonged periods of neurogenesis and neuronal maturation leading to signatures of neoteny at birth. Recent studies have supported the hypothesis that uniquely human brain traits emerged in part through modulation of gene expression during neurodevelopment. However, direct comparisons of human and non-human primate neurodevelopment at cell-type resolution are still limited by the availability of embryonic and fetal human and great ape samples over a robust developmental time course. We are approaching this question in vitro by generating human and chimpanzee iPSC-derived neural organoid models of the thalamus and comparing gene expression dynamics across species at single-cell resolution. Using a neural network-based label transfer method, we directly predict the regional and cell type identity of human and chimpanzee organoid-derived neurons using a ground truth embryonic and fetal human brain scRNA-seq atlas, thereby validating that thalamic organoids recapitulate human neuronal subtypes. Reconstructing gene expression dynamics using trajectory inference supports this conclusion that both human and chimpanzee thalamic organoids generate homologous thalamic cell types. By implementing this robust validation pipeline, we detect human-biased gene signatures with cell type-specific expression in vivo and evidence of chromatin contact by human accelerated regions (HARs) in cultured forebrain neurons, with significant enrichment of these HAR contacts in inhibitory interneurons of the rostral thalamus. By aligning developmental trajectories of organoid-derived thalamic neurogenesis across species, we identify gene sets positively regulating neuronal maturation in chimpanzee neurons and negatively regulating neuronal maturation in human, reflecting signatures of species-specific developmental timing. These findings have the potential to further elucidate human-specific gene regulatory programs in neurodevelopment and unique features of the human thalamus.

TARGETING MICROGLIAL ALTERATIONS IN ALZHEIMER'S DISEASE: EXPLORING THE IMPACT OF APP AND PSEN1/2 MUTATIONS IN HUMAN NEURAL ASSEMBLOIDS

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss and neuronal damage. Neuroinflammation, particularly involving microglia, has emerged as a promising target for new therapies. This research addresses how genetic mutations disrupt microglial function in AD and explores therapeutic strategies targeting these changes. The objective was to examine the interaction between APP and PSEN1/2 mutations and microglial function, focusing on the enhanced phagocytic capacity of healthy microglia and their role in neuronal health in AD cortical organoids. Using induced pluripotent stem cells carrying APP, PSEN1, or PSEN2 mutations, alongside wild-type controls, an assembloid model was developed with microglia-containing organoids. This model allowed a detailed investigation of microglia-neuron interactions in both healthy and AD-like conditions. Several assays were conducted, including morphological analysis, immunostaining, phagocytosis assays, gene and protein expression, and quantification of neurite elongation and synaptic puncta. The results showed over 90% of microglial progenitors expressed CD45+ and CD11b+ markers. Differentiated microglia displayed classical markers such as CD68, CX3CR1, TREM2, IBA1, PU.1, CD11b, and P2YR12. Findings suggest PSEN2 N141L and APP V717G mutations lead to altered microglial morphology, with reduced branch points and process length compared to wild-type controls. Zymosan phagocytic activity significantly decreased in PSEN2 N141L and APP V717G mutants, while PSEN1 M233L showed no such deficit. Additionally, APP V717G mutants displayed reduced amyloid-beta phagocytic activity. Early differentiation of cortical organoids revealed a polarized structure resembling neuroepithelium, with proliferative neural progenitor cells (expressing Ki67, SOX2, and PAX6). Over time, organoids grew in size, with an increased proportion of mature neurons (expressing Beta-3-tubulin and MAP2). APP V717G mutants showed reduced proliferation. Microglia remained viable within organoids for over 30 days of co-culture. In conclusion, AD-risk gene mutations significantly affect microglial function and disease progression. These initial findings highlight the potential for therapeutic strategies targeting microglia in AD. Further research will deepen the understanding of AD mechanisms and help identify novel therapeutic targets.

MULTIPLEXED AND SCALABLE CELLULAR MAPPING TOWARD THE STANDARDIZED THREE-DIMENSIONAL HUMAN NEUROANATOMY

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The advent of three-dimensional histological methods has advanced studies of cellular-resolution anatomy of the brain. The use of whole-mount staining and tissue clearing has promoted systems-level identification of cells underlying brain functions in mouse models. However, application of these methods to studies of human brains has been difficult due to their structural variability and the lack of standardized quantitative metrics. Here we report a rapid and scalable staining/imaging technique, termed mFISH3D, that enables single-cell-resolution imaging of mRNAs of more than ten genes in a large mammalian brain. To apply mFISH3D to postmortem human cerebral cortex, we have reconstructed morphogenic tracks of cortical growth, and used the tracks to provide a framework for quantitative assessment of cytoarchitecture. The morphogenic track was reconstructed from cortical arteries, whose orientation correlates highly with the trace of radial glial cells. The workflow-enabled the objective quantification of biological heterogeneity among cortical regions. We propose these techniques for standardization of 3D histology of the human cortex and the cerebral organoid to provide reproducible measurements of cell-type-specific neuroanatomy.

MODELING JUVENILE BATTEN DISEASE USING BRAIN REGION-SPECIFIC ORGANOIDS: INSIGHTS INTO PATHOLOGY AND THERAPEUTIC STRATEGIES

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Batten disease is a devastating genetic lysosomal storage disorder that affects children globally, with a prevalence of approximately 1 in 100,000 births and a higher incidence in Scandinavia. Juvenile Batten disease, or juvenile neuronal ceroid lipofuscinosis, is the most common form and is associated with loss-of-function mutations in the CLN3 gene, which encodes a lysosomal transmembrane protein of unknown function. Juvenile Batten disease is characterized by progressive vision loss starting between ages 4 and 12, neurodegeneration resulting in cognitive decline and motor impairment, and premature death typically occurring between ages 15 and 30. Currently, there are no available cures for this fatal condition. Pathological hallmarks of the disease include the accumulation of intracellular autofluorescent storage material, considerable neuronal loss, glial reactivity, and retinal degeneration.

To systematically model CLN3 disease, we generated induced pluripotent stem cells from both CLN3 patients and controls, with most affected individuals carrying at least one allele with a 966 bp deletion, and established various region-specific organoids, including dorsal cortex, hippocampus, midbrain, hypothalamus, retinal and neuromuscular junction. Notably, CLN3 organoids were able to recapitulate key features of the disease. Our preliminary data indicate that CLN3 deficiency significantly impacts brain developmental processes, such as impaired proliferation and structural organization of progenitors and neurogenesis. Moreover, the abnormal accumulation of the lysosomal protein LAMP1 in CLN3 organoids suggests compromised lysosomal function.

We have explored the therapeutic potential by treating these organoids with selected candidate drugs and rAAV-mediated gene therapy strategies by evaluating their efficacy and safety in the CLN3 patient-derived organoid models. Additionally, we are conducting single-cell transcriptomics and proteomics to further characterize phenotypes, underlying mechanisms, and treatment responses in these models.

This study underscores the potential of brain organoids as powerful tools for modeling juvenile Batten disease, providing insights into pathophysiology and avenues for developing targeted treatments.

MODELING ARX-RELATED EPILEPSIES USING CORTICAL AND GANGLIONIC EMINENCES ORGANOIDS

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Infantile spasms, with an incidence of 1.6 to 4.5 per 10,000 live births, are a relentless and devastating childhood epilepsy marked by severe seizures but also leads to lifelong intellectual disability. Alarmingly, up to 5% of males with this condition carry a mutation in the *Aristaless-related homeobox* (ARX) gene. Our current lack of human-specific models for developmental epilepsy, coupled with discrepancies between animal studies and human data, underscores the gap in knowledge and urgent need for innovative human models, organoids being one of the best available. Here, we used human neural organoid models, cortical organoids (CO) and ganglionic eminences organoids (GEO) which mimic cortical and interneuron development respectively, to study the consequences of PAE mutations, one of the most prevalent mutation in *ARX*. ARX^{PAE} produces a decrease expression of *ARX* in GEOs, and an enhancement in interneuron migration. That accelerated migration is cell autonomously driven, and it can be rescued by inhibiting CXCR4. We also found that PAE mutations result in an early increase in radial glia cells and intermediate progenitor cells, followed by a subsequent loss of cortical neurons at later timepoints. Moreover, *ARX* expression is upregulated in COs derived from patients at 30 DIV and is associated with alterations in the expression of *CDKN1C*. Furthermore, ARX^{PAE} assembloids had hyperactivity which were evident at early stages of development. With effective treatments for infantile spasms and developmental epilepsies still elusive, delving into the role of ARX^{PAE} mutations in human brain organoids represents a pivotal step toward uncovering groundbreaking therapeutic strategies.

ISOLATION OF FUNCTIONAL SYNAPTIC STRUCTURES FROM HUMAN BRAIN ORGANOIDS

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Synapses are essential for neuronal function but are tiny and difficult to study. Synapses are therefore often enriched from brain tissues in the form of isolated nerve terminals, called synaptosomes. Synaptosomes are detached and resealed synaptic boutons, generated when brain tissue is homogenized in an iso-osmotic buffer. Synaptosomes are viable, metabolically active, capable of regenerating their membrane potential and can be depolarized with e.g., KCl stimulation, resulting in neurotransmitter release. Synaptosomes are traditionally enriched from rodent or postmortem human brain tissue, but ethical issues and availability challenges the studies of functional, alive human synaptosomes. However, with the emerging field of Neural organoids (NOs) a possible new source for harvesting intact and alive human nerve terminals to study human synaptic transmission has emerged.

In the present study we aimed to develop a method for the enrichment of resealed, alive synaptic structures and growth cones from NOs. We developed a differential centrifugation (DC) protocol and used mass spectrometry-based quantitative proteomics to document the enrichment of synapse specific proteins. The method was validated by enrichment of synaptosomes from adult human and mouse brain tissues. We furthermore applied the DC protocol on two established NO models – human guided dorsal forebrain organoids (FBOs) after 100 days of differentiation and air-liquid interface cerebral organoids (ALI-COs) after 90 or 150 days of differentiation. Here we showed a clear enrichment of growth cone and synapse specific proteins. TEM evaluation of the DC fractions from ALI-COs at day 90 and 150 showed the presence of growth cone structures. At day 150 of differentiation, TEM analysis also revealed the presence of synaptosomes, however to a lower extent than observed using for example Percoll gradients, which was likely due to the immaturity of NOs compared to adult brain tissue. Furthermore, we used TiO_2-based phosphoproteomics to investigate the changes in protein phosphorylation upon KCl stimulation of the isolated synaptosome fractions. Our results indicate viability of the isolated synaptic structures/growth cones. Overall, the DC protocol can be used for enrichment of synaptosomes from various brain tissues, especially useful for low sample amounts, and for proteomic studies of normal and disease-related synapse development in human NOs, especially when applying the ALI-CO culture system.

16P11.2 DELETION AFFECTS THE TRANSCRIPTOME AND THE LIPIDOME OF HUMAN IPSC-DERIVED NEURONS AND BRAIN ORGANOIDS

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16p11.2 deletion (16pdel) syndrome is caused by a heterozygous deletion of 740kb DNA region encoding multiple genes playing important roles in brain development and function. Patients with the 16pdel suffer from developmental delay and features of autism spectrum disorder. The molecular mechanisms mediating these symptoms are still poorly understood. To improve our understanding of these mechanisms, we developed 2D neuronal cultures and 3D brain organoids from human 16pdel iPSC clones generated by the CRISPR-Cas9 genome editing method versus their isogenic controls and comprehensively analyzed their transcriptome, lipidome, and electrophysiological activity. Here, we show that 16pdel extensively affects gene expression in organoid neural stem cells, intermediate neuroprogenitors, neurons, and astrocytes, in both cell type-specific and non-specific manners. Among the affected genes are those that regulate neurogenesis, mitochondrial and lipid metabolism, and protein translation, suggesting that 16pdel may affect energy metabolism and synthesis of key components of cellular and organelle membranes. In turn, lipidomics analysis of both neuronal cultures and brain organoids found substantial decrease in the abundance of several lipid classes, particularly the triglycerides and phosphatidylglycerols. Mass spectrometry imaging was used to study the effect of 16pdel on the spatial distribution of metabolites and lipids within the organoids at different time points. Altogether, the comprehensive profiling of brain organoids in 16pdel at the molecular level will contribute to our understanding of the mechanisms leading to 16pdel syndrome phenotype.

IDENTIFYING HUMAN-ENRICHED UNIPOLAR BRUSH CELLS IN THE DEVELOPING HINDBRAIN

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Hindbrain neural progenitor cells play key roles in the evolution of the human brain, brain development, and the origin of pediatric brain cancers. The human rhombic lip progenitor zone is uniquely expanded into a rhombic lip subventricular zone not seen in mice or macaques, which generates disproportionately more *TBR2*-expressing progenitors[1,2]. The genetic determinants of this expansion are unknown. Developmental arrest of hindbrain neural progenitors is hypothesized to cause medulloblastoma (MB), a malignant childhood hindbrain cancer lacking targeted molecular therapies. Of the four molecular consensus subgroups recognized by the WHO, ~35% of cases ("Group 4 MB") resemble developing *TBR2*-expressing neurons stuck in a proliferative state. There are no *in vitro* models of Group 4 MB, limiting research ability.

To identify cell states unique to human neurodevelopment and potentially the cell-of-origin for Group 4 MB, we integrated published single-cell transcriptomes[3–5] from the developing human and mouse rhombic lip (human: N=20; 26,094 cells; 11-21 post-conception weeks; mouse: N=37; 55,449 cells; E10-P14; 16,755 orthologues; canonical correlation analysis). We reclustered *TBR2*-expressing unipolar brush cells (UBCs) and identified two cell populations disproportionately enriched in humans (UBC1 and UBC2; log2(FD)>1 and Q<0.05, scProportionTest). UBC1 appear to be less differentiated than UBC2 (Monocle 3), demonstrate upregulated FOXP2 and CNTNAP2 (Seurat), and upregulated genes converge on neurodevelopmental pathways (g:Profiler, $Q < 0.05$). Top regulons predicted to drive UBC1 cell state include OTX2, SOX4, SOX11, and BACH1 (pySCENIC). In agreement with previous research[6], we find *SOX4* and *SOX11* upregulated in Group 4 MB single-cell transcriptomes[3], and SOX4 and SOX11 are among the top regulons in a cluster of Group 4 MB cells (pySCENIC). We are generating hIPS-derived cerebellar organoids[7] to ascertain if perturbing these transcription factors alters neurogenesis in this model. This work may identify gene regulatory networks that drive evolutionarily-recent aspects of human hindbrain development, and may help create a laboratory model for Group 4 MB.

1. Haldipur, P. et al. *Science* **366**, 454–460 (2019). 2. Hendrikse, L. D. et al. *Nature* **609**, 1021–1028 (2022). 3. Vladoiu, M. C. et al. *Nature* **572**, 67–73 (2019). 4. Sepp, M. et al. *Nature* **625**, 788–796 (2024). 5. Aldinger, K. A. et al. *Nat. Neurosci.* **24**, 1163-75 (2021). 6. de Bont, J. M. et al. *Neuro. Oncol.* **10**, 648–660 (2008). 7. Atamian, A. et al. *Cell Stem Cell* **31**, 39–51.e6 (2024).

MAPPING FUNCTIONAL ENHANCERS IN THE DEVELOPING HUMAN HINDBRAIN

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The rhombic lip neurogenic niche generates all glutamatergic neurons in the hindbrain. The human rhombic lip has an expanded subventricular zone not seen in mice or macaques[1]. Disrupted differentiation of this region is hypothesized to give rise to subtypes of medulloblastoma (MB), the most common pediatric CNS cancer[2-3]. Barring specific instances, the functional consequence of most non-coding genetic variation in these tumours remains unknown. Enhancers are context-dependent DNA elements that regulate gene expression to establish cell identity, and can be reprogrammed in tumours to drive aberrant expression. The goal of this project is to identify functional enhancers in human neurodevelopment and those of evolutionary or disease relevance.

To map enhancer locations in these small cellular compartments, we generated DNA methylomes from the human rhombic lip ventricular zone ($SOX2^{+}KI67^{+}$) and the subventricular zone ($SOX2^{-}KI67^{+}$) using laser-capture microdissection and Enzymatic Methyl-seq (N=9 methylomes, 16.2 +/-1.6 post-conception weeks). 9,855 loci were differentially methylated (DMR) between the two compartments (DSS), and the top three transcription factor motifs enriched in these loci were known neurodevelopmental regulators NEUROD1, NEUROD2, and ATOH1 ($q < 0.05$; AME; 334 TFs total). To characterize DMRs, we integrated the methylomes with human fetal cerebellum H3K27ac and H3K4me3 ChIP-seq (N = 4 maps). Consistent with enhancer enrichment, we found that DMRs were enriched in H3K27ac peaks and were depleted in H3K4me3 peaks, relative to length-, GC-, and mappability-matched loci (permutation, $p < 0.001$). DMRs were also enriched in human accelerated regions ($p < 0.001$) and in recurrent copy number aberrations in 485 Group 3 and Group 4 MB tumours[4] ($p < 0.001$). To functionally validate these enhancers, we are now conducting a pilot lentivirus-based massively parallel reporter assay in hiPSC-derived neural progenitors, with 1,900 non-promoter DMRs. This work will lay the foundation to understand gene regulatory networks driving human hindbrain neurodevelopment and dysregulation in disease.

1. Haldipur, P. et al. Science 366, 454–460 (2019).
2. Hendrikse, L. D. et al. Nature 609, 1021–1028 (2022).
3. Smith, K. S. et al. Nature 609, 1012–1020 (2022).
4. Northcott, P. A. et al. Nature 488, 49–56 (2012).

CEREBRAL ORGANOID AS A TOOL TO EVALUATE THE NEURAL OPIOID TOXICITY

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Organoids represent a model of choice for the study of developmental processes, neurodegenerative and genetic pathologies but also the impact of molecules on brain development. If the use of analgesic treatment with opioids effectively relieves pain, their increasingly widespread and sometimes misused use now poses a public health problem with risks of addiction and adverse effects. The long-term impact on brain development has not yet been evaluated.
By taking advantage of the development of cortical organoids during a long kinetics of more than 70 days, we evaluated the action of Tramadol and its main metabolite (M1) at different concentration on both the structure of organoids and the expression of specific markers as well as on the global transcriptomic profiles.
Following these treatments, it appears that structural alterations are observed, in particular with the disappearance of rosettes, specific to the three-dimensional organization of organoids as well as modification of the distribution of early and late markers such as SOX2, PAX6, NESTIN, OLIG2, etc. among others. Transcriptional analyzes confirm significant changes in gene expression profiles both at moderate doses, but also at quite low doses like those found in patients.
With these new data, it appears that tramadol and its metabolite M1 very significantly modify and disrupt the development of cortical organoids, which could suggest a similar effect during the development of the fetal brain. These alterations could have a long-term impact on the brain and associated cognitive faculties.

GENERATING BRAIN ORGANOIDS FROM STEM CELL-DERIVED EMBRYO MODELS

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Our research interests are broadly focused on studying the roles of centrioles and cilia in establishing correct cellular neuroanatomy required for brain development and function. We aim to use brain organoids to specifically understand how centrioles regulate spatial events of neuronal cell divisions and how aberrant centriole function leads to microcephaly. Centrioles also function as basal bodies of primary cilia that mediate signaling events which are perturbed in ciliopathies. To this end, we are attempting to generate brain organoids from stem cell-derived mouse embryo models. Such embryo models recapitulate embryogenesis to develop progenitors of all brain regions amongst other developmental and organogenesis hallmarks. We are optimizing the establishment of brain organoids from the central nervous system progenitor organ of these embryo models to enable their continued growth in culture to mimic brain development. Given the evolutionary conservation of key centriolar components, many centriolar mutations and aberrations have clinical relevance manifesting as microcephaly and ciliopathy disorders. This makes disease modelling in human brain organoids an attractive complementation to our studies in the mouse brain model.

CORTICAL ORGANOID MATURATION OVER 670 DAYS USING SINGLE CELL RNA-SEQUENCING

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Cortical organoids have emerged as valuable model systems for studying human brain development and disease. Commonly used 3-6 month differentiation protocols reliably produce cell types of the early to mid-fetal cortex, including radial glia, intermediate progenitors, and upper and lower layer excitatory neurons. However, neurons are characteristically immature at these time points, and later born cell types such as astrocytes and oligodendrocytes are rarely detected. While previous studies have conducted longer differentiations (up to 600 days) to study later periods in human development, these approaches used bulk RNA-sequencing that lacked cell type-specific resolution or insights into proportional shifts among cell types over time. We sought to understand neuronal maturity and response to electrophysiological stimulation as well as changes in cell type composition across time in long-term differentiated organoids.

We tracked the development of cortical organoids over 670 days (1.8 years). Organoids were differentiated using a mini-spinning bioreactor based protocol and collected at multiple time points (14, 56, 84, 460, 533, 600, and 670 days of differentiation) for single-cell RNA sequencing (scRNA-seq) to assess changes in gene expression and cell type proportions. We subjected long-term differentiated organoids to either vehicle or KCl to stimulate electrophysiological activity. In total, we analyzed 234,981 cells from 47 organoids after quality control. As detected in previous work, differentiations up to 84 days were composed almost entirely of decreasing proportions of radial glia and increasing proportions of excitatory neurons across time. After prolonged differentiation beyond one year, astrocytes (GFAP, AQP4, VIM) predominated the organoid composition with a low but detectable proportion of excitatory neurons (~6.8%). These cell type proportions were reproducible across organoids of a given age (~6 organoids per time point), but changed across ages.

Overall we reveal that organoids recapitulate known developmental ordering of cell type birth with neurons predominating before astrocytes. We also show that unmodified differentiation protocols have limited representation of neurons after 1 year of differentiation, suggesting they may be outcompeted by astrocytes for resources or require increased concentrations of neurotrophic factors in the media. These results enhance our understanding of the cellular trajectory of cortical organoids during extended differentiation and highlight key considerations for optimizing differentiation timelines depending on the experimental goals.

LABEL-FREE CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF IPSC-DERIVED NEURAL ORGANOID DEVELOPMENT AND MATURATION

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Induced pluripotent stem cells (iPSCs) have provided an invaluable cell source for regenerative medicine and tissue engineering, enabling high-throughput recapitulation of complex human biology in vitro. Rapid advances in iPSC technology have led to widespread adoption for the development of in vitro neural electrophysiology models for drug discovery and safety screening. More recently, neural organoids generated from human induced pluripotent stem cells (iPSCs) have emerged as a model of the human brain to study the complex neural network activity observed during early brain formation. The objective of this work was to develop and validate a live-cell analysis workflow for the characterization of neural organoids in vitro. Whole-vessel live-cell imaging with the Omni was used to monitor iPSC colony formation and expansion in real-time. The Omni iPSC module was used to determine iPSC colony coverage and count to ensure consistent iPSC passaging and prevent spontaneous differentiation. Next, iPSC aggregates, known as embryoid bodies (EBs) were differentiated towards neural organoids. The Organoid analysis module was used to track the size and shape of EBs undergoing neural differentiation. On day 50+, neural organoids were transferred to a multiwell microelectrode array (MEA) plate and allowed to attach. Impedance measurements were used to quantify the attachment of the organoids to the substrate and microelectrodes, as a measure of cell viability and electrode coverage. Broadband (1 – 5000 Hz) electrophysiological data was acquired and then separately processed for action potential detection (200 – 5000 Hz) and low frequency oscillations (1 – 50 Hz). The emergence and maturation of neural organoid electrophysiological activity was tracked via these measurements of spiking activity and low frequency oscillations, coupled with the long-term monitoring of size via live-cell imaging. This new workflow combining live-cell imaging and MEA measurements supports the continued development of in vitro 3D models of neural function.

DEVELOPMENT OF A HUMAN STEM CELL MODEL OF NEUROFIBROMATOSIS TYPE 1 FOR ASSESSMENT OF OPTIC PATHWAY PHENOTYPES.

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Neurofibromatosis type 1 (NF) is a pediatric neoplasia of the nervous system, caused by germline mutations of the neurofibromin gene. Among characteristic pathological phenotypes, the development of optic pathway gliomas (OPGs) affects visual acuity or cause blindness among other ophthalmic features in symptomatic patients. Although transgenic rodent models of NF1 OPGs have helped elucidate disease pathogenesis, significant differences exist in the retina and optic nerves of rodents and primates, including differences in NF1 disease pathology. To address this shortcoming, we have developed a human induced pluripotent stem cell (iPSC) model that mimics many features of the optic nerve and supporting glial cells as a novel tool to characterize OPG-associated clinical heterogeneity and prognostic markers at cellular level. We introduced NF1-relevant single nucleotide polymorphisms (SNPs) into lines of iPSCs using CRISPR/Cas9 editing, resulting in paired disease models and isogenic controls. These cell lines were then differentiated to yield retinal organoids and astrospheres following established procedures to acquire retinal ganglion cells (RGCs) and astrocytes cultures, respectively. These RGCs and astrocytes were characterized for changes associated with the NF1 gene variants via gene and protein expression. The iPSC lines with NF1-associated gene variants were successfully generated and further analyses of these cell lines assess for the possibility of off-target effects of gene editing. These cell lines were also edited to express an RGC-specific mNeonGreen reporter for RGC identification, as well as the mThy1.2 cell surface antigen for subsequent RGC purification. RGC purification from retinal organoids results in highly enriched population of RGCs from all cell lines tested. Likewise, astrocytes were differentiated from both cell lines at high purity, which after maturation expressed variety of astrocyte specific markers. The NF1 variant astrocytes were found to be more proliferative compared to their isogenic controls. Initial experiments were then pursued to combine cell types in a novel, in vitro microfluidic platform mimicking some aspects of the optic pathway for the analysis of NF1 phenotypes. Experiments in progress will further assess changes in RGC morphological features such as neurite complexity as well as functional properties. Taken together, these results demonstrate the generation of human iPS cell lines with patient relevant NF1 gene variants as a disease model for neurofibromatosis type 1, as well as the use of these cells to begin to explore features associated with the OPG phenotype.

DEVELOPMENT OF A MULTIMODAL IMAGING-BASED PHENOTYPING PLATFORM

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Brain organoids are the current state of the art of human models for studying neurodevelopmental and neurodegenerative disease. They capture important aspects of brain tissue development and organization and have significantly advanced our understanding of how pathological conditions arise. Neurological disease phenotypes are highly complex and manifest on different scales, from the molecular to the tissue level. However, the variability of brain organoids in size and shape, as well as their limited accessibility for live-cell imaging, hinder the application of quantitative methods that can acquire, analyze and integrate imaging information on multiple scales. To address this issue, we have developed a multimodal imaging-based phenotypic platform for the detection and integration of complex phenotypes in cortical micropatterned organoids. Our micropatterned organoids are highly spatially reproducible and scalable, and facilitate the detection of subtle disease phenotypes as they are amenable to non-invasive live-cell imaging and unbiased image analysis pipelines. We employ a combination of live-cell labelling probes to investigate neuronal activity and lipid accumulation as well as label-free fluorescence lifetime imaging (FLIM) of auto-fluorescent metabolites to measure neuronal and astrocytic bioenergetics in isogenic Apolipoprotein E (APOE) models. APOE is responsible for the transport and redistribution of lipids throughout the body including in the central nervous system. There are three major isoforms of APOE – APOE2, APOE3 and APOE4. The latter represents the strongest genetic risk factor for the development of sporadic late-onset Alzheimer's disease (AD). APOE4 has been shown to impair lipid exchange between astrocytes and neurons, dysregulate neuronal calcium homeostasis and disrupt cellular metabolism. In our study we aim to elucidate the interplay between these observations and across the main cell types involved, which remains poorly understood. We find that our APOE isogenic micropatterned organoid models occupy discrete areas in the phenotypic space with the main differences observed in development of synchronized neuronal activity.

POU3F2 ACTS AS A MASTER REGULATOR OF NEURODEVELOPMENT AND NEURAL HOMEOSTASIS THROUGH ITS MODULATION OF WNT-MEDIATED PROGENITOR EXPANSION AND NEURONAL MATURATION

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Loss-of-function mutations in the transcription factor POU3F2 have been identified in individuals with neurodevelopmental disorders. To elucidate the mechanistic role of POU3F2 in human neurodevelopment, we induced POU3F2 disruption in human neural progenitor cells (NPCs) and in post-mitotic neurons. Mutation of POU3F2 in NPCs causes reduced baseline canonical Wnt signaling and decreased proliferation, resulting in premature specification of radial glia. Additionally, POU3F2 levels across genetically diverse NPCs significantly associate with baseline canonical Wnt signaling. Through a series of unbiased analyses, we show that SOX13 and ADNP are transcriptional targets of POU3F2 which mediate POU3F2's effects on Wnt signaling in human NPCs. Reduction of POU3F2 in postmitotic neurons resulted in an upregulation of pathways related to synaptic maturation and a concurrent increase in synapse area, as measured via immunostaining and colocalization of presynaptic and postsynaptic marker puncta. Despite this, POU3F2MUT iNs were found to exhibit reduced neurite outgrowth during early maturation and reduced neuronal activity, as measured by multi-electrode array. Finally, we describe five individuals with autism spectrum disorder that harbor loss-of-function mutations in POU3F2, enhancing the genetic evidence for its critical role in human neurodevelopment. Together, these studies define POU3F2 as an activator of canonical Wnt signaling and mechanistically link two high-confidence autism genes, ADNP and POU3F2, in the regulation of neurodevelopment.

CEREBRAL ORGANOID SENSORY AND MOTOR INTERFACING

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Background Cerebral organoids afford an innovative way to study 3D neuronal networks, learning, and adaptation. Cerebral organoids, assembloids, and living neurons express the genetic and developmental pathways of the donor, allowing access to the genetic spectrum of human intelligence via multiple donors.

Proposal Biologically combine human creative capabilities, the speed/efficiency of computers, and artificial intelligence to create faster-than-human decision making. Make cultured neurons more akin to a living brain. Introduce sensory stimulation via cameras, microphones, and somatosensory devices. These visual, auditory, and tactile cues are the same interactions given to severely disabled patients via computer brain interfacing, restoring their sight, audition, and tactile sensation. One might argue these cues are essential for fetal and cultured neuronal development. To complete the feedback loop, artificial intelligence analyzes motor/output responses from the organoids in the form of interfaced light bulbs, vibration, and sound technology. Sequential steps can lead to a process of self-awareness.

Methods Interface organoids with a camera (for “eyes” to the world), a light bulb (for self-expression), and a sensor near the light bulb (for interactive behavior with a researcher). The sensor near the light bulb triggers an electric pattern delivered to the organoid. This delivered pattern represents touch. During culture, cerebral organoids naturally produce spontaneous bursts of activity. One pattern of spontaneous motor activity from the organoid will be assigned the task of lighting a light bulb. The researcher will cover the light bulb with their hand after the bulb lights up. The organoids camera (eyes) conveys many things to the organoid. It conveys when the organoid lights its own bulb, when the researcher covers that bulb, and how the two things are related to the feeling of being touched. Testing the concept of mirror neurons, several sensors will be interfaced. As the researcher pets a cat, in parallel they will set off the series of sensors conveying the sense of being pet. Use multiple designated steps to elicit a sense of self, and the awareness of the world separate from self.

Conclusion I will explore the foundations of consciousness, develop associative learning, and establish basic communication to assess the potential functionality of neuronal networks within cerebral organoids. Bridging bioengineering, sensory substitution, neuroprosthetic technology, and child development education, this research advances our understanding of plasticity in cultured neurons to facilitate bioengineered neural systems into computing technology and seeks to advance neural prosthetics.

RESTORING DEVELOPMENTAL DYNAMICS IN BOTTOM-UP SOMATOSTATIN INTERNEURON CIRCUITS IMPROVES SENSORY DEFICITS IN FRAGILE X MICE.

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Early-generated circuits are critical for the maturation of cortical network activity and the formation of excitation/inhibition (E/I) balance. This process involves the maturation of specific populations of inhibitory neurons. Parvalbumin (PV)-expressing neurons are involved in sensory-driven cortical activity in adulthood and have been associated with E/I impairments observed in neurodevelopmental disorders. However, somatostatin-expressing (SST) neurons have recently been shown to regulate PV neuron maturation by controlling neural dynamics in the developing cortex. SST neurons are involved in transient bottom-up circuits during development: they transiently both receive thalamocortical inputs and form translaminar outputs to layer 4 sensory neurons. Yet the implications of transient connectivity in neurodevelopmental disorders are unknown. Here, we show that bottom-up inputs and outputs of SST neurons are persistent rather than transient in a mouse model of Fragile X syndrome. The restoration of the transient dynamics using chemogenetics corrects for fragile X-associated dysfunctions in circuit maturation and sensory-dependent behavior. Overall, our findings unveil the role of transient dynamics in controlling downstream maturation of sensory functions.

DCHS1 MODULATES FOREBRAIN PROPORTIONS IN MODERN HUMANS VIA A GLYCOSYLATION CHANGE

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Understanding the evolution of human cognition and social behavior is key to appreciating what makes modern humans unique. Comparisons with Neandertal and Denisovan genomes reveal that some genetic changes, although small, likely had significant effects on brain structure and function. Studying these differences helps to trace the evolution of our advanced cognitive and social abilities. In this study, we focused on a single nucleotide variant (SNV) in the *DCHS1* gene that is unique to modern humans and results in the loss of a conserved N-glycosylation site found in Neanderthals and other primates. Given that post-translational modifications (PTMs) such as N-glycosylation are critical for protein function and the role of *DCHS1* in brain development, we hypothesized that this loss may have affected modern human brain development. To test this, we used CRISPR/Cas9 to reintroduce the ancestral *DCHS1* variant into human induced pluripotent stem cells and generated neural organoids to model early brain development. Organoids with the ancestral variant exhibited a reduced neocortex-to-striatum progenitor ratio, as confirmed by single-cell RNA sequencing (scRNA-seq). This suggests that the loss of N-glycosylation in modern humans may have enhanced neocortical development at the expense of striatal structures, potentially supporting advanced cognitive abilities. Furthermore, the loss of N-glycosylation altered the interaction of DCHS1 with EPHA4, a key player in neuronal development, which may have contributed to the remodelling of neural progenitor populations and increased the neocortex-to-striatum ratio in modern humans. Comparative anatomical studies support this, showing that modern humans have a relatively larger neocortex compared to the striatum. This shift in brain structure has likely played a critical role in the evolution of complex cognitive and social behaviors. Our study is the first to demonstrate that a SNV affecting a post-translational modification in modern humans has affected brain development. These findings underscore how specific genetic changes have shaped the evolution of the modern human brain and provide a molecular basis for understanding how these changes have contributed to the emergence of advanced cognitive and social functions in our species.

DIFFERENTIATING THE EFFECT OF MUTATIONS FROM THE GENETIC BACKGROUND IN DRIVING NEURODEVELOPMENTAL AND NEURODEGENERATIVE DEFECTS IN COCKAYNE SYNDROME MODELED WITH NEURAL ORGANOIDS

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Cockayne syndrome (CS) is a rare monogenic disease with the unique feature of combining neurodevelopmental defects, neurodegeneration, and premature aging. The extent of clinical severity is highly heterogeneous ranging from congenital microcephaly to early adulthood onset. In a few cases, patients do not exhibit neurodegenerative and progeroid defects, while displaying UV-hypersensitivity similarly to CS patients, a condition known as UV-sensitive syndrome (UVSS). Despite well-characterized mutations impairing CSA or CSB proteins —central to DNA repair, transcription, and other critical cellular functions —the molecular mechanisms driving the variability in disease progression remain elusive. This unique clinical paradigm offers a powerful opportunity to unravel factors leading to premature ageing-related neurodegeneration, in the context of mutation and loss of the same protein. Using human iPSC-derived neural organoids (NOs), we have established and validated patient-derived models to investigate the contributions of CSB mutations in a human-relevant system, addressing the limitations of animal models.

By reprogramming skin fibroblasts from patients of varying clinical severity into iPSCs and differentiating them into NOs, in the lab we observed that CS organoids exhibit neural maturation defects (i.e. altered cytoarchitecture and defective neural rosettes, described in another abstract) that correlate with the clinical severity. Importantly, UVSS-derived NOs do not display these defects, highlighting the critical role of factors beyond the CSB mutation itself in the development of the disease.

To address the question of the heterogeneity of disease, isogenic iPSC-derived NOs from CS and UVSS patients, as well as healthy individuals were generated by introducing or correcting the respective CSB mutations. The CRISPR/Cas9 editing protocol was optimized to ensure the survival of single cells post-editing, enabling the successful generation of monoclonal populations.

Preliminary immunostaining with markers for neural progenitors, immature, and mature neurons revealed that “healthy” organoids carrying the CSB mutation did not exhibit significant alterations, in striking contrast with patient-derived CS organoids. This suggests that the genetic background, rather than the mutation alone, plays a key role in the manifestation of neural defects. Ongoing molecular and cellular analyses aim to identify the pathways that either trigger or protect against severe neurodegeneration, with the ultimate goal of developing targeted therapeutic strategies for CS and related neurodegenerative disorders.

DEFINING MOTOR NEURON PATHOLOGY IN CERVICAL SPINAL CORD INJURY USING A HUMAN SPINAL ORGANOID MODEL: FROM CELLULAR BIOMARKERS TO MULTIELECTRODE ARRAY ELECTROPHYSIOLOGY

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Approximately 17,000 new cases of cervical spinal cord injury (cSCI) occur annually in the U.S. Over 60% of patients experience significant spinal motor neuron (MN) loss that leads to severe impairment of vital motor functions such as reaching, grasping, and breathing, due to muscle denervation, atrophy, and fibrosis. Our clinical data indicate MN loss is detectable in 80% of patients via electrophysiology, with half experiencing severe loss that devastates the segmental neuromuscular system. However, the mechanisms underlying MN death after cSCI are poorly understood, limiting treatment options like diaphragm pacing and nerve transfers. To investigate these mechanisms, we developed a 3D in vitro model using cervical spinal organoids (cSCOs) derived from human embryonic stem cells (hESCs).

Traumatic cSCI causes rapid narrowing of the spinal canal, either through brief impulses typical of central cord syndrome or sustained compression from spine fractures, both disrupting normal spinal cord function. To simulate these events in vitro, we cultured cSCOs from HB9-GFP-expressing hESCs. Using a custom-built machine with an electromagnetic voice coil, we compressed the cSCOs to 65% of their height in 30 milliseconds. Culture media were collected two hours post-injury to quantify cell death biomarkers, including lactate dehydrogenase (LDH) and neurofilament light chain (NF-L). Additionally, cSCOs were stained with LysoTracker and imaged 24 hours post-injury to quantify lysosomes. Injured cSCOs were transferred to multielectrode array plates for electrophysiological recordings every other day using Maestro Pro (Axion BioSystems).

Cell death assays showed significantly elevated LDH and NF-L levels, along with increased lysosome presence, in compressed cSCOs compared to controls. Electrophysiological analyses revealed an initial decline in weighted firing and bursting frequencies immediately following injury, compared to uninjured cSCOs. However, four days post-injury, both parameters exhibited a marked increase, suggesting a dynamic response in neuronal activity during the recovery phase.

This model offers a platform to investigate the influence of patient genotype on MN death after cSCI, with implications for predicting patient prognosis and enhancing treatment options. Future studies will focus on genetic factors modifying neurotrauma outcomes and screening potential neuroprotective drugs.

INVESTIGATING HUMAN ASTROCYTE-INDUCED SYNAPTOGENESIS

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Astrocytes, the most abundant type of glial cells in the brain, are pivotal in synaptic development and function. Research using invertebrate and rodent models has consistently shown that astrocytes facilitate synapse formation by secreting synaptogenic factors, a process that is conserved across species. However, their increased size, neuronal contacts, and synaptogenic capabilities set human astrocytes apart. Foundational studies have shown that human astrocytes engrafted in the forebrain of mouse pups significantly enhanced plasticity in learning compared to wild-type controls, suggesting that human astrocytes maintain a heightened synaptogenic function. However, it is unknown what mechanisms underpin this phenomenon. We first confirmed that media conditioned by human astrocytes significantly increases synapse formation in human induced pluripotent stem cell (hiPSC)-derived neuronal cultures. Next, we used preliminary integrative analysis combining RNA-sequencing and secretome data from primary and hiPSC-derived human astrocytes to reveal 216 genes associated with upregulated pathways in human astrocyte secretome compared to those in mice. To determine what molecules are enriched in the human astrocyte secretome compared to mouse, in ongoing work, we are employing quantitative proteomics to uncover the specialized armoire of human astrocyte-secreted molecules. Moreover, we are screening these novel human astrocyte-secreted factors for their ability to amplify synapse formation and function, employing a combination of in vitro and in vivo techniques in both rodent and human cells. The successful completion of this work will elucidate human-specialized secreted proteins that drive and enhance synapse formation, ultimately improving our understanding of human brain cell biology, development, and evolution.

LONGITUDINAL STUDY OF MICROGLIA LIFE IN HUMAN FOREBRAIN ORGANOIDS

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Microglia, the immune cells of the central nervous system (CNS), originate from macrophage progenitor cells generated in the yolk sac. These cells migrate and differentiate into the brain around the fourth gestational week. Therefore, the integration of these non-ectodermal cells into brain organoids is essential for studying neuroinflammation-related neurodegenerative diseases and brain aging. The protocol to obtain regionalized brain organoids (RBO) directs neural differentiation and maturation through dual SMAD inhibition, which suppresses the endogenous differentiation of microglia. Several protocols have been published to include microglia in RBOs, but there are still significant challenges in determining the optimal timing for microglia inclusion to achieve a functional immunocompetent brain organoid.
In this study, we used forebrain organoids and iPSC-derived microglia to conduct a longitudinal investigation into the in vitro conditions that mimic microglial invasion in the developing brain. The key variables we considered included the age of the forebrain organoids, the developmental stage of iPSC-derived microglia, the timing of differentiation within the forebrain organoids, and the culture media used. We assessed various morphological parameters (such as microglia number, shape morphology, proliferation, and activation state) as well as functional parameters (including electrophysiology, phagocytic activity, and 2-photon imaging) to evaluate microglial functionality. Following our longitudinal analysis, we propose a new protocol to generate immunocompetent forebrain organoids. Our results indicate that microglial differentiation should begin only after the inclusion of macrophage precursor cells into the organoids and suggest that astrocytes play a crucial role in ensuring the proper functionality of microglia.

REAL-TIME NON-DESTRUCTIVE MONITORING OF HUMAN CEREBRAL ORGANOIDS VIA THE NANOLUC-HALOTAG REPORTER SYSTEM

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This study introduces a NanoLuc-HaloTag reporter system controlled by neuron-specific promoters to enable real-time monitoring of human neural stem cells, neurospheres, and cerebral organoids during differentiation and maturation. Adeno-associated virus (AAV) vectors were used to deliver these reporters, with AAV1 and AAV6 identified as the most effective serotypes for transducing human neuronal cells. Fluorescence imaging via HaloTag expression allowed for spatial and temporal analyses of neuronal differentiation, providing insights into the localization and subcellular distribution of FOXG1, a transcription factor critical for brain development. Additionally, quantification of a secreted form of NanoLuc in the culture medium correlated with FOXG1 levels, demonstrating the reporter system's sensitivity to endogenous gene expression during neural differentiation. Changes in synapsin expression were also observed, underscoring the system's ability to track neuron maturation. Cellular processes were effectively tagged with HaloTag ligands, enabling real-time visualization of neurite outgrowth. A single application of the HaloTag ligand generated consistent fluorescence signals for at least 45 days, facilitating non-invasive, continuous observations. By utilizing both luminescence and fluorescence modalities, this approach enhances the monitoring and quantification of protein dynamics and gene expression over extended periods, providing insights into neuronal interactions and brain cells' overall health and functionality. This system offers advantages over traditional techniques, improving the resolution and throughput of non-destructive assays based on 3D neural modeling, ultimately advancing brain research and drug screening.

INVESTIGATING HIV-INDUCED NEUROIMMUNE DYSREGULATION USING 3D- BRAIN ORGANOID MODEL

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Microglial dysfunction has long been associated with HIV-1 neuropathogenesis, yet the underlying cellular and molecular mechanisms remain unclear. Comprehensive analyses of postmortem frontal cortex (FC) tissues from HIV-1-positive and negative individuals indicate a higher microglial cell density in HIV-positive brains compared to HIV-negative controls. Additionally, microglial inflammatory nodules were observed, along with evidence of dendritic pruning and phagocytosis. Given that synaptodendritic damage is the hallmark of HIV-1-associated neurocognitive disorders (HAND), we propose to investigate the role of microglia in HIV neuropathogenesis using a multicellular 3D brain organoid model.

We developed an iPSC-derived neuroimmune organoid to evaluate this phenomenon in a more physiologically relevant context. Our preliminary results show that this model mimics human cortical cytoarchitecture and cellular composition and is amenable to HIV infection. Furthermore, *in vitro* phagocytosis assays using iPSC-derived microglia revealed a significant increase in the phagocytic capacity of pHRodo-labeled synaptosomes by HIV-infected or exposed microglia.

Our results suggest that HIV-1-infected and activated microglial activity parallels dendritic simplification, possibly through phagocytosis. This synaptodendritic damage could be one of the leading causes of loss of synaptic plasticity and cognitive impairments, observed in HIV-1-positive individuals. We are conducting further studies using our multicellular organoid model to examine the host and viral factors involved in microglial dysregulation and their contributions to the morphological and functional consequences in neurons.

MODELING NEURODEVELOPMENTAL AND PREMATURE AGEING DEFECTS IN NEURAL ORGANOIDS: REVEALING DEFECTIVE CORTICOGENESIS AND THERAPEUTIC RESCUE THROUGH OXIDATIVE AND NITROSATIVE STRESS MODULATION

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Cockayne syndrome (CS) is a genetic disorder characterized by a combination of neurodevelopmental defects, neurodegeneration, and premature ageing, all caused by a single gene mutation. These symptoms have traditionally been attributed to defective UV-induced DNA damage repair due to mutations in the CSA or CSB proteins. However, a subset of patients with similar genetic mutations display UV sensitivity but are strikingly unaffected by neurodegeneration and premature aging, and the disease is then called UV-sensitive syndrome (UVSS). The molecular mechanisms underlying this resilience remain unclear.

We have shown that defects and altered pathways in Cockayne syndrome cells[1], including epigenomic changes in neural genes[2], are strongly linked links to physiological ageing, suggesting that CS is a robust model for studying studying age-related degenerative processes.

Using iPSC-derived neural organoids (NOs) from patients with varying disease severities —including resilient UVSS — we demonstrate aberrant corticogenesis independent of DNA repair deficits, and directly correlating with clinical symptom severity. CS-affected NOs exhibit impaired neural rosette formation, accelerated early neurogenesis, and incomplete neuronal differentiation, leading to abnormal corticogenesis.

Remarkably, modulating oxidative and nitrosative stress rescued these neurodevelopmental abnormalities, restoring proper neural rosette formation and neurogenesis. Our data point to transcriptional dysregulation driven by oxidative and nitrosative stress, rather than DNA repair deficits, as the central mechanism underlying the disease pathology.

Our findings reveal a novel mechanistic link between stress response pathways and neurodevelopmental perturbations leading to early-onset neurodegeneration. These findings have implications for novel therapeutic insights for neurodegenerative and premature aging disorders.

[1]Crochemore et al, 2019, Nature Comms, PMID: 31811121
[2]Crochemore at al, 2023, Aging Cells, PMID: 37688320

TCF4 MUTATIONS CAUSE DYSREGULATION OF THE OLIGODENDROCYTE LINEAGE IN A HUMAN MODEL OF AUTISM SPECTRUM DISORDER.

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Pitt-Hopkins Syndrome (PTHS) is a rare form of autism spectrum disorder characterized by severe developmental delay. PTHS is caused by de novo mutations in TCF4, a key transcription factor in multiple neurodevelopmental programs. Previous studies using PTHS mouse models showed dysregulation in the density and maturity of oligodendrocytes which resulted in myelination deficits, however, confirmation of these phenotypes in a human context is currently lacking. We have generated patient-derived iPSCs that harbor various types of mutations in TCF4 that result in the expression of putative dominant-negative proteins due to point mutations in the bHLH domain, or haploinsufficiency due to de novo stop codons. Oligodendrogenesis is achieved with a 90-day 2D differentiation protocol. All experiments have been performed with 2 clones per iPSC line and at least 3 independent replicates.mRNA levels of pan-oligodendrocyte markers OLIG2 (Ctrl vs Haploinsufficiency $p=0.0015$, Ctrl vs Point mutant $p=0.0019$) and SOX10 (Ctrl vs Haploinsufficiency $p<0.0001$, Ctrl vs Point mutant $p<0.0001$) were downregulated after 75 days in culture. Moreover, oligodendrocyte progenitor cells (OPCs) marker PDGFRa was reduced in both genotypes (Ctrl vs Haploinsufficiency $p=0.0006$, Ctrl vs Point mutant $p=0.0011$) and CSPG4, encoding for the protein NG2, was reduced in the point mutant lines (Ctrl vs Point mutant $p=0.0011$). After induction of maturation, the expression of SOX10 continued to be diminished (Ctrl vs Haploinsufficiency $p=0.0135$, Ctrl vs Point mutant $p=0.0254$). At the same time, mature markers such as MOG and pre-myelinating marker BCAS1 show a slight reduction in mRNA expression in both genotypes. These results suggest TCF4 to be a critical regulator of OL development, particularly during the expansion of the progenitor pool, and therefore could predict myelination deficits as a potential pathophysiological mechanism underlying neurodevelopmental abnormalities in PTHS.

THE ROLE OF ST6GAL1 IN EARLY NEURODEVELOPMENT

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Sialylation is a key post-translational modification involved in many biological functions, particularly in the central nervous system (CNS), where it modifies glycan chains on membrane proteins and sphingolipids. Mutations in genes related to sialic acid metabolism are risk factors for several neurodevelopmental and psychiatric disorders. Sialic acid has also been hypothesized to play a role in neurodegenerative diseases.
Sialic acid can be attached to galactose or N-Acetylgalactosamine in different linkage positions: 2-3, 2-6, and chains of polysialic acid in 2-8 linkage. A few studies have provided evidence for a high proportion of 2-6 linked sialic acid in the human brain, compared to that of other hominids. Sialic acid are attached to galactose or N-Acetylgalactosamine on glycan chains by sialyltransferases, of which Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) performs the majority of 2-6 sialylation linkage to galactose. Neuronal ST6GAL1 levels are increased in humans compared to other hominids. In vivo studies of ST6GAL1 knock out mice have shown that ST6GAL1 has limited involvement on early neural development in mice but the sialyltransferase may be of particular importance for human neurodevelopment. To our knowledge, the effects of 2-6 sialyltransferases on neurodevelopment has not previously been studied in a human model system.
We will study the role of ST6GAL1 in early human neural development by growing brain organoids from human induced pluripotent stem cells, with and without knockout of ST6GAL1. The organoids will be differentiated for up towards 200 days. Data from organoids harvested after 6, 20, and 40 days of differentiation will be included in the poster. The proteome and sialiome of the organoids will be analyzed with a mass spectrometry based in-house developed workflow. Based on these results, confirmatory western blotting, and immunocytochemistry analyses will be performed. Immuncytochemical stainings will also be performed to evaluate the cellular composition and organisation of organoids.
The results from our study will inform us on if and how one type of sialylation regulates and controls neurodevelopment. By better understanding the role of 2-6 sialylation in the developing brain, we will gain an improved understanding on how sialylation may influence human diseases of the CNS.

FUNCTIONAL CHARACTERIZATION OF HIPSC-DERIVED STRIATAL ORGANOIDS FROM DYSTONIA PATIENTS USING HIGH-DENSITY MICROELECTRODE ARRAYS

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Introduction: Dystonia is a hyperkinetic movement disorder, characterized by involuntary muscle contractions that result in abnormal movements or postures. Its aetiology is frequently linked to single-gene mutations leading to network dysfunctions in the deep brain nuclei of the basal ganglia. In particular, the striatum, which plays a critical role in motor control through its extensive connectivity with cortical and subcortical regions, has been implicated in the pathophysiology of dystonia. However, the exact contribution of intrastriatal network dynamics to the functional alterations observed in dystonia patients remains poorly understood.
Objectives: In this study, we employ patient-derived striatal organoids to investigate various forms of monogenic dystonia. We combine this approach with high-density microelectrode array (HD-MEA) technology, which allows for non-invasive extracellular electrophysiological recordings at a high spatiotemporal resolution over extended time. Our goal is to perform a systematic functional characterization of striatal organoids derived from dystonia patients and healthy subjects to enable detailed electrophysiological phenotyping, identify disease-specific biomarkers, and gain insights into the underlying molecular mechanisms driving functional abnormalities.
Methods: Striatal organoids were generated from induced pluripotent stem cells (iPSCs) of patients with monogenic or idiopathic dystonia and healthy subjects with a novel protocol adapted from published methods. At 50 days in vitro, striatal organoids were sectioned and cultured at air-liquid interface (ALI) to promote long-term viability. After 2 weeks at ALI, slices were plated on HD-MEAs and spontaneous neuronal activity was recorded. Finally, an established analysis pipeline was applied to infer a wide range of extracellular electrophysiological features from striatal organoid recordings and to characterize striatal organoids at the subcellular, cellular, and network level.
Results and conclusions: Combining human iPSC-derived striatal organoids with HD-MEA electrophysiology represents a significant methodological advancement in understanding the functional alterations contributing to dystonia. Preliminary results obtained from long-term HD-MEA recordings of developing striatal brain organoids reveal robust spontaneous electrical activity, including single-cell spiking and network bursts. Additionally, we found that organoids could be reliably maintained on HD-MEAs over several weeks in vitro, suggesting that our protocol is suitable for modeling functional network formation and synaptic functionality in striatal organoids over extended time. These findings support the validity of the organoid model and provide a solid foundation for further investigations into potential electrophysiological phenotypes and biomarkers of a human cellular model of dystonia.

MECHANICAL PROPERTIES OF HESC-DERIVED SPINAL CORD ORGANOIDS

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Neural organoids are frequently integrated with devices that pierce them, grip them, load them, or otherwise mechanically interface with them to control their growth, acquire data, or apply environmental stresses. Quantitative mechanical design of such devices is currently difficult because the mechanical properties of neural organoids are not well-understood. To address this deficit, we used microindentation to measure the mechanical properties of spinal cord organoids across a range of ages. A Chiaro microindentation instrument (Optics 11 Life) was used to apply elastic and viscoelastic microindentation protocols to spinal cord organoids derived from human embryonic stem cells (hESCs) at four ages: 78 (n=9), 179 (n=8), 304 (n=9), and 333 (n=4) days old. The indenter tip was a sphere of radius 253 μm. Elastic measurements involved four indentations to a peak load of 0.6 μN at the top center of each organoid. The Young's modulus, E, was determined by fitting the force-displacement relationship from Hertz's solution for the contact of two elastic spheres to the experimental force-displacement data. A ramp and hold stress relaxation protocol was used to measure viscoelastic properties. The organoid was indented to a depth of 15 μm and held at that position for 180 seconds while force was measured continuously. The relaxation function of the organoid, G(t), was convolved with Hertz's solution for the force using a hereditary integral to create an expression for the force history. This expression was fitted to the experimental force and displacement histories in Matlab to determine the coefficients of a 3 term Prony series defining the relaxation function. Organoid size and shape in the horizontal plane were quantified by fitting an ellipse to the edge of the organoid in an image from an inverted microscope. The height of the organoid was quantified by finding the top of the organoid and the base of the dish using the microindenter. The height and semi-major axes in the horizontal plane were used to compute the sphericity, a parameter that equals 1 if the organoid is spherical and becomes increasingly less than 1 as the organoid becomes more ellipsoidal. Young's modulus, size, and sphericity varied with age but not in a monotonic fashion. Young's modulus rose as sphericity increased and fell as size increased. The organoids were highly viscoelastic, i.e., their apparent stiffness declined dramatically over time. This novel, non-destructive method for characterizing spinal cord organoid biomechanics will inform their use in experimental devices and biomechanical simulations.

RIGOR AND REPRODUCIBILITY OF CELLULAR AND ELECTROPHYSIOLOGICAL ANALYSIS IN HUMAN BRAIN ORGANOID RESEARCH

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Cortical organoids derived from human induced pluripotent stem cells (iPSCs) have become increasingly useful in the study of human brain development and neurodevelopmental disorders because they resemble aspects of in-vivo human brain formation. With the increasing applicability of organoids in neuroscience research, it is crucial to establish a standardized quantitative framework that allows for rigor and reproducibility within and across laboratories. To address this challenge, together with the IDDRC consortium, we have conducted an in-depth analysis of published quantitative methods for brain organoids. We have recommended minimum and ideal standards for reproducible quantification of organoids. Finally, we have applied some of these methods and recommendations to assess cortical organoids differentiated from iPSCs of individuals diagnosed with fragile X syndrome, the most commonly inherited neurodevelopmental disorder. With an established recommended quantitative framework for organoid research, we expect to increase reproducibility across laboratories, increasing the usefulness of organoids in the study of brain diseases.

INVESTIGATING NEUROINFLAMMATION IN BIPOLAR DISORDER USING IPSC-DERIVED GLIAL MODELS

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Bipolar disorder (BD) is a severe and complex psychiatric condition that affects approximately 3% of the global population, marked by recurrent episodes of mania and depression. Although the precise etiology of BD remains elusive, increasing evidence suggests that neuroinflammation may be a key factor in its pathogenesis. Numerous studies have consistently demonstrated elevated levels of pro-inflammatory cytokines in the peripheral blood and cerebrospinal fluid of individuals with BD. However, post-mortem brain studies have produced conflicting data regarding astrocyte and microglia numbers, as well as their activation states. These neuroinflammatory processes are thought to contribute to several core features of BD, including mood dysregulation, cognitive impairments, and treatment resistance. Despite these findings, the exact mechanisms by which neuroinflammation contributes to the development and progression of BD are still poorly understood.

In this study, we used induced pluripotent stem cell (iPSC) technology to model BD in a developmental context by generating astrocytes and microglia from patient-derived iPSCs. Our results revealed an exaggerated inflammatory response to pro-inflammatory stimuli in BD iPSC-derived glial cells. Furthermore, we published that astrocytes derived from BD iPSCs displayed significant alterations in gene expression related to synaptic function and inflammatory pathways, as well as impaired neurotrophic support for neurons. These findings provide new insights into the role of glial dysfunction in BD, suggesting that disruptions in glial cell function may contribute to both the neuroinflammatory and neurodevelopmental abnormalities observed in this disorder.

INVESTIGATION OF THE ROLE OF DYSLEXIA-ASSOCIATED *KIAA0319* IN HUMAN BRAIN DEVELOPMENT

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Purpose – Dyslexia is a reading disability that impacts the brain's ability to process written language. Previous research has identified KIAA0319 as a gene associated with dyslexia susceptibility. However, the specific role of KIAA0319 in neurodevelopment and how its variants contribute to dyslexia remain unclear. This study aims to explore the role of KIAA0319 in neurodevelopment using a cortical organoid model derived from human embryonic stem cells (hESCs).

Method – To investigate KIAA0319's role in neurodevelopment, we utilized a CRISPR-Cas9 system to knockout KIAA0319 in hESCs. Differentiation protocols were implemented to create cortical organoids from both wild-type and KIAA0319 knockout hESCs. We characterized the cortical organoids through imaging and gene expression analyses.

Results – The knockout of KIAA0319 in the cortical organoid model resulted in significantly reduced expression of the KIAA0319 gene. The knockout organoids exhibited disrupted morphology and were smaller than their wild-type counterparts. Additionally, these organoids showed an 8-fold increase in the expression of the neural progenitor marker PAX6 and a 70% decrease in the mature neuron marker MAP2, indicating that neuronal differentiation is impaired in the absence of KIAA0319.

Conclusions – These findings suggest that KIAA0319 is involved in neurogenesis, which may contribute to defects in neuronal migration. Future research will focus on examining global gene expression changes and neuronal connectivity in KIAA0319 knockout cortical organoids to gain deeper insights into how the knockout affects neuronal differentiation and migration. This research is crucial for enhancing our understanding of the mechanisms underlying dyslexia.

DEVELOPMENT AND CHARACTERIZATION OF VASCULARIZED BRAIN ORGANOIDS: INSIGHTS INTO HIV-1 INDUCED DYSREGULATION OF BLOOD-BRAIN BARRIER AND VIRAL ENTRY INTO CNS

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Advances in stem cell biology and tissue engineering have facilitated the creation of brain organoids from induced pluripotent stem cells (iPSCs), providing valuable models for studying brain development and neurodegenerative diseases. However, the lack of vascularization in these organoids limits their viability, as well as nutrition and oxygen delivery. Therefore, we aimed to develop vascularized brain organoids using both normal iPSCs and iPSCs overexpressing the ETS translocation variant 2 (ETV2). This model will also allow us to investigate how HIV-1 disrupts the blood-brain barrier (BBB) and affects vascular endothelial cells in the central nervous system (CNS). iPSC cocultures were differentiated into brain organoids using a modified protocol to enhance vascularization. The organoids underwent immunostaining with the endothelial marker CD31 and tight junction proteins to assess the presence and functionality of endothelial cells. For comparative analysis, sections of HIV-positive and HIV-negative human brain tissue from patients with known systemic inflammation were stained with CD31 and Claudin to identify alterations in endothelial marker expression and blood-brain barrier (BBB) integrity. Our results demonstrate the successful development of vascularized brain organoids, exhibiting robust expression of the endothelial marker CD31, which indicates functional vasculature. Comparative analysis with human brain tissue sections from HIV-infected individuals revealed alterations in tight junction proteins, endothelial cell activation, and changes in blood-brain barrier (BBB) permeability. These findings highlight the impact of HIV-1 infection on endothelial cells. The generation of vascularized brain organoids offers a valuable model for studying endothelial function and blood-brain barrier (BBB) pathology. This research highlights the potential of brain organoids in modeling the effects of infectious diseases on the BBB and in developing targeted therapeutic strategies to mitigate HIV-induced disruption of the barrier.

MAPT MUTATION EXACERBATES PATHOLOGY IN A CORTICAL ORGANOID MODEL OF TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) increases the risk of tauopathy, as does the V337M mutation in the microtubule associated protein tau (MAPT). This study hypothesizes that these effects synergize. Since the cortex is the primary site of post-traumatic tauopathy, we reproduced deformations typical of TBI in cortical organoids using a custom-built device and measured resulting pathology. We first characterized the model by studying the response of cortical organoids derived from the WTC-11 induced pluripotent stem cell (iPSC) line to trauma of increasing severity and how that response evolved over time. Mitochondrial membrane potential (quantified using tetramethylrhodamine (TMRM) staining) declined with increasing trauma severity. The decline appeared to be reversible as there was a trend towards recovery over a 7-day time course. Next, we studied the influence of MAPT mutations using 4 isogenic iPSC line pairs: two that isolated the V337M mutation, and two that isolated the IVS10+16 mutation. V337M and IVS10+16 both increase the risk of frontal temporal lobe dementia in patients. V337M is a coding mutation present in all forms of tau. IVS10+16 is a splice site mutation that does not affect the coding sequence of tau but increases expression of 4R tau. 4-month-old cortical organoids derived from these lines were traumatized by compressing and releasing them in a period of 30 milliseconds by either 25% or 50% of their initial height. At the 48-hour timepoint, trauma was found to reduce mitochondrial membrane potential and cell viability (quantified with calcein AM staining) and caused the release of lactate dehydrogenase (LDH), a marker of cell damage. The V337M mutation increased the release of LDH after trauma. Trauma increased tau hyperphosphorylation (quantified with western blots for CP13 and PHF1 normalized to total tau) and this effect was amplified by the V337M mutation. The influence of the IVS10+16 mutation was more modest and inconsistent than that of the V337M mutation. Next, we studied 6-month-old V337M isogenic organoids. These organoids were subject to severe trauma (compression by 50% of initial height). In the absence of trauma, the mutation did not affect cell viability, mitochondrial membrane potential, or LDH release. Trauma reduced cell viability and mitochondrial membrane potential and increased LDH release and these effects were amplified in mutants, indicating synergy between the V337M mutation and trauma. These results suggest that genetic predisposition may help explain variability in neurodegenerative outcomes among TBI patients and create opportunities to study the molecular mechanisms of post-traumatic neurodegeneration. It may one day be possible to estimate the risk of bad outcomes for people exposed to TBI (e.g. athletes, military personnel, elderly people with poor balance) based in part on MAPT mutations.

α7 NICOTINIC ACETYLCHOLINE RECEPTORS REGULATE RADIAL GLIA FATE IN THE DEVELOPING HUMAN CORTEX

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Exposure to nicotine (NIC) during pregnancy is associated with a reduction in fetal cortical grey matter volume, driven by vulnerable cell types and molecular pathways that are not clearly understood. There is evidence of acetylcholine signaling to fetal cortical germinal centers from cholinergic tracts, but the effect of activation of nicotinic acetylcholine receptors (nAChRs) in progenitor cells and radial glia (RG) of the developing human cortex remains unclear. We find two nACHR subunits, CHRNA7 and the human-specific subunit CHRFAM7A, expressed in SOX2+ progenitors, with CHRFAM7A highly enriched along RG apical endfeet. To explore potential functions of cholinergic signaling, we exposed dissociated primary cortical cultures to nAChR agonists, including NIC, or performed nAChR knockdown followed by bulk and single-cell (sc) RNA-sequencing. ScRNAseq revealed that downstream effects of NIC exposure included upregulation of semaphorin-plexin axon guidance associated genes in ventricular RG, outer Radial Glia (oRG) and excitatory neurons (ENs) at GW15-16. We also observed an upregulation of genes associated with chromatin silencing, DNA methylation, and DNA alkylation changes in RG at GW 19-22. Many genes critical for cortex development including DCHS1, SHANK3, LAMA5, NLGN2, LRP1, KIF1A, were downregulated in ENs following NIC exposure. While some DEGs are downstream of both CHRNA7 and CHRFAM7A, a large fraction of DEGs were unique to each nAChR subunit, suggesting a diversification of the regulatory networks controlled by CHRFAM7A during human cortical evolution. Using immunostaining, we observed that activation of nAChRs through agonists increased the number of SOX2+, HOPX+, and KI67+ RG and decreased NEUN+, deep layer CTIP2+ and upper layer SATB2+ neurons. ShRNA-mediated nAChR knockdown produced inverse phenotypic changes, with a reduction in RG cells and an increase in neuron number. Our results suggest that prenatal NIC exposure is able to change RG cell fate, which we hypothesize may be causing some of the clinical phenotypes observed in the offspring of smokers.

INSIGHTS INTO CELLULAR CONNECTIVITY AND NEURAL DYNAMICS IN PARKINSON'S DISEASE USING VENTRAL MIDBRAIN ORGANOID MODELS

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Cellular connectivity plays a critical role in regulating the central nervous system. Dysregulation of cellular connectivity is shown to be involved in various neurological conditions including Parkinson's disease (PD), which presents significant challenges for both society and healthcare systems due to its progressive nature and the burden it places on affected individuals and caregivers. Here, we utilized Ventral Midbrain Organoid (MiVO) models derived from induced pluripotent stem cells (iPSCs) of wild-type (SFC156-03-01, SFC856-03-04) and GBA1 mutation carrier lines (MK071-3, MK088-1) to explore the complexity of cellular connectivity in PD. MiVO were analysed for the expression of neural stem cell and mid-brain gene expression, focusing on Nestin, MAP2, TH, and EN1 markers. Our results indicate differential gene expression between GBA1 mutation and control lines at various developmental stages, providing insights into neural stem cell dynamics and neuronal differentiation relevant to PD. Further analysis using multi-electrode array (MEA) recordings showed that the GBA1 mutation lines exhibited significantly higher spontaneous neural activity compared to the control lines. This increased neural activity in the GBA1 mutation lines might be due to a compensatory mechanism, where the neural circuits attempt to maintain function in response to the loss of dopaminergic neurons that happens in PD. This increased activity could also be linked to underlying neuroinflammatory processes that are known to exacerbate PD. Future work involves the integration of MiVO with the vagal nerve, which is part of the enteric nervous system, using advanced microfluidic platform that allows the connectivity of organoids from different origins. Our approach aims to create a more comprehensive model that can mimic the complex interactions between the brain and gut. These models offer deeper insights into the mechanisms underlying PD, as well as its associations with both genetic and environmental factors.

MODELING CORTICAL SURFACE AREA HYPEREXPANSION IN AUTISM USING INDUCED PLURIPOTENT STEM CELL (iPSC) DERIVED ORGANOIDS

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Early cortical surface area hyperexpansion from 6-12 months of age has been observed in infants who later develop autism within the Infant Brain Imaging Study (IBIS), the largest longitudinal study of infants (>250 participants) at high familial risk for autism. While this research has provided critical insights into early brain development, the cellular and molecular mechanisms driving this overgrowth remain unknown.

To address this, our lab has generated induced pluripotent stem cells (iPSCs) from 115 IBIS participants: high-likelihood individuals who developed ASD (HL+), high-likelihood individuals who did not develop ASD (HL-), and low-likelihood individuals who did not develop ASD (LL-). These individuals have undergone extensive longitudinal neuroimaging between 6-24 months, comprehensive behavioral assessments, and rare and common variant genotyping.

We hypothesize that ASD cortical surface area overgrowth is driven by increased proliferative neural progenitor cells making early symmetric cell fate decisions during prenatal development. Using these iPSCs, we are generating human cortical organoids (hCOs) differentiated to 84 days. Our organoid differentiations are ongoing and we are blinded to diagnostic groups. However, preliminary data indicate stronger consistency of organoid cross-sectional area within donors compared to across donors (ICC= 0.909 (Day 14)). Larger cross-sectional area at 14 days and lower qPCR mesoderm scores at the iPSC stage predict higher organoid survival at 84 days, linking pluripotency to differentiation outcomes.

For lineage tracing, we are utilizing an ultra-high complexity lentivirus-based lineage tracing method called STICR ("Single Cell RNA Sequencing Compatible Tracer for Identifying Clonal Relationships"). We confirmed transduction of organoids through GFP fluorescence and optimized the Multiplicity of Infection (MOI) for Day 15 organoids, achieving a barcode diversity of 44 million assessed through sequencing.

By combining organoid models, single-cell RNA sequencing, lineage tracing, and clinical data, we aim to better understand the cellular and molecular mechanisms of ASD-related brain development in a deeply phenotyped iPSC population.

INVESTIGATING LINE-1 ROLE IN NEURODEGENERATIVE DISEASE

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LINE-1 retrotransposons make up roughly 17% of the human genome and have been implicated in various processes from influencing genomic evolution to potential roles in cancer and aging.
Interestingly, LINE-1 retrotransposons seem to play an important role in neural development, helping protect progenitor cells from premature differentiation, promoting preferential differentiation to neurons versus glia etc.
Conversely, transposable elements, including LINE-1, have been found to be de-repressed in multiple brain disorders, such as Alzheimer's Disease and Amyotrophic Lateral Sclerosis, with as of yet unknown role. LINE-1 has also been implicated in the age-related inflammation, known as '*inflammaging*'.
We aim to study the impact of LINE-1 during *in vitro* neuronal differentiation and in cultured mature neurons, using an artificial LINE-1 construct named "ORFeus", which has a unique DNA sequence, but a comparable amino acid sequence to native LINE-1 species. Our approach will utilize human H1 ES and KOLF2.1J iPS cells, differentiated to neurons either through growth-factor induced NSCs or through viral overexpression of NGN2, as well as human IMR-90 fibroblasts transdifferentiated into neurons via viral overexpression of NGN2 and ASCL1, without generating iPS cells.
We anticipate this work to contribute to our understanding of age-associated neurodegeneration and hopefully towards treatments to halt or even reverse it.

INCREASED GABAERGIC NEUROGENESIS IN HUMAN CORTICAL ORGANOIDS WITH SCHIZOPHRENIA-ASSOCIATED *SETD1A* MUTATIONS

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Schizophrenia (SCZ) is a debilitating psychiatric disorder, affecting approximately 1% of the global population. Estimates of the heritability rate of SCZ range around 80%, suggesting a strong genetic component. Loss-of-function mutations in *SETD1A* have been linked to both SCZ risk and other neurodevelopmental phenotypes. SETD1A is involved in chromatin remodeling through its function as a H3K4 methyltransferase, but how *SETD1A* loss-of-function mutations lead to altered brain function remains unclear. In the present study, we aim to gain insight into functional consequences of loss of SETD1A function through the neural differentiation of human induced pluripotent stem cells (hiPSCs) carrying SCZ-associated mutations in *SETD1A*. Genome editing was used to create isogenic hiPSC clones that carry either a frameshift or a splice variant mutation. These lines were differentiated to 2D neural networks and adherent cortical organoids. After differentiation, we noticed a two-fold increase in the number of GAD67-positive interneurons in the *SETD1A*$^{+/mut}$ neural networks compared to their isogenic controls, relative to the overall number of neurons, in three independently differentiated batches. We also differentiated these *SETD1A*$^{+/mut}$ hiPSCs and their isogenic controls to adherent cortical organoids (ACOs), which show a semi-3D structure with radial outgrowth and rudimentary cortical layering. In this ACO model, we also observed a two-fold increase in Gad67-positive interneurons, relative to the number of neurons. Taken together, these results could shed light on how *SETD1A* loss-of-function mutations affect interneurons, and could lead to novel therapeutic strategies.

GLIOMA-DRIVER MUTATIONS DISRUPT NEURAL LINEAGE DIFFERENTIATION IN AN iPSC-BASED BRAIN TUMOR MODEL

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Gliomas are a heterogeneous and aggressive group of brain tumors classified into subtypes based on genetic alterations such as IDH1 mutations, 1p/19q co-deletions, EGFR amplification, and histone H3 mutations. A hallmark of gliomas is their ability to hijack early neural developmental programs, resulting in malignant cells that resemble neuronal, astrocytic, and oligodendrocytic lineages. Understanding how glioma cells acquire stem/progenitor characteristics and transition between cellular states offers crucial insights into tumor biology and potential differentiation-based therapies. Our comprehensive pan-glioma transcriptome analysis, integrating single-cell and bulk RNA-seq data, identified seven lineage-specific glioma cellular states, which, while found across various glioma subtypes, exhibit different enrichment patterns. For example, IDH-mutant tumors are enriched with cells exhibiting oligodendrocyte lineage characteristics, along with a smaller subset of astrocyte lineage cells showing elevated APOE expression. In contrast, IDH-wildtype tumors are primarily composed of astrocyte lineage cells with low APOE expression. Pediatric H3-K27M mutant gliomas are enriched with oligodendrocyte lineage cells expressing high levels of APOD, whereas H3-G34R tumors show a higher prevalence of astrocyte lineage cells and DCX+ neuronal lineage cells. Interestingly, APOD+ oligodendrocyte lineage cells are also found in adult gliomas, with a significantly higher frequency in peri-tumoral regions compared to tumor cores, suggesting these populations may play a role in tumor invasion. To further investigate the impact of glioma-driver mutations on neural lineage differentiation, we utilized CRISPR-based gene editing in human iPSCs to model gliomas with various mutations. Our findings reveal that IDH1 mutation inhibits differentiation into astrocytic lineages while promoting neuronal differentiation. In contrast, EGFRvIII overexpression strongly impairs neuronal differentiation. Additionally, H3-G34R mutation dramatically suppresses the expression of OLIG1 and OLIG2, key regulators of oligodendrocyte lineage differentiation. These findings offer new insights into how glioma-driver mutations influence neural lineage differentiation and highlight potential therapeutic targets for treating gliomas driven by different genetic alterations.

HOMEOSTASIS OF MITOCHONDRIAL POPULATIONS IN HUMAN NEURONS

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Mitochondria in the human brain face unique challenges. In adulthood and particularly throughout development, the human brain demands exceptionally high energy levels. Moreover, due to the postmitotic nature of neurons, neuronal mitochondria have to undergo lifelong adaptation and rejuvenation.
Besides ensuring general cellular energy homeostasis, mitochondria in neurons contribute to various essential processes associated with the transfer of information. Recently, several studies have suggested that distinct populations of mitochondria fulfill these different functionalities. Yet, how mitochondrial populations in neurons are generated during development and what mechanisms ensure lifelong maintenance of mitochondrial homeostasis are largely unknown. Further, while most previous studies investigated neuronal mitochondrial populations in excitatory hippocampal neurons in rodents, it is particularly interesting whether similar populations likewise occur in human neurons and if their composition differs in specific cell types in the brain.
We address these questions by performing live-cell super-resolution imaging of mitochondria in cultured human neurons. Here, we present an initial description of mitochondrial population homeostasis in excitatory and inhibitory human neurons over a developmental time course. As a next step, we would like to extend this characterization with data acquired from human cerebral organoids. Furthermore, we plan to use proteomics and RNA sequencing to delve into the molecular composition of individual mitochondrial populations. Our project will contribute to deciphering the functional relevance of mitochondrial populations in neurons and might help us understand the consequences of failures in mitochondrial homeostasis during disease.

CELLXPRESS.AI – PLATFORM FOR ROBUST AND AUTOMATIC BRAIN ORGANOID GENERATION.

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In recent years, brain organoids have become an invaluable tool not only to understand the developing brain, but also to generate disease model systems that cannot be studied in animal systems. Despite the progress in protocol maturity, culture heterogeneity remains a challenging issue due to the long cultivation time, difficult differentiation protocols and non-optimal pluripotent stem cell culturing.
To increase organoid quality, we focused on (1) iPSC culture robustness (2) organoid differentiation robustness (3) rigorous application of quality control tools. We addressed these items by creating a set of streamlined protocols on the CellXpress.ai automated stem cell and organoid cultivation platform. Protocol improvements included liquid handling steps, labware optimization for organoid and media handling, as well as machine learning tools to ensure quality control of the samples. Entry point of this automation suite was the development stem cell cultivation protocols, tailored to support stem cell maintenance as well as upscaling protocols to automatically prepare microplates for differentiation. Stem cell culturing quality was improved by image-based health assessment and to identify differentiated cells. Organoid generation robustness was achieved by leveraging the proprietary „smart media module" to autonomously allow media exchange of different differentiation cocktails over long periods of time. Organoid quality was tightly monitored by label free organoid segmentation and classification over multiple weeks, across orders of magnitudes. Lastly, oxygenation and nutrient availability could be increased by cultivating organoids on an automated build in shaker inside the incubator.
Summary: We successfully demonstrated that the CellXpress.ai platform can be used to robustly culture iPSC lines and to differentiated iPSCs into different brain organoids. By using the CellXpress.ai platform it was possible to execute multiple different workflows for different model systems simultaneously.

HOTPOCKETS: NOVEL DEVICES FOR NON-DESTRUCTIVE, LONG-TERM ELECTROPHYSIOLOGICAL RECORDING OF ENTIRE HUMAN BRAIN ORGANOIDS

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The most common approaches to measuring electrophysiological activity in organoids are attachment of a spherical organoid to 2D multi-electrode arrays (MEA) or electrodes inserted into the organoid. These popular approaches have considerable limitations in that they require attachment factors or may damage the organoids, thus limiting their re-use. Several recent technological innovations have created 3D MEAs using buckled 3D scaffolds or grow organoids on mesh-like MEAs. While these represent a substantial improvements, these new techniques still have several limitations including i) low electrode density, limiting coverage of all electrophysiologically active neurons, ii) fixed dimensions, which precludes compatibility with the volumes of organoids generated from different widely used protocols, iii) the ability to add or remove the organoid without damage, which is critical for long-term culture because most organoid protocols increase viability through maintenance in a spinning bioreactor used to increase media diffusion into the center of organoid. We developed a morphable MEA device, called the HotPocket, to record electrical activity around an entire organoid, containing 63 recording electrodes (each 50 um in diameter with impedance ranging from 0.2-0.3 Mohms), that does not require attachment factors so is suitable for placing and removing organoids. The HotPocket is made of parylene encapsulated gold with platinum coating at the recording sites, which are biocompatible materials, and a high elasticity layer that ensures close contact of the electrodes with the organoid. Cortical organoids show spontaneous activity as measured through the HotPocket, which is increased in a dose-dependent manner upon glutamate stimulation (10 to 40 uM), and reduced through application of a sodium channel blocker (TTX). Organoids can be removed from the HotPocket and show consistent activity when placed again days or weeks later. Longitudinal recording after placing and removal into the HotPocket show decreased circularity of the organoid that returns to baseline levels after 1 day and no changes in cell death. The device is capable of holding organoids 2-3.5 mm in diameter, consistent with the mature organoid size of most differentiation protocols. Overall the HotPocket is a novel device that can be used to measure electrophysiological activity at high resolution, longitudinally in 3D organoids.

RARE VARIANTS IN THE GENETIC BACKGROUND DRIVE PHENOTYPIC VARIABILITY VIA GENE NETWORKS IN HUMAN PLURIPOTENT STEM CELL MODELS OF NEURODEVELOPMENTAL DISORDERS.

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It is challenging to establish genotype-phenotype correlations in neurodevelopmental disorders (NDDs) due to extensive genetic causes and heterogenous clinical manifestations, which makes it difficult for genetic counseling, understanding the molecular mechanisms, and developing therapeutic or intervention strategies. Using the 16p12.1 deletion, a moderate-risk factor with variable expressivity, as a paradigm, we proposed a “two-hit” model to explore the correlations, which suggests secondary variants in the genetic background interact with the primary disease-associated variant to shape the phenotypic trajectory. Here, we used human iPSC models combined with a multi-omics approach to test the hypothesis as well as to elucidate the mechanisms conferring genetic susceptibility and phenotypic variability. The patient cohort of this study contains 24 individuals from three large families with whole-genome sequencing (WGS) and deep clinical phenotyping. Affected individuals present a wide range of phenotypes with variability across generations and families, including autism, anxiety, schizophrenia features and head-size abnormalities. 31,867 rare single nucleotide variants, 10,810 rare deletion and 6,747 rare duplication structural variants (≤0.1% frequency) were identified in the genetic backgrounds. In total, 12,520 genes including 661 SFARI genes such as *PTEN* and *NRXN1* were annotated. We performed neural conversion on iPSCs derived from 14 individuals from these three large families and healthy donors as well as the CRISPR/Cas9-mediated deletion line. RNA sequencing on iPSC, neural progenitor cells (NPCs), immature neurons, and mature neurons, along with ATAC-seq on iPSCs and NPCs, revealed alterations in both transcriptomic profiles and chromatin accessibility landscapes driven by susceptible and family-specific genetic backgrounds. Additionally, distinct cellular dysregulations were observed, varying by family. For example, in one family, deletion carrier lines exhibited significantly elevated VGAT signals at the mature neuron stage, indicating the excitatory/inhibitory neuron imbalance. However, deletion carrier lines from another family showed hyperproliferation at the NPC stage, correlating with their head-size phenotype. We also discovered that genes impacted by 19 classes of rare variants were involved in gene networks with the deletion region, influencing various cellular functions and signaling pathways such as cell cycle, axion guidance, Wnt signaling and Notch signaling. Overall, our study provides a framework for dissecting the complex genetic architecture and mechanistic underpinnings of NDDs. These strategies are essential for deriving genotype-phenotype correlations, providing insights into genetic diversity, individual susceptibility and guiding the development of personalized therapeutic approaches in the future.

STEM CELL-DERIVED 3D CO-CULTURE TO INVESTIGATE THE DETERMINANTS OF HUMAN MICROGLIAL INTEGRATION, IDENTITY AND FUNCTION

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Microglia are a subset of macrophages that reside in our brain, which surveil their surroundings and can orchestrate potent responses to brain damage and immune stimuli. Emerging from the yolk sac during primitive hematopoiesis, microglia invade the brain and establish residence during early stages of embryogenesis. Brain residence is essential for microglia to adopt their mature functional form and reciprocally crucial for normal brain development. Little is known about the mechanisms underlying these coordinated developmental processes. To recapitulate this developmental trajectory ex vivo, and study the coordinated development of brain and microglia, we have established human pluripotent stem cell-derived 3D co-cultures that include microglia. Changes in microglia morphology, behavior and transcriptomes were observed. We then engineered distinct environment mimicking either different CNS regions or different brain cell types and have been testing their effects on microglia residence. In addition, we are also examining the effects of microglia depletion/re-addition and infection on the 3D co-culture. The knowledge gained from this study will continue to support our disease-modeling efforts and improve our capability of generating bona fide microglia for therapeutic applications.

MODELLING THE NEUROPATHOPHYSIOLOGY OF LYSOSOMAL STORAGE DISORDERS: IPSC-DERIVED HUMAN NEURONAL PROGENITORS FOR THE STUDY OF INFANTILE GM1 GANGLIOSIDOSIS.

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Introduction: GM1 gangliosidosis (GM1) is an inherited neurodegenerative disease caused by mutations in the GLB1 gene encoding the enzyme β-galactosidase (β-gal). Absent or insufficient β-gal activity results in the accumulation of ganglioside GM1 in tissues, particularly in the central nervous system, resulting in progressive neurodegeneration.
Methods: Skin biopsy and primary culture of fibroblasts from a patient affected by GM1 Gangliosidosis. Reprogramming in Pluripotent Stem Cells (iPSCs) by Sendai Virus and differentiation into Neuronal Progenitors (GM1-NPc). The isogenic control was generated by CRISPR-CAS 9 method.
Results: iPSC-derived GM1-NPc recreated, in vitro, the biochemical and molecular phenotype of GM1 Gangliosidosis with absent β-gal activity and increased/malfunctioning of lysosomes. The accumulation of the ganglioside Gm1 at the neuronal level and its critical role in the pathogenesis of the disease were evaluated. The pathophysiological effects of GM1 cells were compared with the isogenic control line derived from the patient's iPSCs and edited by the Crispr-Cas9 technique. We tested the action of the substrate-specific inhibitor butyl-deoxynojirimycin (NB-DNJ), which alleviated the disease-related phenotypes of GM1-NPCs in vitro. Furthermore, the efficacy of the molecular chaperones on the enzymatic activity of the reverted NPC and of the healthy control was evaluated.
Discussion: GM1-NPCs are a valuable cellular model of GM1 gangliosidosis in vitro and appear to be essential for the genotype-phenotype correlation of the identified variants, especially for those of dubious interpretation. GM1-NPc offers a fundamental contribution to developing, screening and validating new drugs for the treatment of GM1 Gangliosidosis.

Grant: this work received funding from Bando Regione Toscana 2018 (Project ‘Lysolate’); PRIN 2022 (20228SLVY).

HUMAN BRAIN ORGANOIDS UNVEIL PATIENT-SPECIFIC NEURONAL ACTIVITY LANDSCAPE IN MECP2 DUPLICATION SYNDROME

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Neurological disorders affect over 3 billion people worldwide with ~15% of cases occurring in children, where these disorders are often of neurodevelopmental origin. Unfortunately, there is currently no cure for developmental disorders and existing treatments mainly focus on symptom management. However, every individual responds to therapy differently, emphasizing the need for personalized medicine. Human brain organoids have emerged as a transformational tool for advancing personalized medicine, offering unprecedented insights into the pathophysiology of neurological diseases and enabling the development of tailored therapeutic interventions. Derived from human pluripotent stem cells, brain organoids closely replicate the structure, organization, and function of the human brain, making them invaluable for modeling brain disorders. In this study, we used brain organoids to investigate the electrophysiological properties of neurons in MECP2 Duplication Syndrome, a severe genetic neurodevelopmental disorder primarily affecting boys. MECP2 duplication impairs brain function and leads to seizures, generalized somatic and autonomic hypotonia, and intellectual disability. Previous studies in mouse models of MECP2 duplication have suggested that the aberrant gamma-aminobutyric acid (GABA) pathway disrupts the excitatory/inhibitory balance in the brain by impairing inhibitory activity. Therefore, our goal was to examine the excitability and GABAergic activity in human brain organoids derived from patients with MECP2 Duplication Syndrome, to better understand the neuronal dysfunction in this syndrome and lay the groundwork for future drug discovery efforts to normalize it. We used a GCaMP+-GFP line, generated via lentiviral transduction of iPSCs from both patients and healthy controls. To assess baseline neuronal activity over time, we performed multiplexed electrophysiological recordings over seven weeks using multielectrode arrays (MEA). Neuronal excitability was tested by delivering electrical stimulation trains (50-100 Hz) and GCaMP-GFP signals were measured using two-photon microscopy. We also evaluated the effects of picrotoxin, a GABA-A receptor blocker, on neuronal activity. Our findings revealed a significant increase in excitability and the presence of distinct GABAergic populations with unique electrophysiological responses in MECP2 Duplication Syndrome organoids compared to those from healthy controls. These data provide critical insights into the role of GABAergic mechanisms in MECP2 Duplication Syndrome, paving the way for the development of new therapeutic strategies targeting these disrupted neural circuits.

ELUCIDATING THE ROLE OF PSEUDOURIDINE AND PUS7 IN NEURODEVELOPMENT

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PUS7 is a pseudouridine synthase that isomerizes uridine to pseudouridine (Ψ) in pre-mRNA and mediates prevalent changes in alternative splicing and 3' end processing. Patients with mutations in the pseudouridine synthase PUS7 exhibit neurodevelopmental phenotypes including microcephaly, aggression, intellectual disability, and speech delay. However, the molecular mechanisms by which pseudouridines and PUS7 might impact neurodevelopment are unknown. We utilized inducible neurogenin-2 expression to differentiate hESCs into neurons as a model to elucidate the role of PUS7 in neurodevelopment. Using this system, we found that PUS7 protein levels decrease during differentiation, and a highly neuron specific microexon is included in the mRNA, which alters the PUS7 protein coding sequence. To map pseudouridines that are regulated during neurodevelopment, we preformed direct RNA Nanopore sequencing in hESCs and day 6 iNeurons.We discovered that hundreds of Ψ sites were dynamically regulated, and that some of these targets, such as RBFOX3 and SRRM2, have also been linked to neurodevelopmental disorders. Together, our results reveal that pseudouridines and PUS7 are neurodevelopmentally regulated. Ongoing work is determining the role of PUS7 in gene regulation and uncovering cellular phenotypes mediated by PUS7 in neurodevelopment. This work will identify biological functions of Ψ sites in mRNAs and the molecular and cellular underpinnings of PUS7's role in neurodevelopment.

MAPPING HUMAN CORTICAL DEVELOPMENT THROUGH COMBINED TISSUE ENGINEERING AND SYNTHETIC BIOLOGY APPROACHES

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The human cerebral cortex exhibits remarkable cellular diversity, established through precisely orchestrated developmental events that are critical for cortical circuit functionality. While human induced pluripotent stem cell (hiPSC)-derived brain organoids offer unprecedented opportunities to study early cortical development and disease-related perturbations, they have notable limitations. These include lack of stereotypic macroscale anatomic structure, absence of reproducible long-range connectivity, and limited activity-dependent cellular maturation.

To overcome these challenges, we developed a multi-organoid-on-chip platform that combines advanced tissue engineering technologies with unbiased analysis methods to recapitulate in vivo functional connectivity. We implemented TRACT, a ligand-receptor mediated intramembrane proteolytic cleavage system, to monitor synaptic interactions at single-cell resolution and track macro-circuit connectivity dynamics longitudinally.

In parallel, through collaboration with the Elowitz lab at Caltech, we implemented two complementary barcoding approaches for cell lineage reconstruction. Zombie-Memoir enables in situ readout of genomically integrated barcodes, which, when combined with smFISH imaging and seqFISH gene probes, allows simultaneous identification of lineages and cell types while preserving spatial context. Our lineage coupling analysis revealed unexpected high rates of clonal intermixing among progenitor and neuronal subclusters. Additionally, we employed 'hypercascade,' a system utilizing progressively mutating barcode arrays, to enable lineage tree reconstruction through single-cell sequencing. Initial results from two-month-old hypercascade cortical organoids showed successful barcode recovery from nearly 40% of cells, revealing multiple clonally coupled cell types.

Our approach combines tissue engineering and synthetic biology using a newly established multi-brain region organoid model. This framework allows for an in-depth exploration of human cortical development, particularly in the context of establishing long-range connections with other regions of the brain. By integrating these advanced modular technologies, we provide a robust foundation to deepen our insights into both normal processes of neural development and the cellular processes underlying disease states within the developing human cortex.

DECODING SPEECH DISORDERS: INVESTIGATING GENE DISRUPTIONS THROUGH HUMAN iPSC-DERIVED BRAIN ORGANOIDS

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Our abilities to use language have been pivotal in shaping human civilization. For a long time, FOXP2 was the only known gene to be implicated in an inherited severe speech disorder. The rise of sequencing technologies has led to the discovery of a range of further genes with inherited or *de novo* mutations that disrupt childhood speech development. In this study, we harness the potential of 3D cerebral organoids to model the impacts of such gene disturbances on brain development.

In order to investigate this complex phenomenon, we have assembled a catalogue of published *de novo* mutations identified in cases of Childhood Apraxia of Speech, a disorder shown to have a high burden of rare causative variants. Recurrently mutated genes will be selected to either obtain patient-derived induced pluripotent stem cell lines (iPSCs) or generate mutated cell lines through targeted gene-editing. This will expand our lab's current resource of human iPSCs with mutations in key genes like FOXP1/2/4, CHD3 or SETBP1, all previously implicated in the disruption of speech. We study the consequences of the relevant mutations by using these iPSCs to generate patterned striatal or cortical organoids, due to the role of the cortex, striatum, and cortico-striatal circuits in speech development. The generated organoids are evaluated for variations in size, proliferation rate, neurogenesis among different sub-populations, as well as lamination and circuit formation. Single-cell RNA-sequencing and spatial transcriptomics are used to gain deeper insights into the transcriptional landscape and cellular profiles, and how these are disturbed by gene disruptions.

The findings from this project will offer valuable clues about the molecular mechanisms that underpin the emergence of a speech-ready brain, and bring us one step closer in our understanding of the genetically-driven faculties that make us uniquely human.

MECHANISMS GOVERNING HUMAN CEREBRAL CORTEX DIVERSIFICATION IN CORTICAL ORGANOIDS

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How organisms control the final number and identity of cells present in their organs remains elusive. During evolution, the human cerebral cortex has increased dramatically in size and complexity. However, the mechanisms driving human cortical expansion and diversification remain poorly understood. Here, we leverage complex 3D in vitro models of human cortical development, i.e. human cortical organoids, to investigate the mechanisms underlying human cortical cell diversification and expansion. First, we generate a comprehensive single-cell transcriptomic, epigenetic, and spatial atlas of human cortical organoid development, comprising over 610,000 cells, from generation of neural progenitors through production of differentiated neuronal and glial subtypes. We show that processes of cellular diversification in cortical organoids correlate closely to endogenous ones, empowering the use of these model and data to study human fate specification. We define longitudinal molecular trajectories of cortical cell types during organoid development and identify genes with predicted human-specific roles in lineage establishment. Then, we focus on investigating how callosal projection neurons (CPN) of the upper cortical layers have diversified in the human brain. Leveraging our comprehensive human cortical organoid atlas, we first uncover that some degree of CPN transcriptional diversity emerges at early stages of development. Building on this initial finding, we are now using lineage-tracing approaches over extended periods of in vitro organoid culture to further explore the mechanistic underpinnings of CPN expansion and diversification in the human brain. In sum, our work provides a comprehensive, single-cell molecular map of cortical organoid development, identifies predicted novel regulators of human corticogenesis, and begins to unravel previously unexplored mechanisms governing human cortical cell diversity.

HOW DOES THYROID HORMONE AVAILABILITY REGULATE ADULT NEUROGLIOGENESIS IN THE ADULT MOUSE? A NEW ROLE FOR DIO2.

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Thyroid hormones (THs) are critical for neurodevelopment and brain function. Studies in rodents have shown that they strongly influence adult neurogenic regions, especially the subventricular zone (SVZ), the largest neural stem cell niche in the adult rodent brain. THs influence neurogliogenesis, controlling cell cycle progression and progenitor fate commitment to neuronal and oligodendroglial fates.
Type 2 deiodinase (DIO2) is a major factor controlling TH action, as it is the main enzyme responsible for T4 activation to the genomically active T3, and while no inactivating DIO2 mutations have been found in humans, polymorphisms have been linked to several diseases. However, its role in the SVZ remains unclear.
Thus, our study aimed to investigate the function of DIO2 in regulating NSC behavior in the adult mouse SVZ, with a particular focus on the potential function of DIO2 in the release of NSCs from quiescence, a still poorly understood process.
Single-cell RNA sequencing analysis in the adult murine SVZ revealed a strong link between *Dio2* expression and the maintenance of NSC quiescence. Gene expression patterns and RNAvelocity techniques allowed us to establish an inverse correlation between *Dio2* expression and NSC activation, which was later confirmed histologically by RNAscope.
We then examined the expression of several proteins related to adult neurogliogenesis in DIO2-deficient mice (*Dio2* KO) to understand the functional implications of DIO2 action in the adult SVZ. An increase in the density of SVZ SOX2-positive progenitors was observed, but this was not accompanied by an increase in proliferation or neuroglial production, suggesting a hindered transition from NSC to neuroglial stages.
Moreover, these results were validated by testing exogenous deiodinase inhibitors ex vivo using neurosphere assays. This revealed an intriguing role of DIO2 in controlling NSC proliferation, particularly on NSC exit from quiescence, but also on cell fate commitment and differentiation.
Finally, *Dio2* KO mice were also subjected to behavioral testing using olfactory memory and discrimination tests as non-invasive biomarkers of SVZ status. *Dio2* KO mice exhibited impaired olfactory memory, suggesting a functional role for DIO2 in adult SVZ neurogenesis.
In conclusion, we highlight the contribution of DIO2 as a key regulator of adult SVZ neurogliogenesis and point to the need for further experiments to elucidate its implications in brain function and pathology.

HUMAN ADHERENT CORTICAL ORGANOIDS IN A MULTIWELL FORMAT

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In the growing diversity of human iPSC-derived models of brain development, we present here a novel method that exhibits 3D cortical layer formation in a highly reproducible topography of minimal dimensions. The resulting adherent cortical organoids develop by self-organization after seeding frontal cortex patterned iPSC-derived neural progenitor cells in 384-well plates during eight weeks of differentiation. The organoids have stereotypical dimensions of 3 x 3 x 0.2 mm, contain multiple neuronal subtypes, astrocytes and oligodendrocyte lineage cells, and are amenable to extended culture for at least 10 months. Longitudinal imaging revealed morphologically mature dendritic spines, axonal myelination, and robust neuronal activity. Moreover, adherent cortical organoids compare favorably to existing brain organoid models on the basis of robust reproducibility in obtaining topographically-standardized singular radial cortical structures and circumvent the internal necrosis that is common in free-floating cortical organoids. The adherent human cortical organoid platform holds considerable potential for high-throughput drug discovery applications, neurotoxicological screening, and mechanistic pathophysiological studies of brain disorders.

ESTABLISHMENT OF A STEM CELL MODEL TO STUDY PROTEIN AGGREGATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disease associated with CAG (coding for glutamine, Q) repeat expansion in the huntingtin (Htt) gene, resulting in the expression of mutant HTT protein. Stem cell-derived neural cells provide an excellent model system for studying HD pathology. We established a stem cell model of HD using mouse D3 embryonic stem cells (ESCs). The culture of D3 ESC-derived embryoid bodies (EBs) in the serum-free knockout serum replacement (KSR)-containing medium resulted in efficient neural differentiation. The generated neural cells, on day 15 of culture, included neural progenitors (18%), immature neurons (19%), mature neurons (12%), astrocytes (61%), and oligodendrocytes (2%). On day 16 of culture, EBs were dissociated into single cells and plated; on day 17, EB-derived neural cells were transfected with htt 25Q (with 25 glutamine residues) or htt 72Q (with 72 glutamine residues). These plasmids express N-terminal fragment of HTT protein with polyQ repeat lengths of 25 or 72 residues, fused to green fluorescent protein (GFP). The neural cells transfected with htt 25Q showed diffused GFP fluorescence, whereas the neural cells transfected with htt 72Q showed the presence of GFP foci. Therefore, in the stem cell-derived neural cells, the extent of HTT aggregation varied with the polyQ repeat length. This model can be applied for screening and identification of novel compounds that could clear the protein aggregates in HD.

HIGH-THROUGHPUT NEURAL CONNECTIVITY MAPPING IN HUMAN BRAIN ORGANOIDS

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The human brain contains a complex network of ~80 billion neurons. This network changes with age and environment, and it is vulnerable to perturbations. To understand how neural networks form, and why they change in disease, we need to determine connectivity, gene expression, and genotype in the same cell. Current single-neuron connectivity mapping methods cannot cover these modalities, nor are they scalable to 10's or 100's of conditions.

To overcome these limitations in scale and transcriptional information, we have developed a 'connectomics-by-sequencing' method combining barcoded, retrograde transsynaptic rabies tracing with single-cell RNA sequencing to map thousands of synaptic networks with their transcriptomes. We then applied our new method to human cortical organoids, which allow scalable, accessible, and sophisticated 3D human disease modeling.

We identified the connectivity patterns of thousands of neurons across different cell lines and disease conditions. With our data, we described the connectivity preferences of different classes of neurons, linked connectivity to gene expression, and discovered how network formation is influenced by different tissue context, in individual wild type organoids. Next, we clustered individual networks using a latent embedding by an artificial neural network, to identify recurring patterns of connectivity which we termed network motifs..

Finally, we applied our method to our previously established disease model of Tuberous Sclerosis Complex (TSC), which is a neurodevelopmental disorder where cell fate defects cause severe intractable childhood epilepsy. With multiple complementary methods, we now show that it also recapitulates changes in network activity explaining the epilepsy phenotype. Here, we distinguished healthy and diseased network types, and we linked altered connectivity to a defective transcriptional state. Our data have the potential to explain neurodevelopmental disorders on the cortical circuit level, identifying the most affected neuronal subtypes and synaptic connections which can provide a basis for potential therapeutic intervention.

NEXT GENERATION MOUSE ORGANOID MODELS TO COMPLEMENT AND EMPOWER STUDIES OF HUMAN BRAIN DEVELOPMENT IN A DISH

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Natural selection has shaped the gene regulatory networks that control neuronal cell fate specification and terminal differentiation, generating diversity in cell type composition, neural circuit formation, function, and behavior across mammals. However, it remains difficult to parse the specific genetic changes that contribute to this phenotypic diversity and to define the developmental mechanisms underpinning these differences at the molecular and cellular level. Human brain organoids have emerged as an experimental model system for characterizing mechanisms of human brain development, but there are intrinsic ethical and practical limitations that reduce the utility of human brain organoids for mechanistic studies. We propose that mouse brain organoids can complement and empower human brain organoid models, combining the power of mouse genetic tools with the experimental accessibility of organoid models for mechanistic studies.

Towards this end, we have developed a new protocol to reproducibly generate neocortical organoids from mouse epiblast stem cells (EpiSCs) (Medina-Cano et al., *bioRxiv* 2024). Mouse cortical organoids develop with similar kinetics to the mouse cortex in vivo and recapitulate the cellular diversity present in the embryonic cerebral cortex, sequentially generating distinct subtypes of cortical pyramidal neurons, astrocytes, and oligodendrocyte progenitors over ~7-10 days. Importantly, these cell types continue to mature over several weeks in culture, activating postnatal gene expression programs that have been difficult to access in vitro with previous models.

In ongoing studies, we are developing new resources and experimental methods to perform forward genetic screens of cortical development using natural genetic variation present in the Diversity Outbred (DO) mouse stock population (Glenn et al., *bioRxiv* 2024). DO mice exhibit significant, heritable variation in brain size, cellular composition, behavior, and neurological disease phenotypes, and thus can be used to map genetic variants that contribute to quantitative phenotypic variation in brain development. We have generated and characterized a new genetic reference panel of pluripotent stem cell lines (n =230) from the Diversity Outbred mouse Stock. Data from this new platform can complement and inform similar efforts within the stem cell biology and human genetics communities to model the impact of natural genetic variation on phenotypic variation and disease-risk.

GENERATION AND CHARACTERIZATION OF A NOVEL hPSC-DERIVED MEDIAL GANGLIONIC EMINENCE BRAIN ORGANOID MODEL

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Inhibitory interneurons (INs) arise from subcortical structures, most prominently the ganglionic eminences, and migrate into the cortex, where they play important roles in cortical development, brain network formation, and the regulation of cortical excitability. The medial ganglionic eminence (MGE) is a transient structure in the developing brain that serves as the primary origin for a subset of these essential cortically fated INs, including somatostatin (SST) and parvalbumin (PV) INs. Dysfunction of MGE-derived INs is implicated in many neurodevelopmental disorders. The generation of an *in vitro* organoid model that predominantly expresses MGE markers and robustly produces PV INs remains a challenge in the field. Given that regional specification during forebrain development relies on precise morphogen expression, we hypothesized that systematic manipulation of WNT and Sonic Hedgehog (SHH) patterning factors would produce brain organoids that strongly resemble the developing MGE. To test this, we manipulated the timing and concentration of WNT inhibition and SHH pathway activation in our single rosette organoid model to identify the optimal protocol to generate MGE-like organoids. We validated our protocol using human pluripotent stem cell lines derived from female embryonic stem cells, female blood samples, and male fibroblasts. We characterized IN development and specification in our model between 18-250 days in vitro (DIV) using scRNAseq, RT-qPCR, immunostaining, and with functional assays including MEA and patch clamp recordings. Our protocol generates organoids that strongly express MGE lineage markers, including LHX6 and NKX2.1, and produces MGE-derived cortical and subpallial fated INs, oligodendrocytes, and astrocytes. We found robust expression of PV at 200+ DIV, along with expression of $K_v3.1$ and perineuronal nets. Upon fusion with cortically patterned organoids, we observed rapid and extensive migration of INs from the MGE organoid into the cortical organoid. Long term culture of assembloids produced electrophysiological signals with robust synchronous network activity which was altered with bicuculine, as well as spontaneous inhibitory postsynaptic currents in slice patch-clamp recordings. Our MGE-specific organoid model will benefit the brain organoid field by providing a valuable tool to study MGE-derived IN development and for modeling IN-related phenotypes in genetic epilepsies and related neurodevelopmental disorders.*Authors contributed equally

BRAIN-ON-A-CHIP: DEVELOPMENT OF A HUMAN CELL-BASED BLOOD-BRAIN BARRIER AND MIDBRAIN ORGANOID MODEL

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Organ-on-a-chip cell culture models are important to investigate cerebral drug delivery, pathology and brain protection. Microfluidic chip devices allow more complex and physiological modelling of the blood-brain barrier (BBB) and enable the co-culture of multiple human cell types. The use of human brain spheroids and organoids derived from human induced pluripotent stem cells (iPSC) are the latest trend in the pharmaceutical drug testing. Our aim was to create and optimize a new, dynamic BBB-organoid microelectronic device by the co-culture of human endothelial cells, brain pericytes and human midbrain organoids. For modelling the BBB, a co-culture of human stem cell derived endothelial cells and brain pericytes was used (Cecchelli et al, 2014). Human midbrain organoids were differentiated from iPSCs from healthy people and Parkinson's disease patients (Nickels et al, 2020). Our lab-on-a-chip enables visual observation, impedance and permeability measurements across the brain endothelial monolayer and also the introduction of fluid flow to mimic blood circulation (Walter et al, 2016; Kincses et al, 2020; Santa-Maria et al, 2021). The cellular composition of the newly introduced midbrain organoids was characterized by immunostaining for glial and neuronal cells. Barrier integrity of the brain endothelial layer was stable after the addition of the organoids measured by impedance using gold and platinum electrodes and by permeability for fluorescent markers. Brain endothelial intercellular junctional morphology was also healthy and continuous. Targeted nanoparticles carrying a fluorescent cargo were introduced to the system and their passage across the brain endothelial monolayer to the brain organoids was followed. Nanoparticles crossed the BBB and entered the organoids successfully. Changes in gene expression of brain endothelial cells with or without the organoids were also identified by MACE-seq profiling. Here we introduce a novel brain-on-a-chip device, which can be a valuable tool for pharmaceutical testing, pathology modelling and for toxicological studies.

Funding:
The project was supported by the NKFIH OTKA K-143766 and M-ERA.NET2 nanoPD program (NNE-129617), the Secretariat of Lorand Eotvos Research Network (SA-111/2021 to F.R.W.).

OPTOGENETIC MODULATION OF WNT3A SIGNALING IN BRAIN ORGANOIDS & ITS IMPLICATIONS FOR HIPPOCAMPAL-LIKE STRUCTURES

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Brain organoid research is advancing our understanding of brain development by replicating specific brain regions. Morphogens play a pivotal role in orchestrating spatial and temporal patterning during embryonic brain development, guiding the emergence of specialized cell types and regional identities. Leveraging these signalling molecules in vitro facilitates the generation of brain organoids resembling distinct regions such as the hippocampus, essential for learning and memory. WNT morphogens, especially WNT3A, are crucial in hippocampal formation, guiding neural stem cell differentiation into excitatory and inhibitory neurons, thus shaping hippocampal circuitry. Here, we employ a light-inducible gene expression system to activate WNT3A in induced pluripotent stem cells and organoids. The overexpression of WNT3A in embryoid bodies triggers a striking phenotypic transformation in organoids, characterized by the emergence of distinct cell layers resembling hippocampal architecture. Notably, PROX1, a pan-hippocampal marker predominantly expressed in the dentate gyrus, is observed in a defined layer within light-stimulated organoid cells by day 30. Single-cell sequencing of these organoids confirms the expression of other hippocampal markers such as ZBTB20, GAD1, KA-1, NEUROD2, SPARC, and MEIS2. Spatial transcriptomics data produced with Open-ST further confirms the layered organization of these organoids. Beyond fusing WNT3A overexpressing organoids with cortical organoids to create multi-regional assembloids, we take advantage of the optogenetic system to spatially activate a ‘hippocampal-like organizer’ for studying cortical-hippocampal interactions. This method not only advances our comprehension of hippocampal brain organoid differentiation and cortical-hippocampal dynamics, but also presents promising avenues for therapeutic innovation in neurological disorders.

CONSTRUCTION OF HUMAN NUCLEUS BASALIS ORGANOIDS AND CHOLINERGIC PROJECTIONS IN nbM-CORTICAL ASSEMBLOIDS

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The nucleus basalis, also known as the nucleus basalis of Meynert (nbM), which is considered to be one of the major cholinergic output of basal forebrain, have been found to dynamically modulate activity in the cortex. Dysfunction of nucleus basalis-cortical cholinergic circuit led to cognitive impairment, such as Alzheimer's disease (AD) and Down syndrome (DS). Human nucleus basalis cholinergic neurons derived from human pluripotent stem cells provide powerful tools to study cholinergic neurons-associated diseases and cell therapy. Previous studies reported the generation of 2D human basal forebrain cholinergic neurons which failed to recapitulate the spatial organization, cellular diversity, and crosstalk between different regions. Therefore, a better model to recapitulate human nucleus basalis and cholinergic projections in nbM-cortical is desired.
Here we developed a approach for differentiating human pluripotent stem cells into nucleus basalis of Meynert organoids (hnbMOs). We reconstructed hnbM-cortex cholinergic projection by transplanting hnbMOs into immunodeficiency mice to construct chimeric brains and coculturing with human fetal brain. Then we fused hnbMOs with cerebral cortex organoids (hCOs) to form hnbMO-hCO assembloids. We validate the structural and functional connectivity of basal forebrain cholinergic neurons to the cortex in assembloids. An assembloid-chimeric brain was constructed innovatively by transplanting corresponding organoids in the cortex and nbM region to establish a complete human cholinergic projection system. Futhermore, we identified the defects in projection of cholinergic neurons at the morphological and transcriptomic level in Down syndrome patient iPSC-derived assembloids as well as Down syndrome fetal brain tissue. Our work establishes new approach for the study of neurological disorders associated with nbM and nbM-cortical cholinergic neuron circuit.

DECODING TRANSCRIPTIONAL IDENTITY IN DEVELOPING HUMAN SENSORY NEURONS

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Dorsal root ganglia (DRG) are pivotal in the sensory nervous system, harboring the cell bodies of sensory neurons responsible for detecting and transmitting sensory signals. Despite their importance, the cell heterogeneity and cellular mechanisms regulating the development of human DRG remain poorly understood. To address this gap, we constructed a single-cell spatiotemporal transcriptomic atlas of human embryonic DRG spanning from the first to second trimesters. This atlas profiled the cell type diversity and highlighted the extrinsic signaling cascades and intrinsic regulatory hierarchies guiding cell fate decisions, including neuronal/glial lineage restriction, sensory neuron specification and differentiation, and neuron-satellite glial cell (SGC) unit formation. We identified two unspecialized sensory neuron populations (uSN1 and uSN2) emerging from two neurogenesis waves and revealed a human-enriched NTRK3+/DCC+ nociceptor subtype involved in multimodal nociceptive processing. Additionally, we explored the spatial interactions between neuronal subtypes and glial cells and found specific cell interactions that mediate the functional maturation of sensory neuron subtypes. In summary, our research elucidated the multilevel signaling pathways and TF regulatory hierarchies that underpin the diversity of human sensory neurons.

Keywords: human dorsal root ganglia, sensory neuron, neurogenesis wave, transcription factors, single-cell spatial transcriptome, nociceptor, TF-seqFISH

PROBING AND ANALYZING THE ACTIVITY OF SPINAL ORGANOIDS WITH ADVANCED HIGH DENSITY MICROCHIP TECHNOLOGY

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Spinal organoids present a cutting-edge in vitro model to explore the complexities of spinal cord-related diseases, offering a dynamic environment that closely mirrors in vivo conditions. However, obtaining large volumes of high-quality, reliable data from these complex systems remains a significant challenge.

This study addresses this limitation by utilizing 3Brain's high-density microelectrode array (HD-MEA) technology, leveraging its 4096 electrodes to assess electrophysiological activity in both isogenic N6 and CS5 spinal organoids lines. Baseline properties were evaluated, and the organoids' response to carbachol, a cholinergic agonist, was analyzed. Spiking and bursting activity exhibited consistent and reproducible patterns, underscoring the robustness of this model system in conjunction with HD-MEA recordings.

Our findings emphasize the importance of HD-MEA technology in deepening our understanding of the functional characteristics of spinal organoids and their responses to pharmacological treatments. This platform holds significant potential for testing therapeutic interventions and driving forward research into spinal cord-related disorders.

DEVELOPMENT OF BRAIN-GUT ASSEMBLOIDS TO RECAPITULATE INTER-ORGAN PROPAGATION OF PARKINSON'S DISEASE PATHOLOGY

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopamine neurons in the substantia nigra. Recent studies suggest inter-organ communication, particularly the gut-brain axis, plays a pivotal role in PD pathogenesis. Alpha-synuclein aggregates, a key pathological hallmark, may spread through the vagus nerve, and PD is frequently associated with prodromal constipation, which can manifest years prior to the onset of motor symptoms. These findings suggest two PD subtypes: "brain-first," where symptoms originate in the brain, and "body-first," where symptoms begin in the gut, highlighting the need for early detection and intervention targeting both brain and gut to slow disease progression. Although induced pluripotent stem cells (iPSCs) offer a controlled environment to study multiple factors, current iPSC models face challenges in accurately modeling two distinct organ systems. To address this, we aimed to reproduce pathological inter-organ interactions using brain-gut assembloids. We first developed an induction protocol for hindbrain region-specific brain organoids. According to Braak’s staging, alpha-synuclein deposits initially affect intestinal afferents, reach the dorsal motor nucleus of the vagus nerve (DMV), then spread to the locus coeruleus (LC) and, ultimately, the substantia nigra. By optimizing the retinoic acid concentration, we successfully generated organoids containing ChAT/PHOX2B/PACAP-positive DMV-like neurons and TH/DBH/NET-positive LC-like neurons. We then combined these brainstem organoids with iPSC-derived intestinal organoids to create brain-gut assembloids. In the absence of PRPH/PHOX2B-positive enteric neurons in the normal state, the co-culturing of intestinal organoids with enteric neural progenitor cells prior to assembly resulted in the emergence of enteric neurons at the brain-gut border. To confirm the utility of this system for PD research, we conducted alpha-synuclein pseudo-propagation experiments. Adeno-associated virus (AAV) vectors containing the SYN1 promoter-driven human SNCA (alpha-synuclein gene) with FLAG sequence were infected into brainstem organoids, which were subsequently assembled with intestinal organoids. Remarkably, exogenous alpha-synuclein was deposited in the enteric neural filaments, indicating successful propagation from the brain to the gut. This model could be instrumental in elucidating novel mechanisms underlying neurodegeneration and identifying new therapeutic targets through multi-organ interactions.

NMNAT2 MAINTAINS CYTOSOLIC NAD^+/NADH IN MITOCHONDRIA-SPARSE AXON TO EMPOWER GLYCOLYSIS-FUELED FAST TRANSPORT

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Extensively arborized long-range axons constitute brain-wide connectivity. Within the restricted dimension of axonal compartments far away from the cell body, mitochondria distribution is relatively sparse. In the absence of mitochondria, glycolytic enzymes have been shown to associate with fast-moving vesicles to provide the onboard ATP source for fast axonal transport. The redox reactions in the glycolysis pathway are driven by NAD^+ to NADH ratio. However, a relatively high NAD^+/NADH in the cytoplasm requires mitochondrial oxidative phosphorylation to recycle NAD^+ from NADH. How is cytosol NAD^+/NADH maintained in mitochondria-sparse axon remains unknown. Here, by employing genetic-encoded metabolite sensor imaging in live neurons together with time-lapse imaging, we discovered that (1) NMNAT2 serves as the chief molecule for NAD^+/NADH maintenance in distal axonal segments; (2) NMNAT2 sustains axonal ATP through glycolysis independent of mitochondrial oxidative phosphorylation; (3) NMNAT2 enables fast transport in distal axons through glycolysis. Deleting NMNAT2 specifically in cortical glutamatergic neurons *in vivo* leads to transport cargo accumulation in long-range axon projecting areas. Both NMNAT2 transcript and protein levels are largely reduced in postmortem human brains suffering from Alzheimer's disease (AD). To develop neuroprotective strategies against NMNAT2 reduction, we would like to utilize CRISPR-Cas9 tools to introduce various dosages of NMNAT2 reduction in human neurons differentiated from iPSC or directly converted from fibroblasts preserving aging signatures. With the human-relevant NMNAT2-deficient models in hand, we would like to test if rewiring the alternative metabolic pathways mediated by lactate dehydrogenase A, malate dehydrogenase 1, or glycerol-3-phosphate shuttle enhances the resilience of NAD+/NADH balance in distal axons.

DEVELOPMENT OF AN ORGANOID PLATFORM TO STUDY BRAIN DEVELOPMENT IN THE MEDIAL PALLIUM

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The establishment of stem cell culture conditions, combined with advancements in three-dimensional (3D) culture techniques, has enabled the development of organoids. Organoids are 3D multicellular, stem cell-derived microtissues designed to closely mimic the complex structure and functionality of human organs. During mammalian telencephalon development, the medial pallium gives rise to the medial entorhinal cortex, hippocampus, and cortical hem. Notably, the hippocampus and entorhinal cortex play crucial roles in learning and memory and are among the first regions affected in neurodegenerative diseases such as Alzheimer's disease. Here, we describe a method for differentiating human embryonic stem cells into hippocampus-like organoids through medial pallium induction. By guiding stem cells towards a specific fate using precise culture conditions and signaling cues, we can generate organoids that closely resemble key regions of the hippocampus. These hippocampal-like organoids express markers specific to the medial pallium, providing an innovative model to study early hippocampal development. This study presents a significant step forward in modeling complex brain structures in vitro, offering valuable insights into the mechanisms that underlie neurodevelopment and disease progression within the medial pallium.

ALTERED ASTROCYTE MORPHOLOGY AND REACTIVITY IN HUMAN BRAIN ORGANOID MODELS OF TUBEROUS SCLEROSIS COMPLEX

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the *TSC1* or *TSC2* genes, whose protein products negatively regulate mTORC1 signaling. TSC is associated with epilepsy, variable intellectual disability, autism spectrum disorder, and other neuropsychiatric conditions. A hallmark pathology of TSC is the presence of cortical tubers, which are focal brain malformations that arise during embryonic development. Tubers contain dysplastic and abnormal neurons and glia and can often become seizure foci. How cortical tuber cells drive seizures and epileptogenesis is not well understood. Analysis of resected patient tissue has revealed marked signatures of astrocyte reactivity and neuroinflammation within tubers. Based on this, we hypothesize that altered glial cell function, particularly astrocytes, may be a key contributor to seizure pathophysiology in TSC.

To test this idea, we have established human brain organoid models of TSC to investigate how loss of *TSC2* affects neural development. Our single-cell RNA sequencing (scRNA-seq) results from *TSC2-/-* cortical organoids provide insights into the role of astrocytes in TSC pathogenesis. The most pronounced phenotypes we observe are an increase in glial-lineage cells compared to neurons and astrocyte reactivity, which has also been observed in patient cortical tuber samples.

Here, we validate the scRNA-seq results through western blot of whole organoids and find alterations in mTOR signaling and astrocytic proteins, several of which correspond to genes that were shown to be altered in astrocyte clusters by scRNA-seq. To directly investigate the functional and morphological changes in astrocytes, we perform immunopanning of *TSC2-/-* astrocytes from brain organoids. We find that *TSC2-/-* astrocytes from day 250-350 organoids are highly enlarged and have altered expression of astrocytic proteins.

This work supports our observation that complete loss of TSC2 during neural development biases developmental trajectories toward the generation of astroglial cells, which show hallmarks of reactivity at early developmental stages. Also, this work provides the groundwork for future studies investigating the role of astrocytes as a major contributor to *TSC2*-related epilepsy.

PRECISION DRUG SCREENING IN 3D GLIOMA CEREBRAL ORGANOIDS: A NOVEL TRACKING APPROACH

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Glioblastoma multiforme (GBM), the most common primary malignant brain tumor in adults, remains incurable despite extensive research and clinical efforts. The use of preclinical models that fail to accurately replicate the biological complexity of human disease for drug screening has been suggested as a contributing factor to our inability to identify clinically effective drugs for GBM. Our lab has developed the cerebral organoid glioma (GLICO) model, a co-culture of human cerebral organoids and patient-derived glioma stem cells (GSCs). The GLICO model better recapitulates the human GBM microenvironment and intratumor heterogeneity, making it a promising model for improved drug screening.

The use of the GLICO model as a novel patient-specific drug screening assay requires developing tools to precisely assess tumor cell viability in real-time and at multiple time points within a normal cerebral organoid (CO) without disrupting the organoid. Traditional in vitro tumor organoid viability assays, however, fail to distinguish between cell populations and are endpoint-destructive, preventing analysis of tumor response over time.

To address these limitations, we present a novel, non-destructive, and automatable luminescence-based tumor tracking system compatible with both 2D cell-autonomous and 3D co-culture tumor models. We integrate a lentivirus carrying NanoLuc, a small non-secreting luciferase, fused with the PEST degradation tag, into GSCs (NLucP GSCs). The NLucP luminescence serves as an indicator of GSC viability, allowing real-time evaluation of tumor growth. The luminescence is detected using Endurazine, a stable substrate added directly to the GSCs, providing continuous measurement with minimal sample manipulation. Our method successfully tracked NLucP GSC growth both in 2D and within GLICOs for a week without disrupting the integrity of the CO, with luminescence values linearly correlating with established viability assay readouts (R^2 2D=0.99, R^2 GLICO=0.88).

To assess the utility of our system for drug screening, we calculated the IC50s and drug sensitivity scores for three anti-tumor drugs in NLucP GSC 2D cell lines, and subsequently treated corresponding NLucP GLICOs with the same drugs at clinically relevant doses (serum Cmax). Our system detected GSC drug responses in both 2D and GLICOs with high reproducibility across manual and high-throughput settings. Notably, GSCs exhibited differential responses to the same treatments in 2D versus GLICOs, underscoring the significance of integrating more physiologically relevant 3D organoid models into drug screening.

Our system provides a novel, sensitive, and robust tumor tracking platform, enabling real-time, non-destructive measurement of GSC viability within the clinically relevant GLICO model, improving the predictive value of drug screening and precision medicine for GBM.

PREVENTING APOPTOSIS IMPROVES RGC DEVELOPMENT AND SURVIVAL IN HUMAN RETINAL ORGANOIDS

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How cell death impacts the development of the human retina is poorly understood. To model human retinal development, we studied human stem cell-derived retinal organoids. Whereas retinal cell composition, morphology, and neuronal function have been studied in organoids, the temporality and roles of cell death have not been well examined. Our data suggest that retinal organoids proceed through two waves of apoptosis, with the death of retinal progenitor cells (RPCs) early and retinal ganglion cells (RGCs) later. A wave of necrosis in the organoid core affecting RGCs leads to a complete loss of these cells. Inhibition of apoptosis in BAX/BAK double knockout (BAX/BAK dKO) organoids prolonged the survival of RGCs and promoted the maintenance of a subpopulation of RGCs. The RGC density in BAX/BAK dKO organoids declined over time due to a compensatory increase in necrosis. Taken together, inhibition of BAX/BAK-mediated apoptosis prolongs RGC lifespan and promotes the maintenance of specific RGC subtypes in human retinal organoids. As RGCs are susceptible to impairment and death during glaucoma, a leading cause of vision loss, our findings inform organoid design for potential therapeutic applications.

INVESTIGATING HUMAN-ENRICHED CELLULAR DEFICITS IN CDKL5 DEFICIENCY DISORDER USING CEREBRAL ORGANOIDS

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CDKL5 deficiency disorder (CDD) is an X-linked neurodevelopmental disorder caused by pathogenic variants in the CDKL5 gene. Patients with CDD exhibit a wide range of symptoms, including early-onset epilepsy, global developmental delay, intellectual disability, autistic features, visual impairment, and motor impairment. The CDKL5 gene is located at position 22 on the X chromosome and encodes the CDKL5 protein, a member of the serine/threonine kinase family. Studies over the past decade using various cellular and animal models have implicated CDKL5 in various cellular functions, such as cell proliferation, neuronal migration, dendrite development, synapse formation, and synaptic function. However, the mechanisms through which CDKL5 dysfunctions contribute to the pathophysiology of CDD remain unclear. Notably, genetic knockout of CDKL5 in mice fails to reproduce the seizure phenotypes observed in human patients, emphasizing the urgent need for disease-relevant human models of CDD to elucidate the functional roles of CDKL5.
To comprehensively understand the molecular functions of CDKL5 and expedite therapeutic development, it is crucial to identify its direct substrates and analyze its function in clinically relevant cell types and models. Three-dimensional brain organoids generated from patient-derived induced pluripotent stem cells (iPSCs) serve as a robust model for investigating the underlying mechanisms of CDD with a human background. By employing single-cell RNA sequencing and immunostaining techniques, this study uncovered previously unrecognized proliferation and maturation deficits among major neural progenitor stem cells in CDD organoids. Given that CDKL5 is a serine/threonine kinase, the study explored several potential downstream substrates, including the microtubule-binding protein EB2. The findings of this study propose an uncharacterized mechanism underlying CDD pathology and offer crucial insights into the exploration of novel treatment strategies for CDD. This work was supported by the Lo Kwee-Seong Biomedical Research Fund (J.I), Faculty Innovation Award (FIA2020/A/04) from the Faculty of Medicine, CUHK (J.I.), Hong Kong RGC Research Matching Grant Scheme (J.I.) and Hong Kong PhD Fellowship (PF20-43681; Y.Z.). The travel is supported by IBRO Travel Grant (Y.Z.).

DECODING HUMAN CORTICAL DEVELOPMENT: INTEGRATING SINGLE-CELL TRANSCRIPTOMICS ACROSS DEVELOPMENTAL STAGES AND EXPERIMENTAL MODELS

Asia Zonca, Erik Bot, Josè Davila-Velderrain

Human Technopole, Neurogenomics, Milan, Italy

Single-cell genomic technologies have revolutionised the way the human brain is experimentally interrogated. Profiling the transcriptome of individual cells is particularly interesting in cortical development, where brain cell diversity of relevance for human cognitive traits is primarily generated. Broad application of this technology allowed the generation of atlases depicting specific windows of brain development, offering an increased resolution to capture subtle variations characterising the dynamics of development. Moreover, with the recent improvements of in vitro models, nowadays it is possible to mimic aspects of brain development in a dish and to investigate how sequential developmental processes take place in a more controlled environment. However, both single-cell, 2D and 3D in vitro models come with technical challenges and limitations. Single-cell technologies are inevitably noisy and sparse, making the analysis computationally challenging; in vitro models are a simplification of an extremely complex and dynamic mechanism that is difficult to compare to what happens in vivo.
To aid the interpretation of in vitro and in vivo brain development, we present a computational resource that learns, integrates, and interprets de novo unbiased patterns (prototypes) from single-cell transcriptomics data spanning human cortical development. We classified human neurodevelopment into 13 stages based on well-known biological milestones and compiled corresponding single-cell transcriptomic data encompassing ventro-dorsal and rostro-caudal development across stages. We collected and analysed 119 samples, each representing a single time point, for a total of 79 unique ages and 1,617,236 single cells. To account for technical and biological challenges, we subsampled cells from outlier samples, harmonised cellular taxonomy, and learned unbiased prototypes representing aggregates of cells and their transcriptional signatures. To obtain a single, cross-data developmental reference, we developed a method to integrate the prototypical signatures by mapping them to biologically interpretable and constructed a prototype network for joint analysis and visualisation. To better investigate the physiological dynamics of neurogenesis and gliogenesis, we built lineage-specific reference networks to highlight the diversity of the neuronal and glial cells as development progresses. We leveraged the reference network to biologically interpret new data from different experimental models, including 2D and 3D cultures, animal models, and newly generated primary data from both single-cell and tissue-level profiling.

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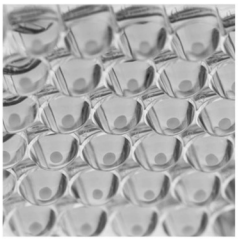

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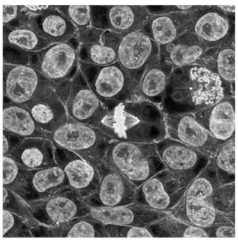

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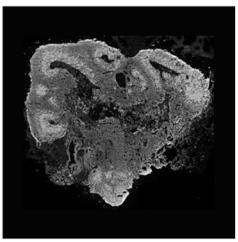

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Talk includes live software demo

To illustrate the practical implications of **CorePlate™**, the session will also feature a live demonstration of data interpretation and analysis. This will offer attendees an in-depth look at how the platform works in real-world applications, showcasing its ability to generate meaningful data from high-resolution datasets. We will provide the audience a clear understanding of how to apply **CorePlate™** technology to study electrogenic cells at an unprecedented level of detail.

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UNLOCKING THE POTENTIAL OF NEURAL ORGANOIDS: A HANDS-ON WORKSHOP WITH MAXWELL BIOSYSTEMS' HD-MEA TECHNOLOGY

Speakers: Silvia Oldani, Marie Engelene Obien and Praveena Manogaran.

MaxWell Biosystems AG, Zurich, Switzerland

Neural organoids derived from human induced pluripotent stem cells (h-iPSCs) are rapidly emerging as promising tools for the investigation of developmental and disease progression. Organoids and 3D models allow for rapid, non-invasive, patient-tailored, and cost-effective drug screenings and disease modelling. Increasingly, assessing the cell type composition, gene expression patterns and physiological function in different types of neural organoids demands new technologies for characterization.
The electrical activity of neurons can now be easily captured, label-free, with MaxWell Biosystems' High-Density Microelectrode Array (HD-MEA) technology. This technology powers both the MaxOne (single-well) and MaxTwo (multi-well) HD-MEA Systems, enabling to observe, record, and analyze electrophysiological properties of electrogenic samples at network, single-cell, and sub-cellular levels at high-resolution. The MaxWell Biosystems' HD-MEA Systems have been shown to provide high-quality, robust, and reproducible data in scientific publications across different applications.

In this workshop, the speakers will:

- Guide the participants on a step-by-step walkthrough for organoid plating on the HD-MEA chip
- Showcase high-resolution functional imaging of organoids on MaxWell Biosystems' HD-MEA platform
- Present results and analysis from organoids modeling different brain compartments
- Demonstrate the advantages of HD-MEA technology for characterizing the physiological function of human brain organoids and for testing compounds

The speakers will showcase how to use MaxWell Biosystems' HD-MEA technology to capture and monitor the electrophysiological properties of brain organoids. This includes a practical demonstration of plating organoids on the HD-MEA chip, followed by showcasing datasets and analyzed results of pre-recorded organoids data. By the end of the workshop, the attendees will learn how to obtain high-quality organoid data and characterize the function of their different samples using the MaxWell Biosystems' HD-MEA technology.

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MaxWell Biosystems is a technology leader providing instrumentation and solutions to boost scientific research and development in neurosciences, stem cell and tissue engineering, ophthalmology, and other fields involving electrogenic cells. The company engineered advanced High-Density MicroElectrode Arrays (HD-MEAs) as the core of easy-to-use platforms, MaxOne (single-well) and MaxTwo (multi-well), that equip scientists to record electrical signals of neurons in in-vitro 2D and 3D models. MaxWell Biosystems' HD-MEA technology allows to capture neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory (CSHL or the Laboratory) is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code's prohibition against discrimination and harassment is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy (*see below)*.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on actual or perceived sex, pregnancy status, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, creed, nationality or national origin, immigration or citizenship status, mental or physical disability status, veteran status, military status, marital or partnership status, marital or partnership status, familial status, caregiver status, age, genetic information, status as a victim of domestic violence, sexual violence, or stalking, sexual reproductive health decisions, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

BREACHES OR VIOLATIONS OF THE CODE OF CONDUCT

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu
- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports may be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code.

The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

If you have questions or concerns, you can contact the meeting organizers, CSHL staff.

For meetings and courses funded by NIH awards:

Participants may contact the Health & Human Services Office for Civil Rights (OCR). See this page for information on filing a civil rights complaint with the OCR; filing a complaint with CSHL is not required before filing a complaint with OCR, and seeking assistance from CSHL in no way prohibits filing complaints with OCR. You may also notify NIH directly about sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported events.

For meetings and courses funded by NSF awards:

Participants may file a complaint with the NSF. See this page for information on how to file a complaint with the NSF.

Law Enforcement Reporting:

- For on-campus incidents, reports to law enforcement can be made to the Security Department at 516-367-5555 or x5555 from a campus phone.
- For off-campus incidents, report to the local department where the incident occurred.

In an emergency, dial 911.

DEFINITIONS AND EXAMPLES

Uncivil/disrespectful behavior is not limited to but may take the following forms:

- Shouting, personal attacks or insults, throwing objects, and/or sustained disruption of talks or other meeting-related events

Harassment is any unwelcome verbal, visual, written, or physical conduct that occurs with the purpose or effect of creating an intimidating, hostile, degrading, humiliating, or offensive environment or unreasonably interferes with an individual's work performance. Harassment is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance

Sexual Harassment includes harassment on the basis of sex, sexual orientation, self-identified or perceived sex, gender expression, gender identity, and the status of being transgender. Sexual harassment is not limited to sexual contact, touching, or expressions of a sexually suggestive nature. Sexual harassment includes all forms of gender discrimination including gender role stereotyping and treating employees differently because of their gender. *Sexual misconduct* is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching
- Groping or sexual assault
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly

MEETING ALCOHOL POLICY

Consumption of alcoholic beverages is not permitted in CSHL's public areas other than at designated social events (wine and cheese reception, picnic, banquet, etc.), in the Blackford Bar, or under the supervision of a licensed CSHL bartender.

No provision of alcohol by meeting sponsors is permitted unless arranged through CSHL.

Meeting participants consuming alcohol are expected to drink only in moderation at all times during the meeting.

Excessive promotion of a drinking culture at any meeting is not acceptable or tolerated by the Laboratory. No meeting participant should feel pressured or obliged to consume alcohol at any meeting-related event or activity.

VISITOR INFORMATION

EMERGENCY (to dial outside line, press 3+1+number)	
CSHL Security	**516-367-8870 (x8870 from house phone)**
CSHL Emergency	**516-367-5555 (x5555 from house phone)**
Local Police / Fire	**911**
Poison Control	**(3) 911**

CSHL SightMD Center for Health and Wellness ***(call for appointment)*** Dolan Hall, East Wing, Room 111 **cshlwellness@northwell.edu**	**516-422-4422** **x4422 from house phone**
Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2000**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400**

GENERAL INFORMATION

Meetings & Courses Main Office

Hours during meetings: M-F 9am – 9pm, Sat 8:30am – 1pm
After hours – See information on front desk counter
For assistance, call Security at 516-367-8870
(x8870 from house phone)

Dining, Bar

Blackford Dining Hall (main level):
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Blackford Bar (lower level): 5:00 p.m. until late

House Phones

Grace Auditorium, upper / lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

Books, Gifts, Snacks, Clothing

CSHL Bookstore and Gift Shop
516-367-8837 (hours posted on door)
Grace Auditorium, lower level.

Computers, E-mail, Internet access

Grace Auditorium
Upper level: E-mail and printing in the business center area
WiFi Access: GUEST (no password)

Announcements, Message Board Mail, ATM, Travel info

Grace Auditorium, lower level

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: **(On your registration envelope)**

Laundry Machines
Dolan Hall, lower level

Photocopiers, Journals, Periodicals, Books
CSHL Main Library
Open 24 hours (with PIN# or CSHL ID)
Staff Hours: 9:00 am – 9:00 pm
Use PIN# (On your registration envelope) to enter Library
See Library staff for photocopier code.
Library room reservations (hourly) available on request between 9:00 am – 9:00 pm

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Local Interest

Fish Hatchery	631-692-6758
Sagamore Hill	516-922-4788
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City
Helpful tip -
Take CSHL Shuttle OR Uber/Lyft/Taxi to Syosset Train Station
Long Island Railroad to Penn Station
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

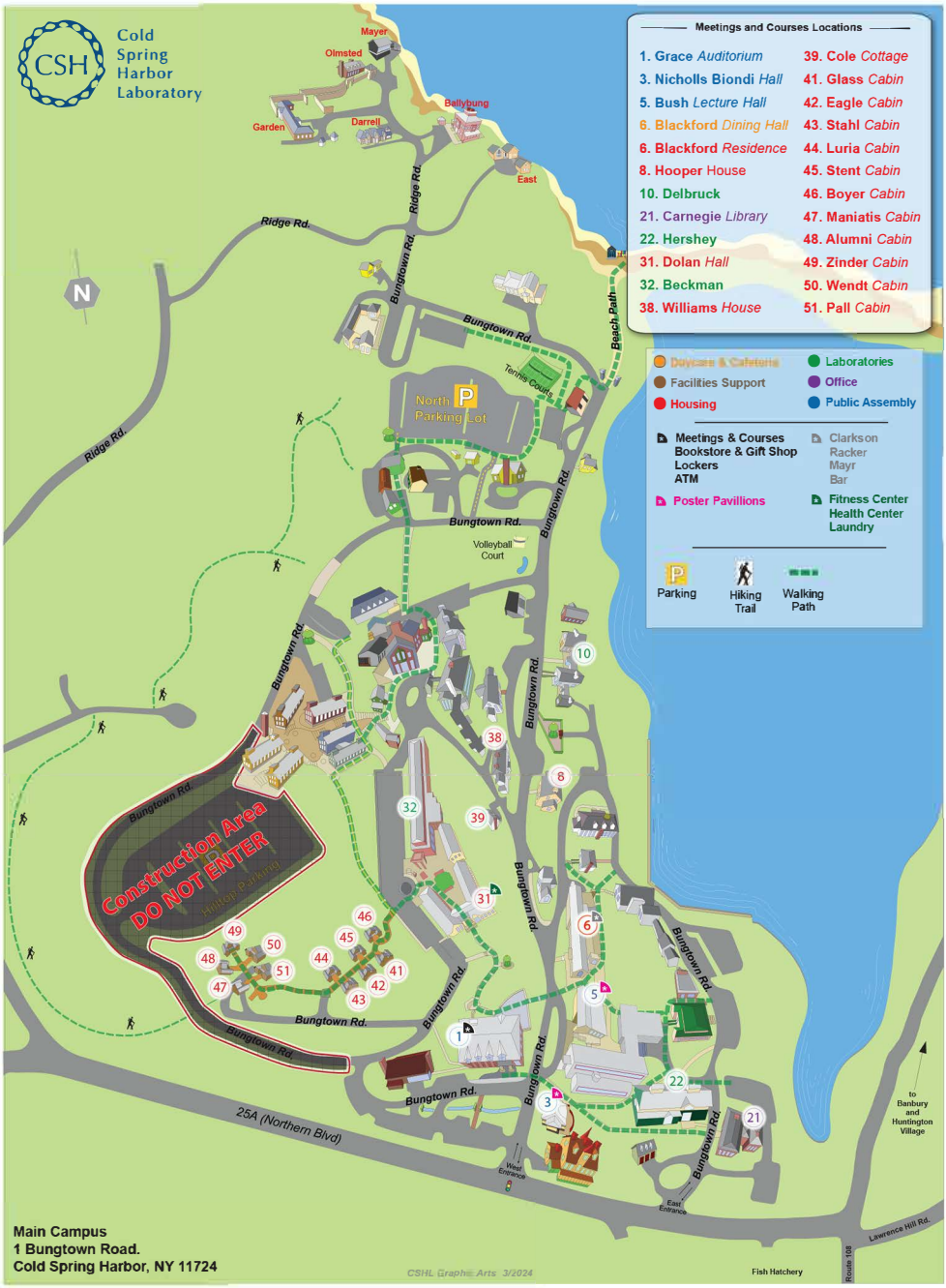
Syosset Limousine	516-364-9681
Executive Limo Service	516-826-8172
Limos Long Island	516-400-3364
Syosset Taxi	516-921-2141
Orange & White Taxi	631-271-3600
Uber / Lyft	

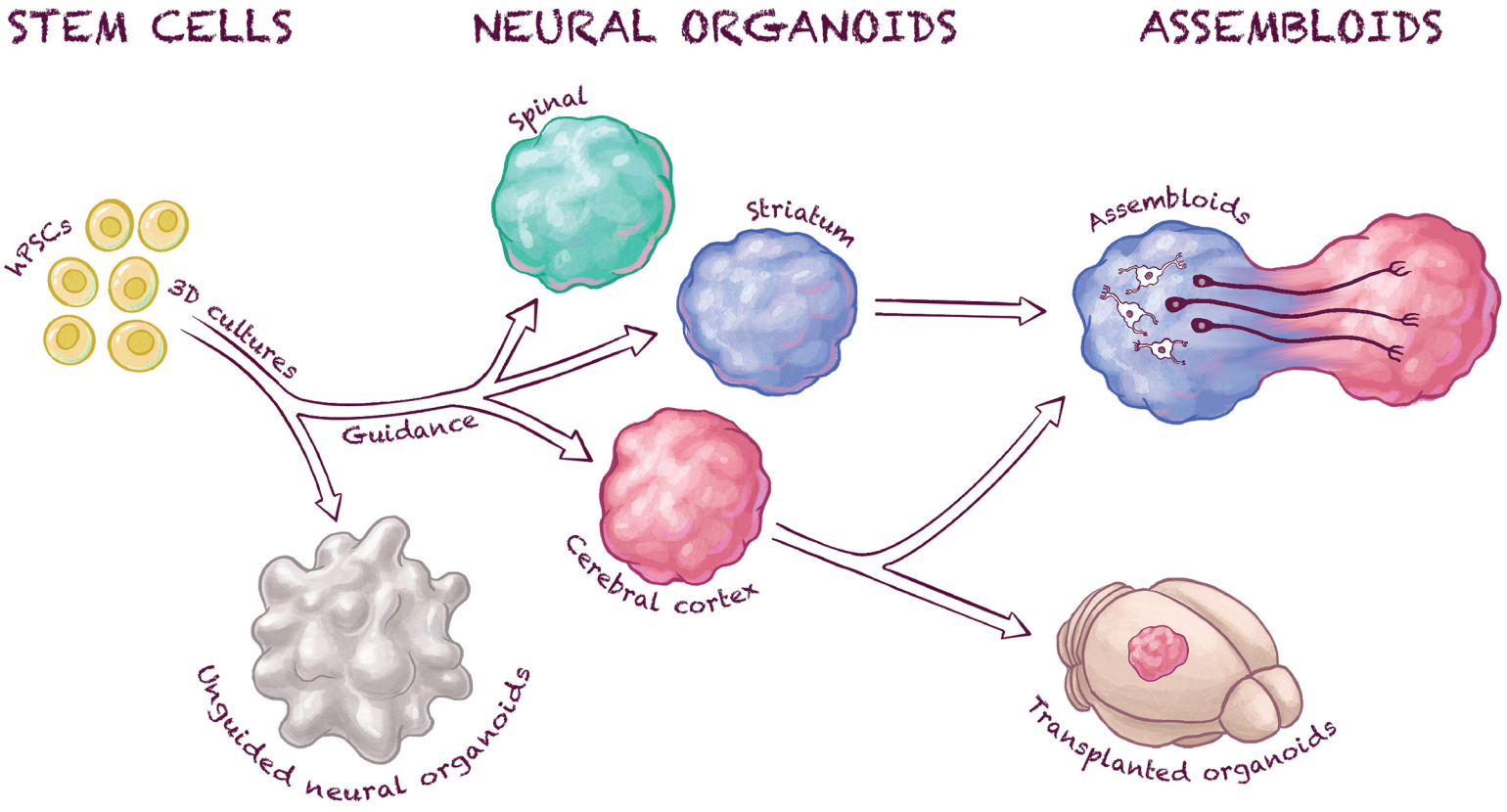
Trains

Long Island Rail Road	718-217-LIRR (5477)
Amtrak	800-872-7245
MetroNorth	877-690-5114
New Jersey Transit	973-275-5555

CSHL Campus Map

CSHL Map




STEM CELLS
NEURAL ORGANOIDS
ASSEMBLOIDS
hPSCs
3D cultures
Guidance
Spinal
Striatum
Cerebral cortex
Unguided neural organoids
Assembloids
Transplanted organoids