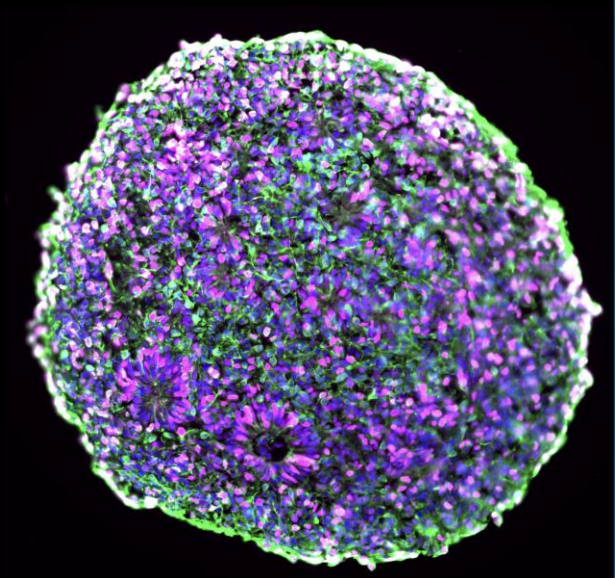

Human Cellular Models and Their Application in RARE Drug Development

Angels Almenar-Queralt, Ph.D.

Assistant Professor

Division of Genetics. Department of Pediatrics

UC San Diego

aalmenar@health.ucsd.edu

Image credit: Surya Venogupal

Agenda

INTRODUCTION

OVERVIEW HUMAN PLURIPOTENT STEM CELL CULTURES

EXAMPLES DISEASE MODELING/THERAPIES

CONCLUSIONS

Agenda

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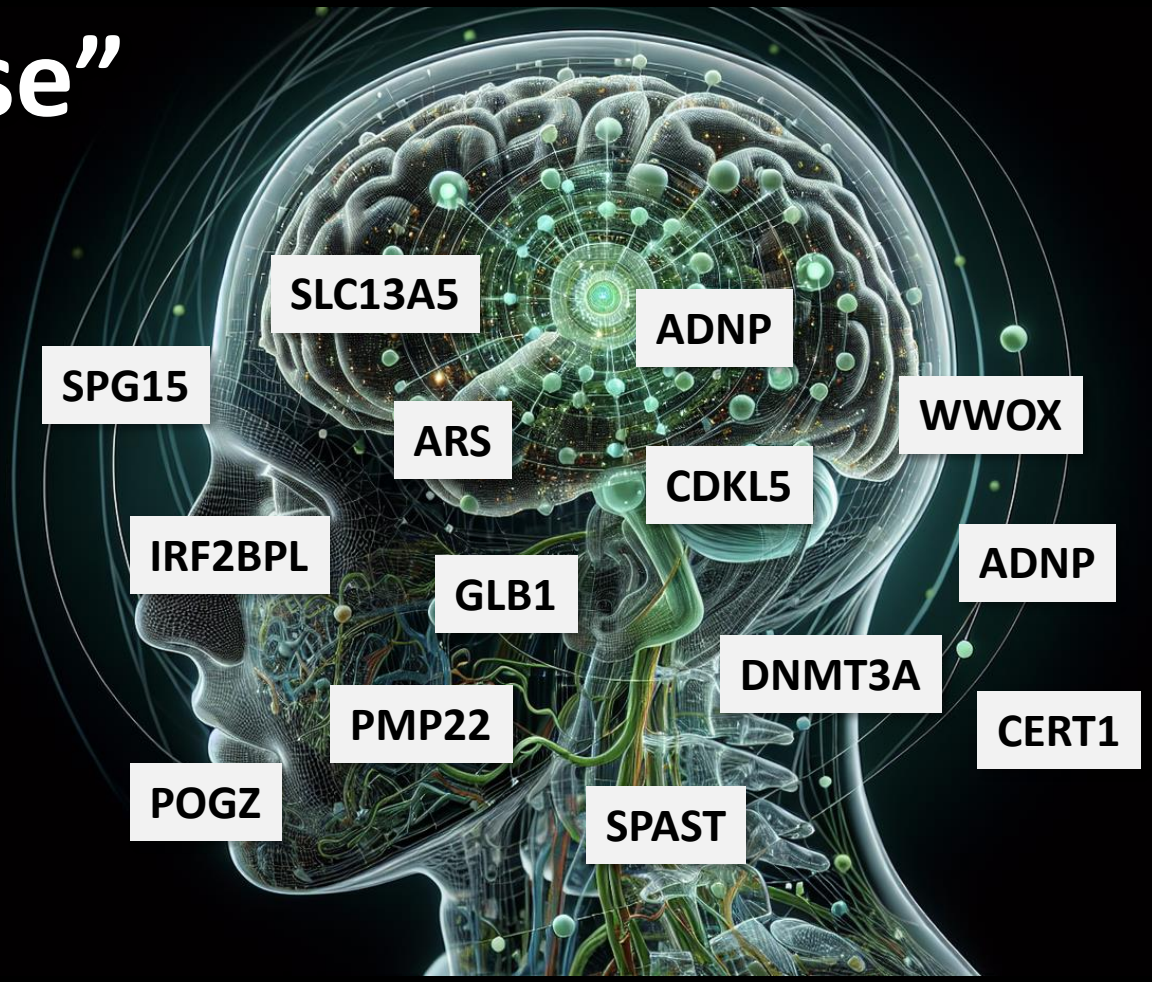
The Complexity of the Human Brain: "A Blessing and a Curse"

- Billions of cells
- Decades to develop
- Highly Vulnerable
- Mutations in thousands of genes can impair neurodevelopment

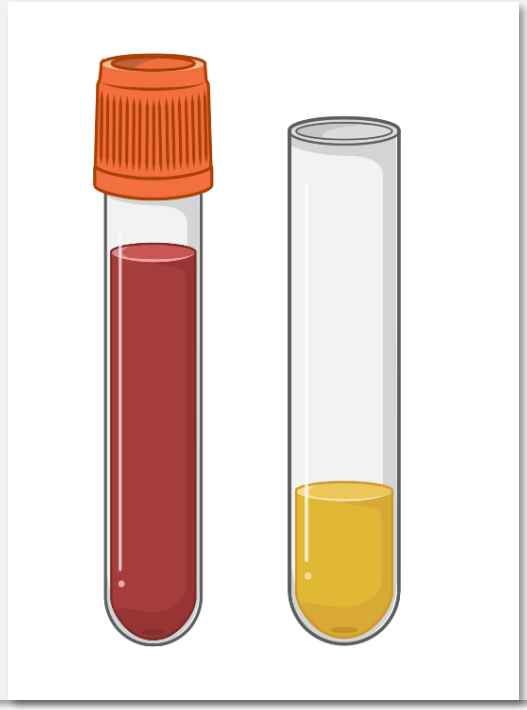


https://due.com/decoding-the-human-brain-body-complexity/

Limitations in Studying the Livi

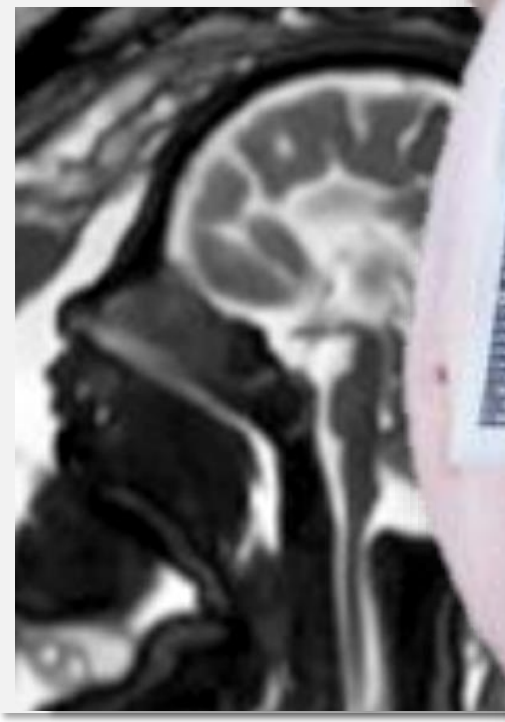
Biomarkers

Indirect Measurements



Imaging

Low Resolut



Limited Predictivity of Animal Models

- Species specific genetics
- Do not fully recapitulate disease complexity
- Shorter lifespans
- Reduced brain size and complexity
- Lack of diversity

Agenda

BACKGROUND m

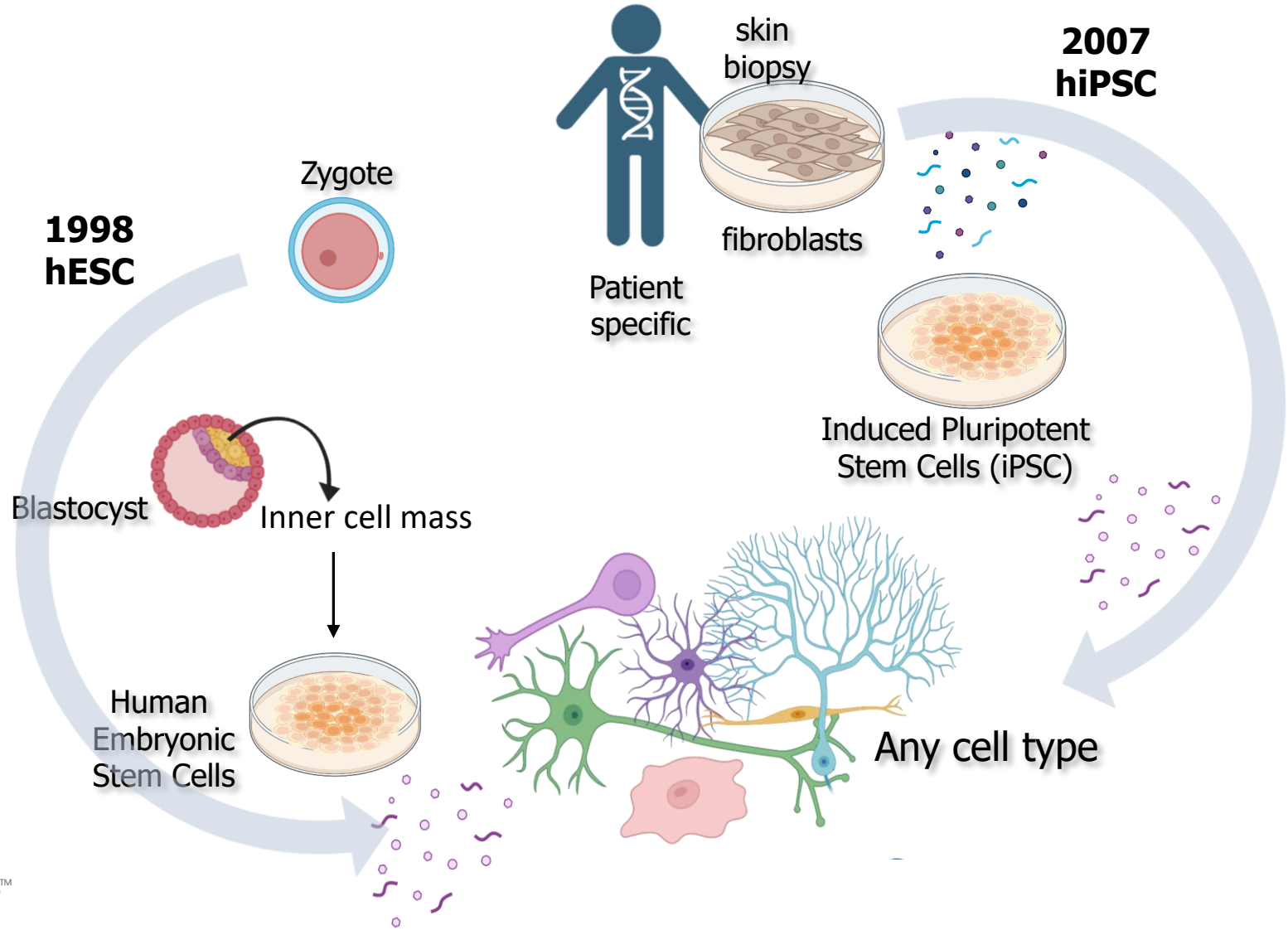
OVERVIEW HUMAN PLURIPOTENT STEM CELL CULTURES

EXAMPLES DISEASE MODELING/THERAPIES

CONCLUSIONS

Human Pluripotent Stem Cells – A Revolution

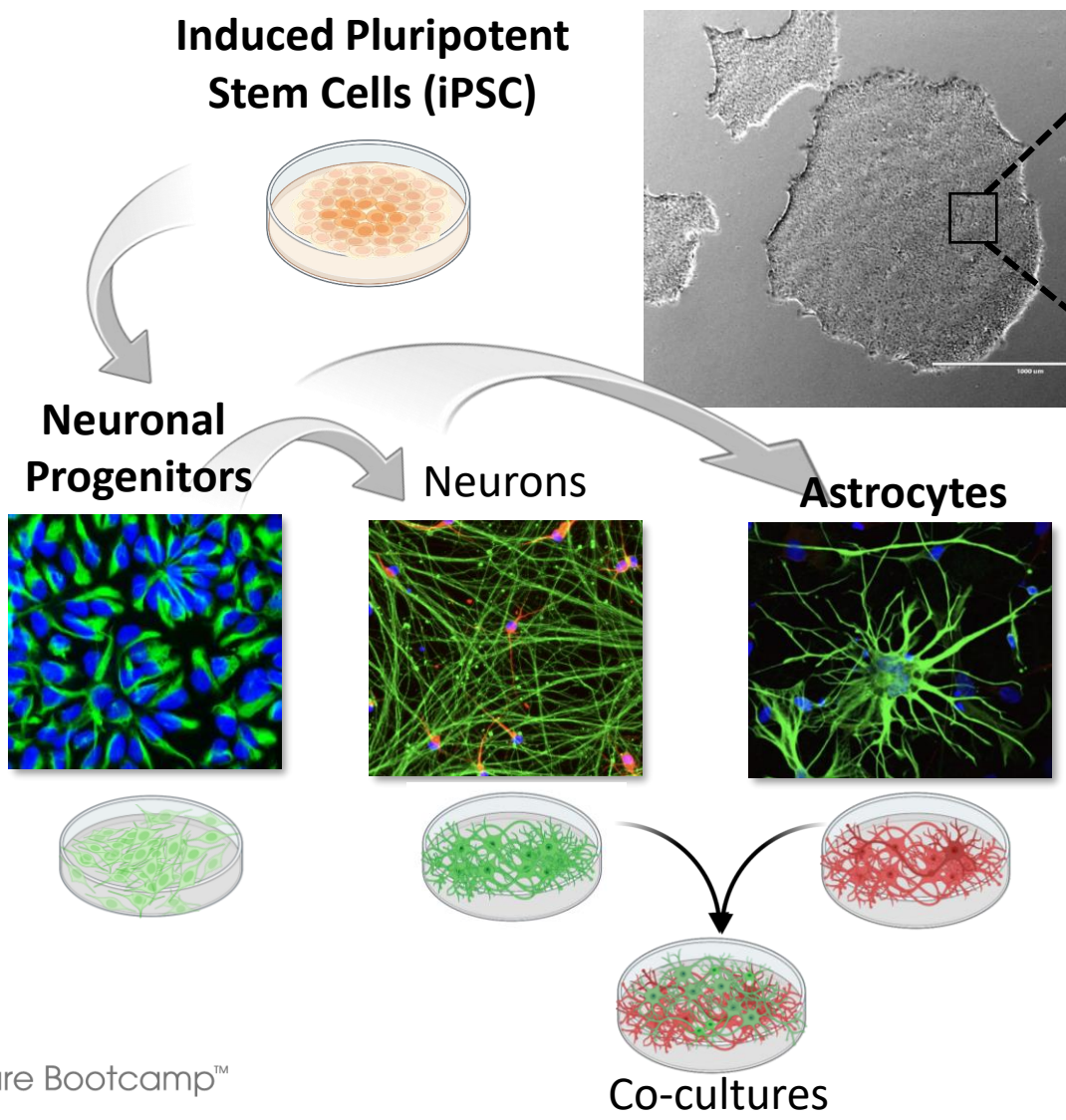
James Thomson

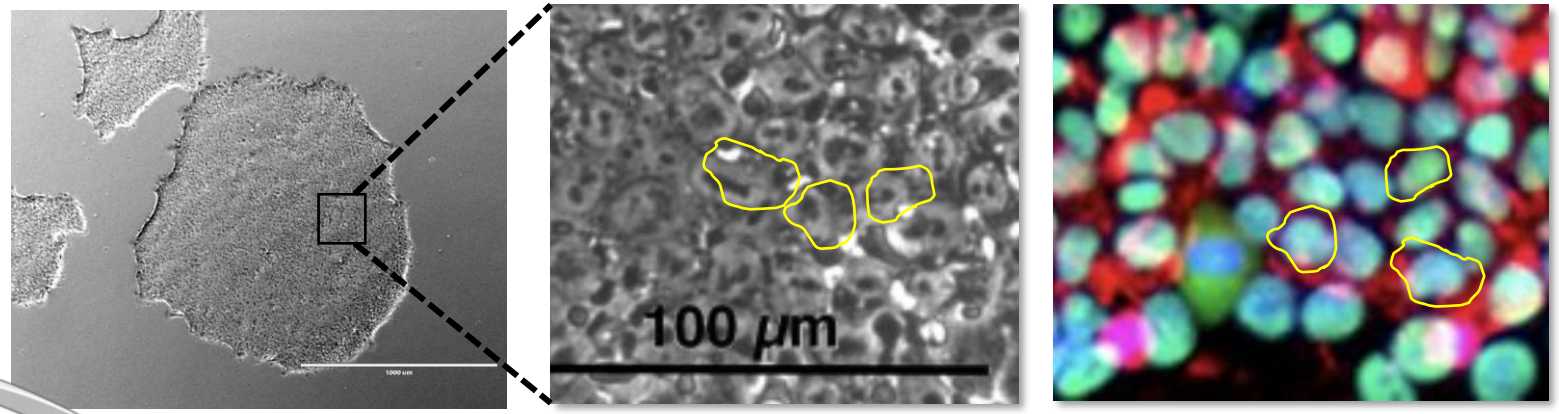


Shinya Yamanaka

2012 Nobel Prize

Two-Dimensional hiPSC-Derived Neural Cultures





Limitations of 2D cultures

- Lack of tissue complexity
- Incomplete cellular maturation
- Homogeneity of cell populations
- Inability to model tissue biomechanics
- Artifact-prone experimental conditions
- Limited lifespan (weeks)

Miniaturizing the Brain Cortex in a Dish

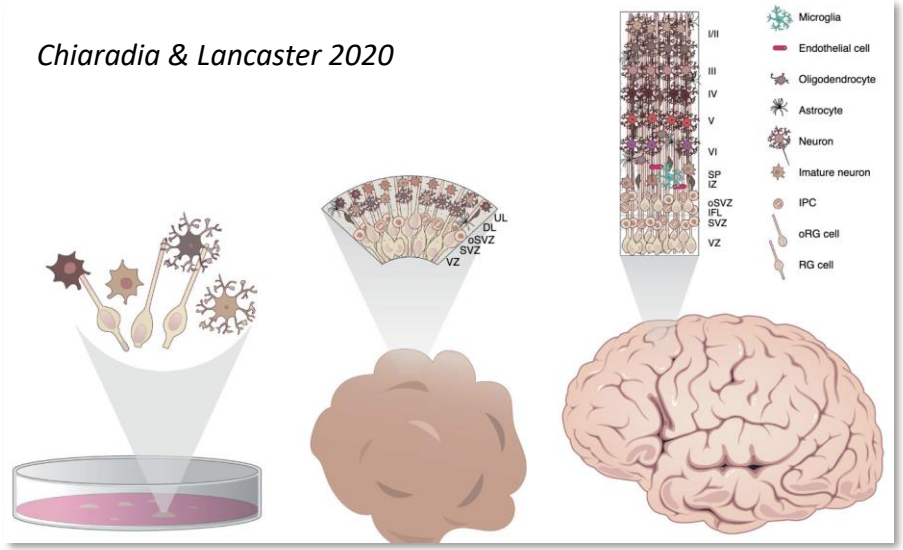
Cell Stem Cell
Article

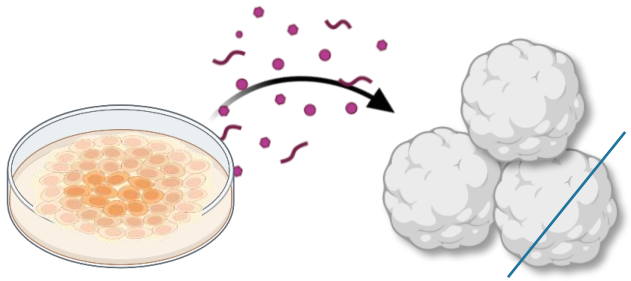
Dr. Sasai's Lab 2008

Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals

Mototsugu Eiraku,[1] Kiichi Watanabe,[1] Mami Matsuo-Takasaki,[1] Masako Kawada,[1] Shigenobu Yonemura,[2] Michiru Matsumura,[1] Takafumi Wataya,[1] Ayaka Nishiyama,[1] Keiko Muguruma,[1] and Yoshiki Sasai[1,*]
[1]Organogenesis and Neurogenesis Group
[2]Electron Microscope Laboratory
RIKEN Center for Developmental Biology, Kobe 650-0047, Japan
*Correspondence: yoshikisasai@cdb.riken.jp
DOI 10.1016/j.stem.2008.09.002

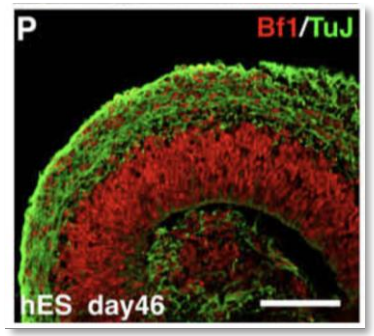
Chiaradia & Lancaster 2020

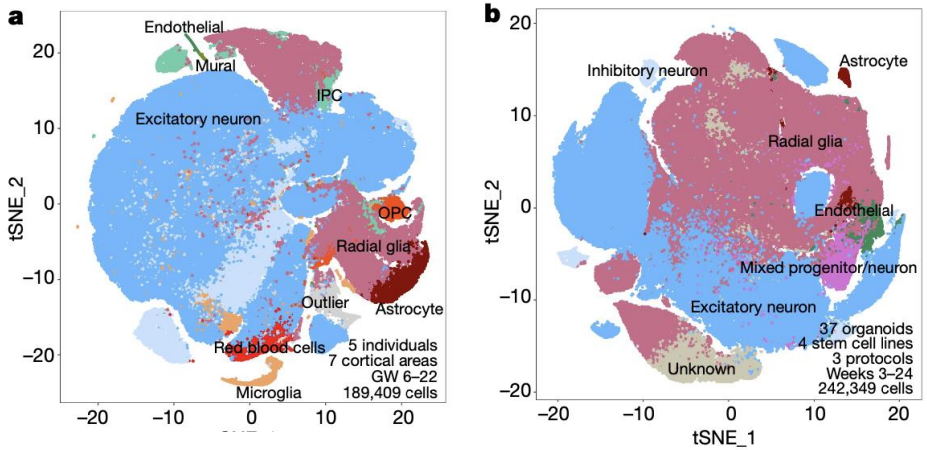




hiPSC

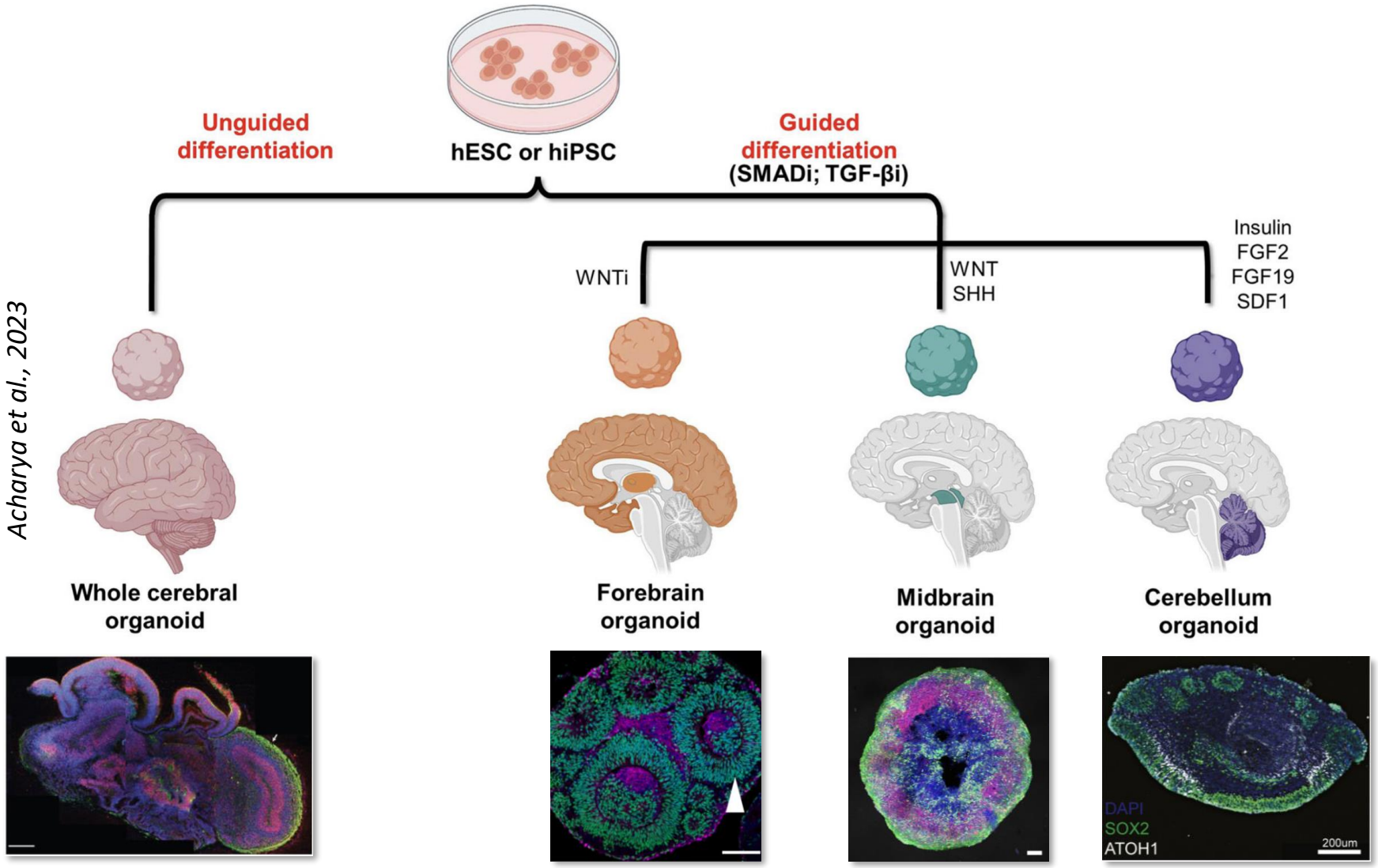
cortical brain organoids





Bhaduri et al., 2020

3D-Neural Organoids: Current Strategies



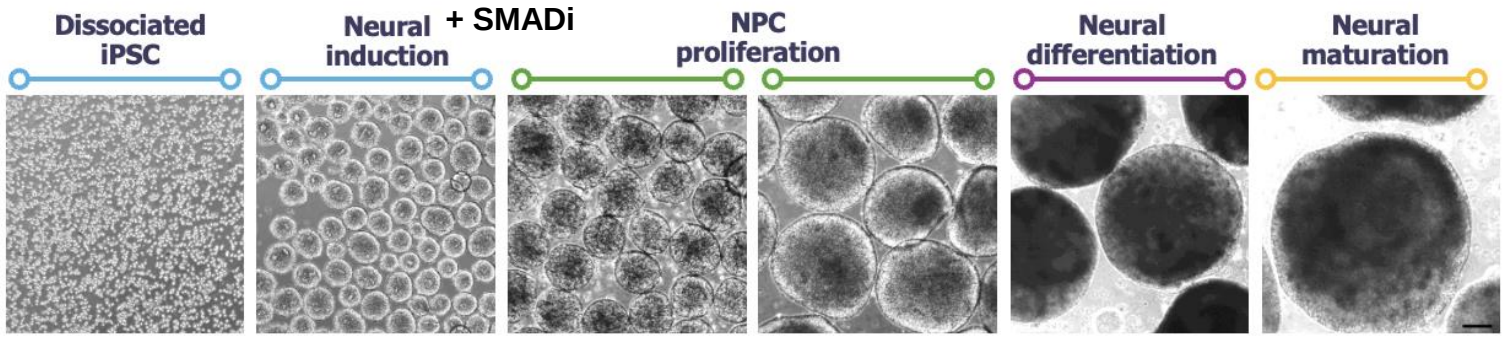
Lancaster et al., 2013

Papes et al., 2022

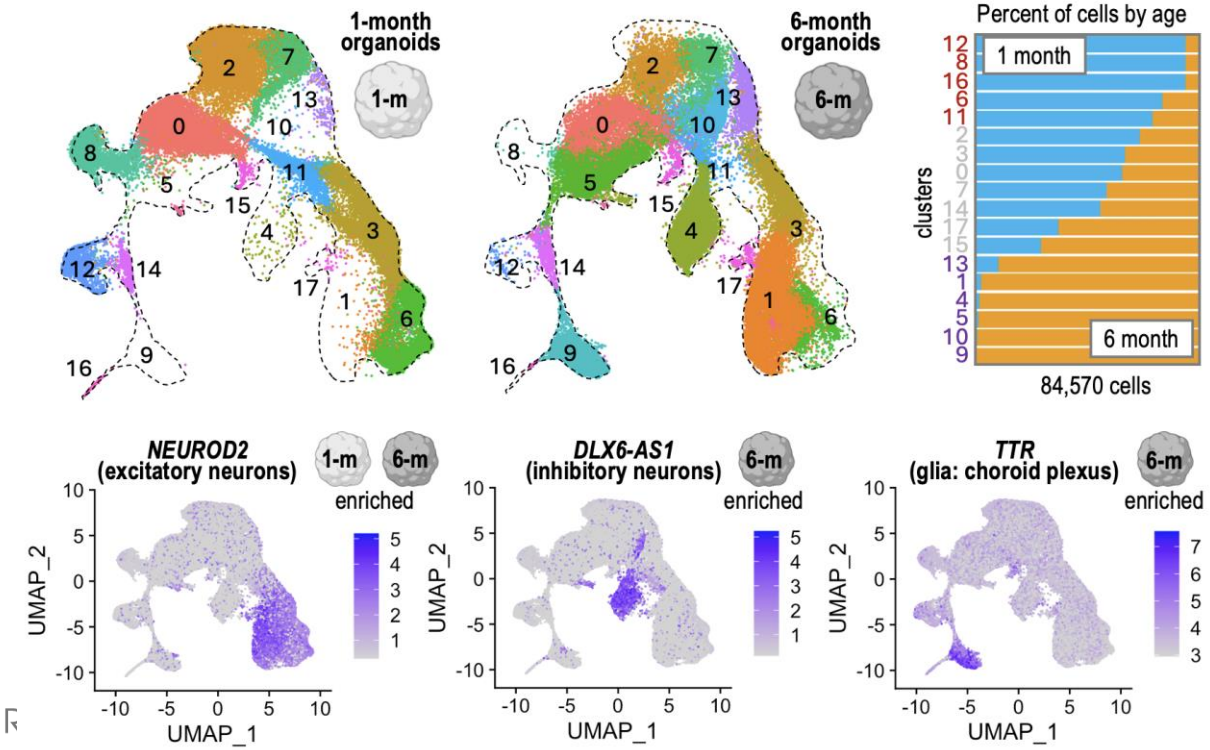
Smits et al., 2019

Atamian et al., 2024

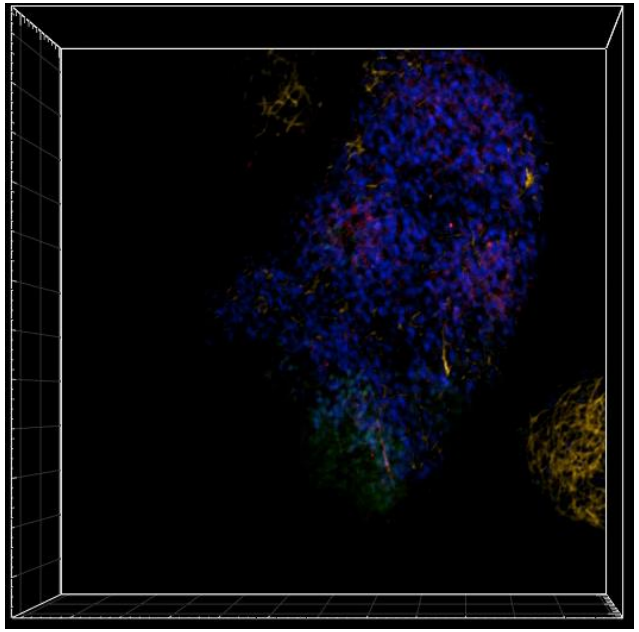
The Muotri Lab Cortical Organoid Recipe



Single cell analysis

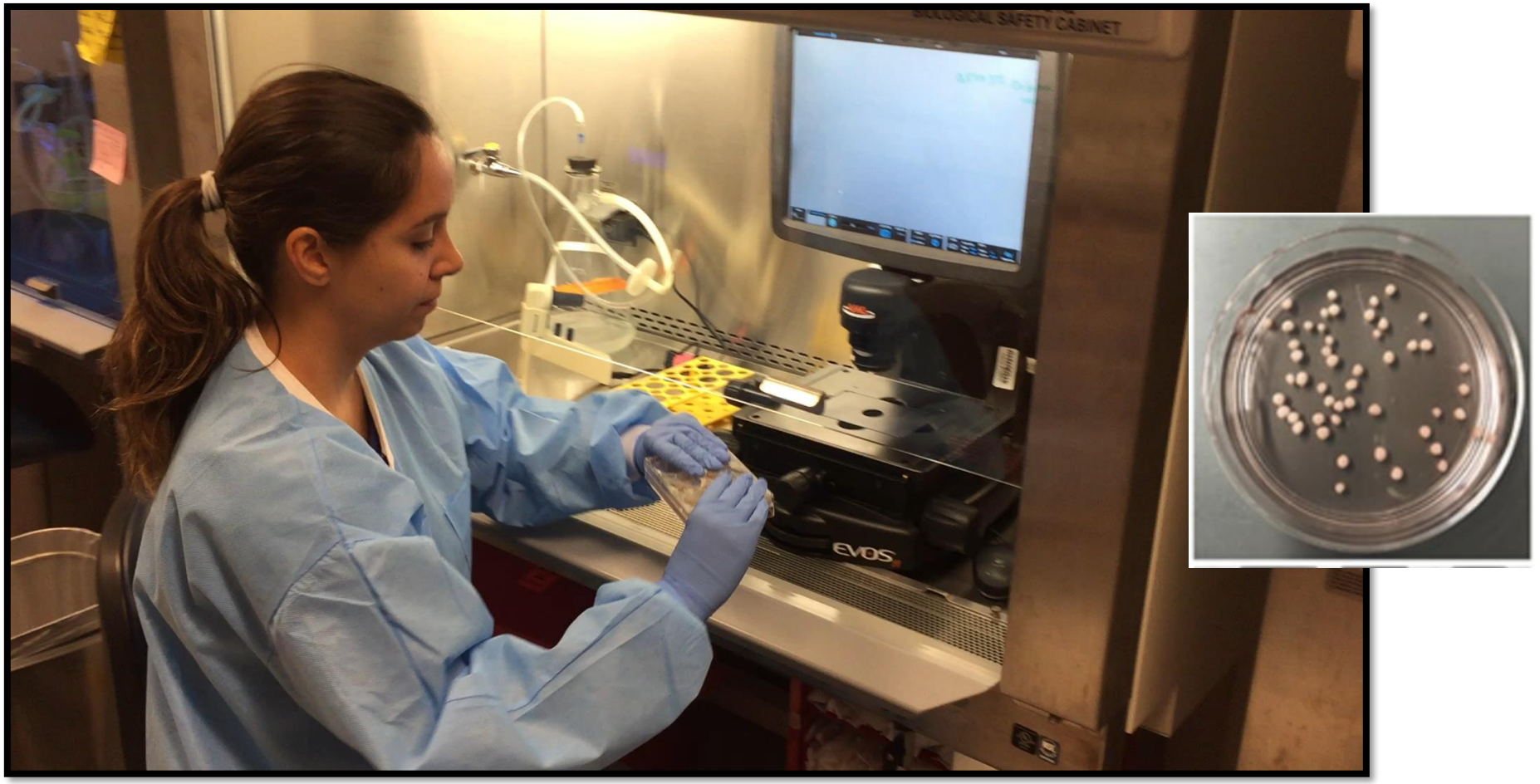


DAPI MAP2 Neurofilament SOX2

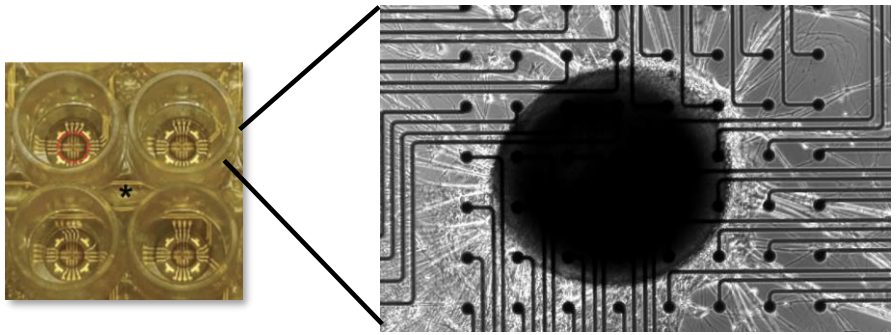


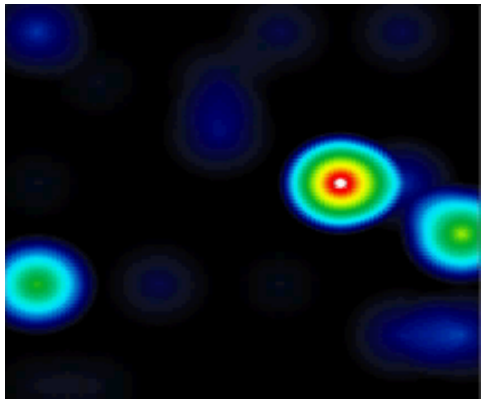
Movie credit: Surya Venogupal

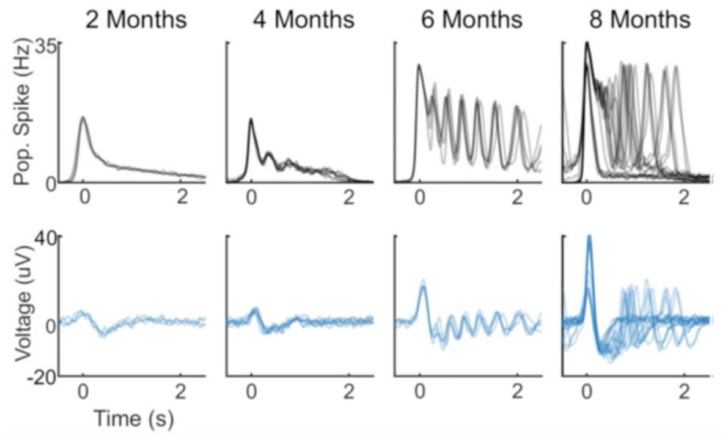
Meet the Muotri Lab Cortical Brain Organoids



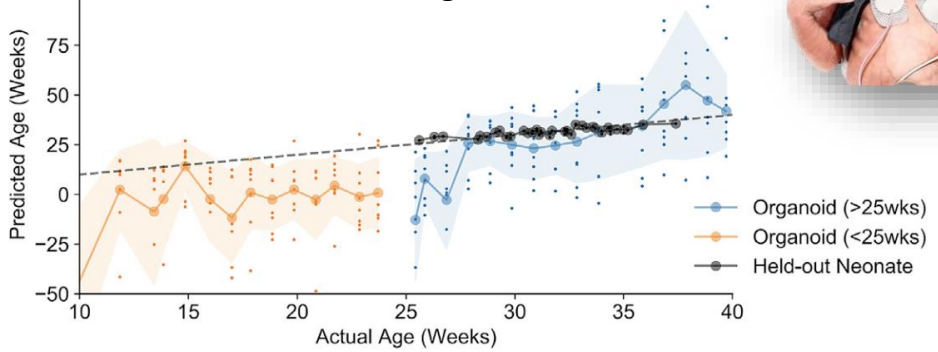
Tracking Network Maturation







Predicted organoid developmental time from neonate regression model



Machine Learning

Trujillo et la., 2019

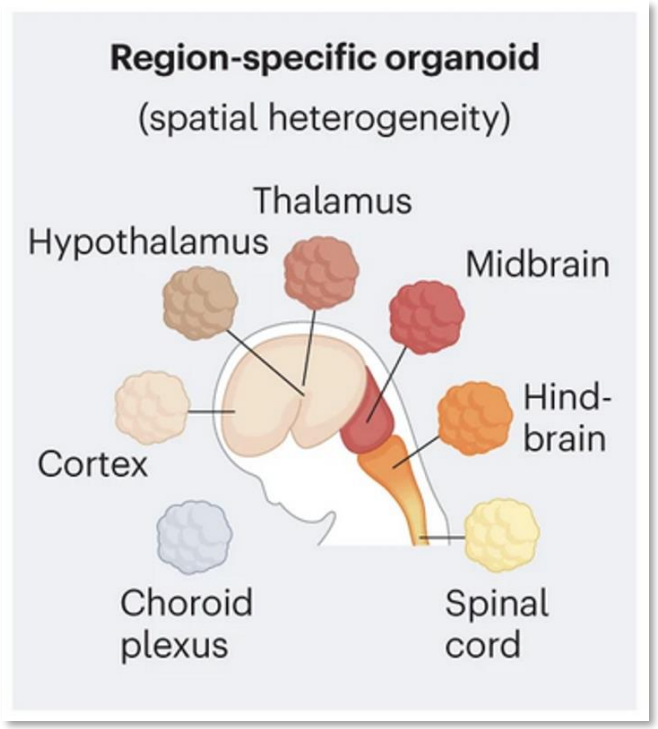
Limitations of 3D-Neural Organoids

- Recapitulate pre-natal features
- Reduced cytoarchitecture complexity
- High inter-organoid variability
- No blood brain barrier
- Missing systemic contribution

>40 neurological disorders modeled

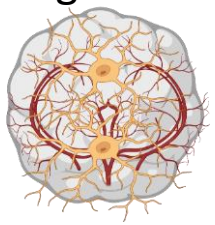
Increasing Complexity In Vitro

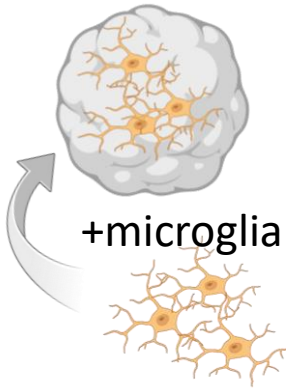
DIFFERENT BRAIN REGIONS



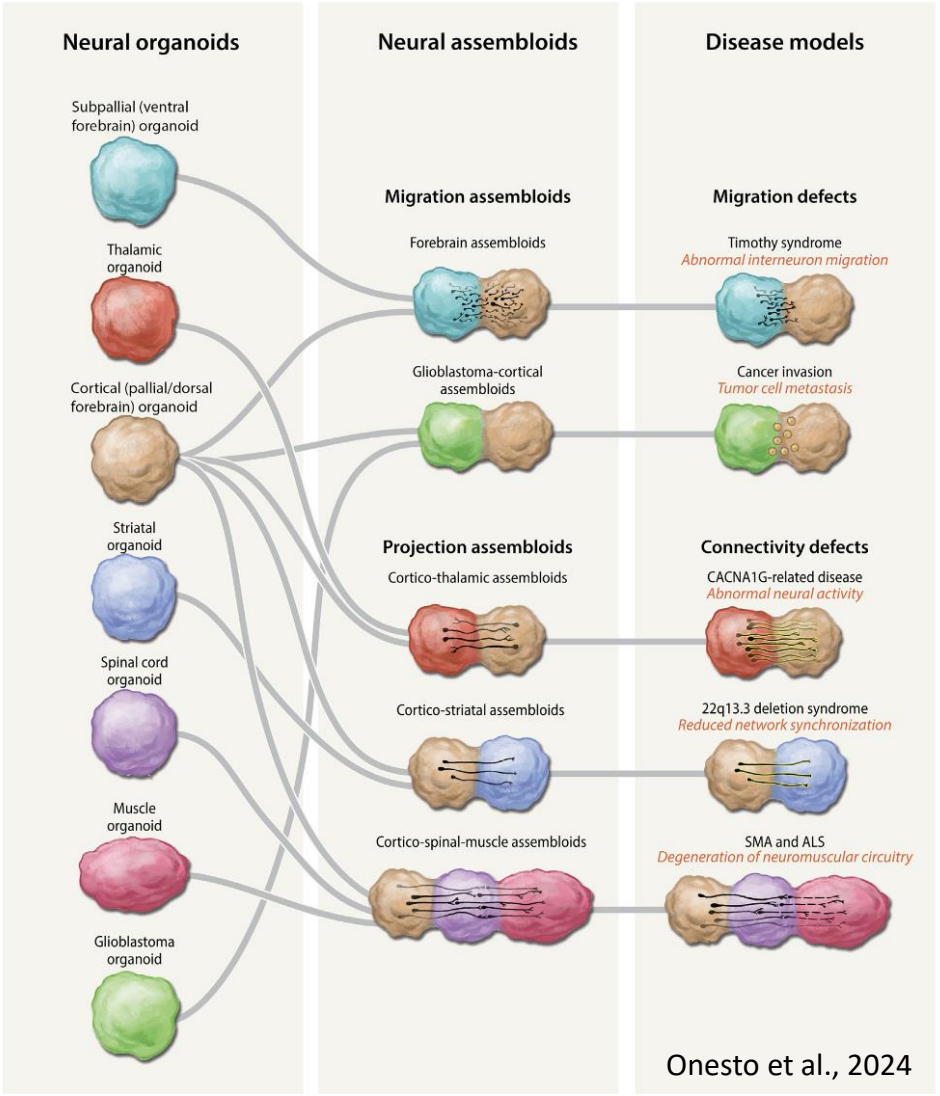
ASSEMBLOIDS

Vascularized organoids



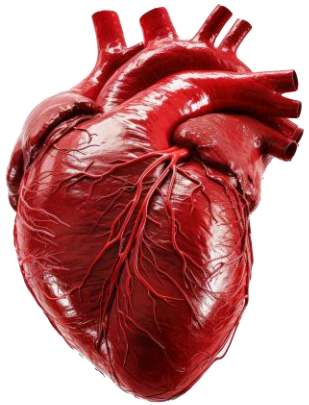


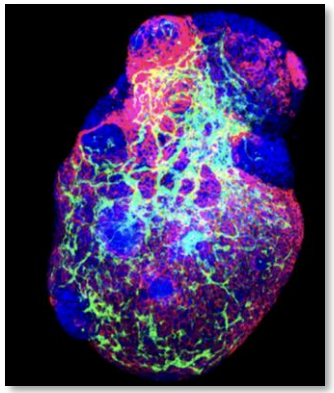
CONECTING DIFFERENT BRAIN REGIONS



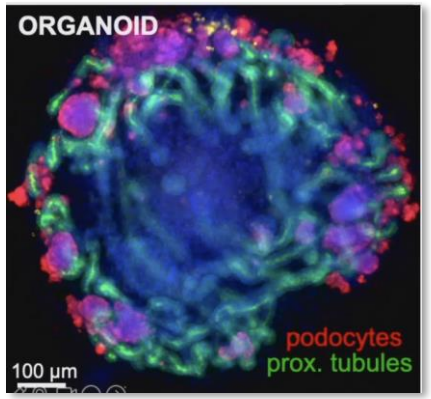
Onesto et al., 2024

Miniaturizing Other Organs in a Dish

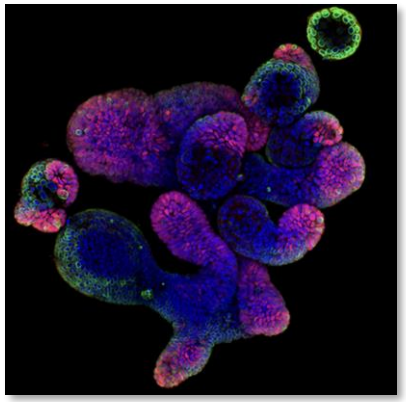




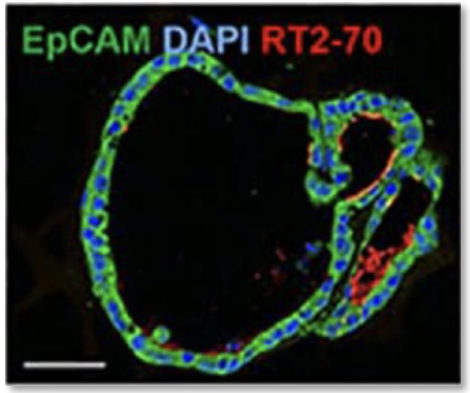
Aguirre Lab 2023



Freedman Lab 2024



https://www.drugtargetreview.com



Fabian Lab 2021

Agenda

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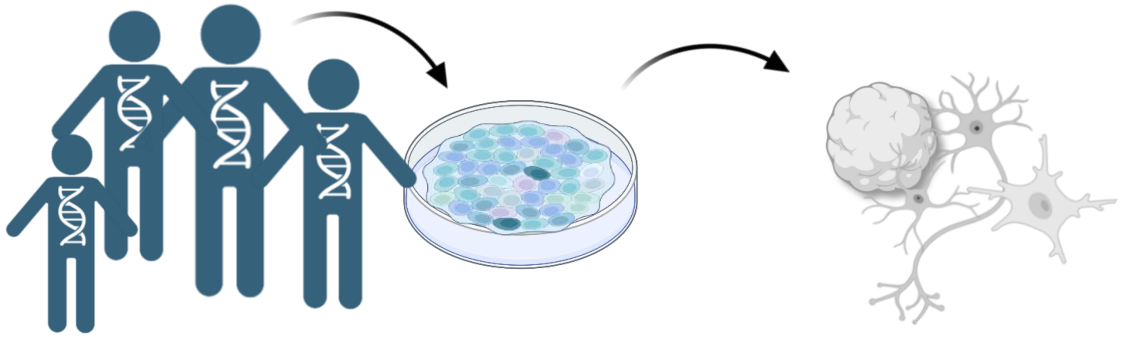
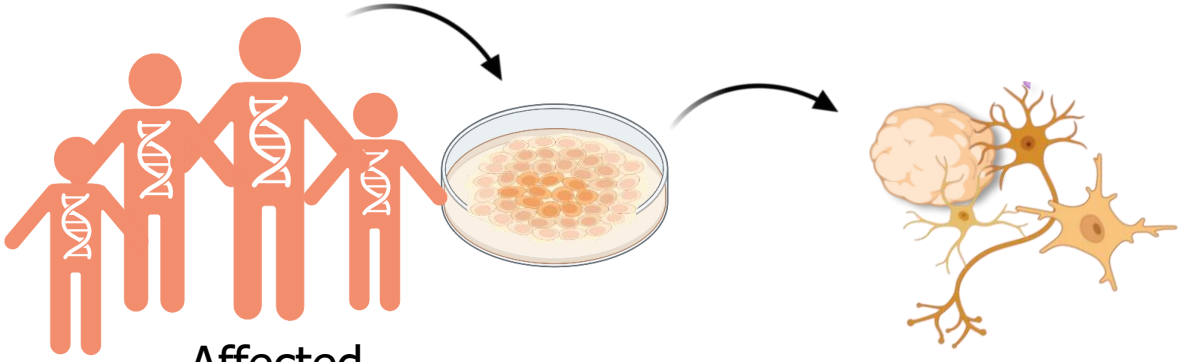
CONCLUSIONS

Identifying Disease Phenotypes

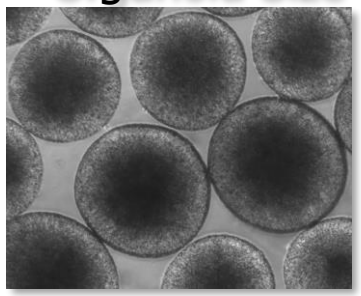


Neurotypical

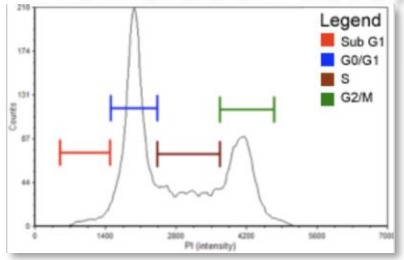


Affected

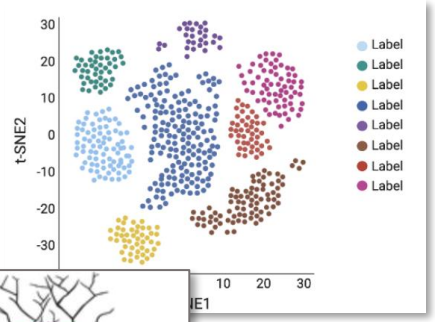
Organoid Size

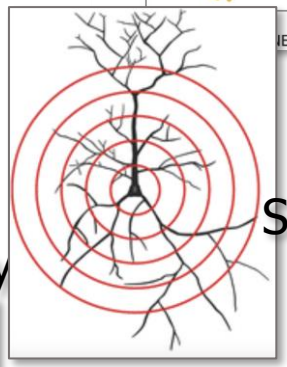


Proliferation assay

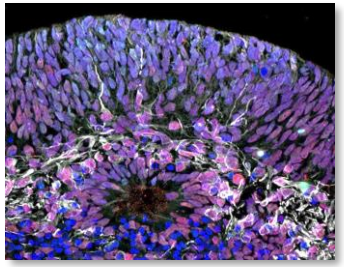


Single Cell -omics



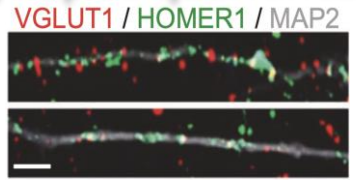


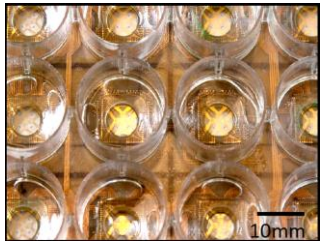
Morphology

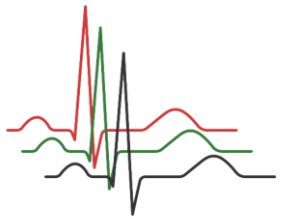


Cytoarchitecture

Synaptic quantification







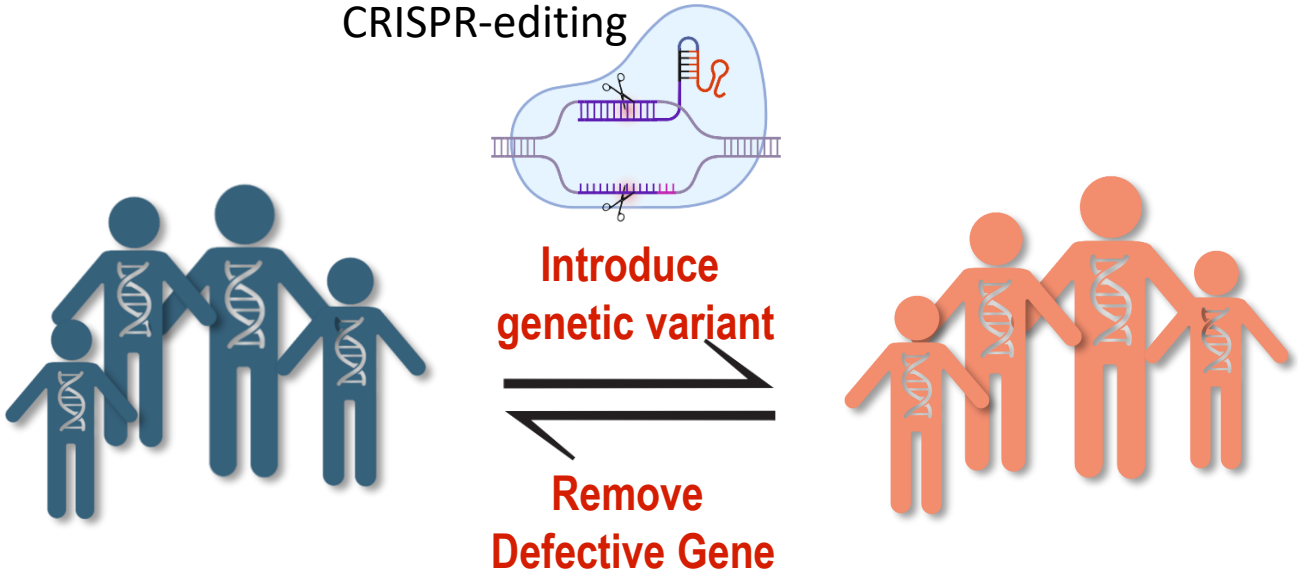
Neuronal Activity

CRISPR – A Revolutionary Tool to Edit Genomes

2020- Nobel Prize in Chemistry

Emmanuelle Charpentier
&
Jennifer Doudna

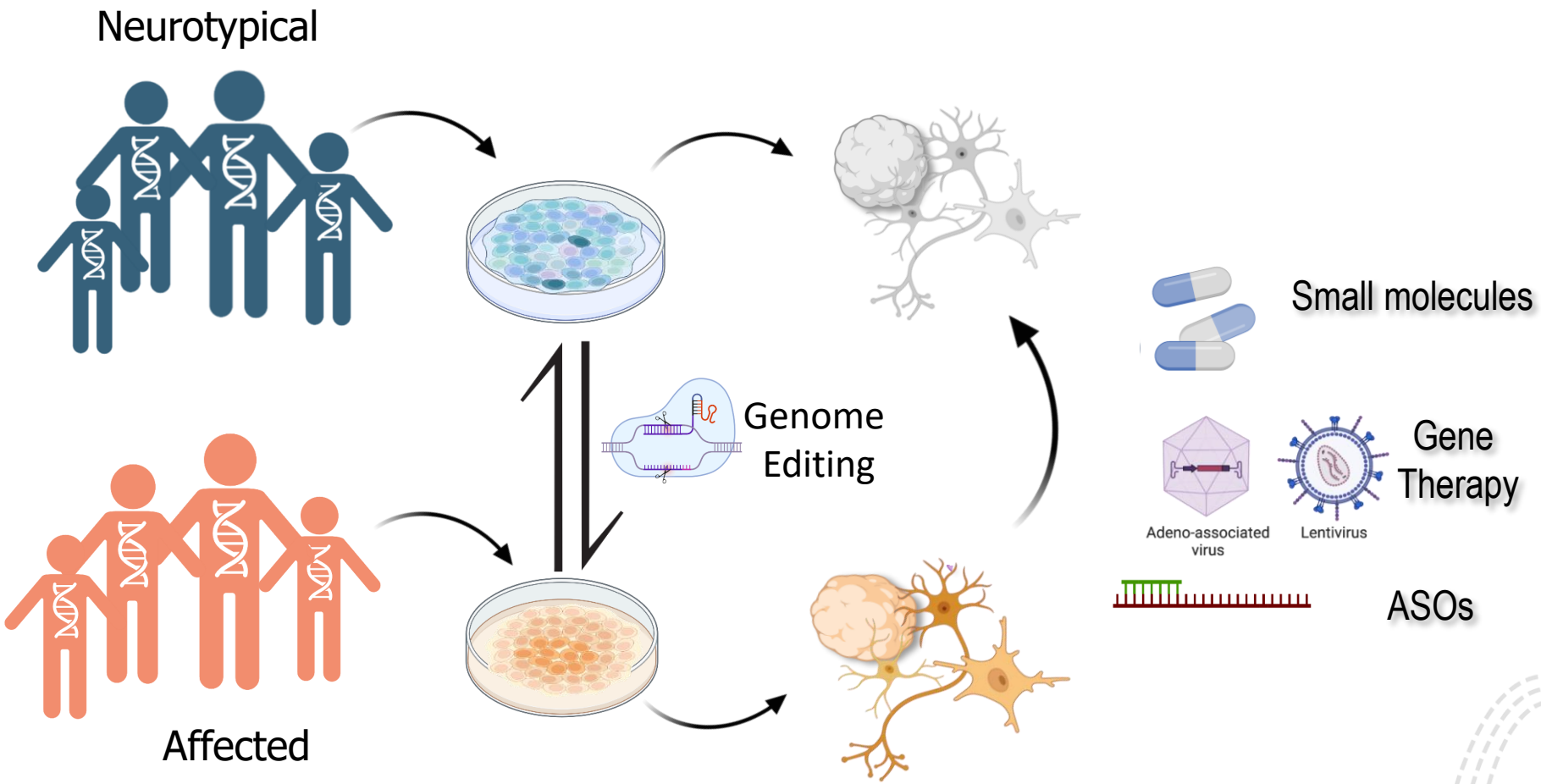
CRISPR-editing



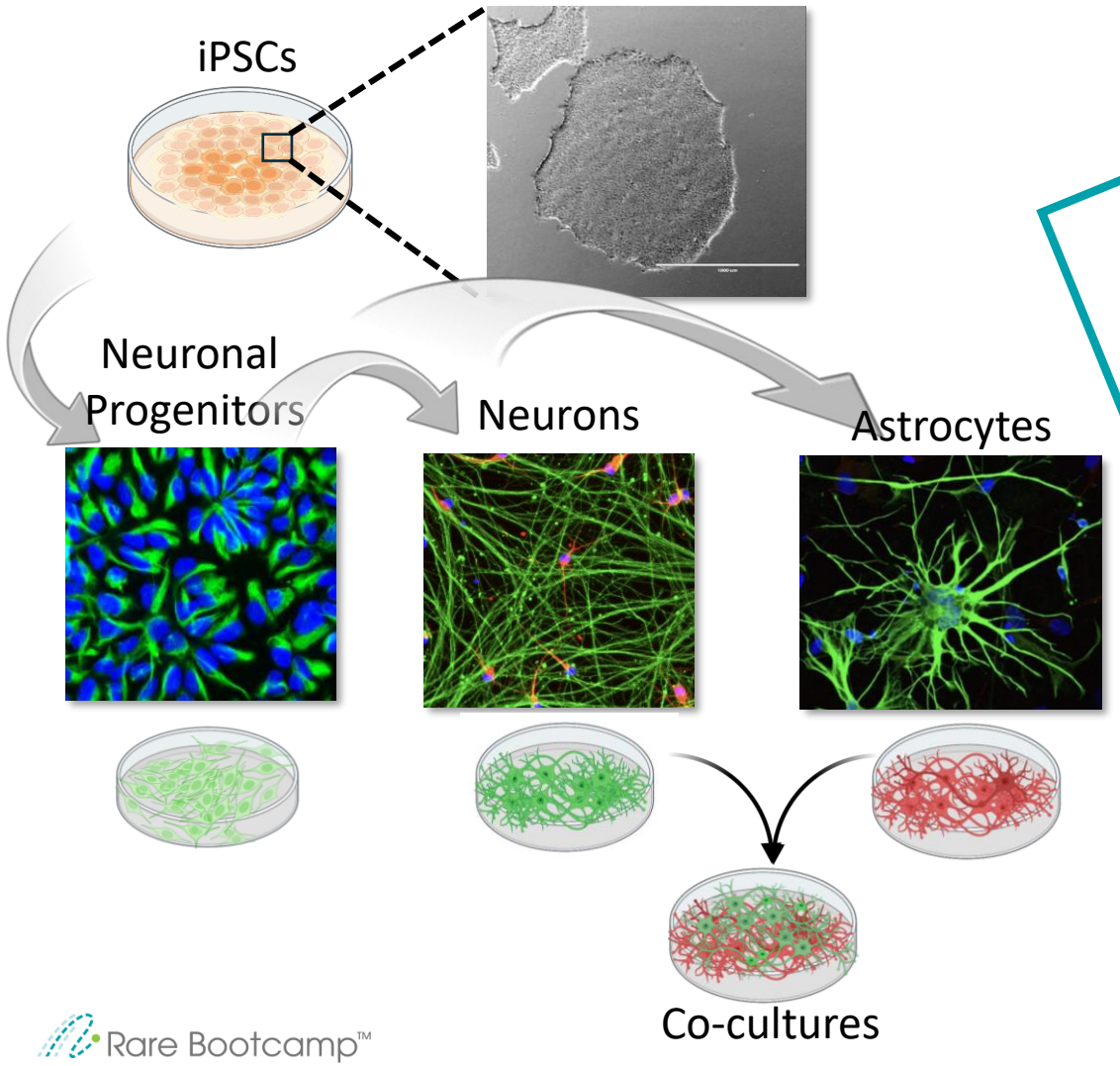
Healthy Donors

Affected Donors

Personalizing Therapies



Autism in Two Dimensions



CDKL5 syndrome

Fragile X syndrome

NRXN1

Rett syndrome

SETD5 Syndrome

ERP44

SHANK2

Angelman syndrome

Timothy syndrome

CHD8 mutations

NLGN4

15q11-q13 syndrome

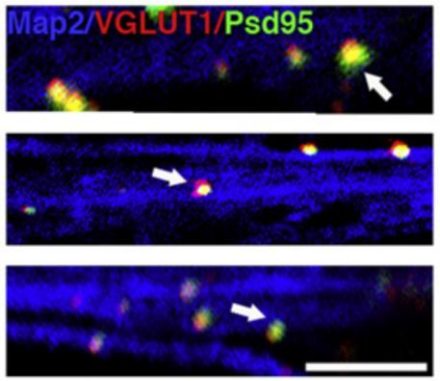
Phelan Mc Dermon syndrome

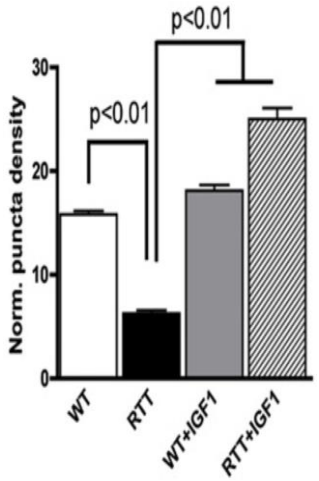
KLEEFSTRA SYNDROME

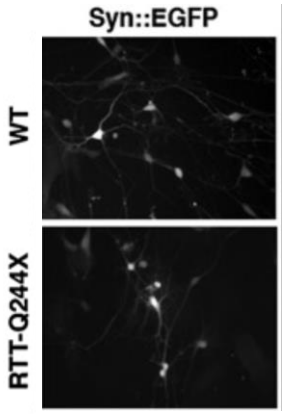
TRP6 mutations

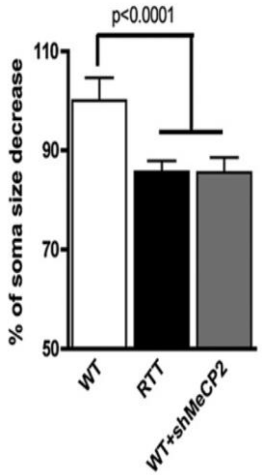
Rare Bootcamp™

Rett Syndrome in Two Dimensions



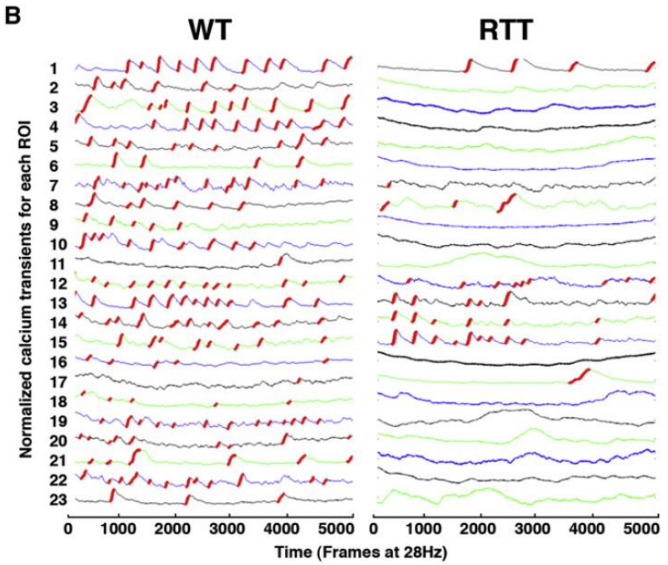


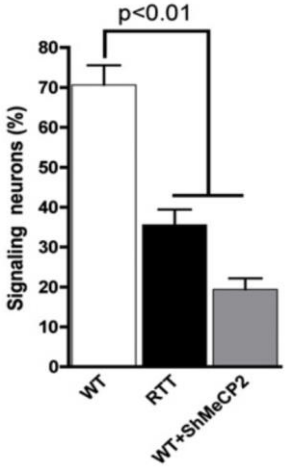




Marcheto et al., 2010. Cell

2023 Daybue™ (trofinetide)

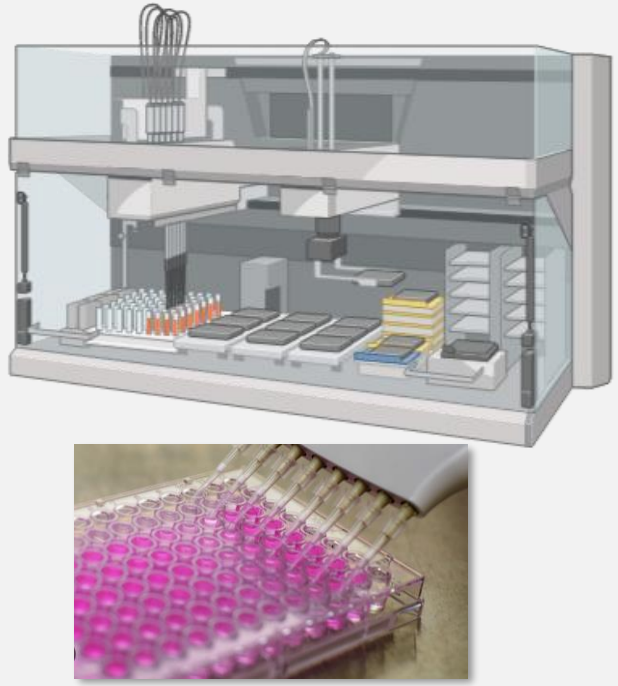




2D Cells as Platforms for Phenotypic Screenings

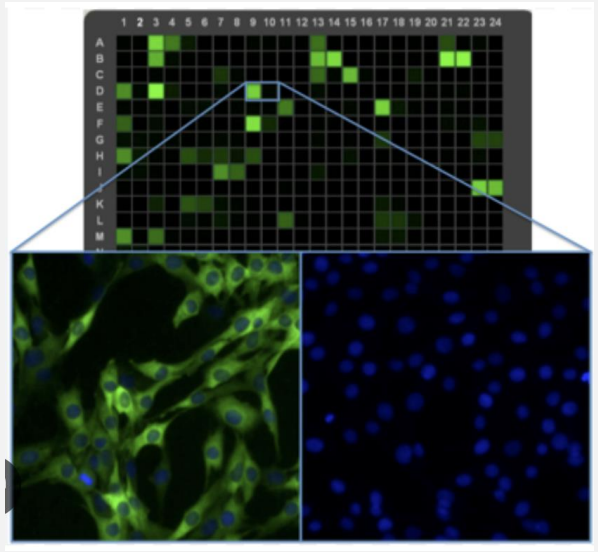
Biomarkers

e.g., Inflammation



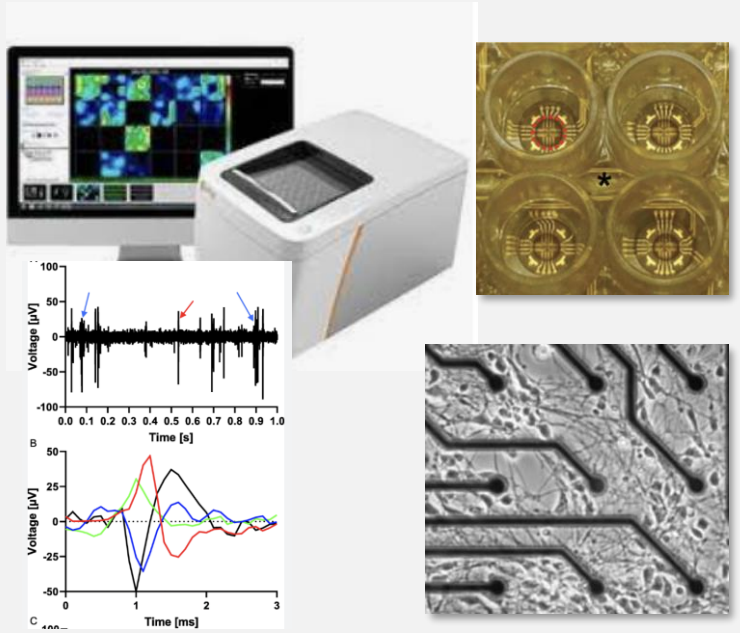
Morphology

High content imaging (e.g., number of synapses)

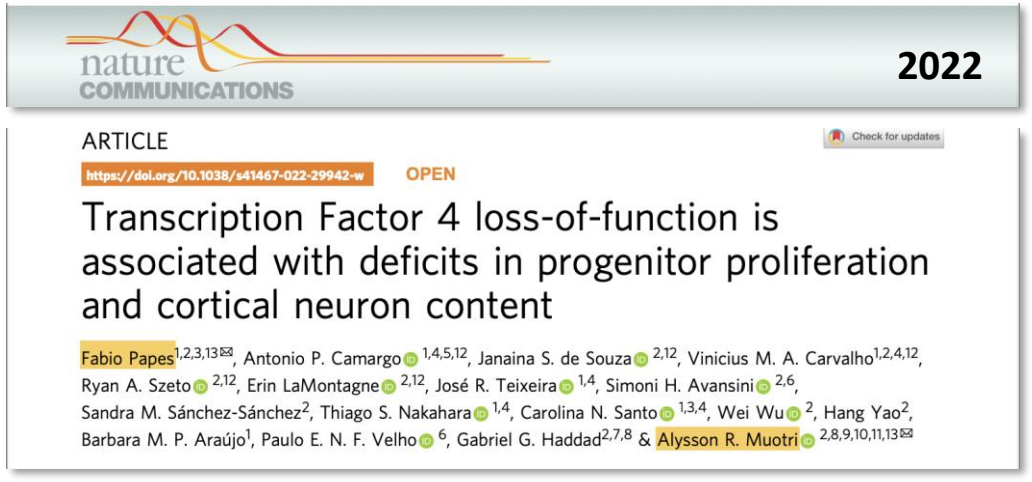


Functionality

Multi-electrode arrays (e.g., Neuronal Activity)



Modeling Pitt-Hopkins Syndrome (PTHS)



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ARTICLE

https://doi.org/10.1038/s41467-022-29942-w OPEN

Check for updates

Transcription Factor 4 loss-of-function is associated with deficits in progenitor proliferation and cortical neuron content

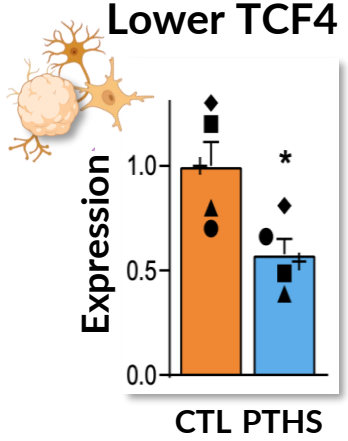
Fabio Papes[1,2,3,13]✉, Antonio P. Camargo[1,4,5,12], Janaina S. de Souza[2,12], Vinicius M. A. Carvalho[1,2,4,12], Ryan A. Szeto[2,12], Erin LaMontagne[2,12], José R. Teixeira[1,4], Simoni H. Avansini[2,6], Sandra M. Sánchez-Sánchez[2], Thiago S. Nakahara[1,4], Carolina N. Santo[1,3,4], Wei Wu[2], Hang Yao[2], Barbara M. P. Araújo[1], Paulo E. N. F. Velho[6], Gabriel G. Haddad[2,7,8] & Alysson R. Muotri[2,8,9,10,11,13]✉

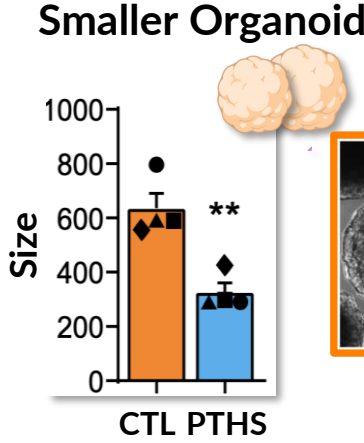
Wide nasal bridge
Flared nasal alae
Full cheeks
Wide mouth full lips
Narrow forehead
Thin lateral eyebrows
Thickened helix
Wide nasal tip
Cupid bow upper lip

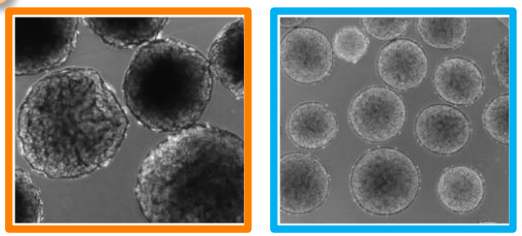
Rare genetic condition
Developmental delay
Intellectual disability
Impaired speech
Breathing difficulties
Motor issues
Epilepsy

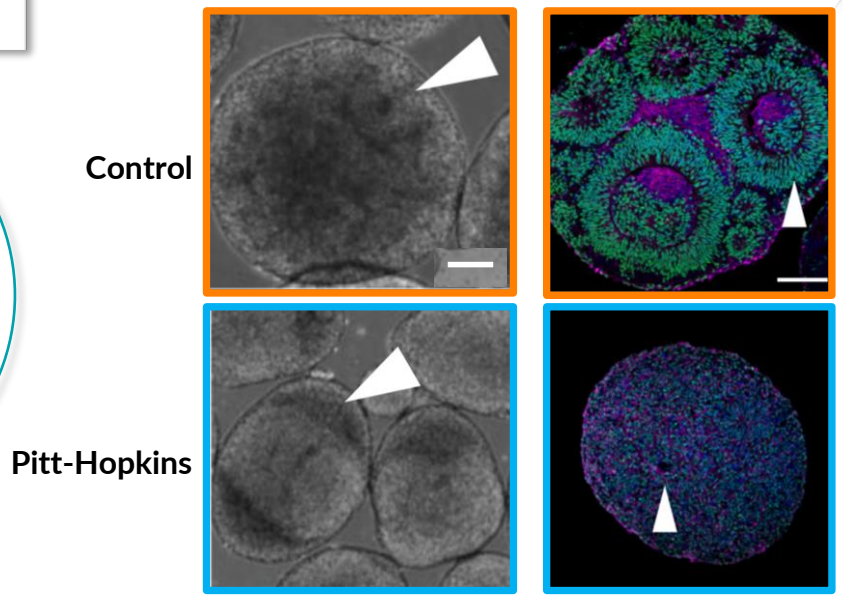
Zollino et al., 2019

Rare Bootcamp™





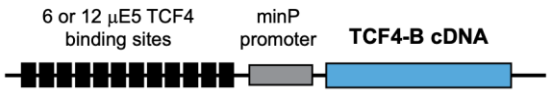




Reduced proliferation
Impaired differentiation

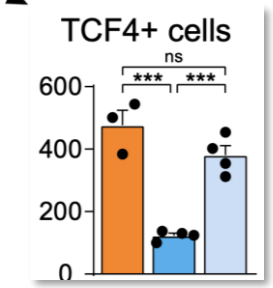
Phenotypes Recapitulated in post-mortem Pitt-Hopkins

Assessing Therapies in Pitt-Hopkins Organoids

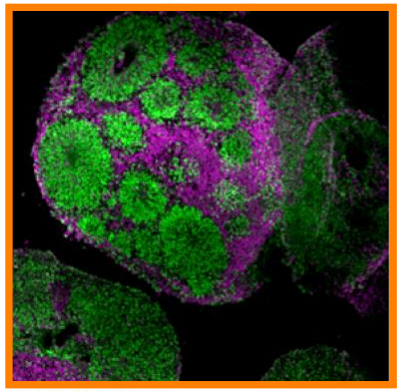


Adenovirus

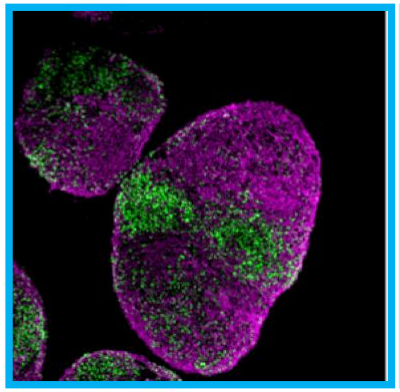
TCF4



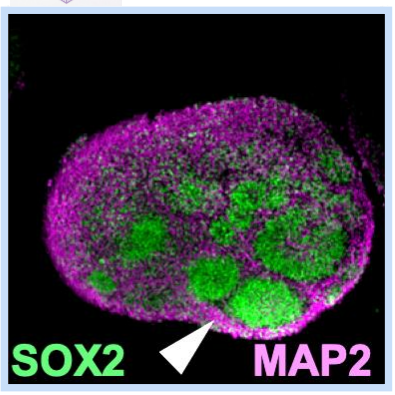
CONTROL

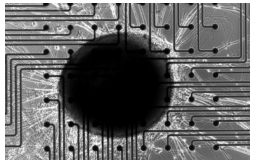


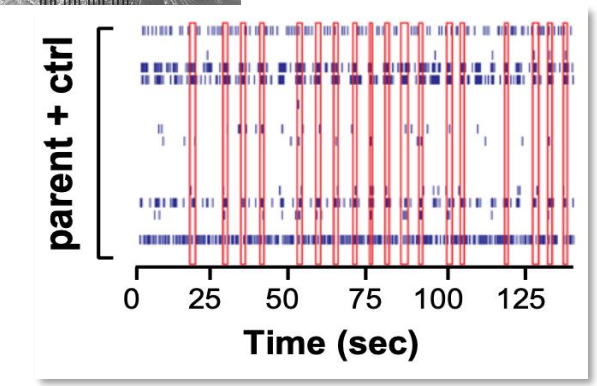
PTHS

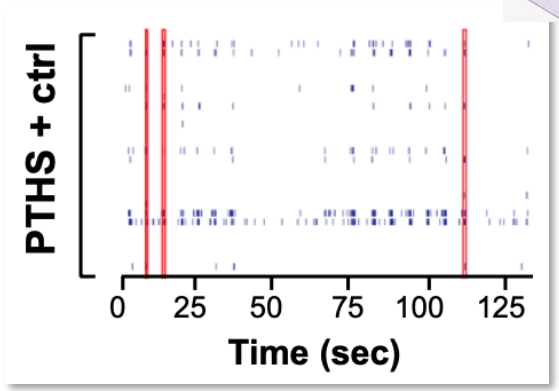


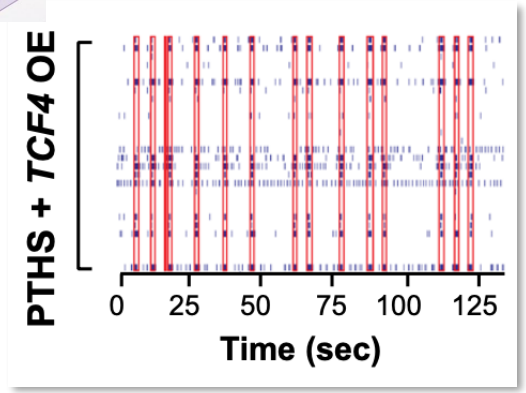
PTHS + AAV











CIRM grant
$4 Million

AAV9-base TCF4 replacement approach

MAHZI therapeutics

Agenda

INTRODUCTION

OVERVIEW HUMAN PLURIPOTENT STEM CELL CULTURES

EXAMPLES DISEASE MODELING/THERAPIES

CONCLUSIONS

Why Establish hiPSC-Derived Disease Models?

FDA Modernization Act 2.0 (2022): This bill allows an application for market approval for a new drug to use alternatives to animal testing including cell-based assays.

- A platform to study disease and biology
- Recapitulate disease phenotypes
- High-throughput screenings: drug candidates/toxicity
- Amenable to genetic engineering
- Personalized medicine: Tailored therapies
- Reduced immunogenicity
- Unlimited cells source
- Minimum ethical concerns
- Cell replacement therapies
- Regenerative medicine

How To Establish hiPSC-Derived Models?

- Recruit several probands with different mutations
- Obtain consent
- Perform biopsies – bank fibroblasts
- Reprogram fibroblasts to hiPSC: 2-3 different clones/mutation
- CRISPR-engineered mutation correction
- CRISPR-introduce mutation in control cells
- Deposit cells in cell repositories
- Fund a stem-cell based labs to establish model to find phenotypes
- Fund labs experts in high throughput phenotypic screening
- Fund labs experts in therapeutic development/testing: AAV, ASOs

Cost & Timeline to Establish Disease Models

YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
$150K Foundation $150K Muotri Lab	$150K Foundation $150K Muotri Lab	$150K Muotri Lab	$1-1.5M R01/CIRM	>$4M CIRM/Company
Generate iPSCs/QC Expand/Bank Organoids QC/phenotype	Complex experiments Phenotype Grant Application	Mechanism Proof-of-concept Grant Application	Confirmatory Publication Grant Application	Pre-clinical Therapies IND

$500 to culture 1 patient-skin fibroblasts
$10-20K to reprogram, QC, and establish one iPSC line
$15K to differentiate one iPSC line
$20K CRISPR-editing/line
+ SALARIES (25-50%) technician/(100%) postdoc or grad student

Rare Bootcamp™

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Thank You

Sponsored by Ultragenyx

Modeling CDKL5 Deficiency Disorder (CDD)

Molecular Psychiatry
https://doi.org/10.1038/s41380-021-01104-2

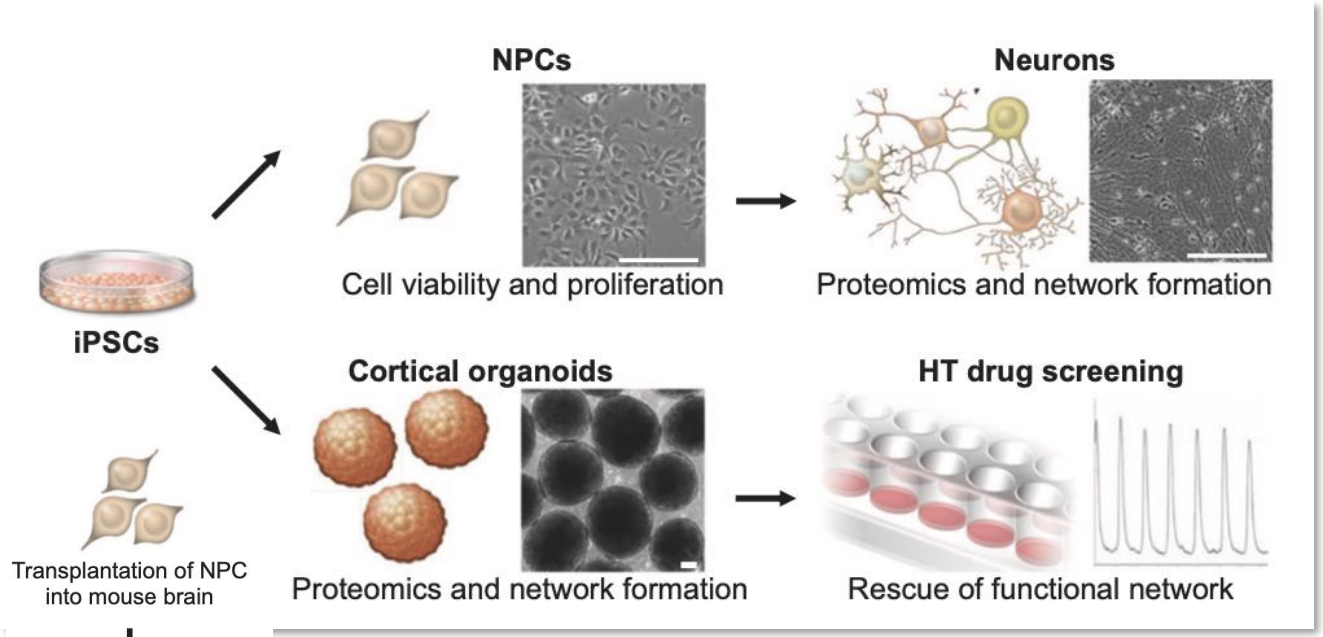
2021

ARTICLE

Altered network and rescue of human neurons derived from individuals with early-onset genetic epilepsy

Priscilla D. Negraes[1] · Cleber A. Trujillo[1] · Nam-Kyung Yu[2] · Wei Wu[1] · Hang Yao[1] · Nicholas Liang[1] · Jonathan D. Lautz[3,4] · Ellius Kwok[1] · Daniel McClatchy[2] · Jolene Diedrich[2] · Salvador Martinez de Bartolome[2] · Justin Truong[1] · Ryan Szeto[1] · Timothy Tran[1] · Roberto H. Herai[5] · Stephen E. P. Smith[3,4] · Gabriel G. Haddad[1,6] · John R. Yates 3rd[2] · Alysson R. Muotri[1,7,8]

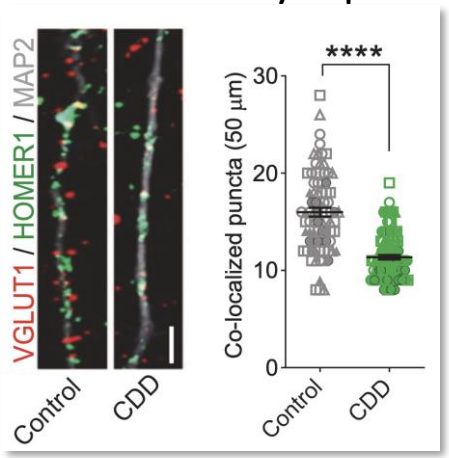
Modified from Fehr et al., 2013



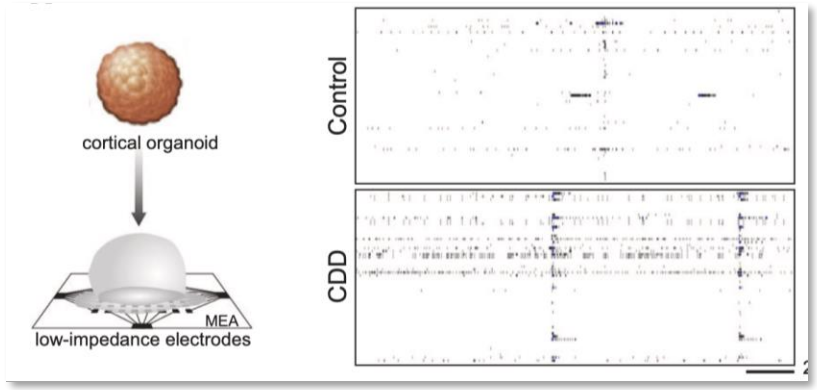
X-linked
Rare Genetic Condition
Developmental delay
Motor dysfunction
Early-onset seizures
Impaired cognition
Impaired Speech

CDKL5 Deficiency: Neuronal In Vitro Dysfunctions

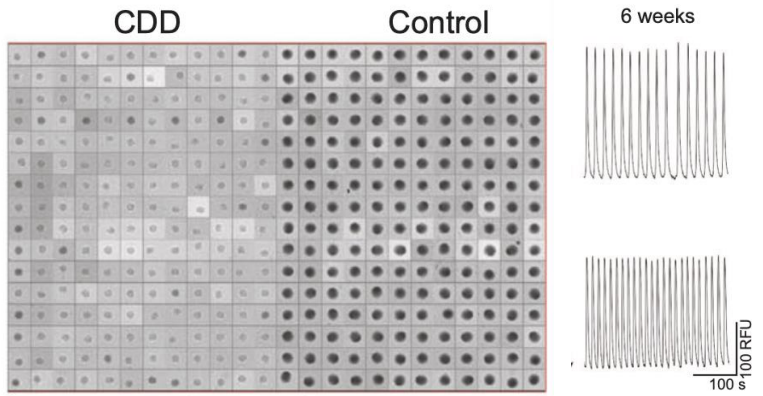
Reduced synapses



Hyperexcitability

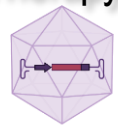


Human neurons

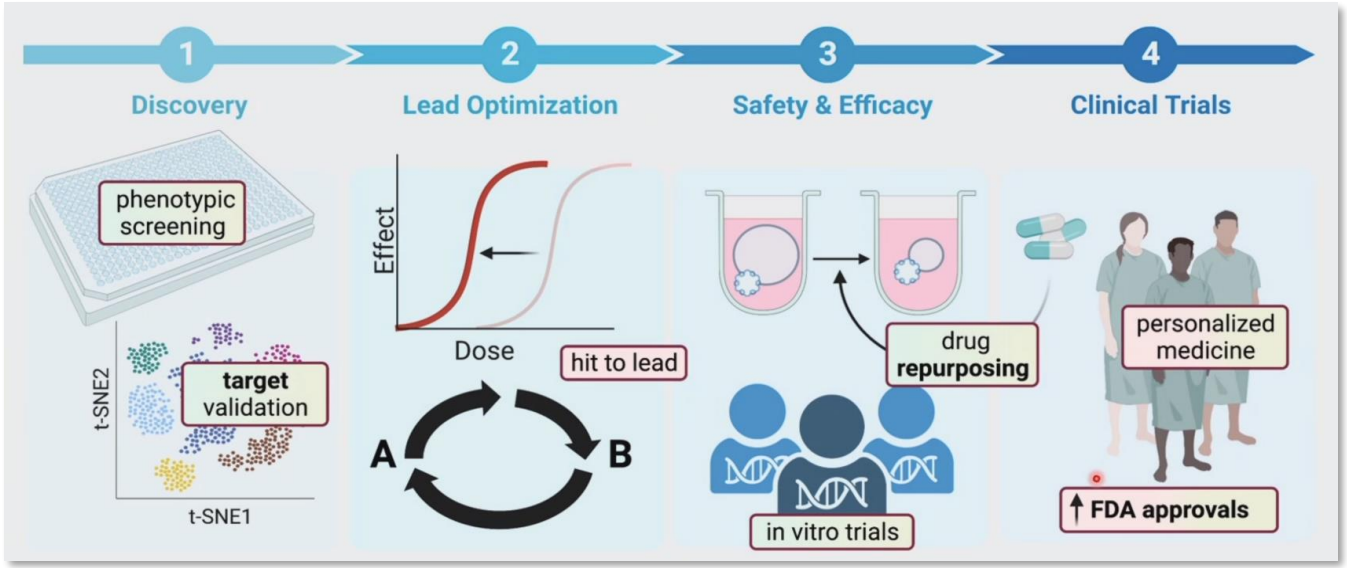


High Throughput Screening

ultragenyx Gene Therapy



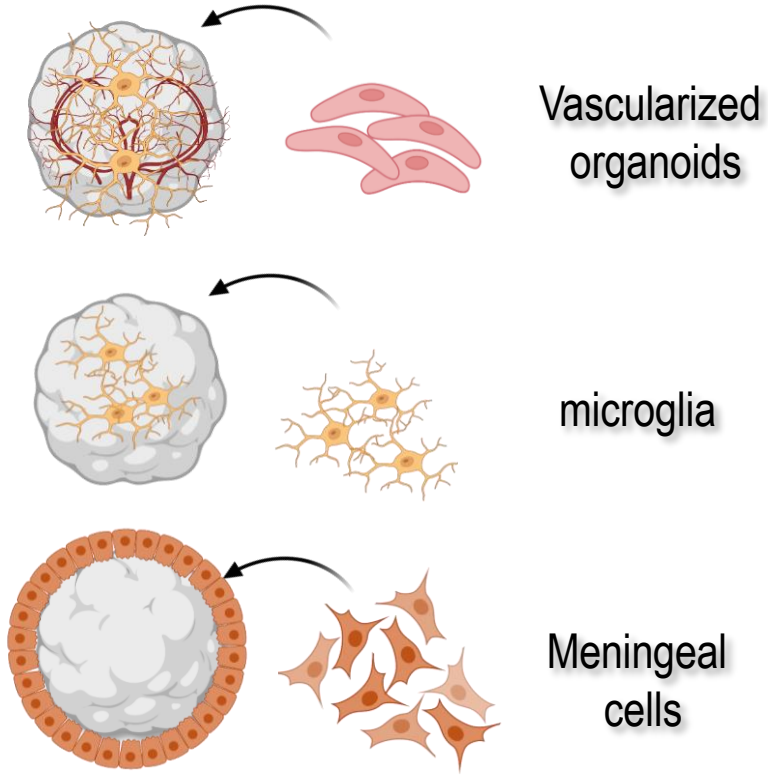
Adeno-associated virus



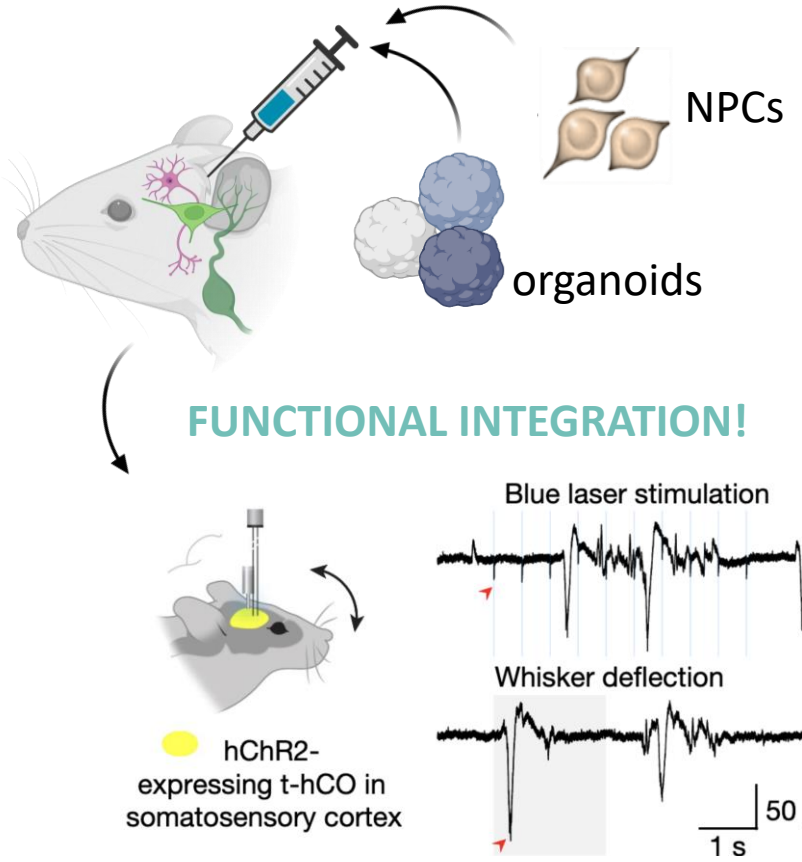
After Dr. Freedman's presentation

Enhancing Maturity by Increasing Complexity

Multi-lineage organoids



In vivo transplantation



https://www.nature.com/articles/d41586-022-02073-4

Autism: A Complex Genetic Landscape

https://www.npr.org/sections/health-shots/2023/10/02/1202749791/new-tool-study-genetics-autism-schizophrenia-brain-disorders

Jill George, NIH

Show this slide as a summary of what the cell types are

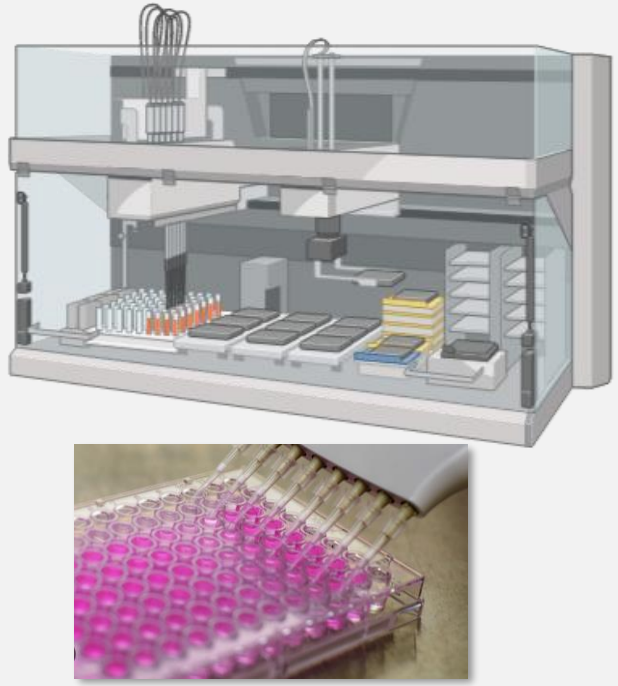
Have a slide showing increasing complexity. Inspired by Dasa's talk

Monolayer → co-cultures → spheroid --> organoids → assembloids

2D Cells as Platforms for Phenotypic Screenings

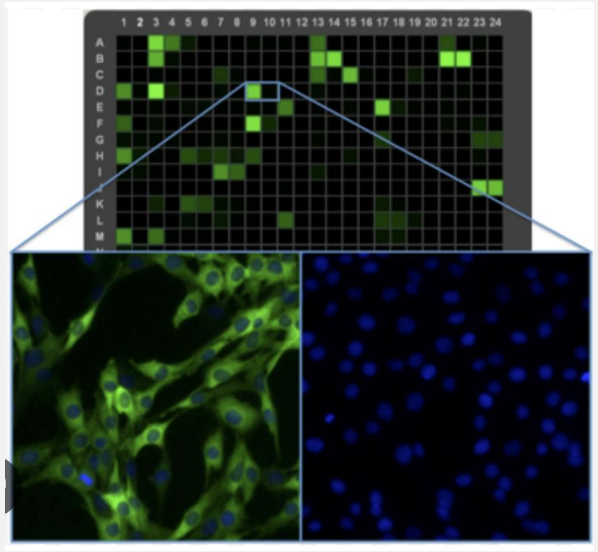
Biomarkers

e.g., Inflammation



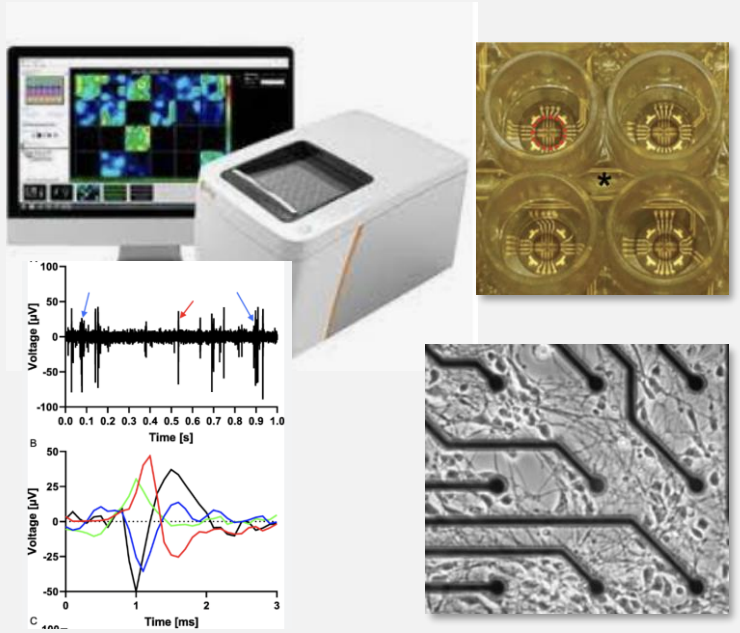
Morphology

High content imaging (e.g., number of synapses)



Functionality

Multi-electrode arrays (e.g.,Neuronal Activity)



Connecting Organs – Organoids-on-chip