Human Brain Organogenesis in a Dish

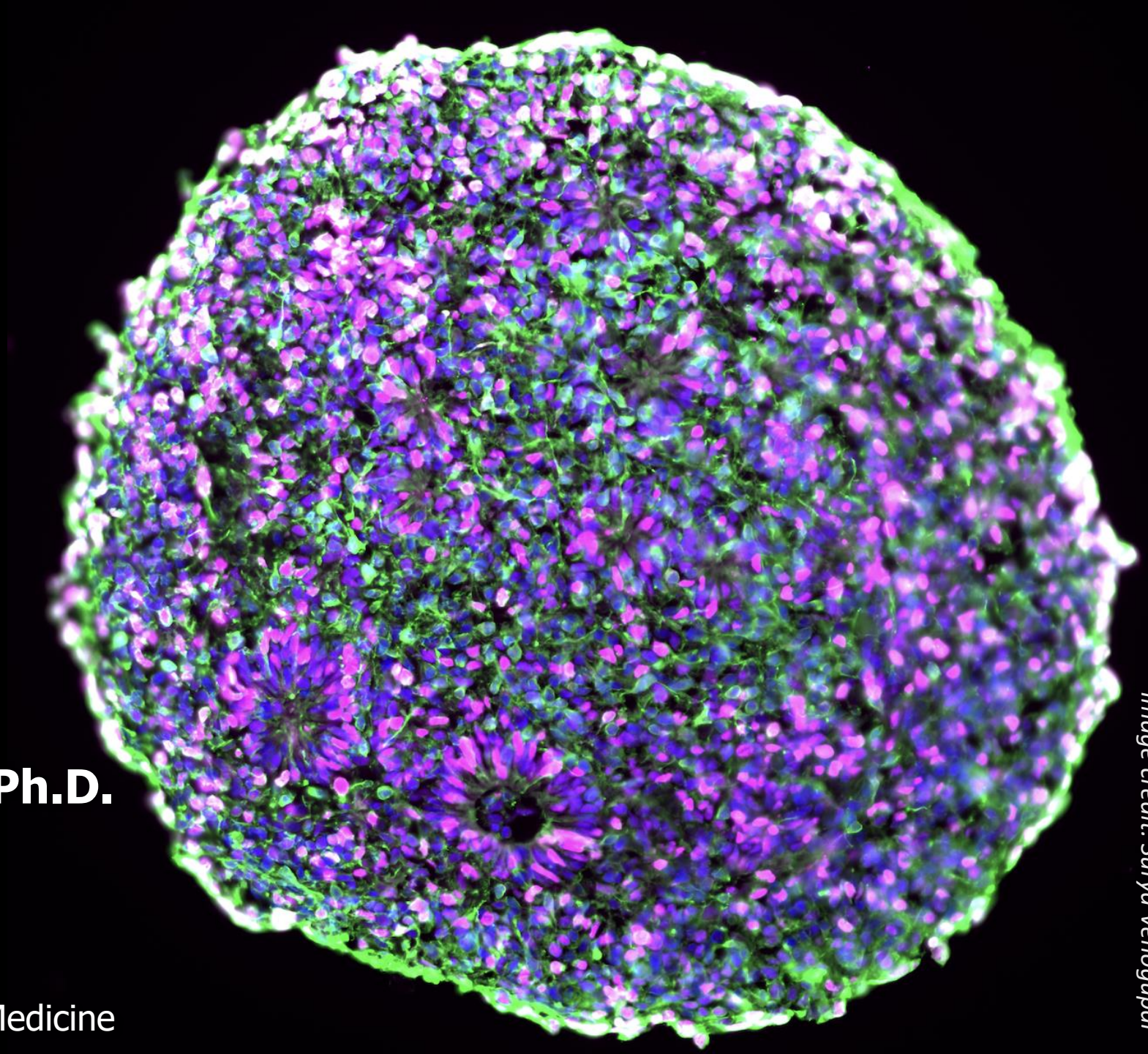
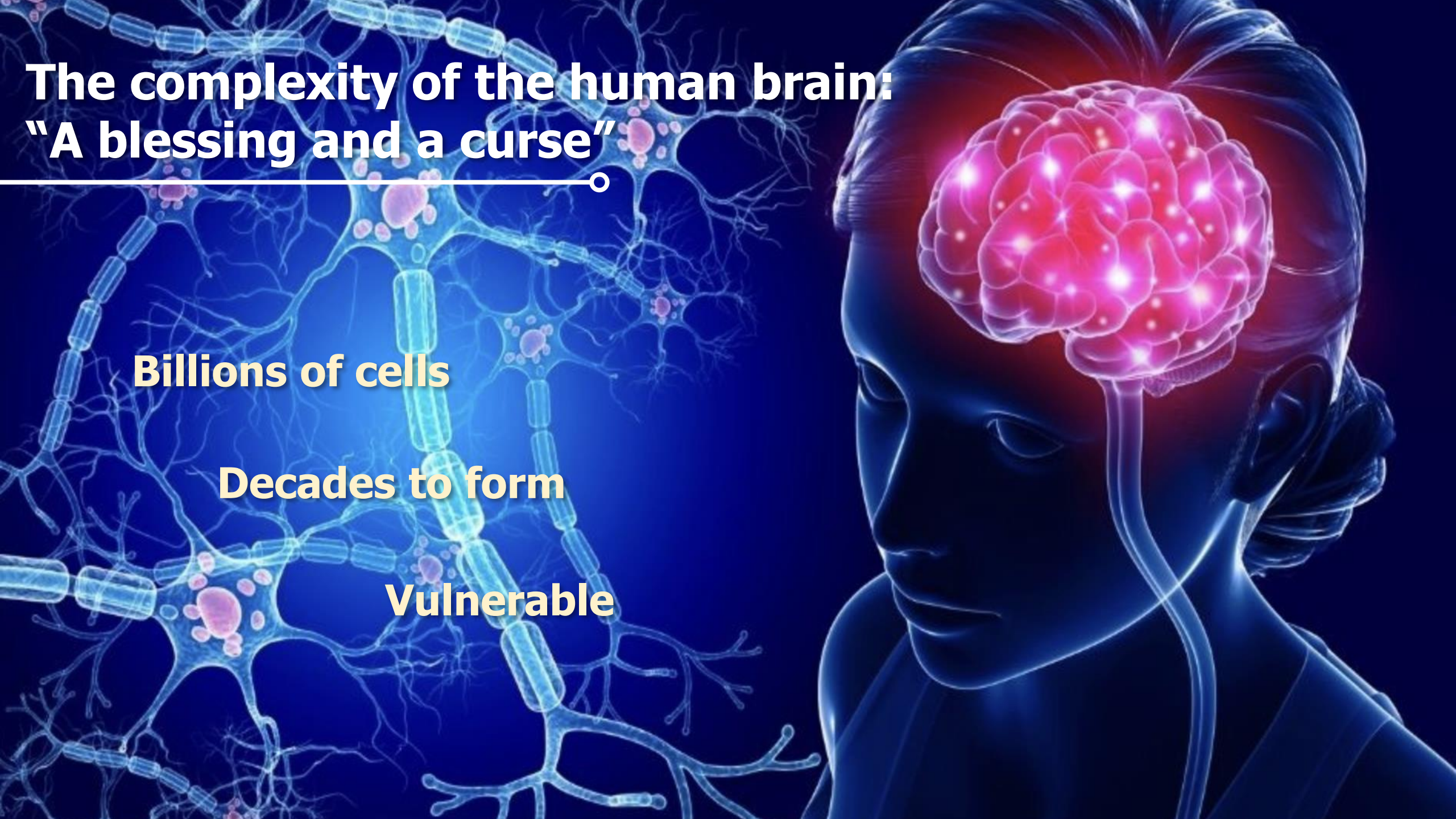


Image credit: Surya Venogupal

Angels Almenar-Queralt, Ph.D.

Dr. Muotri Laboratory
University of California, San Diego
Dept. Pediatrics - Division of Genetics
Sanford Consortium for Regenerative Medicine

The complexity of the human brain: "A blessing and a curse"

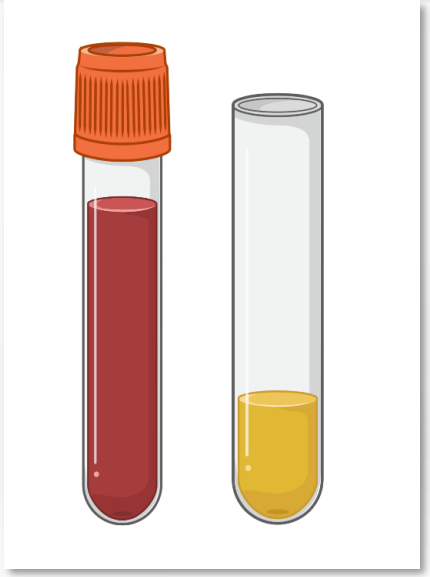


Billions of cells

Decades to form

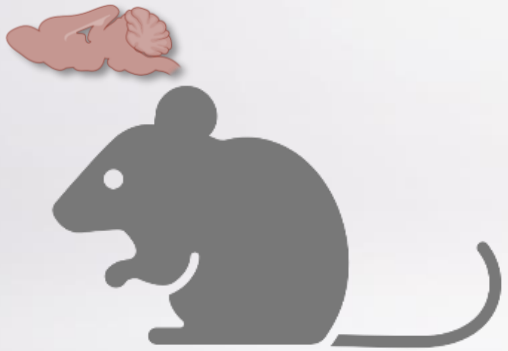
Vulnerable

Inaccessibility to living human brain

Imaging	Biomarkers	Postmortem tissue
		
Low Resolution	Indirect	Snapshots

Rodents vs humans

Multi-organismal
Rapid development
Inexpensive
Pre-clinical model



Size
Complexity
Neuronal density
Time to develop
Genetics
Incomplete disease modeling

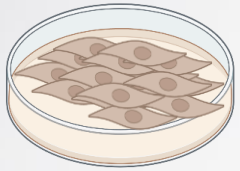
Reverse engineering the human brain

Dr. Yamanaka

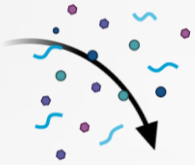
2006
reprogramming

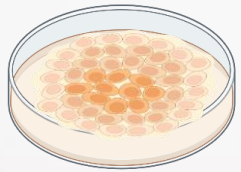
2012
Nobel Prize

skin
biopsy

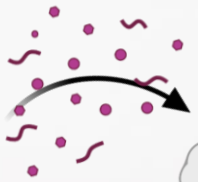


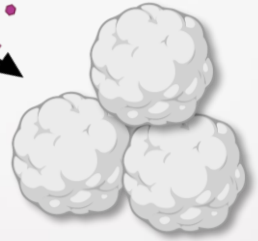
fibroblasts





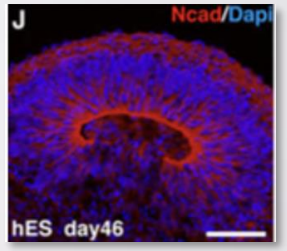
Induced Pluripotent
Stem Cells (hiPSC)



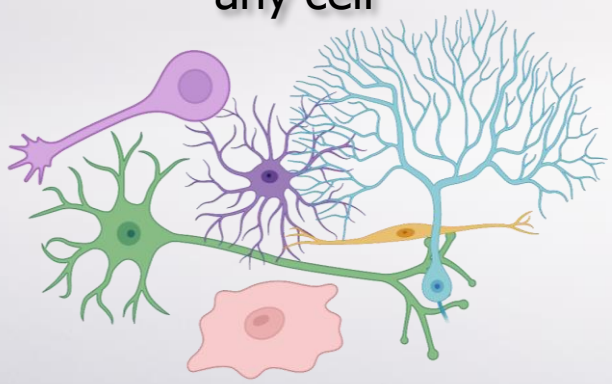


cortical
organoids

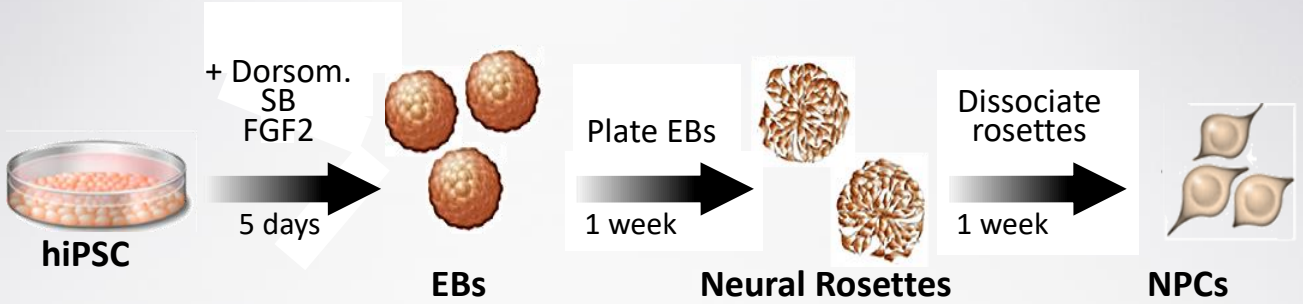
Dr. Sasai
(2008)

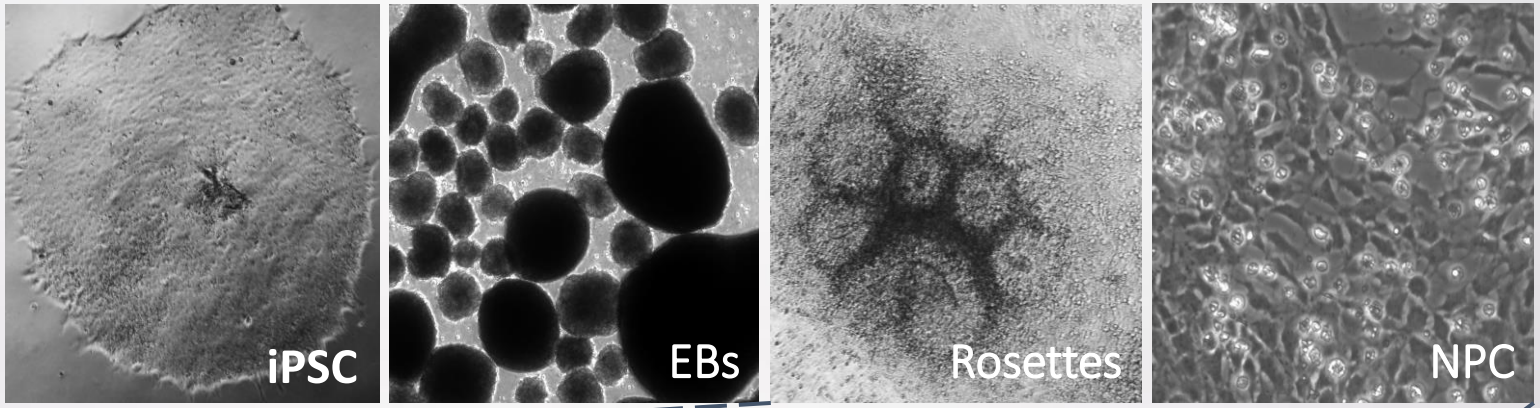


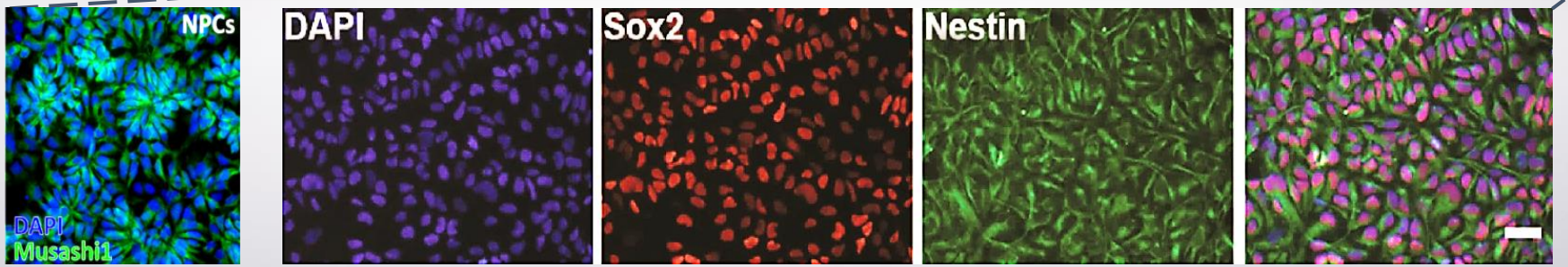
any cell



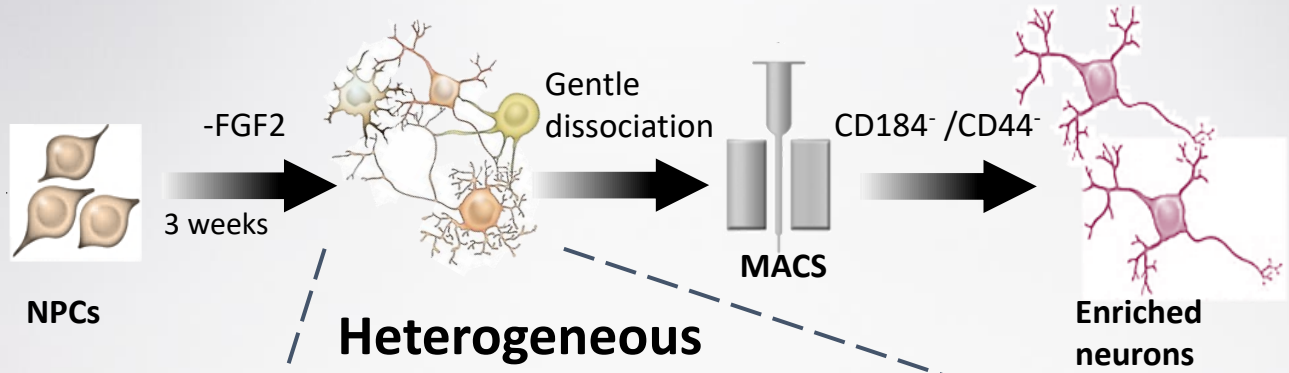
Neural Progenitor Cell (NPC) Production



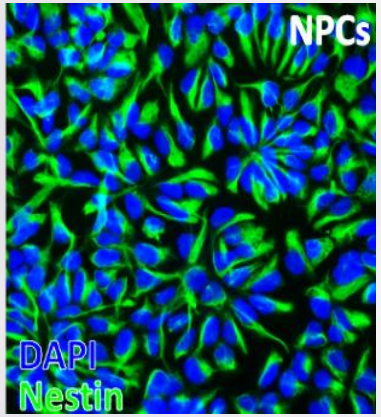


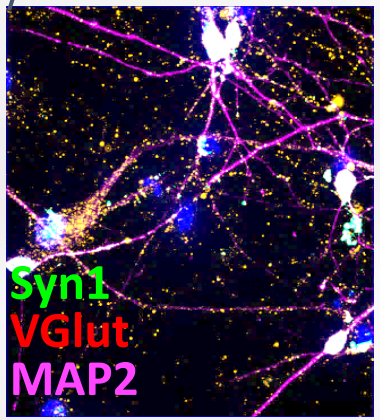


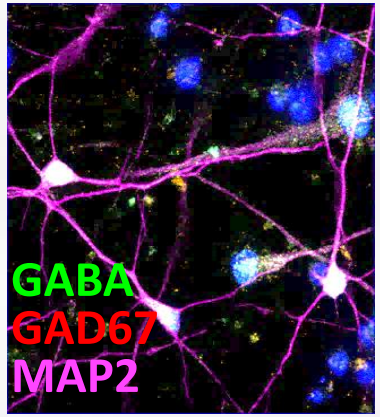
Neuron Production

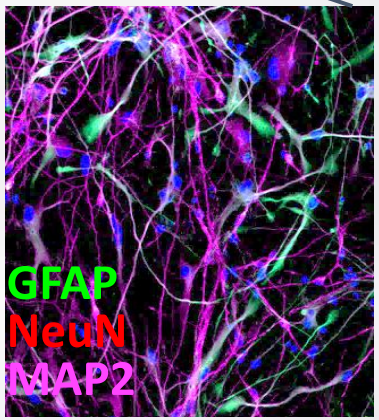


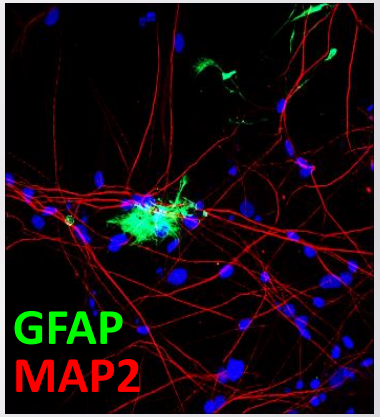
Heterogeneous neural culture





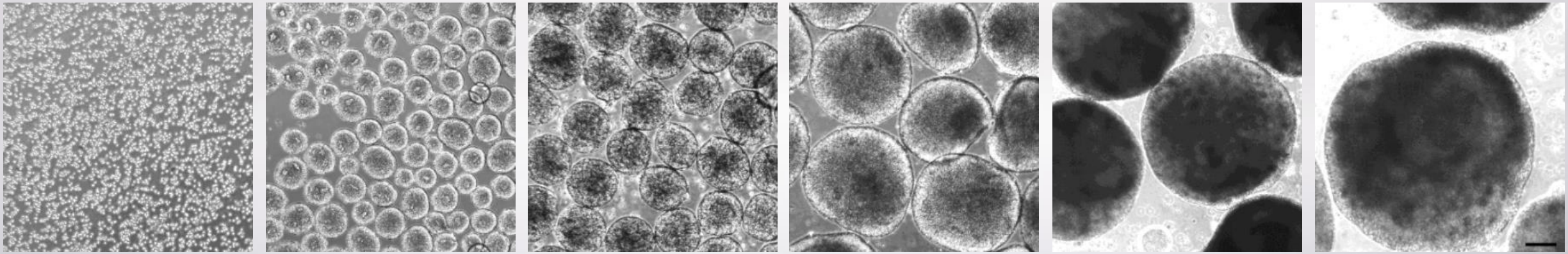




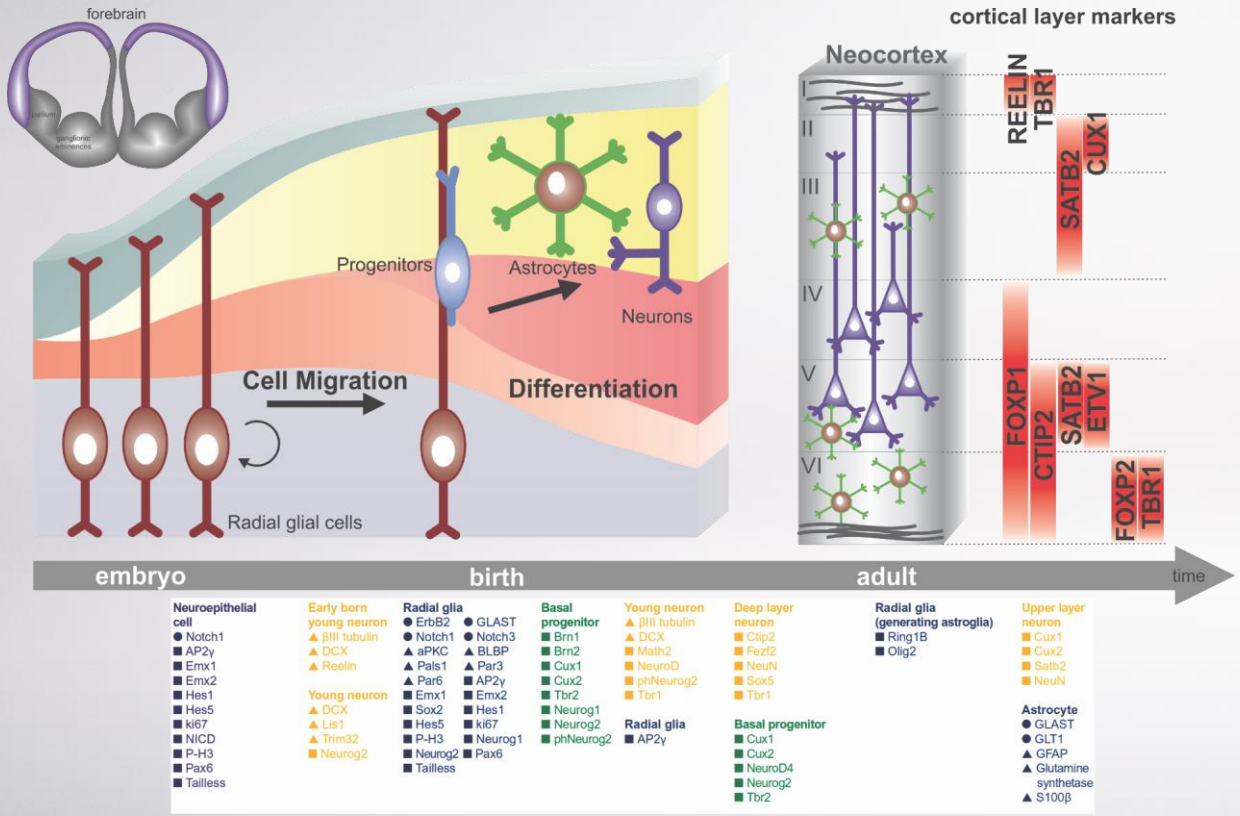


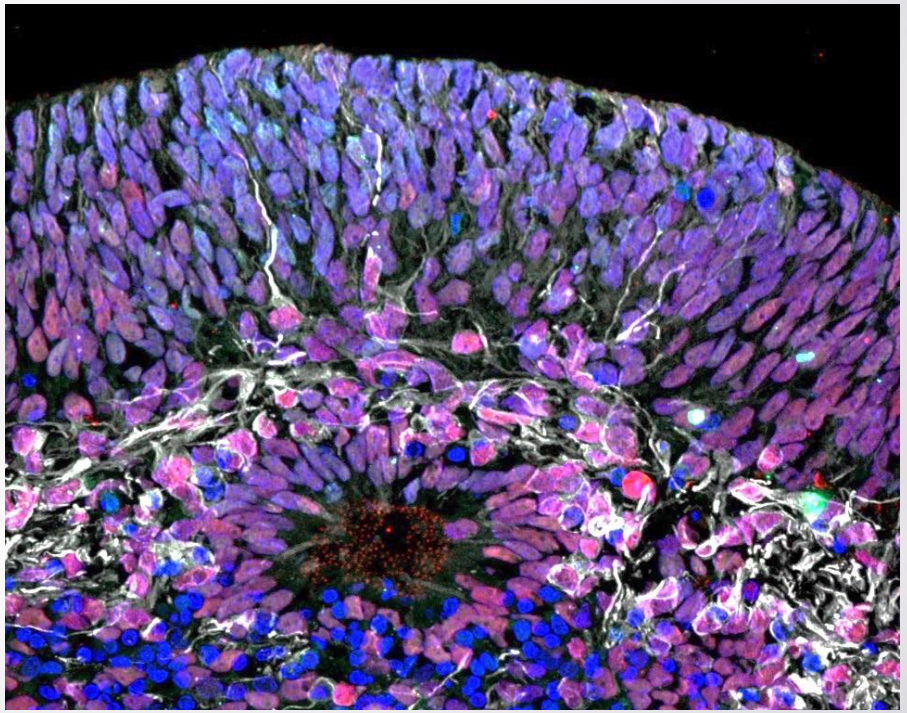
Muotri lab cortical organoid recipe

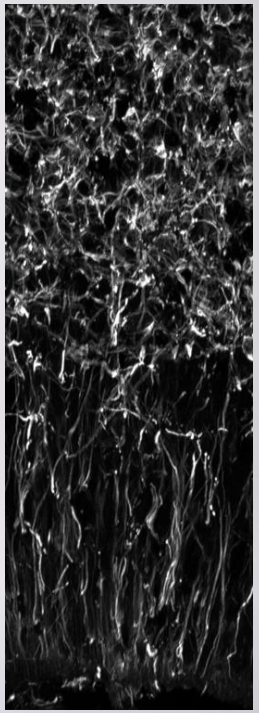
Dissociated iPSC	Neural induction	NPC proliferation		Neural differentiation	Neural maturation
iDEAL Media	Media1	Media2	Media2	Media 3	Media2
2 days	7 days	7 days	7 days	7 days	months



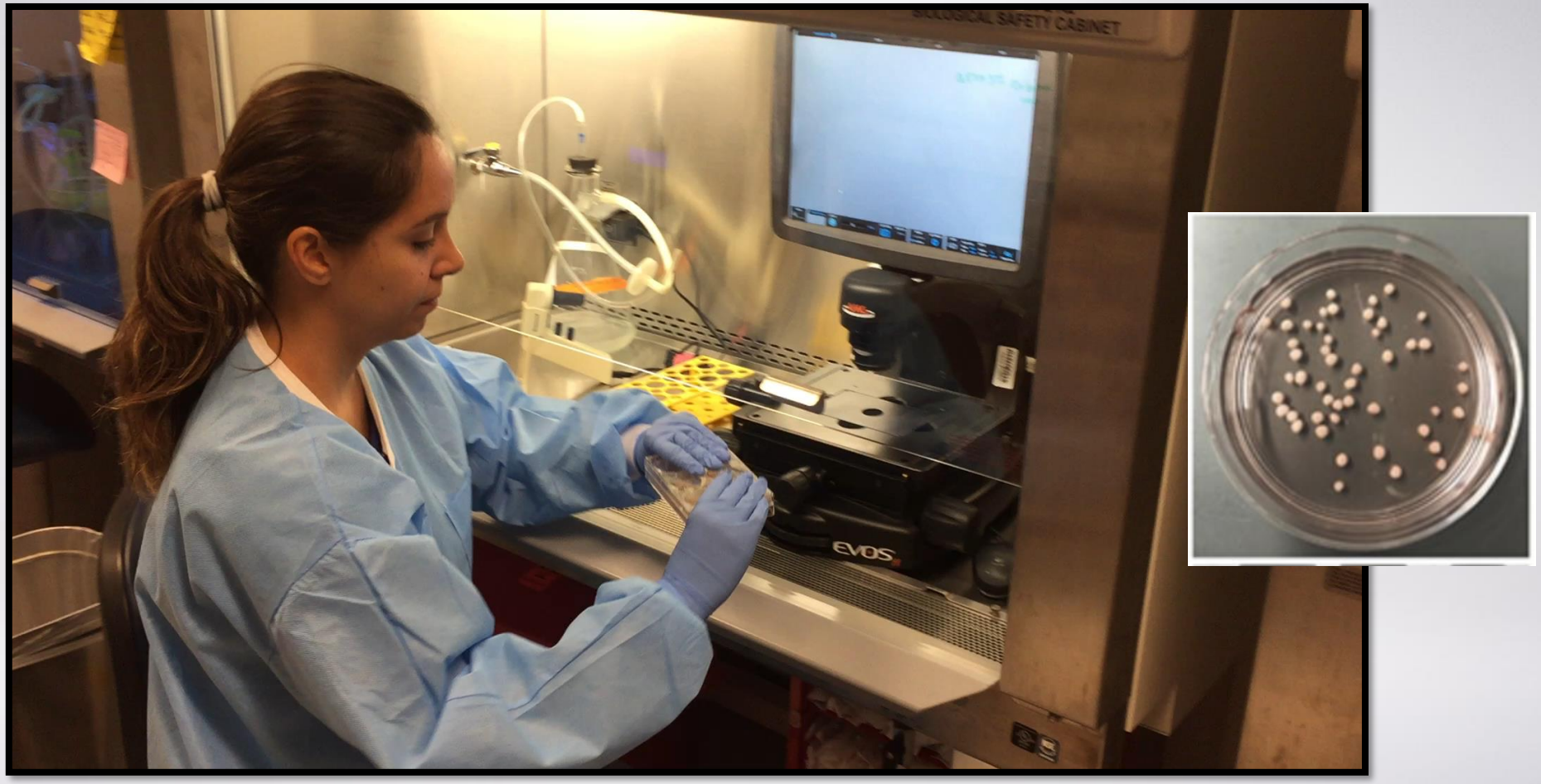
Mimicking cortical organization





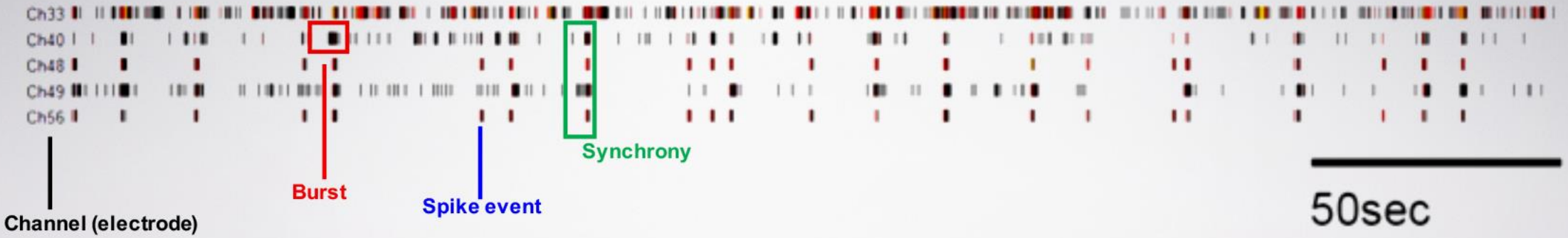


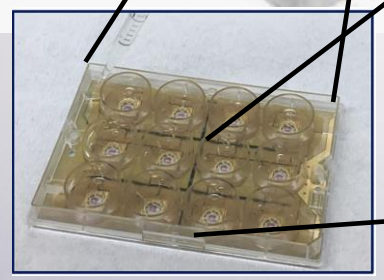
Trujillo 2019

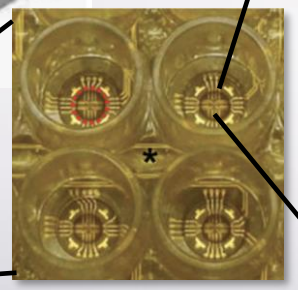

BIOLOGICAL SAFETY CABINET
EVOS

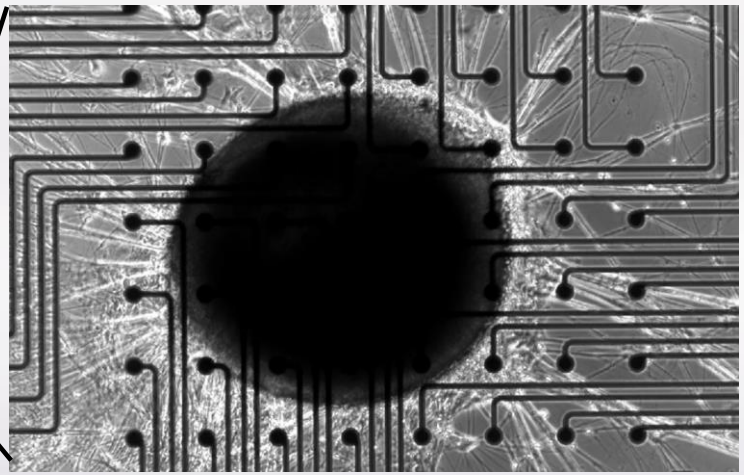
Organoid network activity (MEA)

Raster Plot

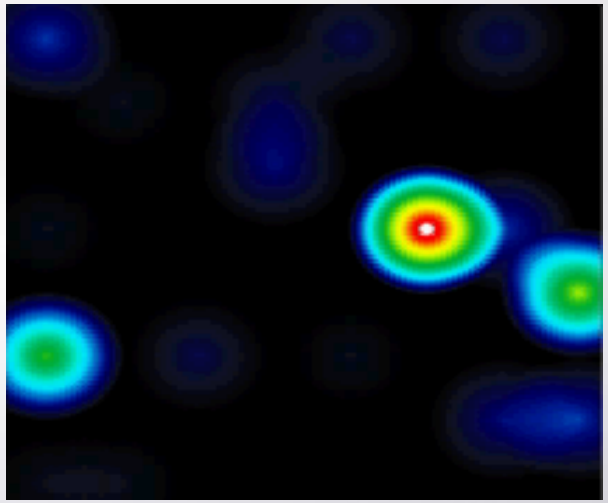






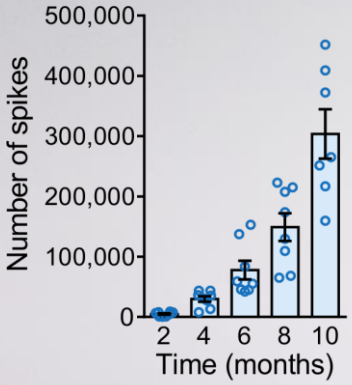


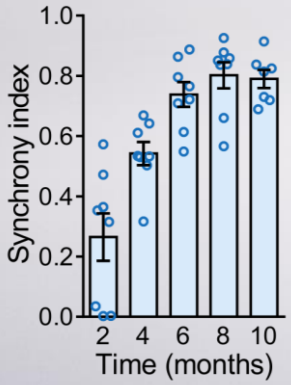
150 um

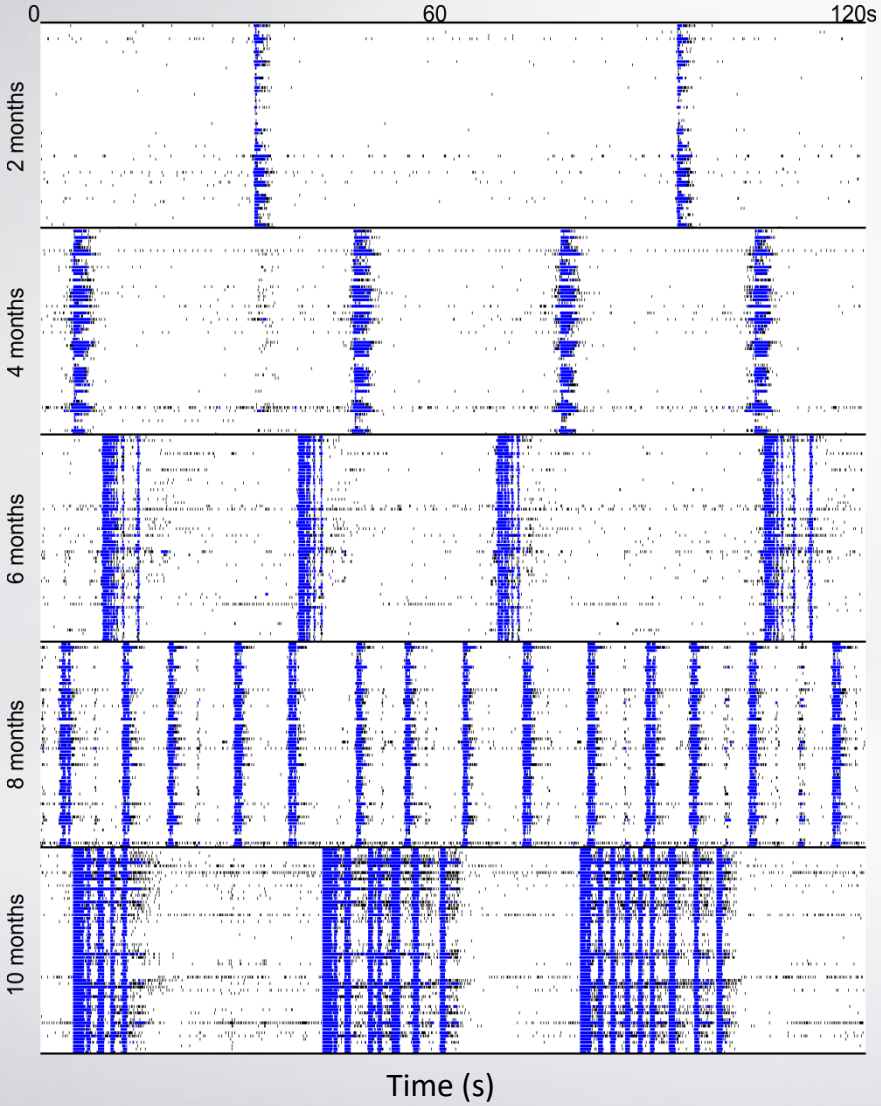


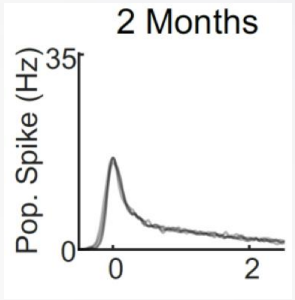
Activity Map

Long-term network activity

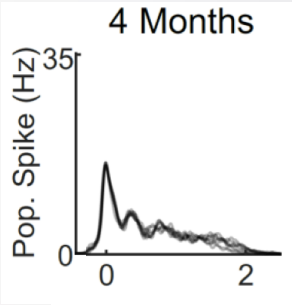




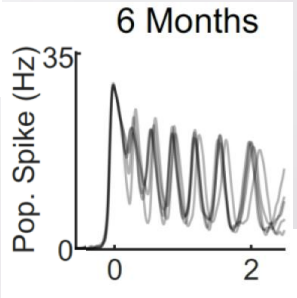


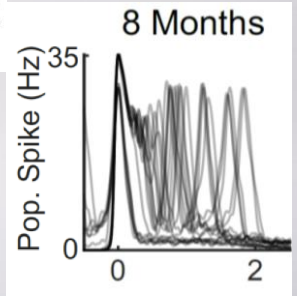


sparse activity with extreme regularity



Repetitive oscillatory patterns

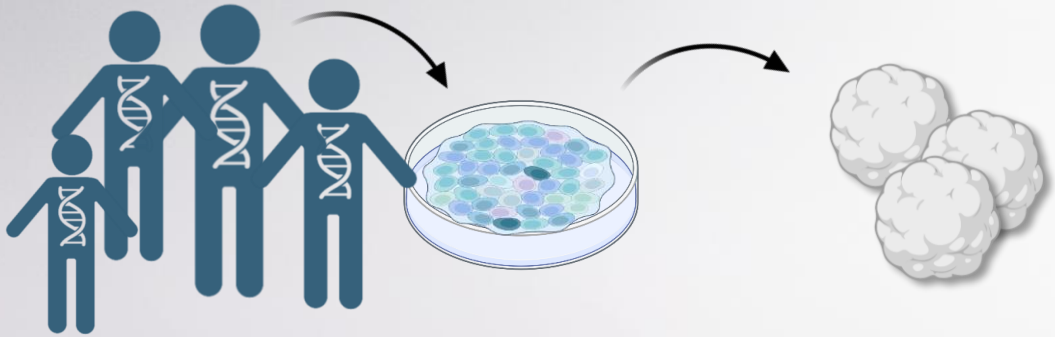




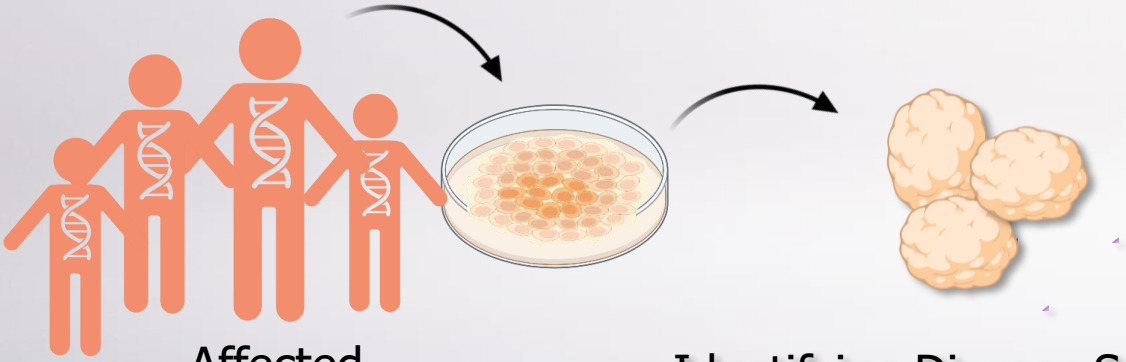
higher spatiotemporal complexity and variability.

Trujillo 2019

Organoids to uncover disease-specific phenotypes



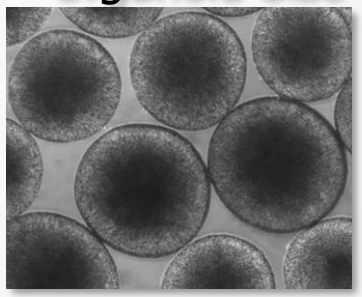
Neurotypical



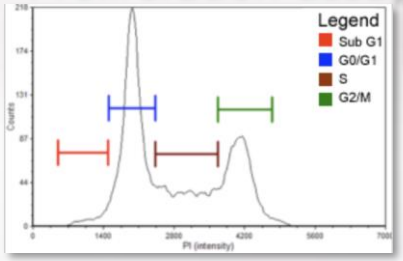
Affected

Identifying Disease-Specific Endophenotypes

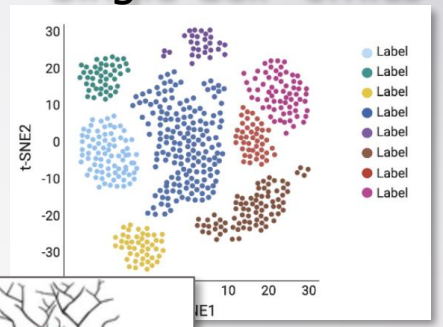
Organoid Size

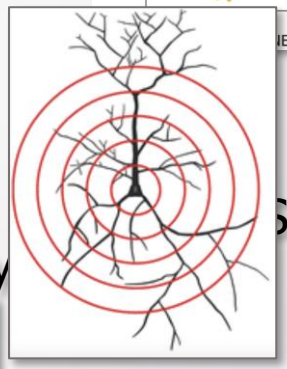


Proliferation assay

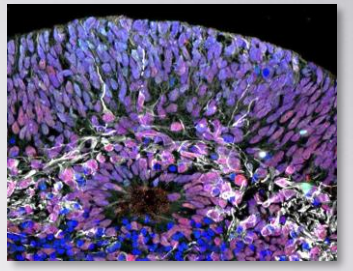


Single Cell -omics



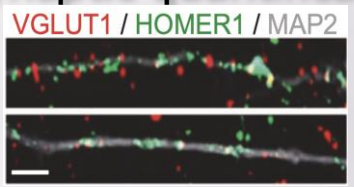


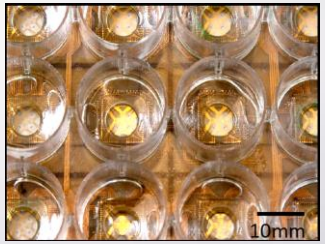
Morphology

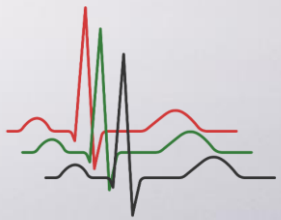


Cytoarchitecture

Synaptic quantification

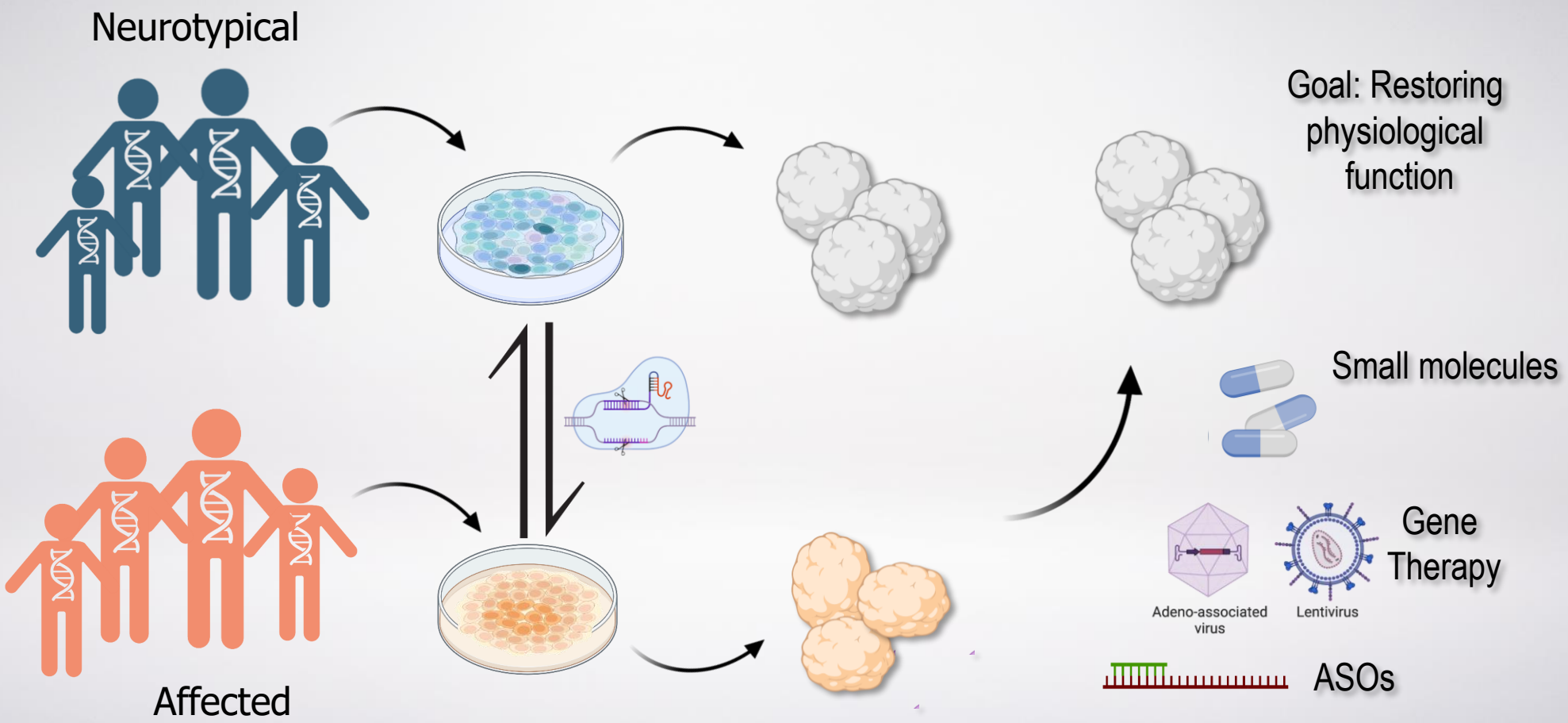






Neuronal Activity

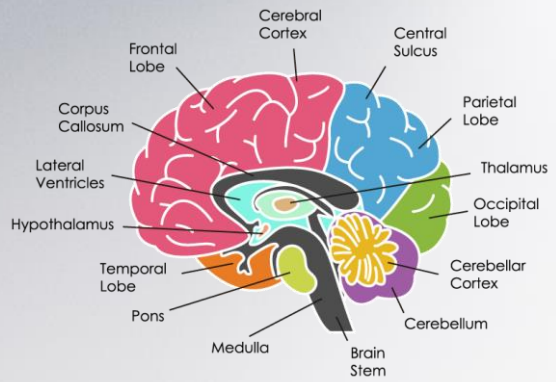
Personalizing neurological disorders using neural organoids to guide treatments

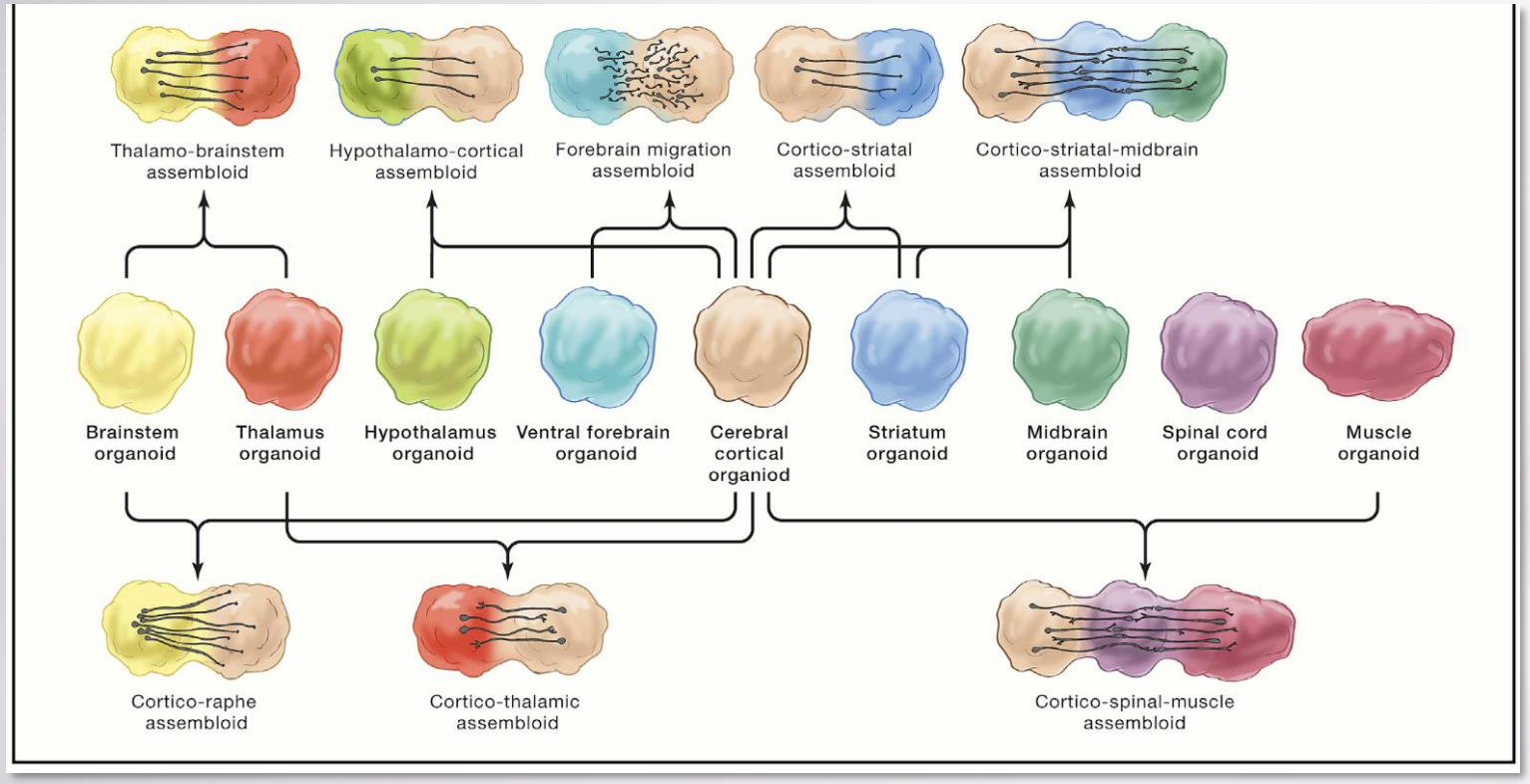


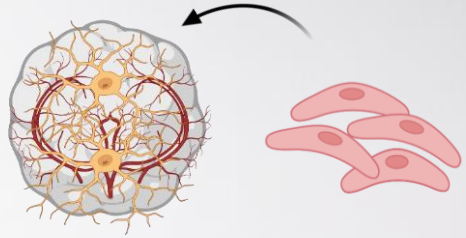
Limitations

*Fetal-like
*Missing cell types (microglia)
*Reduced complexity
*No Blood Brain Barrier
*Not connected to other organs
*Slow to “mature”
*High variability

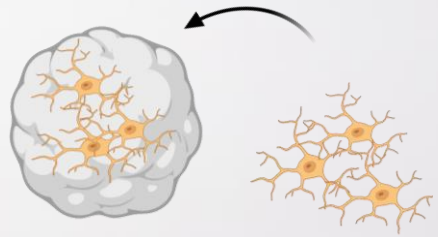
Increasing complexity and maturity



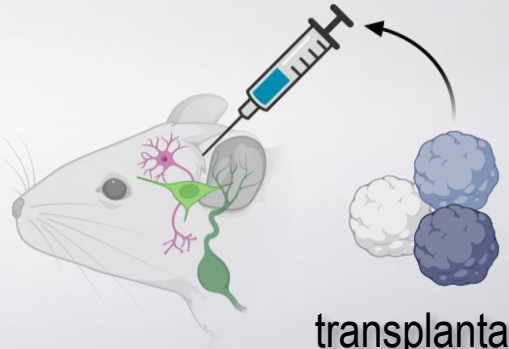




Vascularized organoids



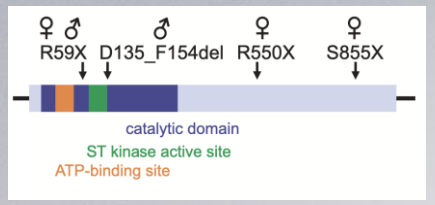
+microglia



transplantation

Modified from Kelley and Pasca 2022

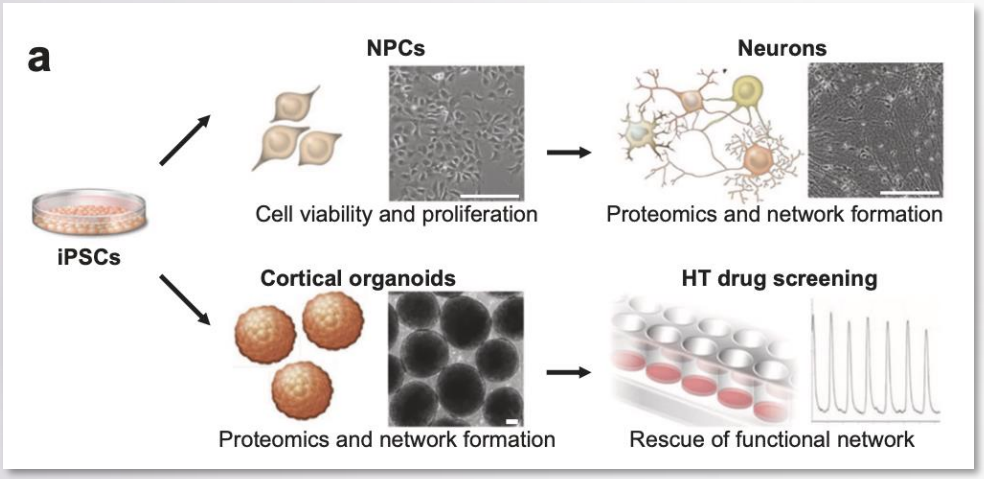
CDKL5 deficiency disorder (CDD)



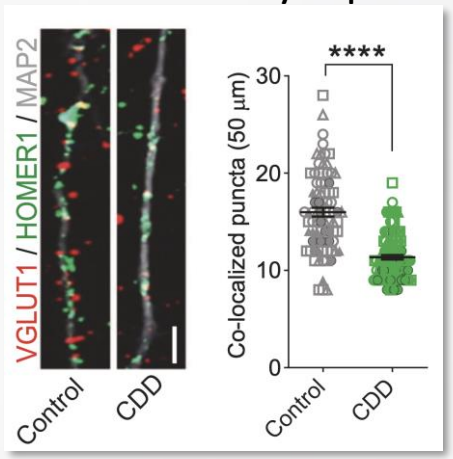
Molecular Psychiatry
https://doi.org/10.1038/s41380-021-01104-2
2021
ARTICLE

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

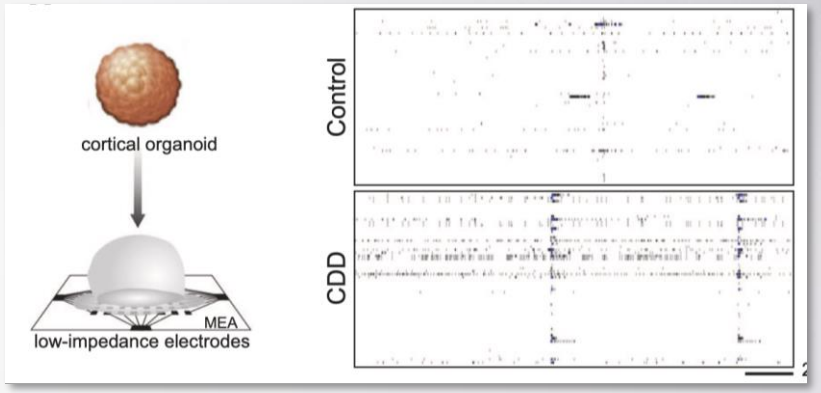
Priscilla D. Negraes[1] · Cleber A. Trujillo[1] · Nam-Kyung Yu[2] · Wei Wu[1] · Hang Yao[1] · Nicholas Liang[1] · Jonathan D. Lautz[3,4] · Ellius Kwok[1] · Daniel McClatchy[2] · Jolene Diedrich[2] · Salvador Martinez de Bartolome[2] · Justin Truong[1] · Ryan Szeto[1] · Timothy Tran[1] · Roberto H. Herai[5]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



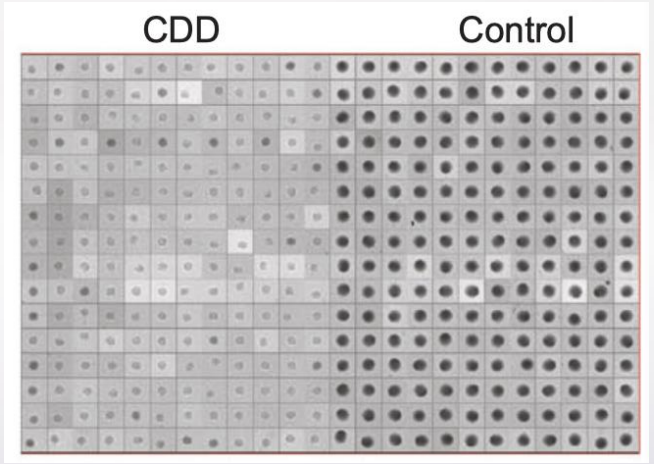
Reduced synapses

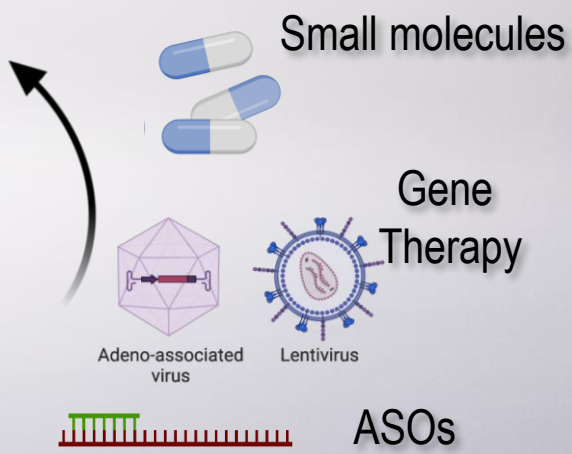


Hyperexcitability



Human neurons





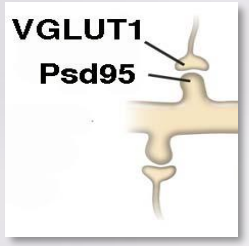
Rett syndrome

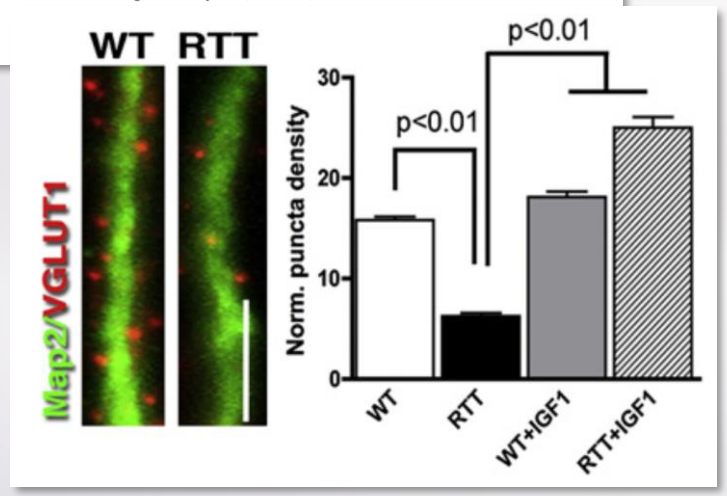
2010

Cell

A Model for Neural Development and Treatment of Rett Syndrome Using Human Induced Pluripotent Stem Cells

Maria C.N. Marchetto,[1,5] Cassiano Carromeu,[2,5] Allan Acab,[2] Diana Yu,[1] Gene W. Yeo,[3] Yangling Mu,[1] Gong Chen,[4] Fred H. Gage,[1] and Alysson R. Muotri[2,*]
[1]The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[2]University of California San Diego, School of Medicine, Department of Pediatrics, Rady Children's Hospital San Diego, Department of Cellular and Molecular Medicine, Stem Cell Program, 9500 Gilman Drive, La Jolla, CA 92093, USA
[3]University of California San Diego, School of Medicine, Department of Cellular & Molecular Medicine, Stem Cell Program, 9500 Gilman Drive, La Jolla, CA 92093, USA
[4]Pennylvania State University, Department of Biology. 201 Life Science Building, University Park, PA 6802, USA
[5]These authors contributed equally to the work
*Correspondence: muotri@ucsd.edu
DOI 10.1016/j.cell.2010.10.016





Insulin Growth Like Factor 1 (IFG-1)

March 2023

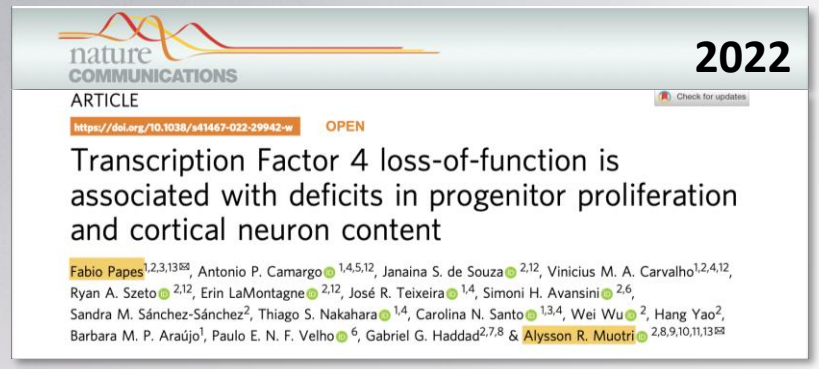
ACADIA® Pharmaceuticals

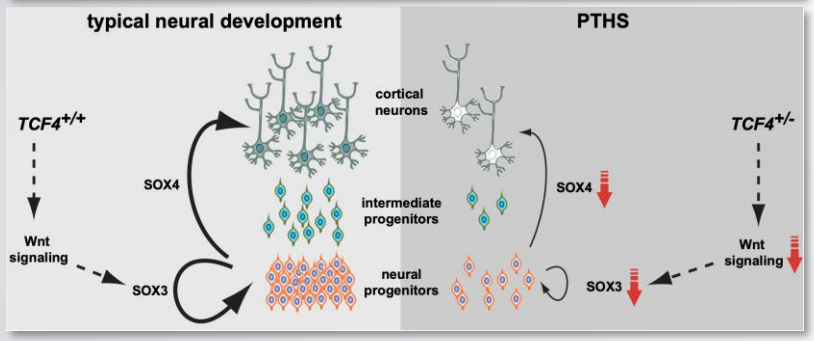
FDA APPROVES TROFINETIDE

The first-ever treatment for Rett Syndrome

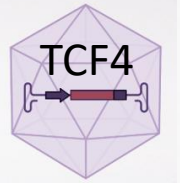
Trofinetide = Modified IGF-1

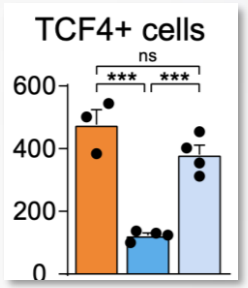
Pitt-Hopkins syndrome – TCF4 deficiency

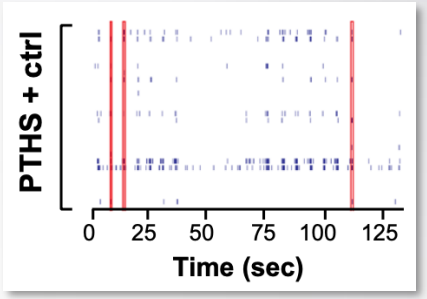


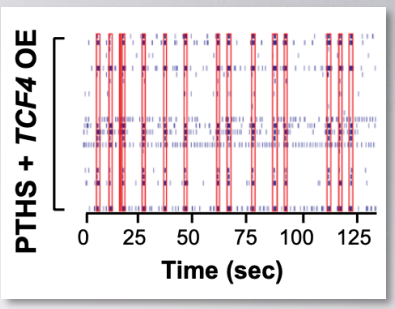


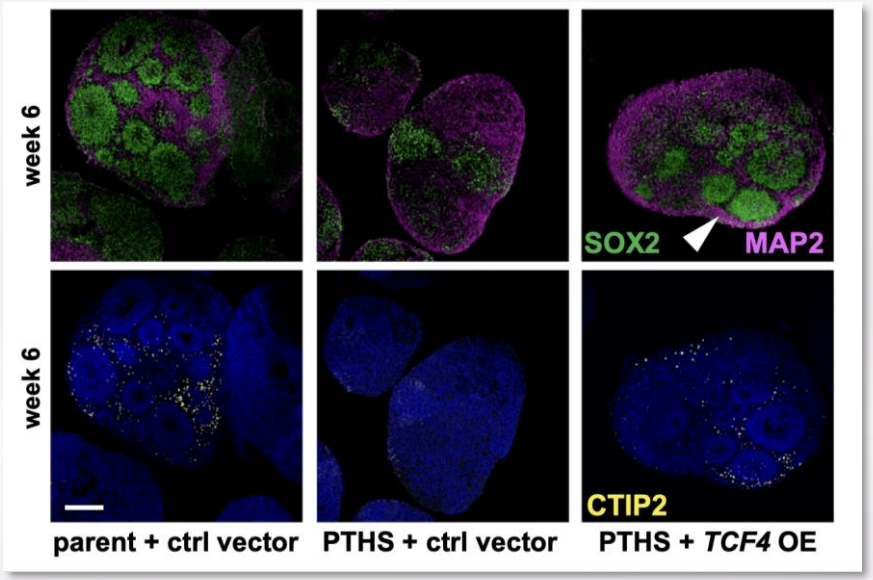
Adenovirus









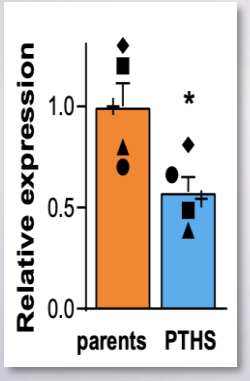


Rescued phenotypes

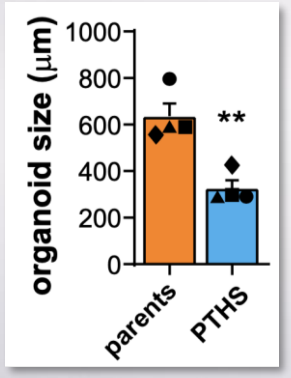
AAV9-base TCF4 replacement approach

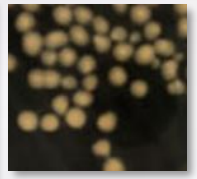
MAHZI therapeutics

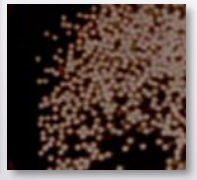
Lower TCF4



Smaller Organoids







How much does it cost? Timeline?

Year 1	Year 2	Year 3	Year 4	Year 5
$100K foundation $150K Muotri Lab	$150K foundation $150K Muotri Lab	$150K Muotri Lab	$800 R01	$300K licensing
Generate iPSCs Basic experiments	Complex experiments Investigate mechanism		Publication Grants	Working on Pre-IND

$500 to establish a skin fibroblast line
$10-15,000 to reprogram, QC and establish one iPSC line
$15,000 to differentiate one iPSC line
% effort of a technician/postdoc

The Muotri Lab