norecopa

UNIVERSITY OF EASTERN FINLAND

Novel human-cell based models to study neurodegeneration

Nordic 3R webinars, 5.-6.5.2021

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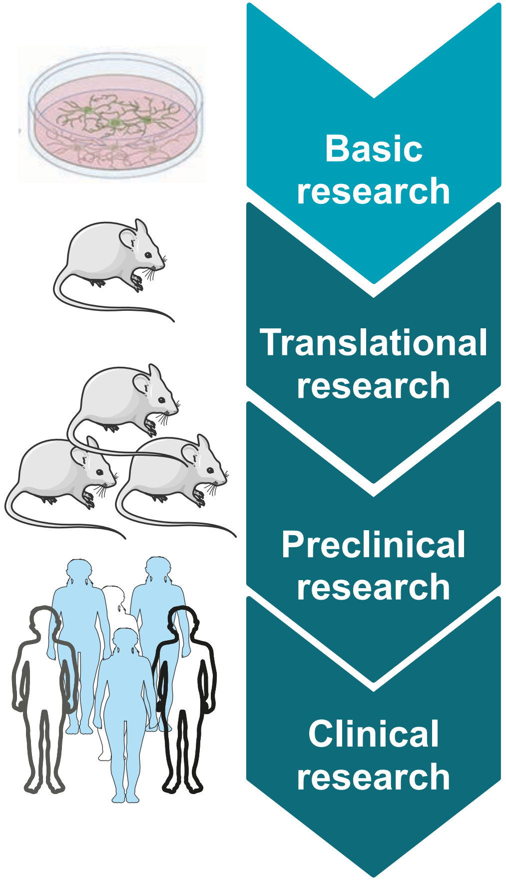
Head of Human brain disease modelling group

Current in vivo approach does not necessary predict human effects



- ➢ Traditional drug success rate from preclinical stage to phase I clinical trials is only 30 % and in clinical trials 10%.
- ➢ Clinical trials of CNS drugs have shown a very low overall success rate (6.2% vs. 13.3% for non-CNS drugs)
- ➢ For AD, only 1 out of 244 experimental compounds in Alzheimer's clinical trials was approved during a 15-year period

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Average cost for approved drug: over $ 2 billion
Average time: 10 years

Neurodegenerative diseases

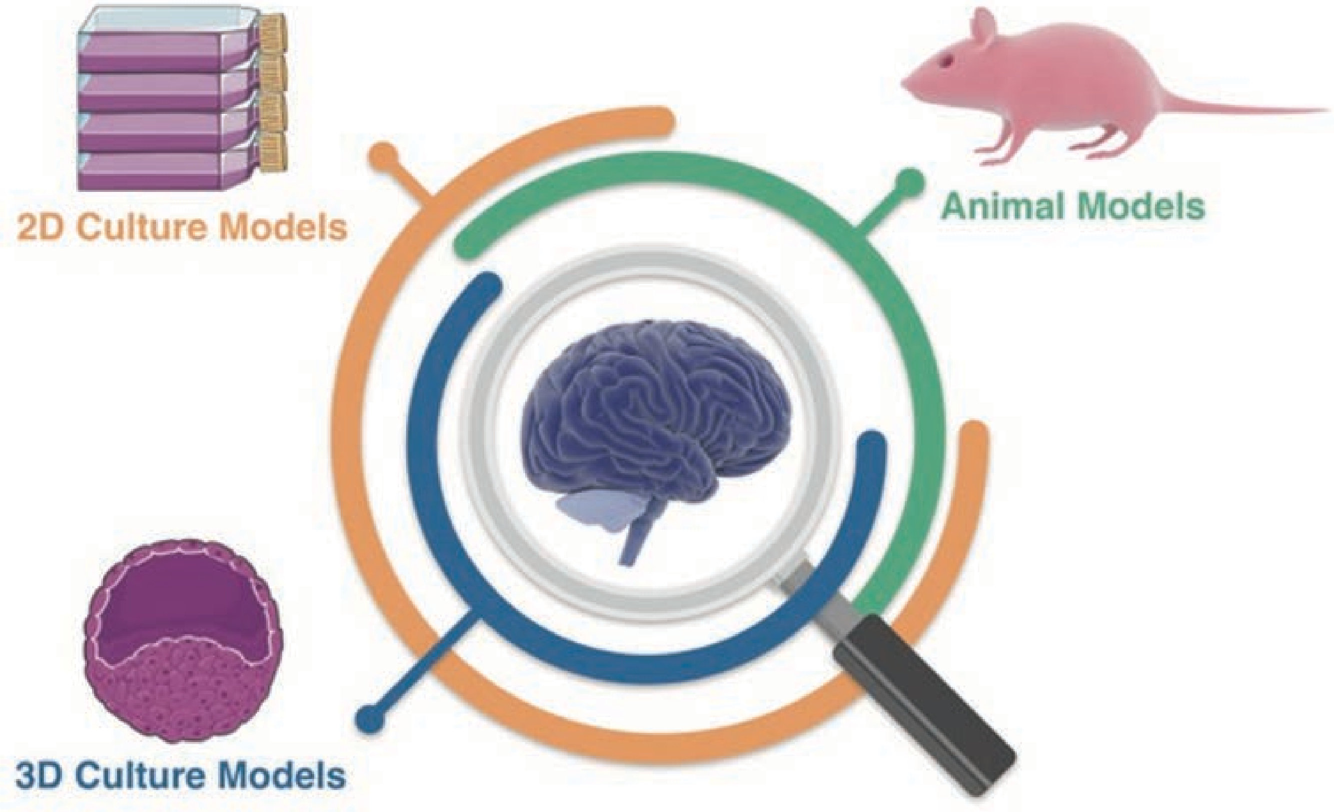
- associated with neuronal loss, cellular dysfunction, and pathological accumulation of proteins in the brain
- this causes problems with movement or mental functioning (called dementias)
- lack of efficient treatment

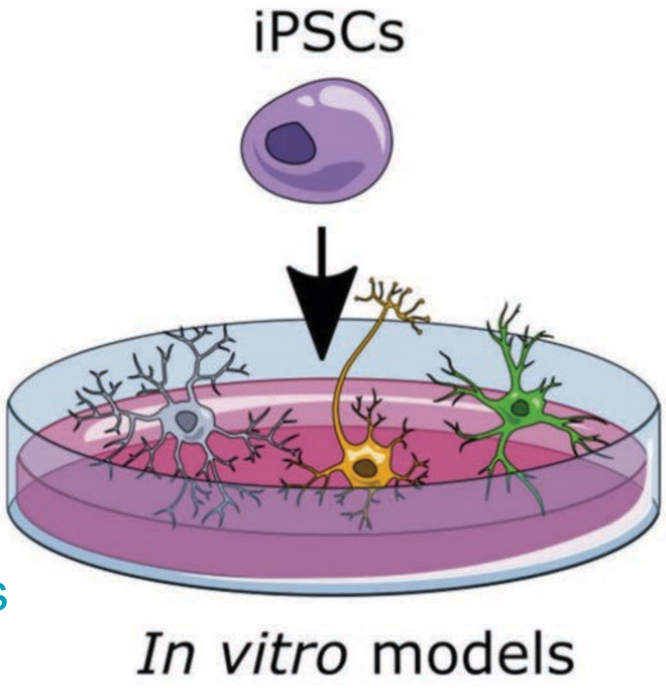
- **The fastest-growing threats of old age include dementia, AD, stroke, PD,** and progressive hearing loss.
- Dementia incidence outpaces all other disorders. The economic burden of dementia is 12 times that of cancer.

FINLAND takes a lead among countries with highest rates of deaths with dementia. **54 people dying per 100,000** in the Finnish population every year due to Alzheimer's and other dementias.

MODELING HUMAN NEURODEGENERATIVE DISEASES

Where do we go from here?





iPSCs can give advantages over traditional animal models in that they more accurately represent the human genome

Albert et al., 2021 *IJMS* 22(9), 4334.

Important AD studies utilizing hiPSC-derived cells

Table 2 Select important AD studies utilizing iPSC-derived brain cells

Model	Reference	Mutation(s)	Significance
Neurons	Israel et al. [89]	sAD, APP^{Dp}	Early study of iPSC modeling; elevated Aβ, p-tau, endosome accumulation in AD neurons
	Shi et al. [98]	Down syndrome	Elevated Aβ secretion, Aβ aggregation, tau phosphorylation in DS neurons
	Wang et al. [39]	Isogenic APOE3, APOE4 and APOE null	Elevated Aβ and p-tau levels, GABAergic neuron degeneration in APOE4 neurons; identification of small molecule APOE4 structure corrector
Astrocytes	Oksanen et al. [128]	Isogenic $PSEN1^{\Delta E9}$	Increased Aβ production and oxidative stress, altered cytokine release and Ca^{2+} homeostasis, reduced neuronal support function in PSEN1 astrocytes
	Lin et al. [38]	Isogenic APOE3 and APOE4	Impaired Aβ clearance and increased cholesterol content of APOE4 astrocytes
Microglia	Lin et al. [38]	Isogenic APOE3 and APOE4	Reduced Aβ uptake from media and fAD organoids, reduced morphological complexity of APOE4 microglia
3D cultures	Choi et al. [56]	Overexpression of $APP^{K670N/M671L}$, APP^{V717I}, $PSEN1^{\Delta E9}$	Robust deposition of Aβ and filamentous tau in vitro; demonstrates that Aβ can cause tau deposition
	Park et al. [170]	Overexpression of $APP^{K670N/M671L}$, APP^{V717I}, $PSEN1^{\Delta E9}$	Triculture model system incorporating iPSC-derived neurons, astrocytes, and immortalized human microglia; recapitulates AD phenotypes, microglial recruitment, and neuroinflammation

Glial cells making up approximately half of the cells in the CNS and along with neurons play an equally central role in neurodegenerative diseases

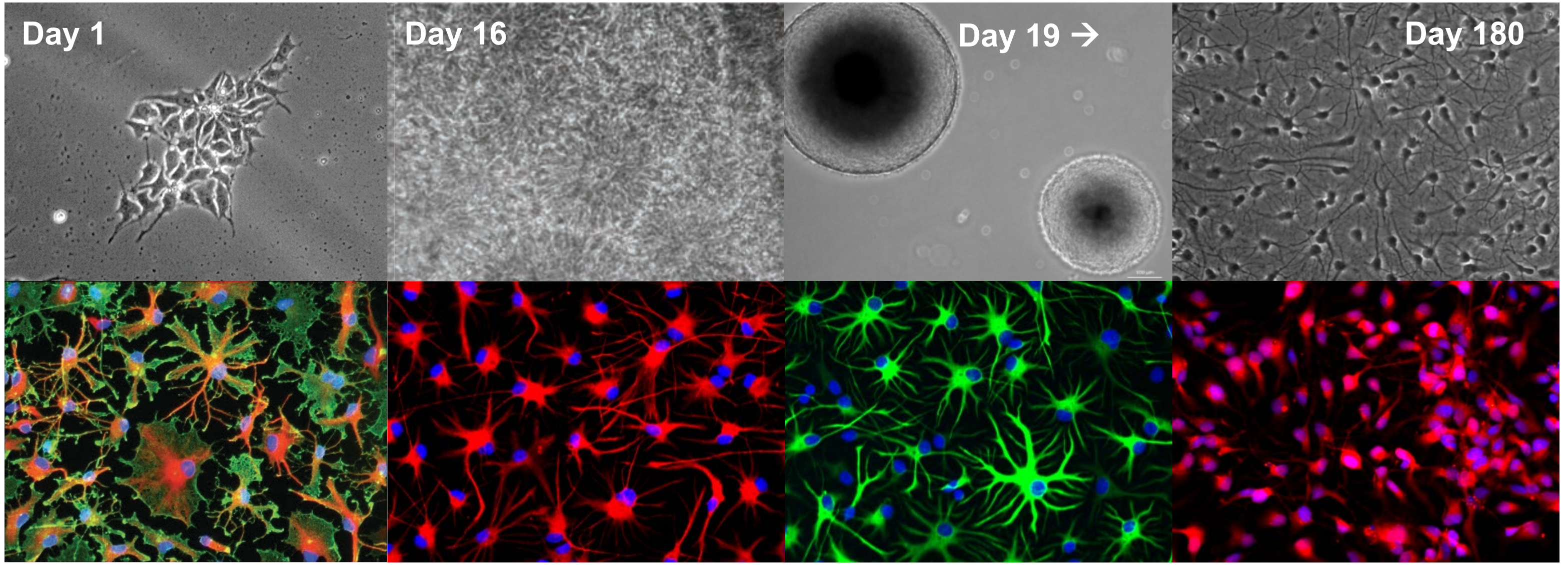
Pennay et al. *Molecular Psychiatry* (2020) 25:148–167

hiPSC cells were generated from healthy controls and *PSEN1 ΔE9* patients.

Astrocytes in the healthy CNS

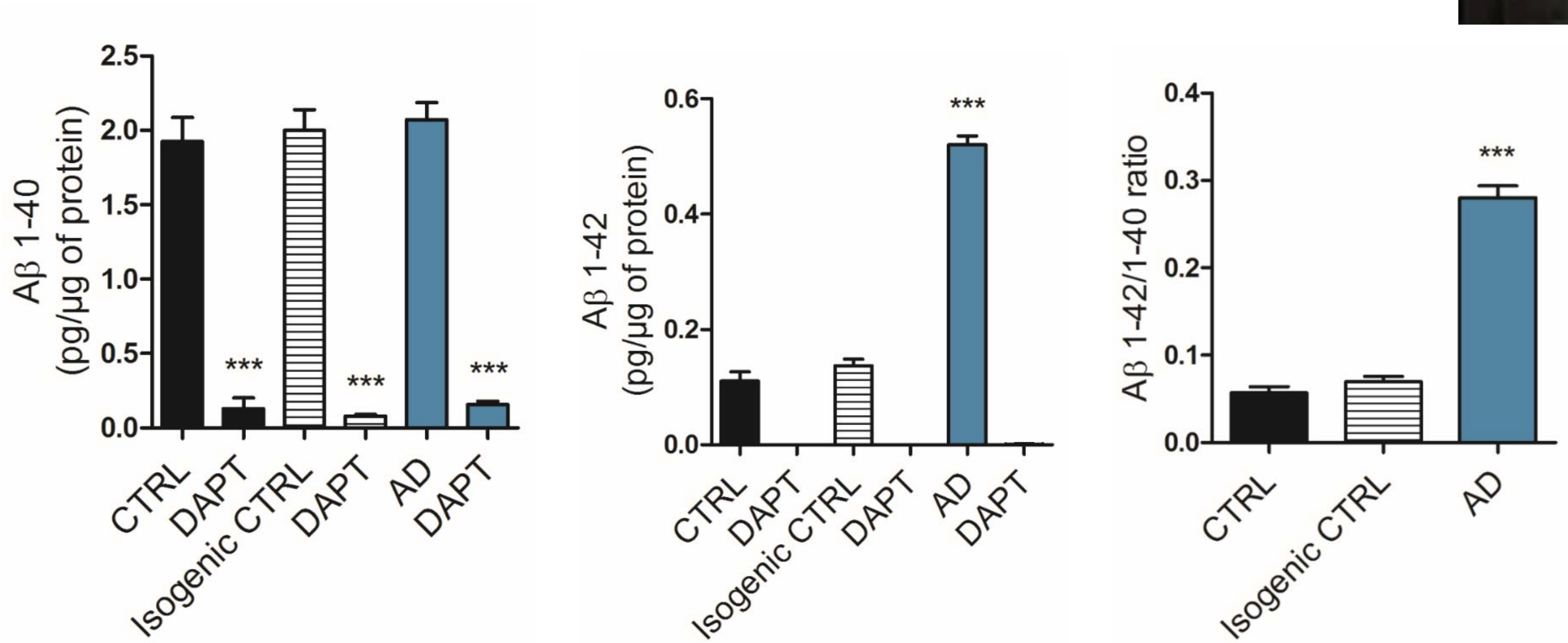
- Maintenance of homeostasis
- Regulation of blood flow
- Regulation of blood-brain barrier
- Recycling of neurotransmitters
- Activity-dependent regulation of synapse number and function
- Regulation of neurogenesis

hiPSCs efficiently differentiate into astrocytes



AQP4/GFAP/dapi | Vimentin/dapi | GFAP/dapi | S100B/dapi

PSEN1 ΔE9 astrocytes exhibit amyloidogenic properties and DAPT efficiently block Aβ production

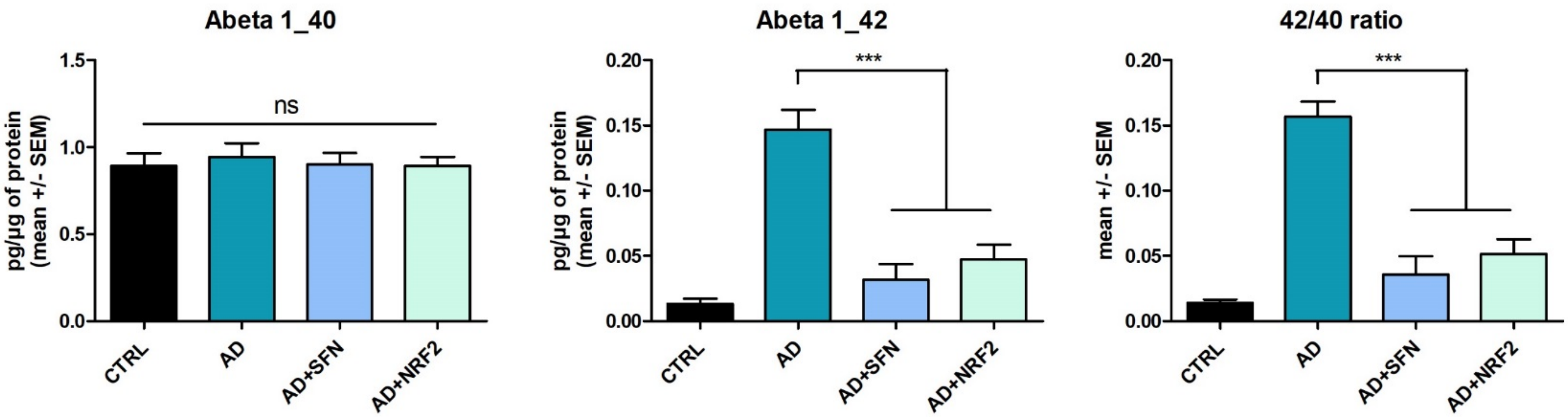


DAPT –gamma secretase inhibitor

Aβ 1-42 – 10% of total, highly fibrillogenic, readily aggregated, and neurotoxic

Oksanen et al. *Stem Cell Reports* 9(6):1885-1897, 2017

Nrf2 induction significantly reduces Abeta 1-42 secretion and leads to a healthier Abeta ratio in AD astrocytes



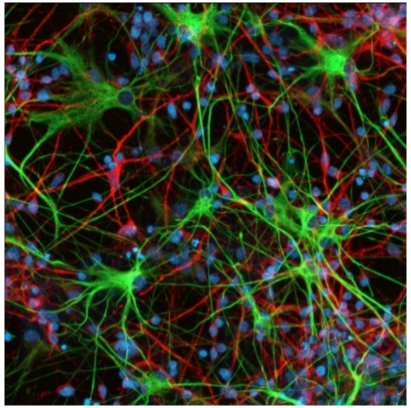
Nrf2 – transcription factor regulating the expression of antioxidant genes/proteins

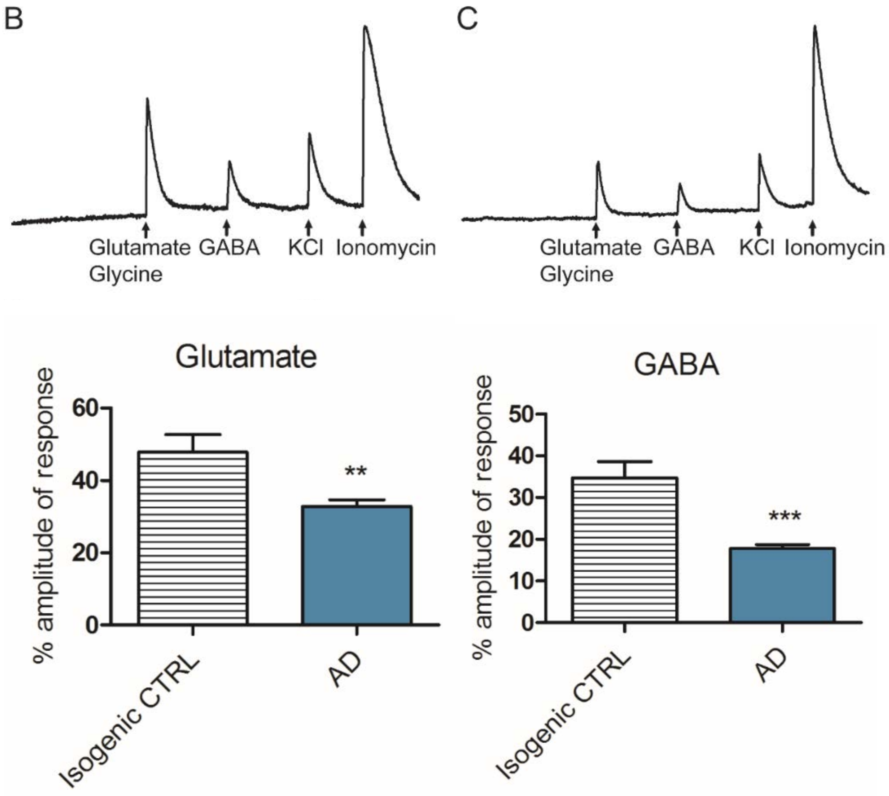
SFN – Sulforaphane

Oksanen et al. *Glia* 68(3):589-599, 2020

3D co-culture model reveals an important role for astrocytes in neuronal activity

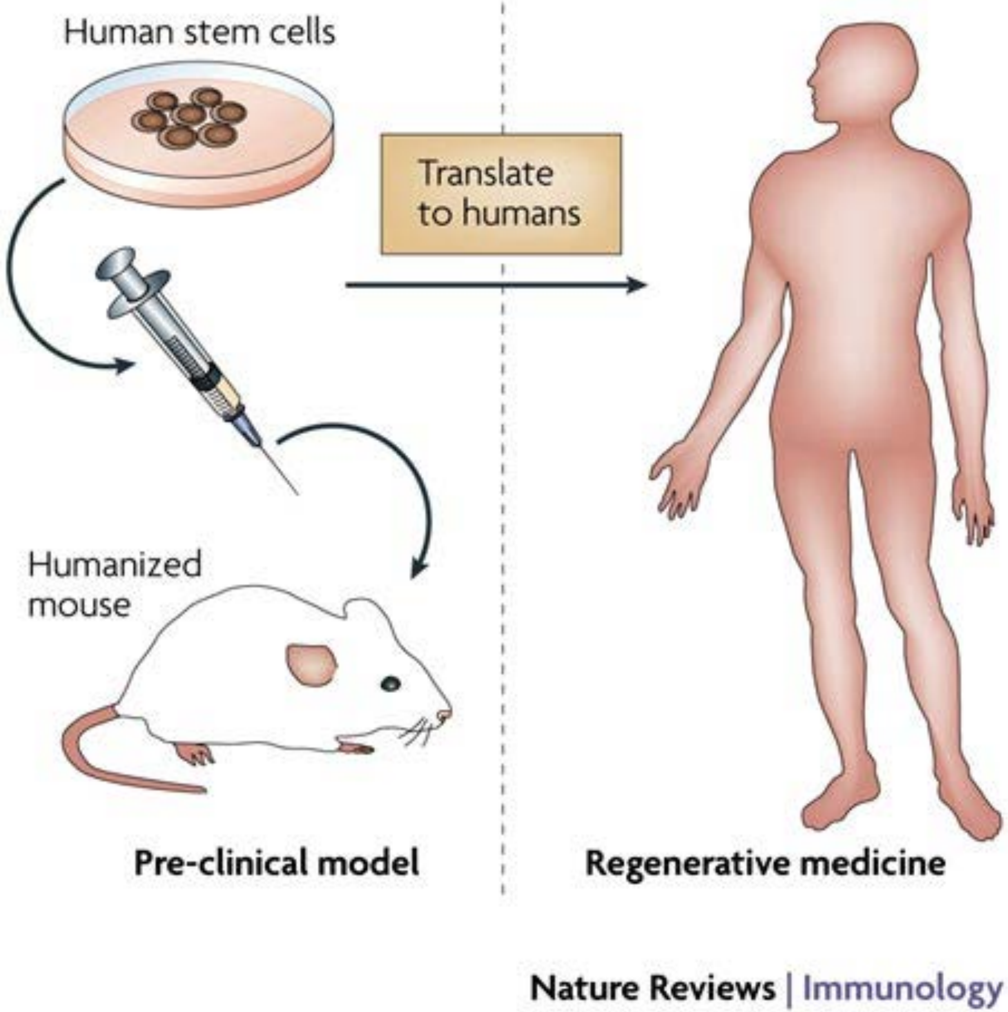
GFAP/MAP2/nuclei





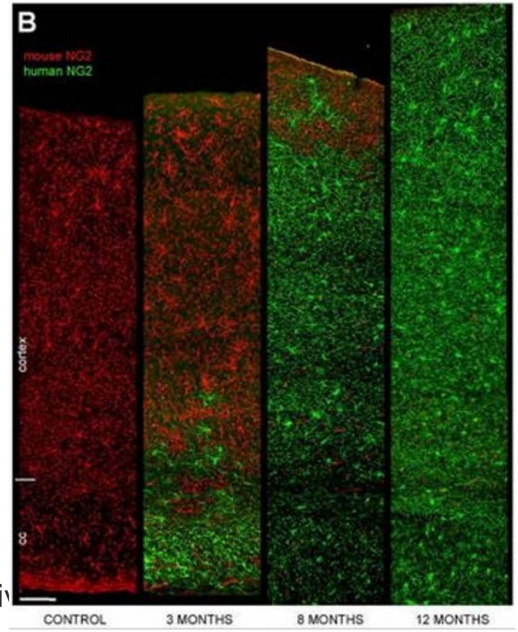
Oksanen et al. *Stem Cell Reports* 9(6):1885-1897, 2017

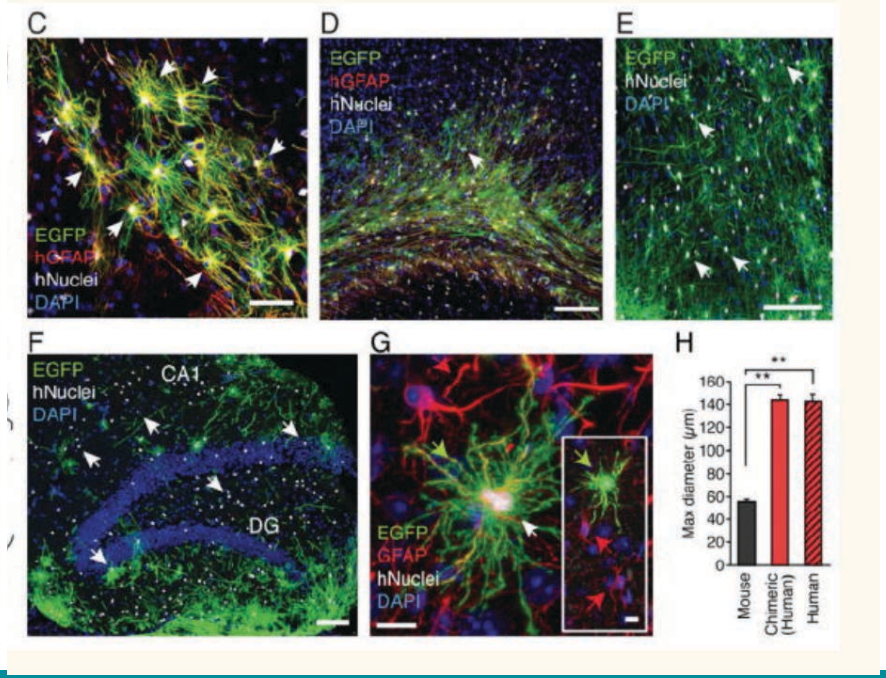
Humanized mouse



Humanized mouse model_forebrain

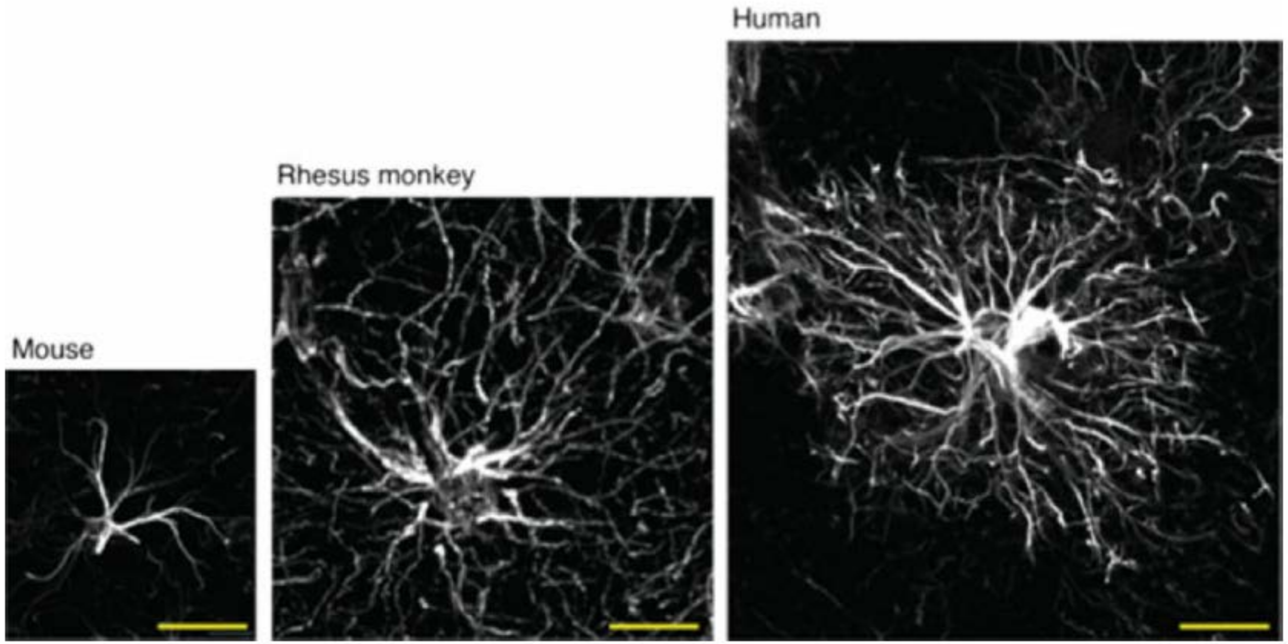
Type of study	Transplanted human cell type	Outcome of transplantation	References
$rag2^{-/-}$ or $rag1^{-/-}$ immunodeficient mice	A2B5+/PSA-$NCAM^{-}$ (from human 17-22-week old fetuses); transpl.to forebrain 2 locations; 100,000 cells	↑ Calcium propagation, gap junction-coupled to host astroglia, ↑ LTP; improved cognition functions (learning and memory)	Han et al. **2013,** Cell Stem Cell 12: 342-353





Human glial progenitor cells outcompete and ultimately replace resident mouse glial progenitor cells.

Human astrocytes are larger and more complex than rodent and other primates

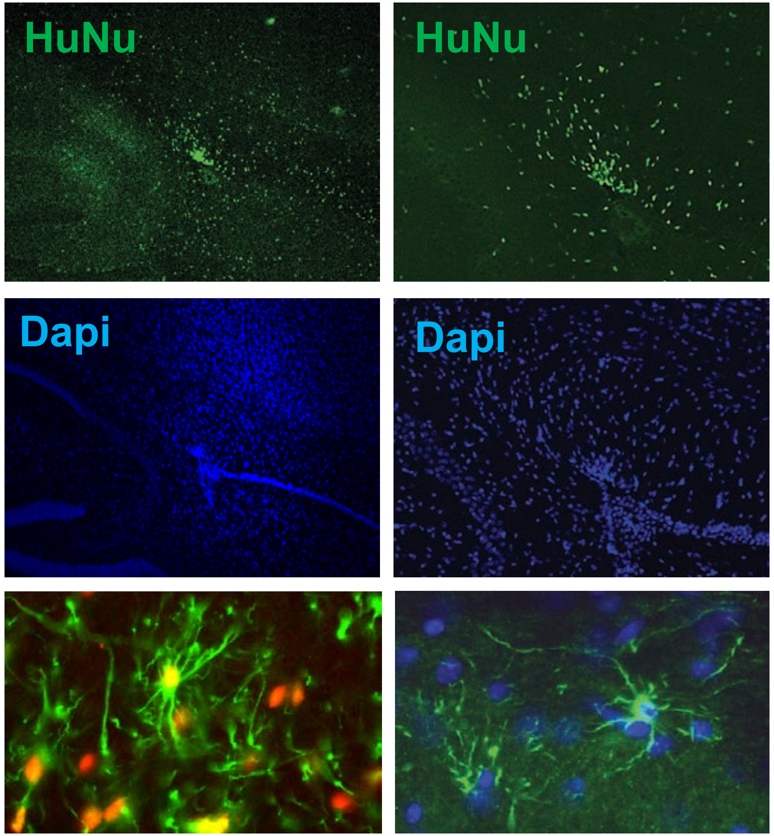


Evolutionary changes in astrocytes contribute to higher cognitive functions in humans

Methods Mol Biol 2012, 814, 23-45.

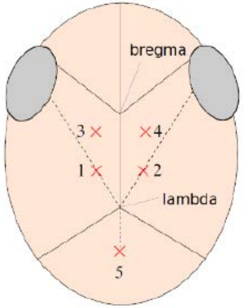
Transplantations of AD astroprogenitors

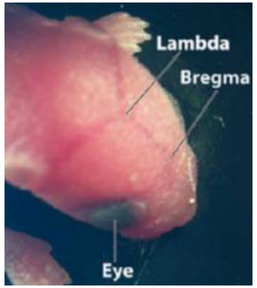
Cells from AD PSEN1 donor transplanted



hGFAP HuNu

hGFAP Dapi



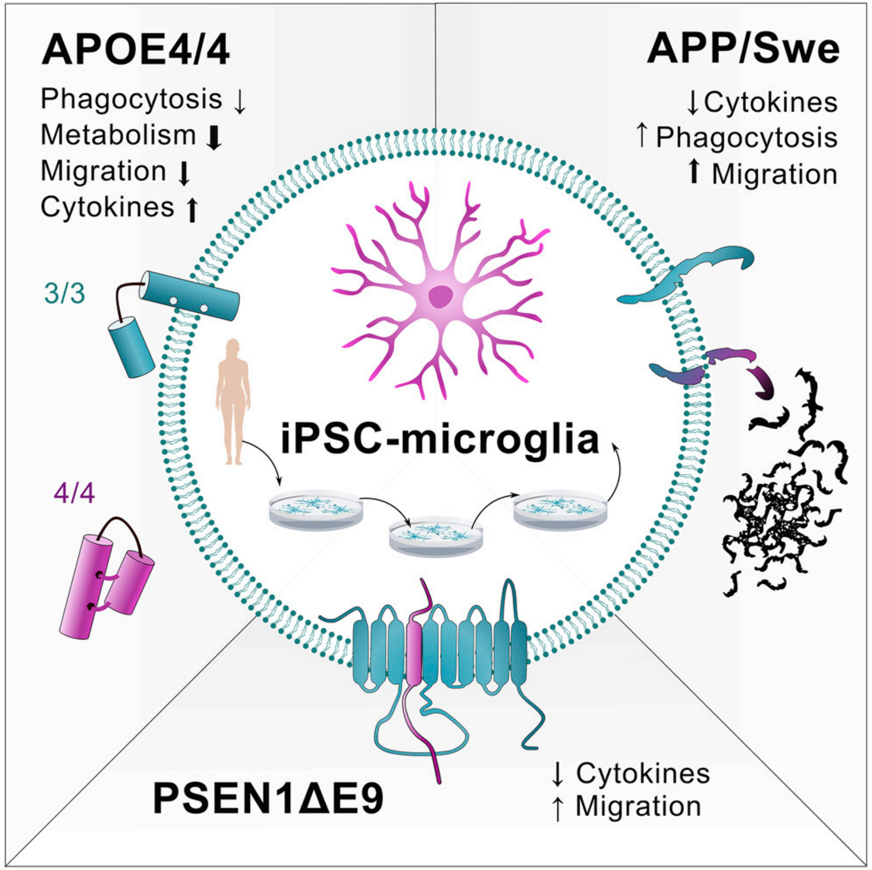


(0.8, 1.0, -1.5; 0.5µl)
(-0.8, 1.0, -1.5; 0.5µl)
(0.8, 2.0, -1.5; 0.5µl)
(-0.8, 2.0, -1.5; 0.5µl)
(0.0, -1.0, -1.5; 1µl)

Rate: 0.05 µl/s
Amounts:
50,000 cells/0.5µl

Koistinaho lab

Disparate phenotypes in AD iPSC-derived microglia



- APOE4 genotype has a profound impact on several functions of microglial cells
- Inflammatory responses are aggravated in cells with APOE4 genotype
- Metabolism, phagocytosis, and migration are decreased in APOE4 microglia-like cells
- Familial mutations APPswe and PSEN1ΔE9 have only minor effects on functionality

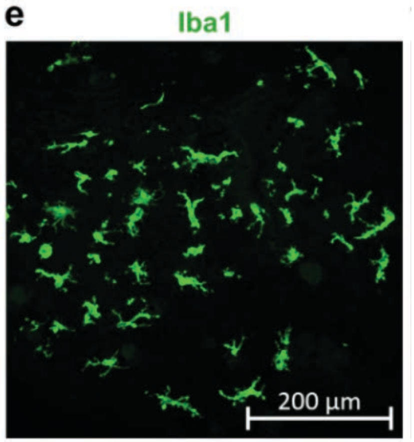
Stem Cell Reports 2019, Vol. 13, 669–683

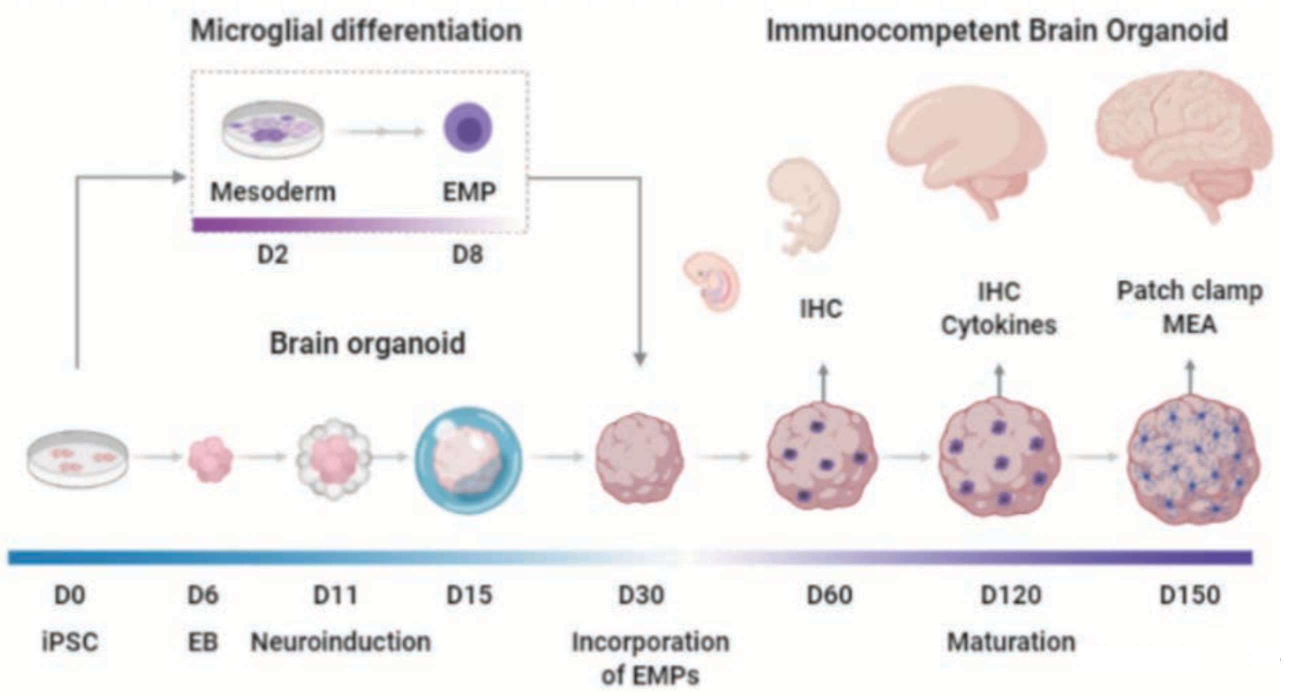
Immunocompetent brain organoids

Microglia orchestrate neuronal activity in brain organoids

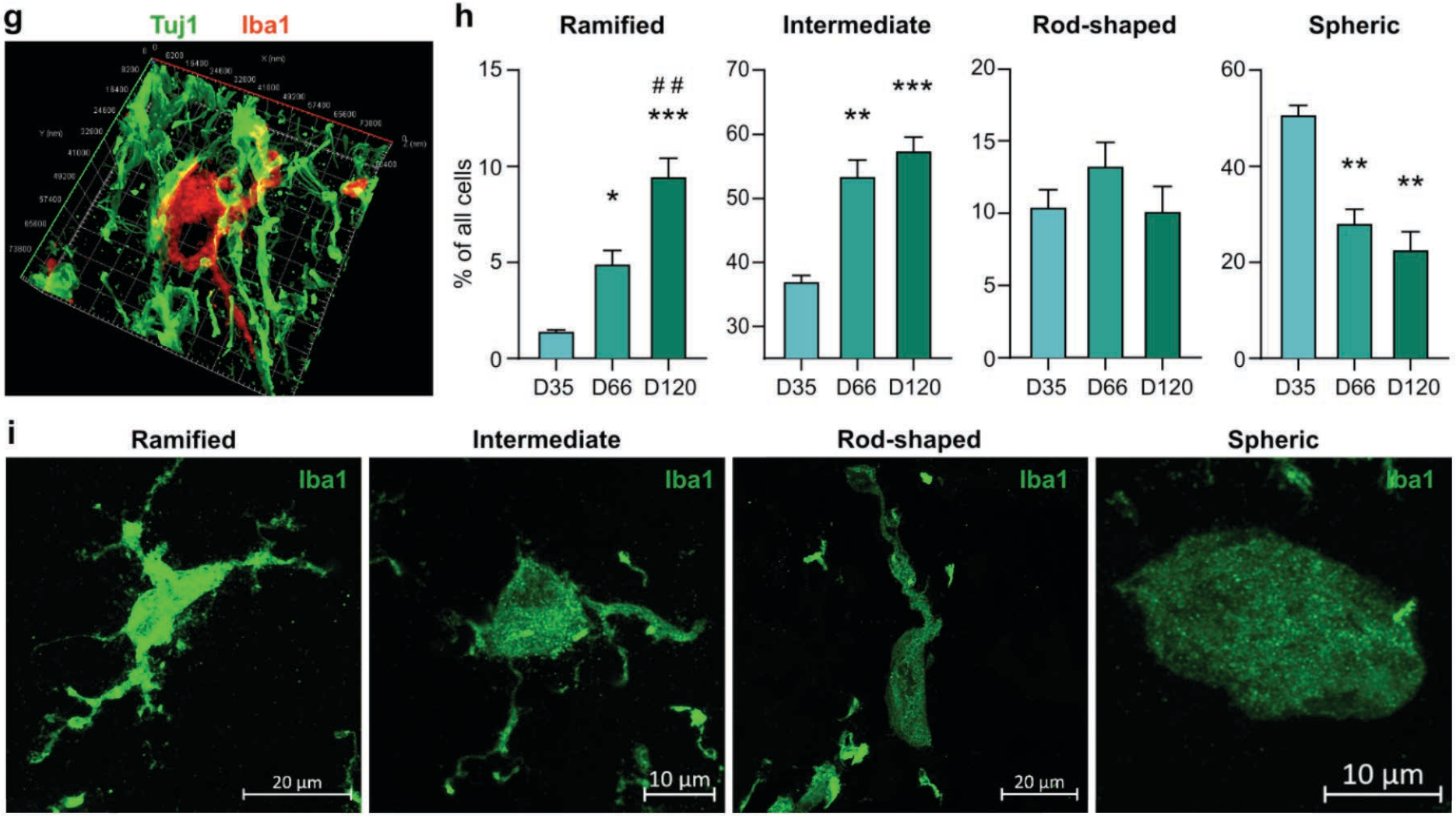
Ilkka Fagerlund, Antonios Dougalis, Anastasia Shakirzyanova, Mireia Gómez-Budia, Henna Konttinen, Sohvi Ohtonen, Fazaludeen Feroze, Marja Koskuvi, Johanna Kuusisto, Damián Hernández, Alice Pebay, Jari Koistinaho, Sarka Lehtonen, Paula Korhonen, Tarja Maln

doi: https://doi.org/10.1101/2020.12.08.416388





A population of Iba1+ cells in taking different morphologies

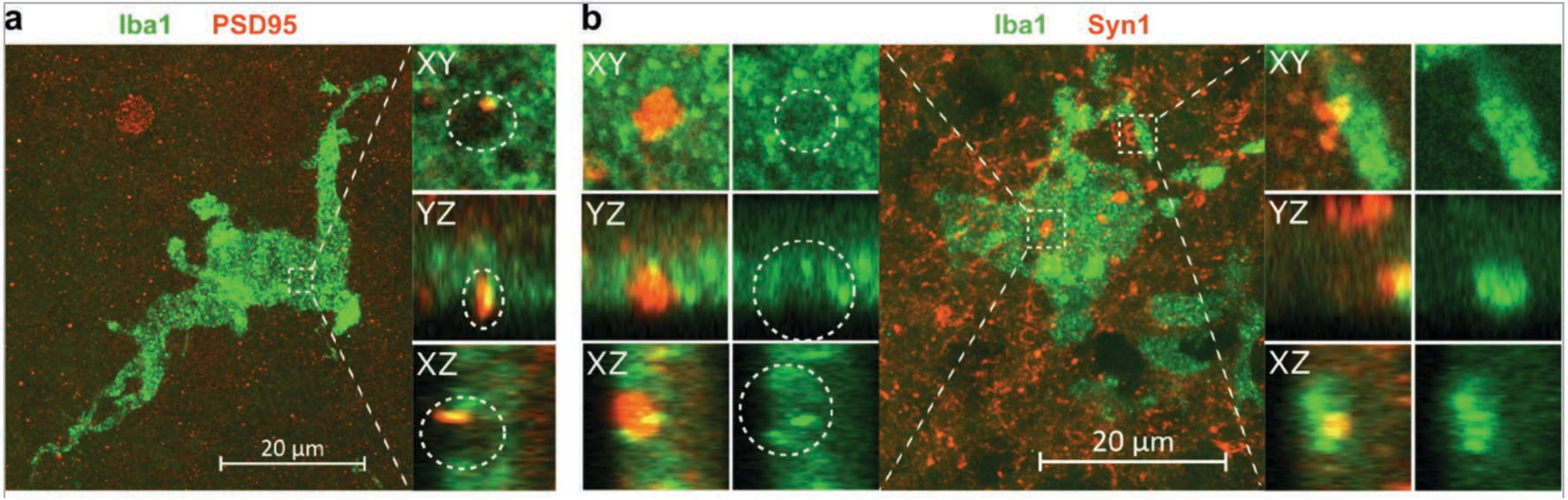


doi: https://doi.org/10.1101/2020.12.08.416388

Incorporated microglia interact with synapses

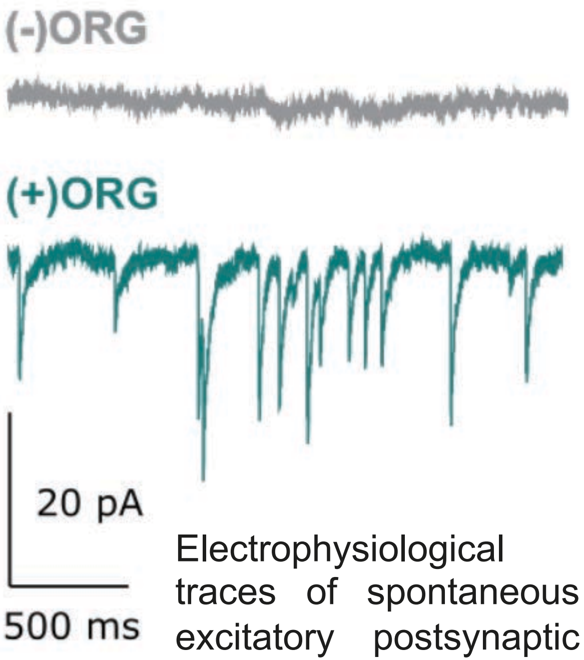
PSD95+ post-synaptic material embedded within a pocket on the surface of an **Iba1+** cell

Syn1+ presynaptic material embedded within a pocket on the soma of **Iba1+** cell and partially internalised by process of the cell



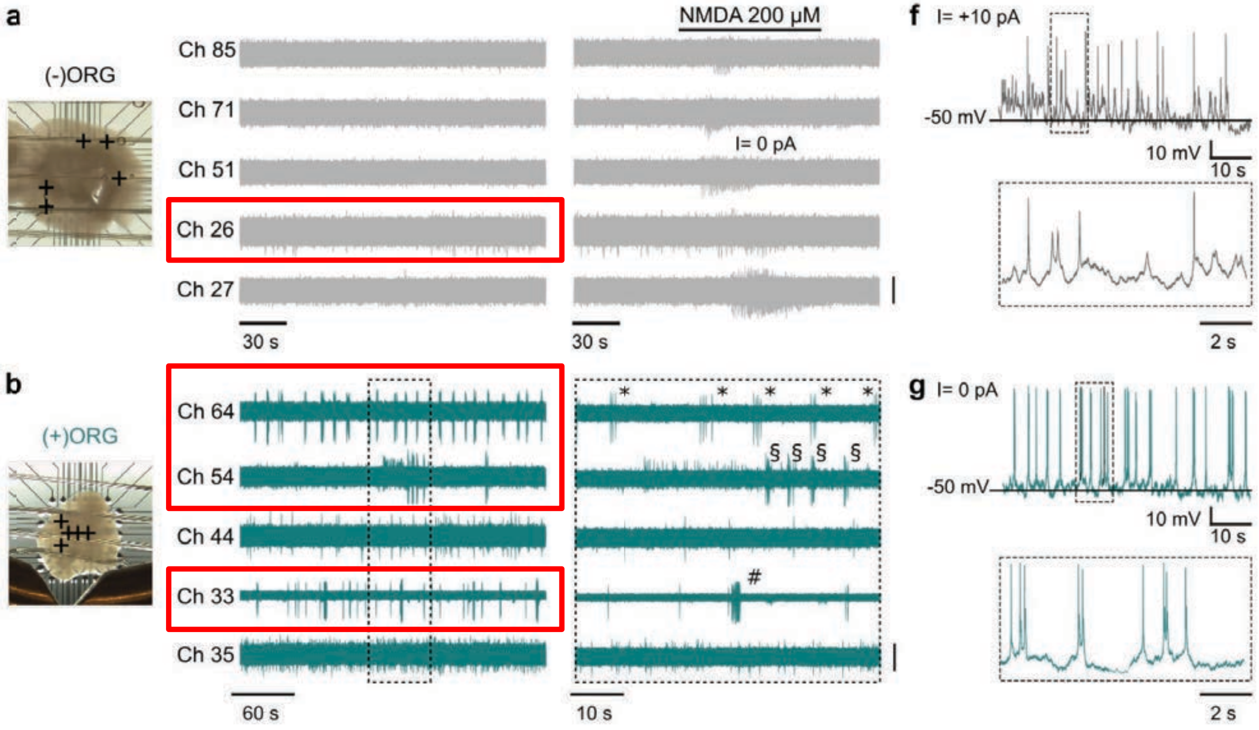
doi: https://doi.org/10.1101/2020.12.08.416388

Microglia empower neuronal maturation



Electrophysiological traces of spontaneous excitatory postsynaptic currents recorded under voltage clamp

Microglia drive neuronal bursting and network activity in (+)ORGs



spiking activity before and after NMDA perfusion

Electrophysiological traces from whole-cell recording

doi: https://doi.org/10.1101/2020.12.08.416388

Summary

Development of human brain cell platforms for improved clinical translation

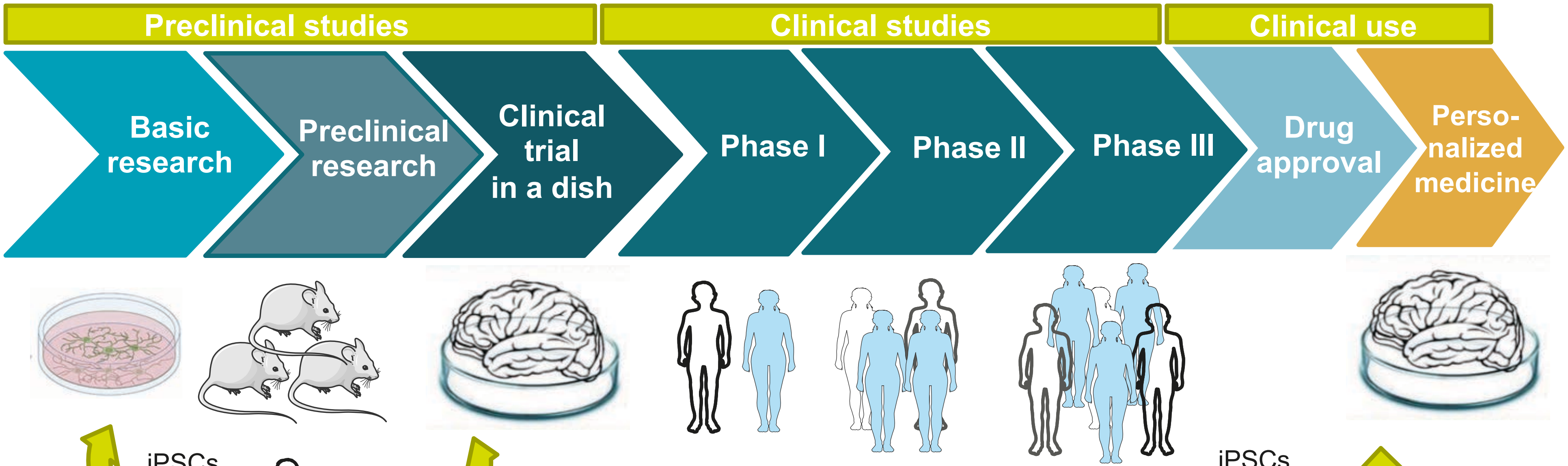
Astrocytes or microglia in 2D system:
- manifestation of disease pathology
- platform for drug trials

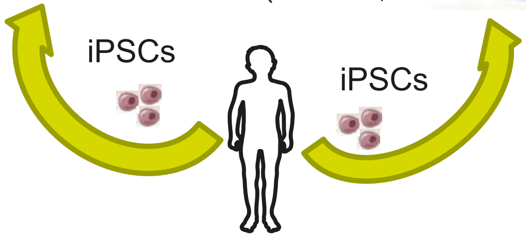
3D co-cultures with neurons:
- mimicking in vivo complexity
- different functional behavior

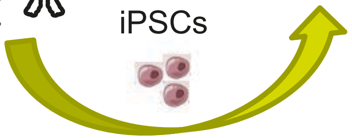
Brain organoids:
- complex cellular interactions
- modelling of brain networks
- immunocompetent (microglia)
- vascularization (endothelial cells)

Humanized models:
- studying contribution to the disease pathogenesis
- elucidate mechanism of neurodegenerative diseases

Summary







Increase the success in clinical studies
Avoid unnecessary clinical studies
Reduce the expenses in drug development

Reduce and partly replace number of animals

Supports development of more efficient drugs and personalized medicine for brain diseases

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Thank you!

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