Modelling neuronal microenvironment dynamics in disease

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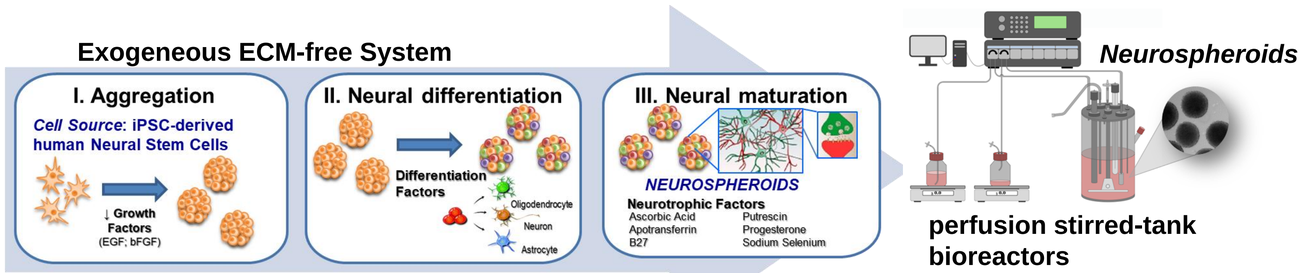
Motivation

The neuronal microenvironment modulates physiological functions and pathological processes, including synaptic function. A major challenge in studying the underlying mechanisms is the lack of human cell models in which the dynamics of the extracellular space is recapitulated without the confounding effects of heterologous ECM components. We hypothesized that once an endogenous neuronal microenvironment is sustained in neurospheroids, the model would recapitulate pathological features of neuronopathic diseases in which astroglyosis and ECM remodeling may be relevant.

The Neurospheroid Methodology

Our group has recently developed a 3D neural differentiation protocol from induced pluripotent stem cells (hiPSC) that does not resource to heterologous matrices. Neurospheroid, composed of neurons, astrocytes and oligodendrocytes are generated and maintained in perfusion stirred-tank bioreactors.

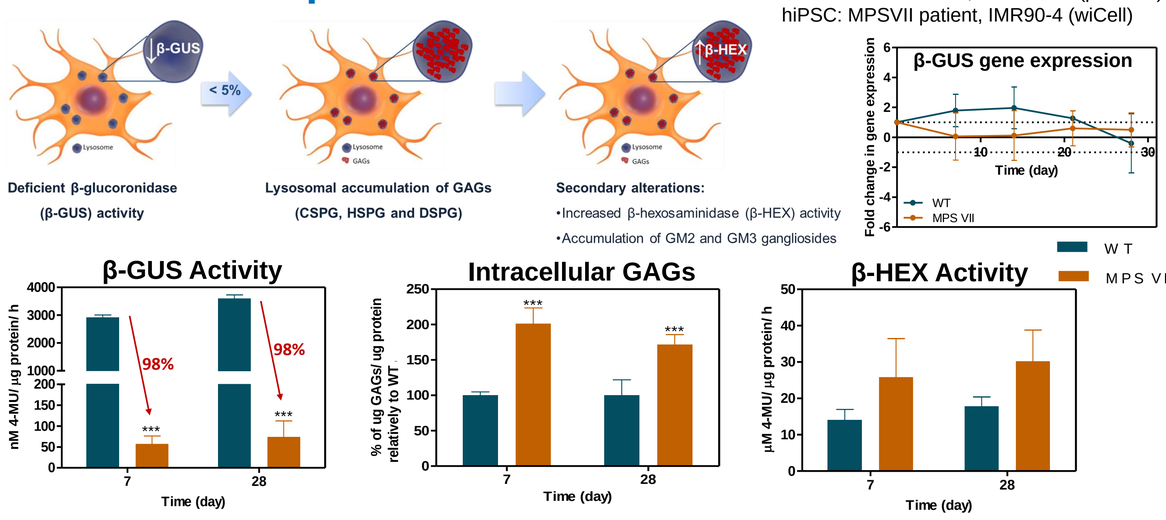


Mucopolysaccharidosis type VII (MPSVII)

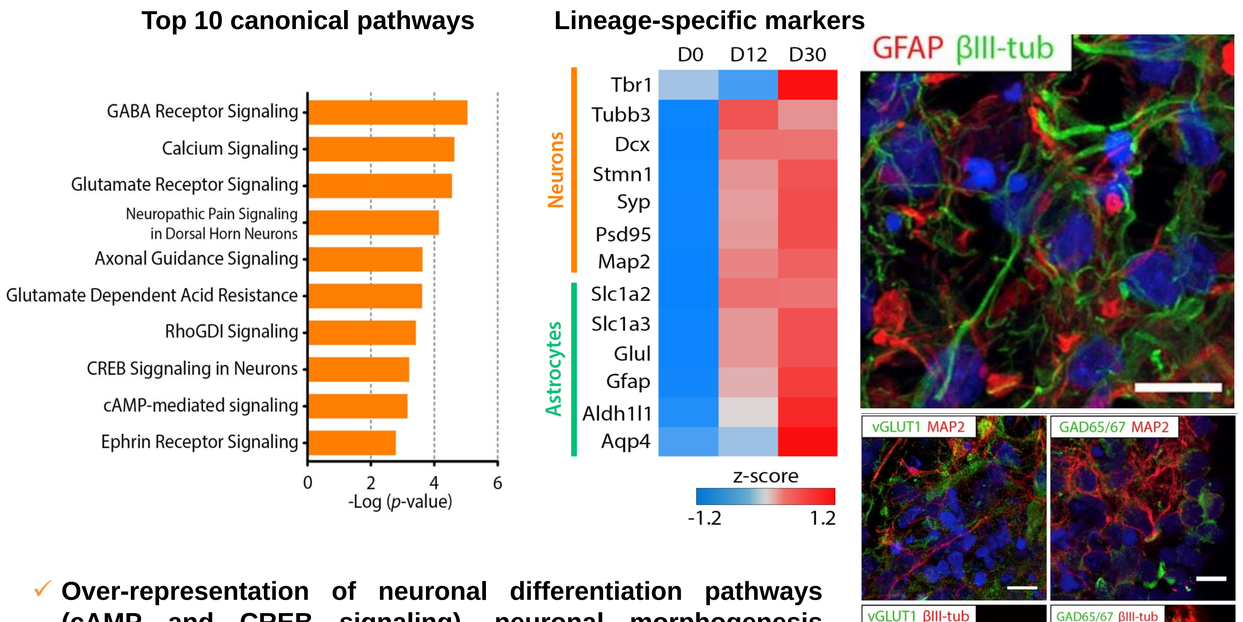
MPSVII is a rare lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, leading to glycosaminoglycan (GAG) accumulation in the brain. GAGs are important components of the extracellular matrix (ECM) in the brain, modulating cell-ECM interactions and affecting neural cell fate and functionality; however, the neuropathological hallmarks of MPSVII are poorly studied and the link between lysosomal defects and neurological dysfunction is not established.

Recapitulation of disease cellular hallmarks in MPSVII Neurospheroids

MPSVII patient mutation:
Chromosome 7.11.21, c. 526 C>T (p.L176F)
hiPSC: MPSVII patient, IMR90-4 (wiCell)



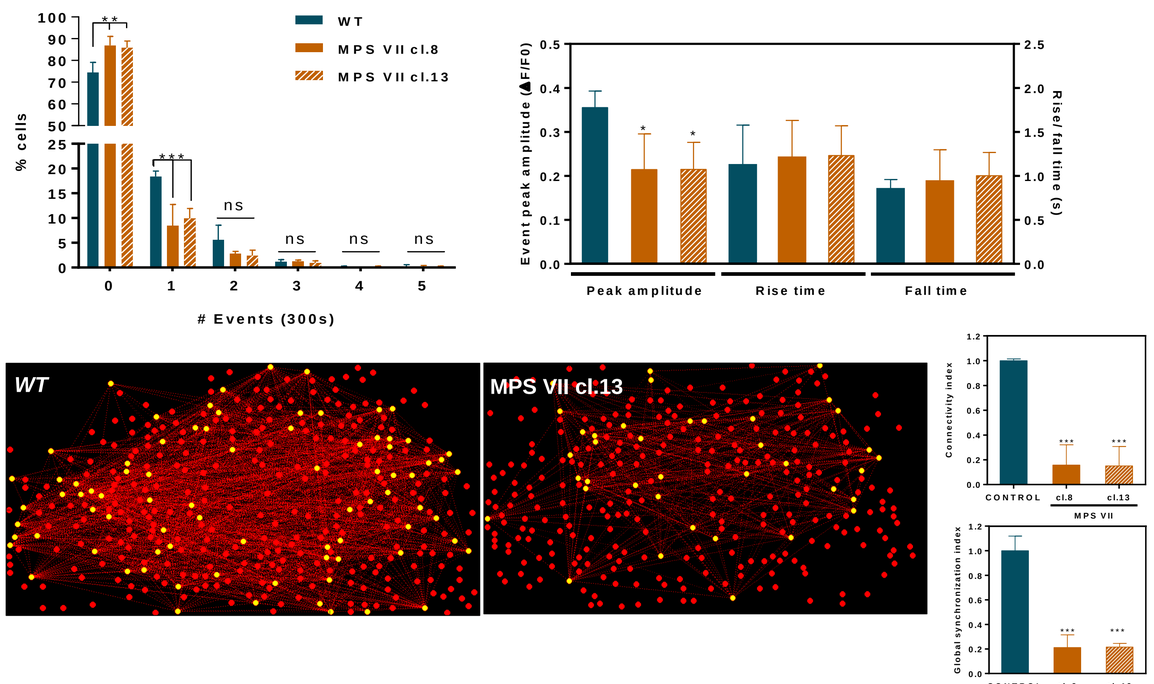
Neurospheroids are composed of neurons and glia



- ✓ **Over-representation of neuronal differentiation pathways (cAMP and CREB signaling), neuronal morphogenesis (Ephrin and RhoGDI signaling), glutamatergic and GABAergic synaptic machinery**
- ✓ **Upregulation of neuronal and astrocytic markers**

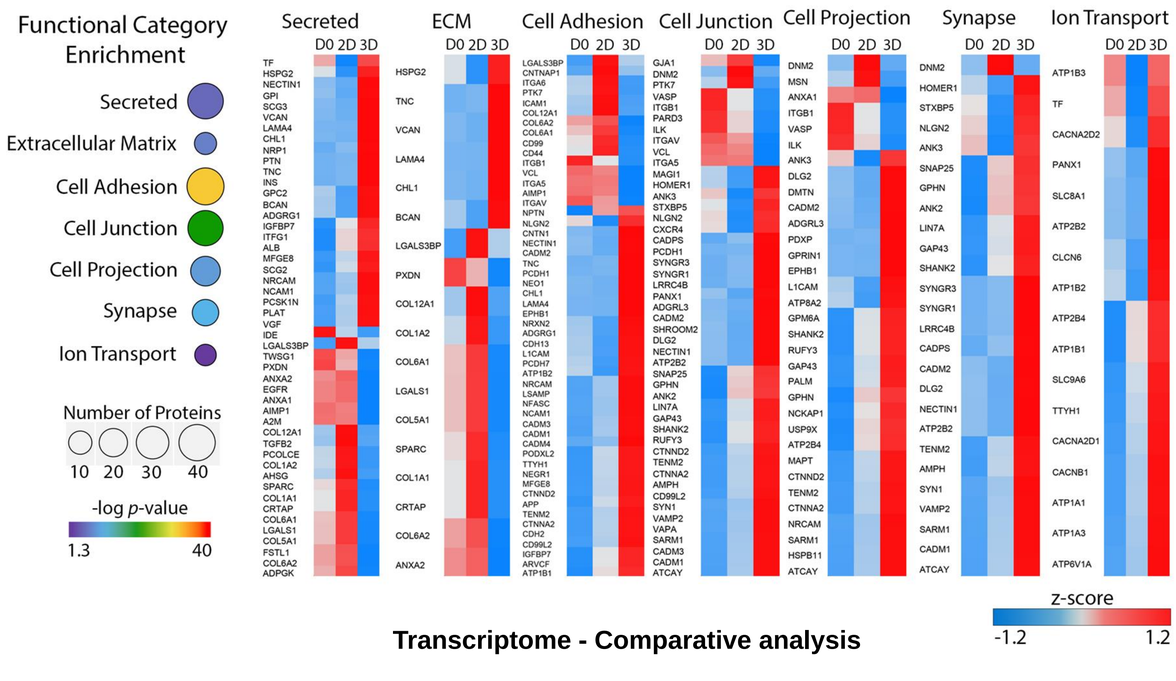
Impaired neuronal functionality in MPSVII Neurospheroids

Spontaneous neuronal activity Neuronal connectivity maps from Ca^{2+} imaging data (FluoroSNNAP software)



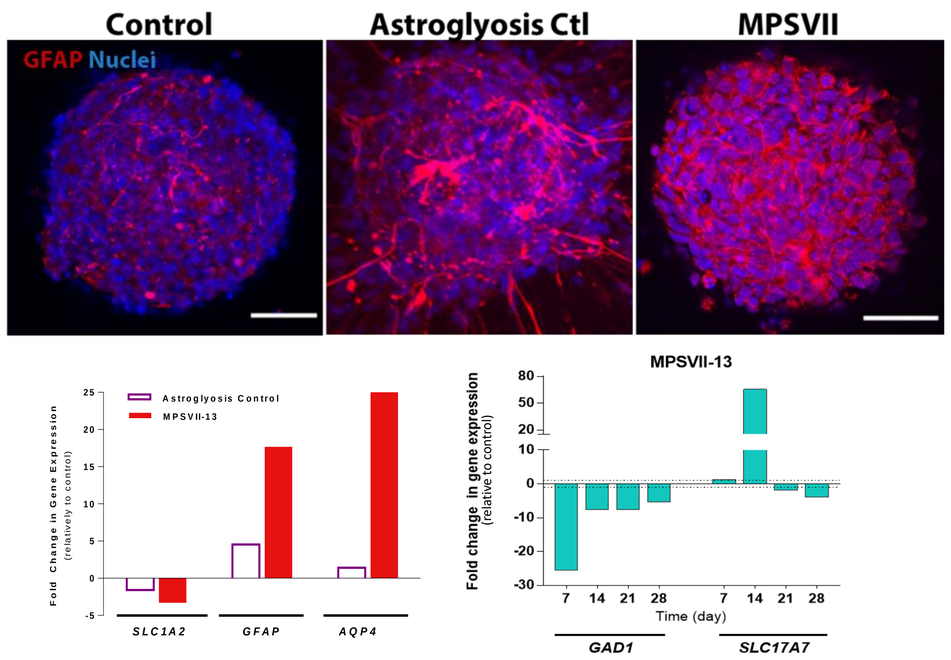
Typical Brain ECM & plasma membrane signatures

Proteome (Tripe TOF SWATH-MS)



Transcriptome - Comparative analysis

Deregulation of neuronal & astrocytic markers



***In MPSVII Neurospheroids*:**

- ✓ **Deficient β-GUS activity; intracellular accumulation of GAGs.**
- ✓ **Decreased expression of Gabaergic markers (GAD67), increased expression of glutamatergic markers (vGlut1 -*SLC17A7*).**
- ✓ **Lower neuronal activity, with impaired connectivity and synchronization of the neuronal networks**
- ✓ **Upregulation of astrocytic markers (GFAP, AQP4), associated with neuroinflammatory processes**

***In Neurospheroids*:**

- ✓ **Higher levels of neural proteoglycans**
- ✓ **Enrichment in CAMs associated with axon guidance and neuronal migration**
- ✓ **Increased expression of synaptic machinery proteins and voltage-gated ionic channels**
- ✓ **Neurospheroid transcriptional program closer to human fetal cortex in terms of ECM than other neural differentiation methods**

Conclusions

The Neurospheroid methodology is suitable for addressing the role of microenvironment perturbations in different neurological disorders, exploring the relevance of neuroinflammation processes in genetic or acute brain injuries

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Simão D *et al.* (2018) *Stem Cell Reports*, 11(2), pp. 552-564
Bayó-Puxan N *et al.* (2018) *Scientific Reports*, 8(1), pp. 1–19.
Simão, D. *et al.* (2016) *Methods in Molecular Biology* , 1502, pp. 129–142