B53. 261.03

Electrophysiological Maturation of hIPSC-Derived Dopaminergic Neurons

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Background & Aim:

→ DA neurons are critically involved in the control of voluntary movement, reward processing, and cognition

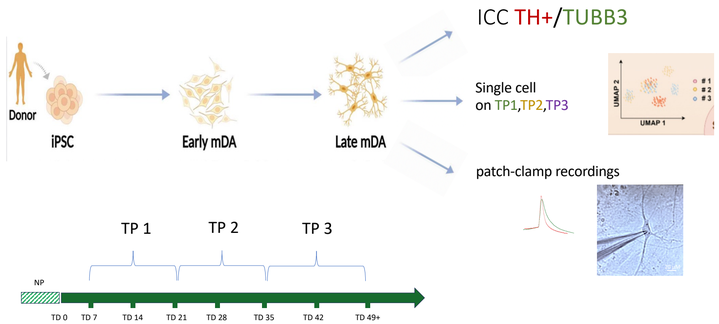
→ The degeneration of some of them in Parkinson's disease underscores the need for reliable human-relevant *in vitro* models

→ Human iPSC-derived DA neurons can bridge this gap, but a detailed understanding of their electrophysiological maturation is essential.

The aim of this study was to characterize the temporal dynamics and defining features of electrophysiological maturation in hIPSC-derived DA neurons, with a focus on pacemaking activity.

Methods:

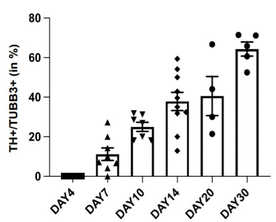
➢ GM23338 and an mCherry-tagged TH-reporter iPSC were differentiated as in *Stathakos et al., 2019*

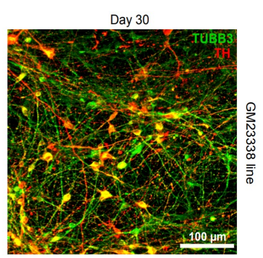


For electrophysiological recordings and transcriptomic analysis, the process of terminal differentiation was split into three time periods

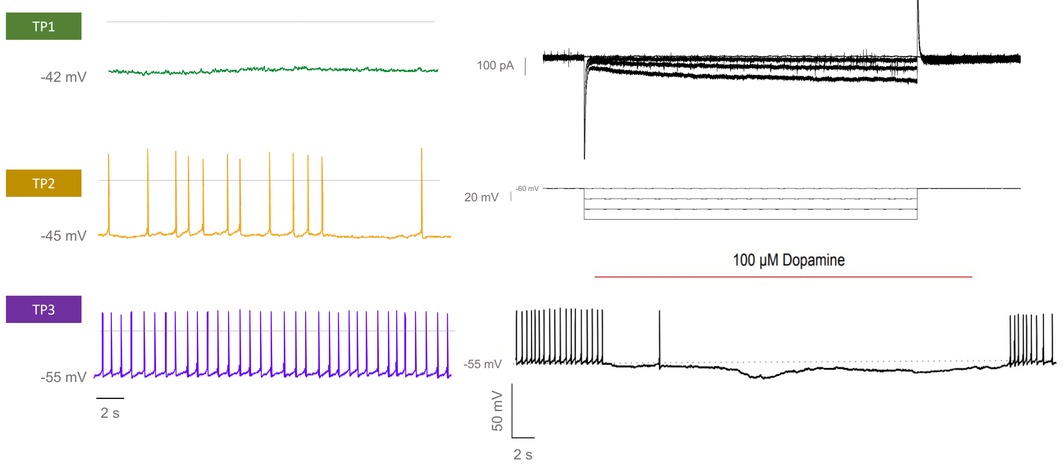
Results:

1) Progressive increase of TH+/TUBB ratio in the differentiation process

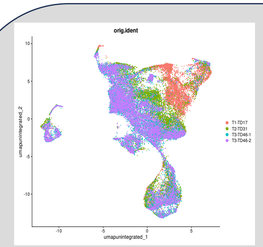




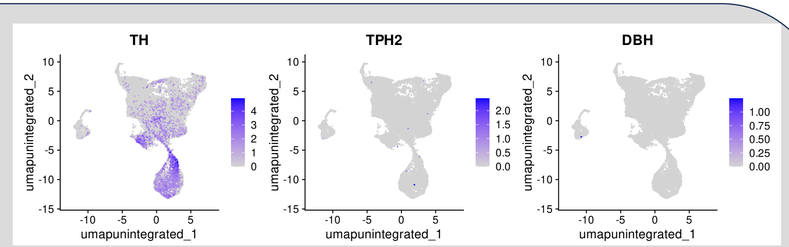
2) Electrophysiological "evolution" and mature mDA profile



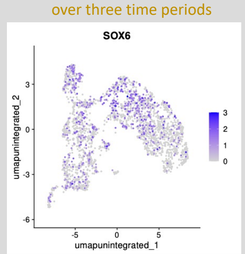
3) Single-cell RNA-seq over three time periods of differentiated mDA

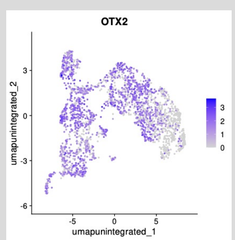


Progression in gene expression over three time periods

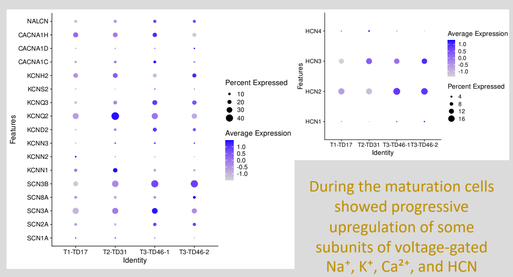


A significant number of cells express TH but not TPH2 or DBH, indicating a predominance of the dopaminergic profile among neurons



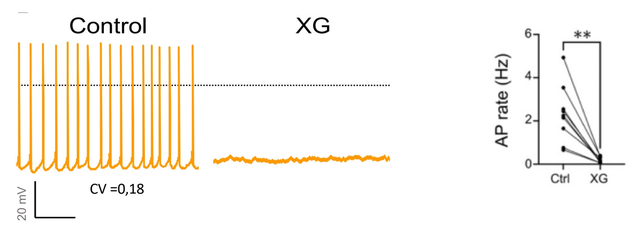


Transcriptomic clustering revealed the coexistence of both substantia nigra (SNc-like, 7,5%) and ventral tegmental area (VTA-like, 40,1%) neuronal identities within the culture.



During the maturation cells showed progressive upregulation of some subunits of voltage-gated Na^+, K^+, Ca^{2+}, and HCN channels.

4) 1-(2,4-xylyl)guanidinium (XG) (see *Jehasse et al., 2021*) specifically blocked slow pacemaking in human mDA



Slowly pacing mDA neurons are completely inhibited by 600 µM XG

Non-reactive irregular fast-firing cells

Conclusions:

By day 40, hIPSC-derived putative DA neurons exhibit hallmark features of mature electrophysiological function — I_h current, regular pacemaking, broad action potentials and dopamine-sensitive D2 autoreceptors—positioning them as a powerful in vitro model for neurophysiological and pharmacological studies. The nature of irregular fast-firing neurons remains to be determined. Their presence may be problematic in the perspective of DA neuron-based grafts in PD patients.

Aknowledgements: This work was supported by grants J.0148.19 and T.0188.22 of the Fonds National de la Recherche Scientifique (FNRS, Belgium) (VS) and by a "Strategic opportunity grant" of Liège University (OP, VS)