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HIPSC-DIFFERENTIATED DOPAMINERGIC NEURONS ARE A USEFUL TOOL FOR STUDYING THEIR NEUROPHYSIOLOGY AND MATURATION

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Dopaminergic (DA) neurons play a crucial role in motor control, motivation, and cognition, with their degeneration in Parkinson's disease leading to severe motor deficits. While rodent models are widely used, species-specific differences necessitate human-relevant models.

Aim. This study investigates the functional maturation of DA neurons derived from human induced pluripotent stem cells (IPS).

Methods. DA differentiation was performed using a mCherry-based TH reporter iPS line. Immunocytochemistry confirmed neuronal identity, while patch-clamp recordings assessed electrophysiological properties, including firing rate, action potential duration, Ih current, and dopamine sensitivity.

Results. TH expression was detected from day 10, reaching 64% by day 30. Electrophysiological maturation followed a distinct timeline, with spontaneous activity emerging around day 20 and stable pacemaking developing by day 40, along with D2 receptor-mediated autoinhibition.

Conclusions. Our findings demonstrate that hIPSC-derived DA neurons attain an adult-like profile by day 40, making them a viable model for studying Parkinson's disease mechanisms and testing potential therapies. Further research will focus on slow pacemaking mechanisms in these neurons.

Keywords: Dopaminergic neurones, human induced pluripotent stem cells, electrophysiology, pacemaking, Parkinson's disease.

Dopaminergic (DA) neurons are essential for modulating motor control, motivation, and various cognitive functions. Predominantly located in the substantia nigra pars compacta and the ventral tegmental area, these neurons (at least part of them) undergo progressive degeneration in Parkinson's disease, resulting in severe motor deficits1. While rodent models are widely used to study DA neurobiology, species-specific differences limit the direct translation of findings to humans2. Consequently, investigating the electrophysiological and functional properties of DA

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neurons derived from human induced pluripotent stem cells (IPS) represents a crucial strategy for advancing our understanding of their physiology and potential therapeutic interventions to prevent their degeneration.

Aim. This work aims to investigate the changes in the functional activity of human DA neurons in the process of its differentiation from hIPSC.

Methods. Differentiation of DA from mCherry-based human TH reporter iPS line was performed according to a published protocol 3 with minor changes. The DA profile of neurons in the process of maturation was confirmed by immunocytochemistry as described earlier 4 with the use of TH (Merck, AB152), βIII tubulin/ TUJ1 (BioLegend, MMS-435P) antibodies. For functional aspects, patch clamp recordings were performed, as described in Borgs et al.4 and Seutin, 20215. SNc DA neurons were identified and assessed based on their electrophysiological properties, including low frequency of firing (0.5–5 Hz), AP duration (width at half-amplitude > 1.35 ms), the presence of a strong hyperpolarization-activated inward current (Ih) and sensitivity to dopamine (100 µM) (hyperpolarization > 5 mV from -60 mV).

Results and Discussion. We found that cells that expressed βIII tubulin/ TUJ1 start expressing TH from day 10 of terminal differentiation (TD), and the percentage progressively increases to 64%

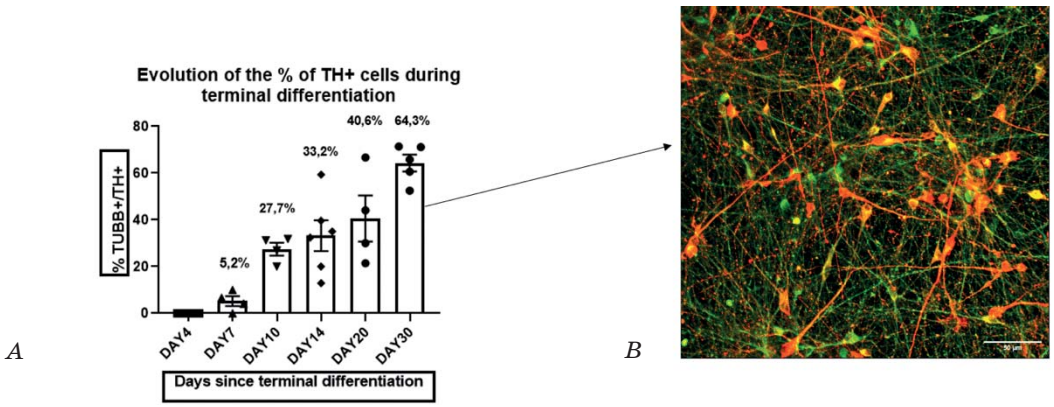

Fig. 1. **(*A*) Percentage of TUBB/TH ratio on day 4, 7, 10, 14, 20, 30 of terminal differentiation of dopaminergic neurons (*n* = 4). (*B*) — immunocytochemistry of neurons on day 30 of terminal differentiation TUBB(green)/TH(red)**

Bar = 50µm

by TD 30 (Fig. 1).

Regarding electrophysiological maturation, the ability to evoke action potentials appeared on days 10–14 of TD, as did the Ih current. After TD 20, cells demonstrated irregular spontaneous activity. At the same time, after TD 40, they had stable pacemaking activity both in the "on-cell" mode (Fig. 2) and in the "whole-cell" mode, which was reversibly blocked by 100 µM dopamine (Fig. 3), which indicates the presence of somatodendritic D2-type receptors. Activation of these receptors by dendritically released dopamine leads to a hyperpolarization mediated by GIRK-type K+ channels and is a hallmark of DA neurons. The coefficient of variation for firing was CV = 0.18; all together, this corresponds to a mature DA profile.

Conclusions. In this study, we characterized the maturation of dopaminergic neurons during their differentiation from human-induced stem cells. We obtained a relatively high percentage of

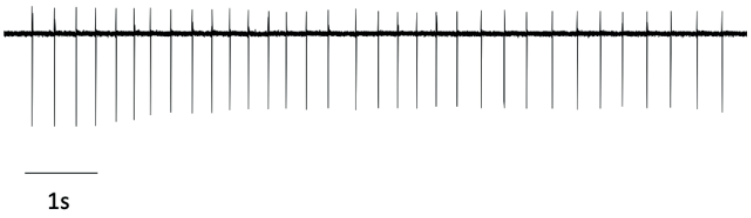

Fig. 2. **Representative cell-attached recording of a spontaneous firing dopaminergic neuron on day 49 of terminal differentiation**

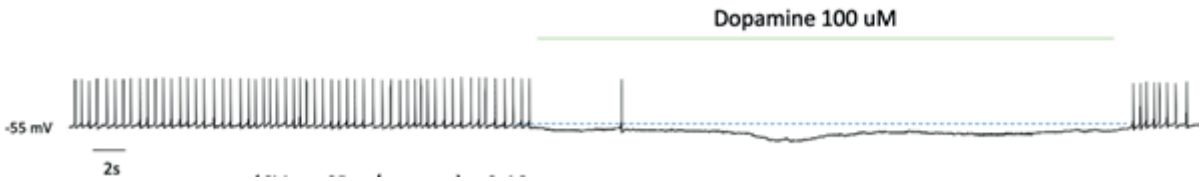

Fig. 3. **Representative recording showing that the firing of a DA neuron is reversibly inhibited by 100 µM dopamine**

TH-positive neurons. We showed that after day 40 of terminal differentiation, they have an adult profile (Ih current, stable regular pacemaking, and the presence of D2 receptors, which is confirmed by the reversible autoinhibition by 100 µM dopamine). Our results demonstrate that hIPS-induced dopaminergic neurons are a viable model for research and, in the future, may be used in a cellular model of Parkinson's disease both for understanding the mechanisms of pathology development and for finding preventive or therapeutic drugs. In addition, this model will be used to investigate further the mechanism of slow pacemaking of these cells, which is a current focus of the lab.

Authors' contribution

OP and LV worked on the cell culture; OP performed the electrophysiological recordings, LV performed ICC, and VS wrote the project and supervised the work. OP, LV, and VS analyzed the data.

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