Compounds with different pharmacological profiles enhance the neurite outgrowth in Human iPSC-derived neurons

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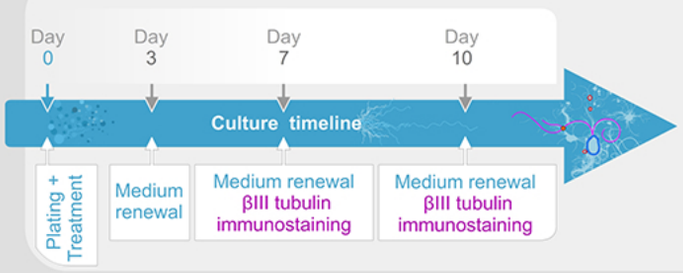
Introduction

There is a vast amount of evidence indicating that neurotrophic factors (neurotrophins) play a major role in the development, maintenance and survival of neurons.
Neurotrophic factors, which repair damaged neurons through stimulation of neurite outgrowth, may be important for the regeneration of the damaged neurons. The development of new compounds which could mimic the neurotrophin effect without their limit appears to be a good strategy for the development of new therapeutics in neurodegenerative diseases.

Objectives

- To study and measure the neurite sprouting in human iPSC-derived neurons (iCell neurons, *Cellular Dynamics International*, Madison) in culture under basal condition
- To assess the neuritogenic responses of iCell neurons to compounds with different pharmacological profiles

Experimental design

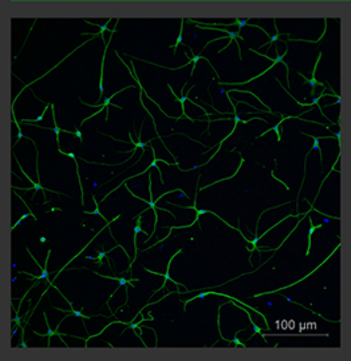


Cryopreserved iCell neurons were thawed and plated according to *Cellular Dynamics International* instructions.

Pharmacological treatments were carried out after the cell adhesion.

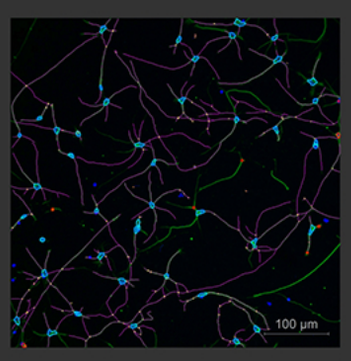
At different timepoints in cultures, iCell neurons were immunostained against βIII tubulin (neuronal marker). Neurite detection and measure were performed high content cell analyzer (Cell Insight, *ThermoFisher Scientific*).

Immuno-labelling



Immuno-labelling of iCell neurons.

The blue colour represents the nucleus (DAPI staining) and the green outlines the neuronal cytoskeleton (βIII tubulin detection).



Example of neuron scaffold detected during neuritogeneis analysis.

Neuronal cytoplasm is outlined by blue circle, neurite is outlined in purple, branch point is indicated by a orange dot and excluded object (e.g., neuron near the image border) is shown in red circle.

Time course of neurite outgrowth in iCell neurons under basal condition

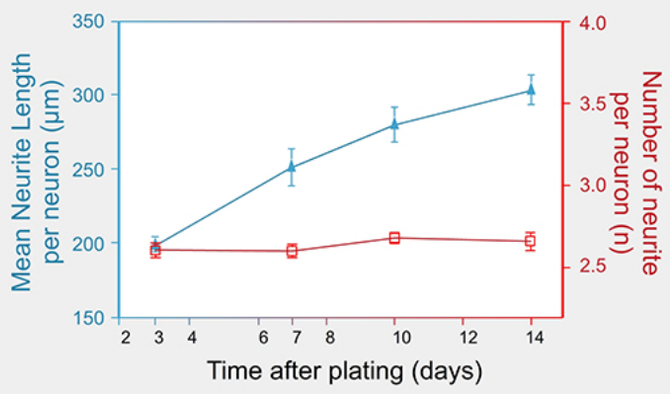


Figure showing the spontaneous neurite outgrowth in human iPSC-derived neurons at different timepoints of the culture.

The results showed a time-dependent increase in the neurite length (▲) with a plateau around 14 days of culture. In contrast, the number of neurite per neuron (□) did not evolve during the culture period.

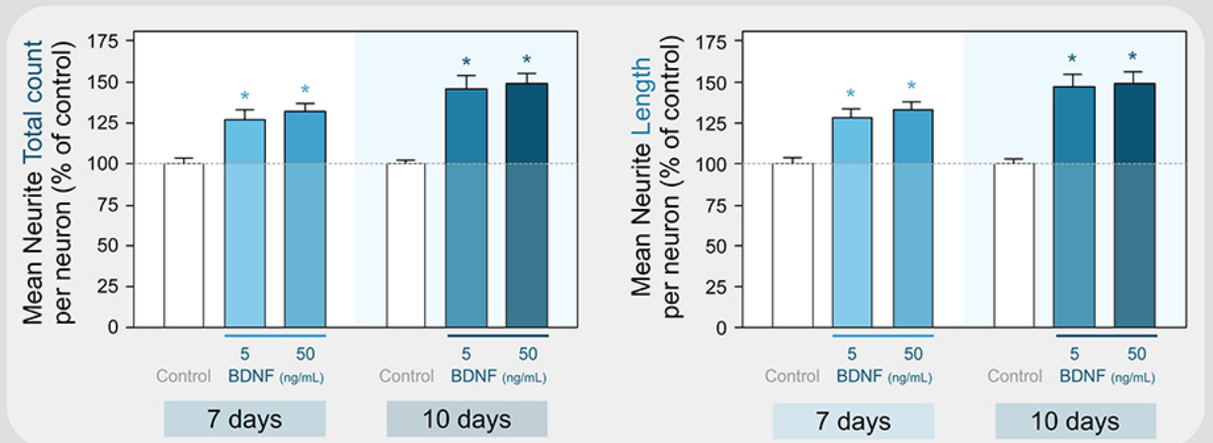
Key points

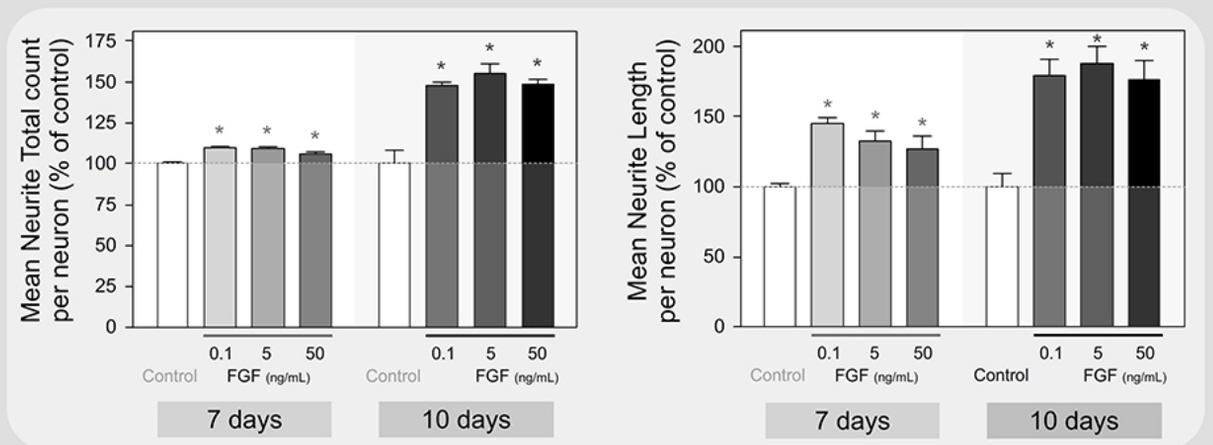
- Spontaneous and time-dependent increase of neurite length but not the number under basal culture condition
- Neurite length was further enhanced in response to pharmacological treatment with neuritogenic potential
- Large dynamic range of neurite outgrowth response at 10 days; suitable timepoint condition for screening purposes

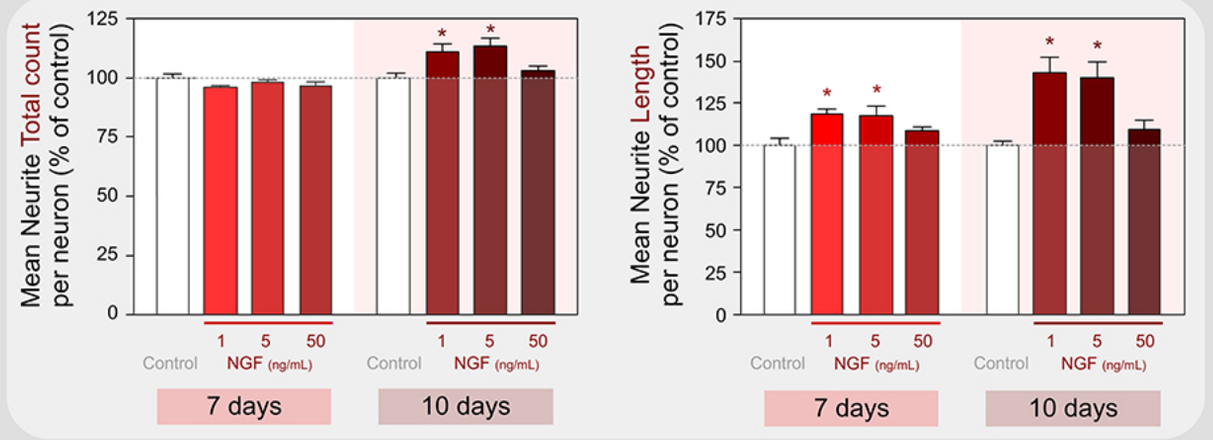
Conclusion

These results suggest that iCell neurons respond to different mechanisms of neuritogenic agents and thus can be instrumental to screen neurotrophic compounds.

Neurite outgrowth response of iCell neurons to treatment with neurotrophin

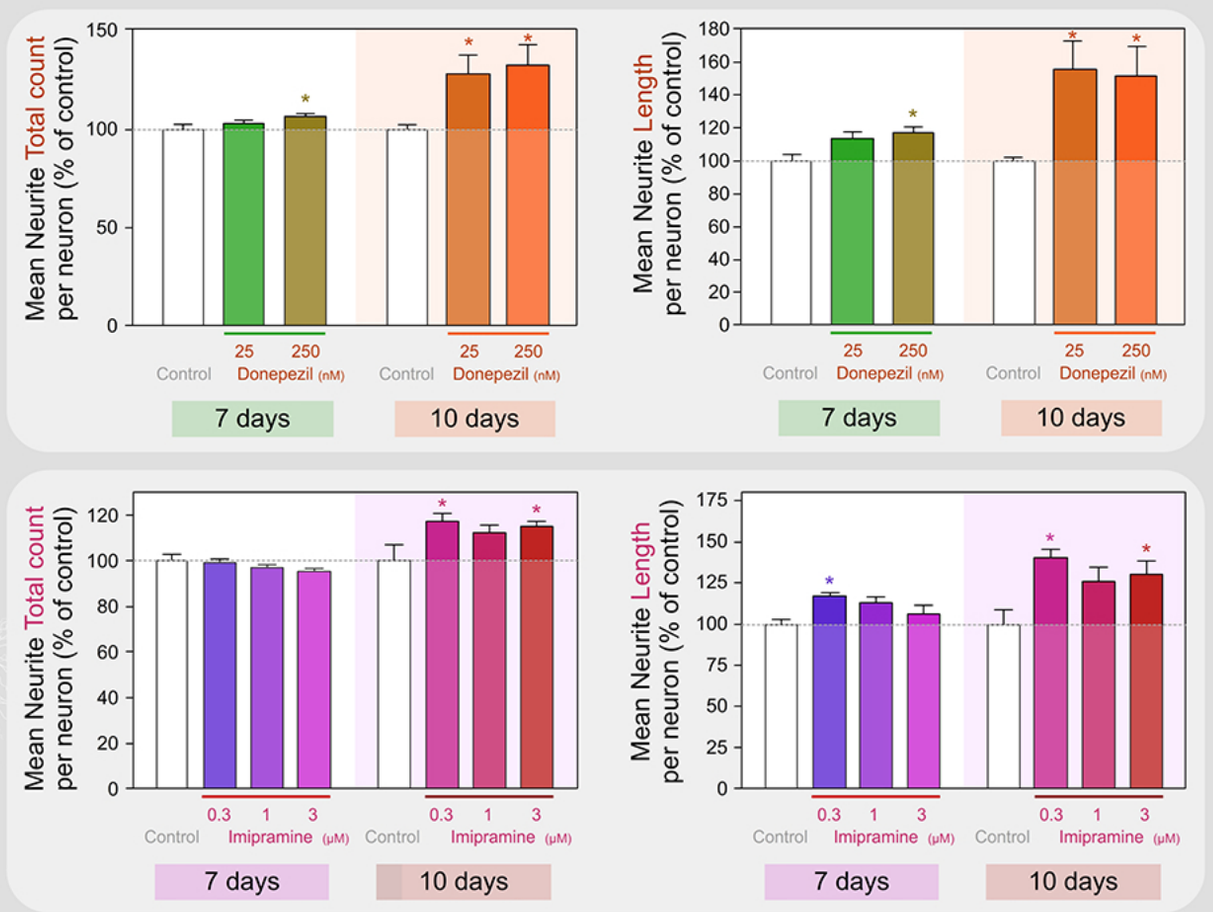






Brain-derived neurotrophic factor (**BDNF**) significantly enhanced the neurite outgrowth in iCell neurons. Similar effect was observed with other family of neurotrophins such as fibroblast growth factor (**FGF**) and nerve growth factor (**NGF**).

Neurite outgrowth response of iCell neurons to small molecules



Donepezil (Acetylcholine esterase inhibitor used in Alzheimer's disease) and **Imipramine** (tricyclic antidepressant used in major depressive disorders) significantly enhanced neurite outgrowth in iCell neurons.