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Human Pluripotent Stem Cell-Derived Neurons Are Functionally Mature In Vitro and Integrate into the Mouse Striatum Following Transplantation

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Abstract

Human pluripotent stem cells (hPSCs) are a powerful tool for modelling human development. In recent years, hPSCs have become central in cell-based therapies for neurodegenerative diseases given their potential to replace affected neurons. However, directing hPSCs into specific neuronal types is complex and requires an accurate protocol that mimics endogenous neuronal development. Here we describe step-by-step a fast feeder-free neuronal differentiation protocol to direct hPSCs to mature forebrain neurons in 37 days in vitro (DIV). The protocol is based upon a combination of specific morphogens, trophic and growth factors, ions, neurotransmitters and extracellular matrix elements. A human-induced PSC line (Ctr-Q33) and a human embryonic stem cell line (GEN-Q18) were used to reinforce the potential of the protocol. Neuronal activity was analysed by single-cell calcium imaging. At 8 DIV, we obtained a homogeneous population of hPSC-derived neuroectodermal progenitors which self-arranged in bi-dimensional neural tube-like structures. At 16 DIV, we generated hPSC-derived neural progenitor cells (NPCs) with mostly a subpallial identity along with a subpopulation of pallial NPCs. Terminal in vitro neuronal differentiation was confirmed by the expression of microtubule associated protein 2b (Map 2b) by almost 100% of hPSC-derived neurons and the expression of specific-striatal neuronal markers including GABA, CTIP2 and DARPP-32. HPSC-derived neurons showed mature and functional phenotypes as they expressed synaptic markers, voltage-gated ion channels and neurotransmitter receptors. Neurons displayed diverse spontaneous activity patterns that were classified into three major groups, namely “high”,

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