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Automatic extraction of axonal arbor morphology applied to h-iPSC-derived neurons

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Neurons derived from human induced pluripotent stem cells (h-iPSCs) offer tremendous opportunities to investigate the mechanisms involved in brain function and to model neurodegenerative diseases. Analyzing the behavior of h-iPSC-derived neurons that represent the phenotypes of human neurological disorders paves the way for the development of physiologically-relevant models and assays for drug discovery. In this framework, we utilize a CMOS-based high-density microelectrode array (HD-MEA, MaxWell Biosystems) to investigate h-iPSC neurons at sub-cellular resolution. Recording extracellular action potentials (EAPs or spikes) of cultured neurons through microelectrode arrays (MEAs) is a well-established technique for extracting valuable features of neuronal function and network connectivity (Obien et al., Frontiers in Neuroscience, 2015). In this work, we obtain electrical images of h-iPSC neurons in 2-dimensional cultures using HD-MEAs, which allows the extraction of the position of axonal arbors on the array. We plated h-iPSC-derived dopaminergic neurons (iCell Dopaneurons, Cellular Dynamics International) on HD-MEA dishes (100-200k cells per dish), both as monocultures and in co-culture with h-iPSC-derived astrocytes (iCell Astrocytes, Cellular Dynamics International). Spontaneous spiking activity was recorded under normal culturing conditions (cells in growth media inside an incubator at 37°C, 5% CO_2). Axonal action potential propagation was electrically visualized by spike-triggered averaging of pre-selected electrodes (Bakkum et al., Nat. Comm., 2013). Electrodes were ranked according to the probability of being in close contact with active neurons. This probability can be computed using the amplitude and firing rate of detected spikes in each electrode. We observed that in average, h-iPSC neuron spike amplitudes are lower compared to primary neurons. Moreover, the spontaneous activity in high-density cultures (>100k cells) presents a caveat of introducing biological interference in the recorded signals. Thus, a new post-processing technique that automatically isolates noise-free single-neuron extracellular action potential (EAP) maps is necessary for reliable extraction of h-iPSC axonal arbor morphologies. We employed a clustering-based algorithm to identify neuronal signals from noise. We compared the extracted neuronal EAP maps with fluorescent images of the corresponding neurons. As a result, we show how the presence of astrocytes facilitates the development of h-iPSC-derived neurons at the functional level, as revealed by the strength of the neuronal signals and extension of axonal arbors. Applications of this method include the characterization of axons in healthy and disease models, as well as the detection of drug effects in pharmacology and toxicity screening.

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