Cells in each culture condition were plated in duplicate wells of a 12-well plate (Axion Biosystems; M768-GL1-30Pt200), with 64 recordings taken twice a week for hPSC culture and once a week for primary neuronal cultures. Only the last 10 minutes of each recording was system (Maestro, Axion Biosystems) at a sampling rate of 12.5 kHz/channel. For all recordings, a Butterworth band-pass filter

# Methods

## (A) Culture of hPSC-derived Neurons

Neural progenitor cells derived from hPSCs (XCL1-NPC) were cultured in STEMdiff™ Neuron Differentiation Medium on poly-L-ornithine-coated MEA plate for 5 days. On day 5, neural progenitor cells were dissociated and single cells were replated onto a PLO-dextran-coated MEA plate in a 5:1 condition. One day after, half of the medium was replaced with differentiation medium (BrainPhys™ Neuron Differentiation Medium). After one day, half of the medium was replaced with maintenance Medium (BrainPhys™ Neurobasal® Medium + NeuroCult™ SM1 + L-glutamine). Half-medium changes were performed every 3 - 4 days throughout the culture period.

## (B) Culture of Primary E18 Rat Cortical Neurons

E18 rat cortical cells were dissociated into single cells and plated onto PLO/dextran-coated MEA plate at 200,000 cells/plate in Neurobasal® Medium with 5% NeuroCult™ SM1 Neurobasal® Supplement, L-glutamine and B27 (0.5x). Half-medium changes were performed every 3 - 4 days throughout the culture period.

## (C) Multi-Electrode Array (MEA) Recording and Analysis

Cells in each culture condition were placed in duplicate wells of a 96-well plate (Axion Biosystems: MTP-96L-30PM100), with 64 recordings taken twice a week for hPSC culture and once a week for primary neuronal cultures. Only the last 10 minutes of each recording was used for analyses using the A4W (3.3) analysis software.