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## Supplemental Information

# A functional hiPSC-cortical neuron differentiation and maturation model and its application to neurological disorders

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**Supplemental Information**

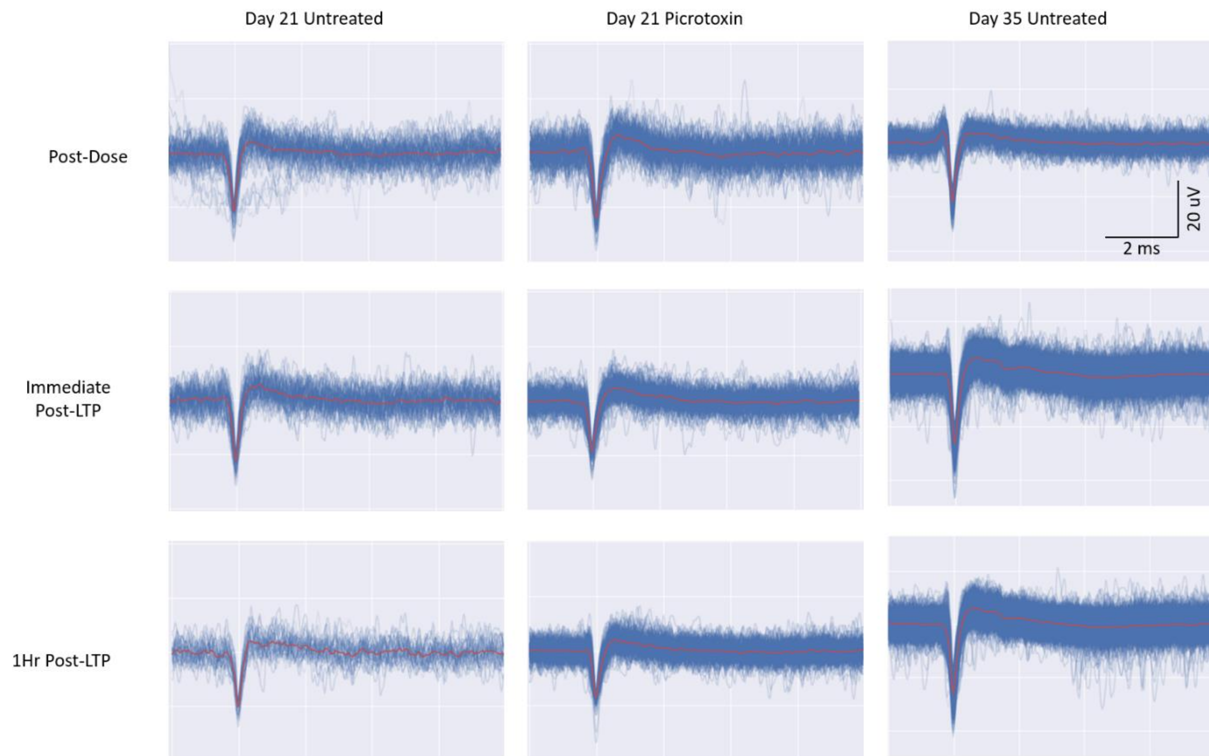


**Figure S1: Waveforms illustrating the effects of picrotoxin on LTP maintenance in immature cortical neurons compared to mature cortical neurons, related to Figure 6 (in main text).** Representative waveforms on day 21 untreated (left), day 21 treated with 100uM picrotoxin, and day 35 untreated (right) illustrating neuronal activity following addition of picrotoxin or vehicle control (top), immediately following LTP induction vis HFS (middle) , and 1 hour following LTP induction (bottom).

**Supplemental Experimental Procedures**

**HiPSC Culture Details:** HiPSCs were maintained by daily full medium exchange mTESR1 culture medium (Stemcell Technologies 85850). Corning matrigel matrix (Corning Life Sciences 354230) was diluted in DMEM F12 (Fisher Scientific 21041-025). Differentiation began at 90% confluency.

**Differentiation Media Formulations:** Differentiation Medium I consisted of N2B medium (equivalent to DMEM F12 with insulin (Millipore Sigma 407709) 20 µg/ml and 1X N2 supplement (Fisher Scientific 17502048)) supplemented with 6 µM SB431542 (Tocris 1614), 0.1 µM LDN193189 (Fisher Scientific 605310), 2 µM DMH-1 (TOCRIS 4126), and 10 ng/mL recombinant human DKK-1 protein (Fisher Scientific 5439-DK-010). Differentiation Medium II consisted of N2B medium supplemented with 6 µM SB431542, 0.1 µM LDN193189, 2 µM DMH-1, and 5 µM cyclopamine (Sigma 239803-1MG). Lastly, Differentiation Medium III (also referred to as "Maintenance Medium") consisted of Neurobasal (Fisher Scientific 21103049), supplemented with 1X Glutamax (ThermoFisher 35050-061), 1X N-2 supplement (Fisher Scientific 17502048), 1X B27 without Vitamin A (Fisher Scientific 12587-010), 20 ng/mL BDNF (Cell Sciences CRB600D), 20 ng/mL GDNF (Cell Sciences CRG400E), 200 nM Ascorbic Acid (Sigma Aldrich A4403), 1 µM cAMP (Sigma A6885), 1 µg/mL Laminin (Fisher Scientific 23017015), and 1X Antimycotic-Antibiotic (Fisher Scientific 15240062).

**Culture Surface Coating for Cortical Neurons: DETA Surface Coated with Poly-L-Ornithine/Laminin Protein Adsorption Surface Coating:** The surface for coverslips was first coated with self-assembled monolayer (SAM) DETA as described in (Gonzalez et al., 2019). Further surface modification on DETA coated surfaces was achieved by immersing the coverslips in 0.01% Poly-L-Ornithine (PLO) (Sigma Aldrich 27378-49-0) at room temperature for 2 hours. They were then rinsed 3 times with 1X PBS (Fisher Scientific 70013-032), followed by the addition of 3.33 ug/mL laminin diluted in 1X PBS. Surfaces were left at 4°C overnight, and subsequently warmed at 37°C for 1 hour prior to the removal of laminin for cell seeding. Patterned surfaces on MEAs were generated by the photolithography combining the cell permissive SAM DETA and the nonpermissive SAM PEG (Edwards et al., 2013). The patterned surface was further coated with PLO/Laminin as described above.

**Cortical Neuron Plating and Maintenance:** Following differentiation, cortical neurons were harvested using 0.05% Trypsin-EDTA (ThermoFisher 15400054) and neutralized by Trypsin inhibitor (Fisher Scientific 17-075-029). Cortical neuron stocks were cryopreserved in 10% DMSO (Sigma Aldrich D4540). Individual vials were thawed using a drop-by-drop method with cold 1X PBS. Cells were then centrifuged at 260g for 3 minutes, and seeded on coverslips at a plating density of 150 cells/mm$^2$ and on MEAs at 500 cells/mm$^2$. Cells were maintained for one week in Maintenance Media. After one week, cells were further maintained in BrainPhys Neuronal Media Kit with SM1 and N2 Supplement A (Stemcell Technologies 05793) that was further supplemented with 20 ng/mL BDNF, 20 ng/mL GDNF, 200 nM Ascorbic Acid, 1 µM cAMP, 0.5 µg/mL Laminin, and 1X Antimycotic-Antibiotic. Cell maintenance consisted of a half-medium change twice weekly from the time of seeding for the duration of the culture.

**Immunocytochemistry:** Cells were fixed in 4% paraformaldehyde for 15 minutes followed by three washes with 1X PBS. Cells were permeabilized in 0.1% Triton for 10 min and subsequently blocked 1 hour in a blocking buffer solution containing 0.5% BSA and 5% goat serum diluted in 1X PBS, then incubated with primary antibodies diluted in blocking solution at 4°C overnight. Primary antibodies used for characterization included vGLUT1 (Sigma Aldrich AB5905), GABA (Sigma Aldrich A2052), Beta-III Tubulin (Millipore AB9354), GFAP (Sigma Aldrich AB5804), Synaptophysin (abcam ab8049), PICK1 (Santa Cruz 9541), NMDAR1 (ThermoFisher MA1-2014), and GLUR1 (Millipore AB1506). Following rinsing, coverslips were then incubated with secondary antibodies diluted at 1:250 in blocking solution at room temperature for two hours. Coverslips were then washed and mounted using a Gold Antifade Reagent with Dapi (Fisher Scientific P36931) and imaged on a spinning disk confocal microscope.

**Flow Cytometry:** Cells were lifted using accutase (ThermoFisher 00-4555-56) and resuspended following centrifugation in FACS buffer containing 1% BSA diluted in PBS. Further methodology for this method is described in Patel et al (Patel et al., 2020). Cytometry analysis was performed for the following antibodies: Live-Dead (using Draq7), ctip2, Beta-III Tubulin, and GFAP. Expression levels were

determined by gating based on fluorophore wavelengths for cells of interest (live cells excluding dead cells and debris).

**Whole-Cell Patch Clamp Electrophysiology:** Measurement of spontaneous firing, repetitive firing, and ionic currents were achieved through whole-cell patch clamp. Each experiment was repeated 3 times per timepoint, and 3 cells were patched per experiment for a total of 9 cells per data point. Cells were recorded in a recording chamber filled with Brainphys medium (buffered with 10 mM Hepes with ~300 mOsm/ kg), maintained at room temperature (22-25$^{o}$C). Patch clamp methodology was performed as described in Caneus et al (Caneus et al., 2020).

**Compound Preparations:** All compounds except lidocaine were prepared in 0.1% DMSO diluted in BrainPhys Neuronal Medium at a working concentration of 10X final desired concentration. Lidocaine was dissolved in sterile water. Compounds used for chemical modulation of neuronal activity included glutamate (Aldrich 855642), lidocaine HCl (Sigma L5647), NBQX (Tocris 0373), picrotoxin (Tocris 1128), bicuculine (Sigma 14340), and valproic acid (Sigma P4543-25G).

**LTP Induction Protocol:** LTP was induced via a high frequency stimulation protocol that consisted of 10 repetitions of 4X biphasic 500mV, 5ms pulses (Abraham and Huggett, 1997).

**MEA Data Analysis:** Analysis of waveform shape and firing rate were done by filtering raw data with a 100 Hz high-pass filter and superimposing all spikes surpassing -5 standard deviations from the mean of the noise (Obien et al., 2015). Raw electrode data recorded from customized MEA chips was filtered using a Butterworth 2$^{nd}$ order 100Hz high-pass filter. Following filtering, electrodes with a firing frequency lower than 0.1Hz were removed from analysis. Spikes occurring at the same microsecond on more than two electrodes were deemed as artifacts and removed. It has been observed that transient noise effects tend to co-occur simultaneously across multiple electrodes, whereas coupled biological events (such as co-firing neurons) co-occur at timestamps that are very close, but which are not identical to each other. Removing events across multiple electrodes with the same timestamp has not been observed to impede the detection of biological activity, as demonstrated by identifying the activity that is removed when neural signaling is abolished by administration of a compound such as lidocaine.