Properties of Human Stem Cell-Derived Neurons in 2D and 3D in Long-term Cell Culture

Hamed Salmanzadeh and Robert F. Halliwell
Thomas J. Long School of Pharmacy, University of the Pacific, Stockton, CA

Laboratory of NEUROPHARMACOLOGY

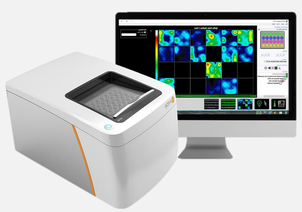
Introduction

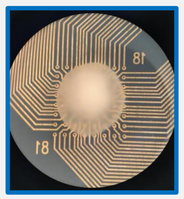
Studies in our lab have addressed the functional properties of receptors and ion channels expressed in neurons derived from human stem cells in 2D culture (e.g., Halliwell *et al.,* 2021).
We are now investigating the molecular, anatomical and electrophysiological properties of stem cell-derived neurons and glial cells in 3D neurospheres (neural organoids). These neural organoids represent a more complex model of early brain development for use in drug discovery and safety testing.

Methods

Cell culture. The hPSC line TERA2.cl.SP12 was cultured under differentiating conditions towards a neuronal phenotype using retinoic acid (10µM). Neural organoids were developed using a STEMdiff™ Cerebral Organoid Kit (Stemcell Technologies).
Immunocytochemistry. Stem cell-derived neurons were immunolabeled with the neural markers βIII-tubulin, MAP2 and the glial cell marker, GFAP.
Multi-Electrode Array Electrophysiology. TERA2.cl.SP12 stem cells were cultured in 6-well MEA plates and allowed to differentiate. Activity of differentiated cells was recorded weekly for 1 year using the Maestro Edge MEA system (Axion Biosystems). In 3D culture, neural organoids were transferred from regular 6-well plates to 6-well MEA plates after 40 days of differentiation.

Neurosphere

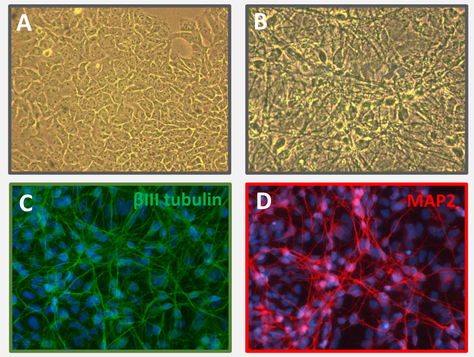




A human stem cell derived neurosphere differentiated for 40 days in vitro on a 64 electrode MEA well.

Conclusion

2D cultures of hPSC-derived neurons are a new tool in drug discovery. 3D neural organoids may represent an even more powerful model in drug development and disease modeling. Further work must be conducted to fully characterize these complex organoids.



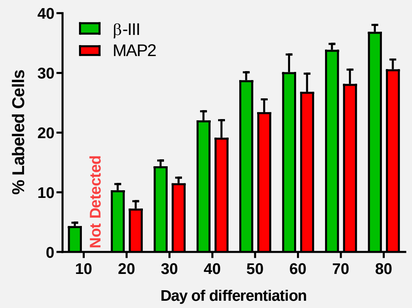


Figure 1. Retinoic acid (RA) promoted neuronal differentiation of TERA2.cl.SP-12. (A) and (B) are phase contrast micrographs showing cells at day 0 (A) and day 80 (B) of neural differentiation. Immunofluorescence images show fixed stem cell-derived neurons labeled with (C) β-III tubulin (green) and (D) MAP2 (red) at 80 days differentiation.

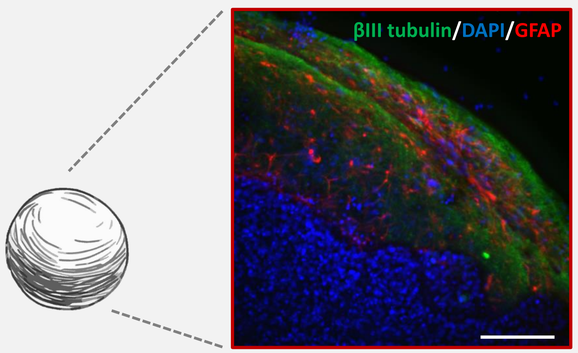


Figure 2. Immunofluorescence image shows fixed neural organoids stained with βIII tubulin (green), GFAP (red) and DAPI (blue) at 130 days differentiation.

Results

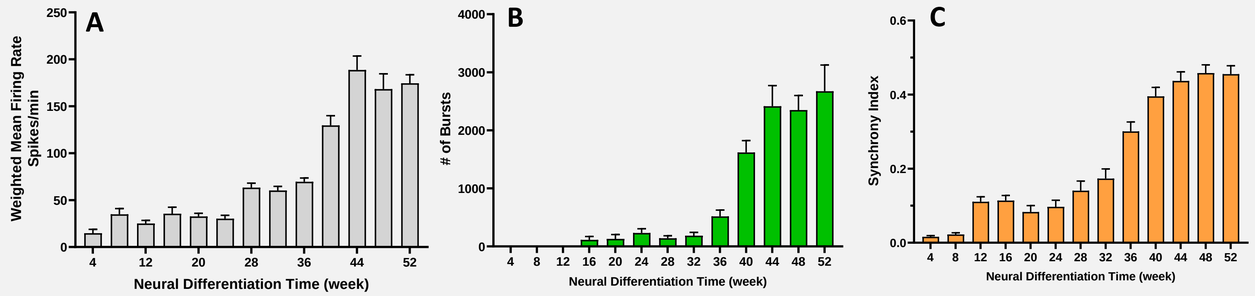


Figure 3. Development of spontaneous firing of hPSC-derived neurons over one year of differentiation. (A) Time course of the average firing rate from 4 to 52 weeks of differentiation, (B) development of spontaneous burst firings and (C) synchrony firing during long-term culture.

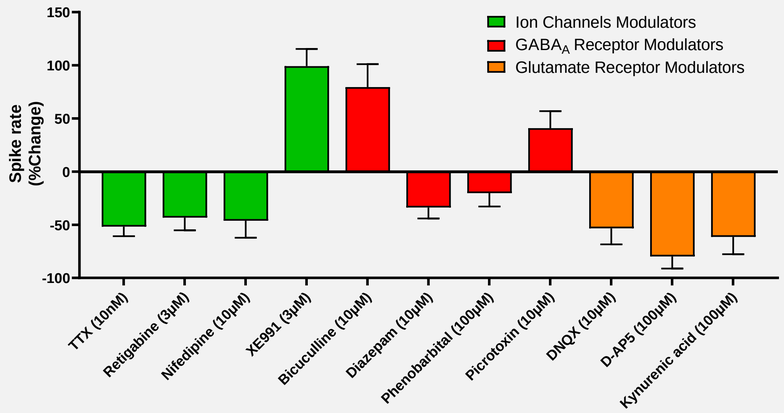


Figure 4. Pharmacological properties of spontaneous firing activity. Administration of ion channel and receptor modulators indicated the presence of voltage-gate sodium and potassium ion channels and neurotransmission in these neural networks, mediated via glutamate and GABAA receptors.

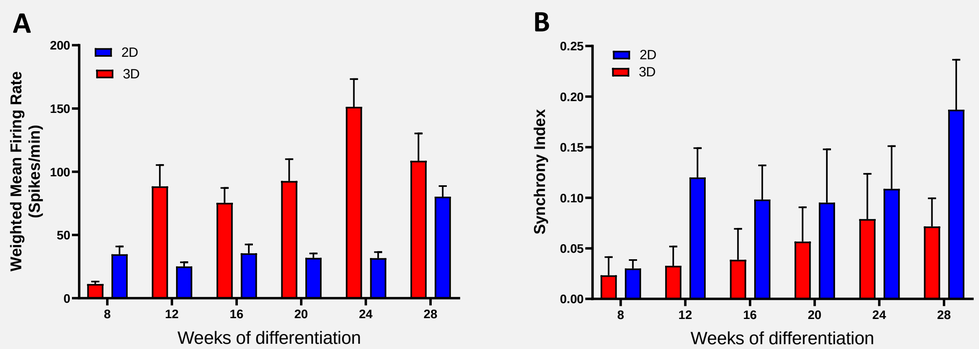


Figure 5. Figure 3. Development of spontaneous firing of hPSC-derived neurons in 3D vs 2D culture. (A) determines the difference in the development of the average firing rate and (B) shows the difference of spontaneous synchronized firings in 2D vs 3D culture over 4 months.