Development of a Functional Assay using Human Brain Organoids to Study Developmental Neurotoxicity

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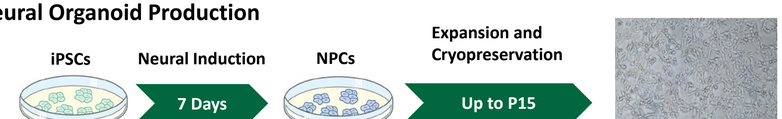
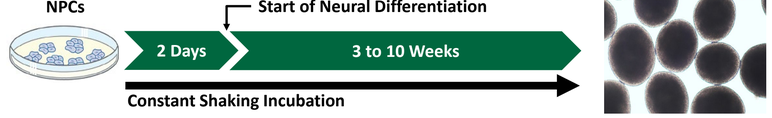
Background and Purpose

- New Approach Methods (NAMs) have been developed to characterize the impacts of exposure to environmental and pharmacological compounds during neurodevelopment that can cause adverse effects such as morphological alterations and/or functional changes in the developing brain.
- Neural organoids are three-dimensional (3D) cell culture systems that mimic the complex structure and development of the human brain and produce recordable spontaneous electrical activity.
- We developed a functional assay to characterize the electrophysiological development of the organoids as well as the response of that activity to an acute exposure of three common pharmacological compounds: picrotoxin (PTX), tetrodotoxin (TTX), and nicotine (NIC).

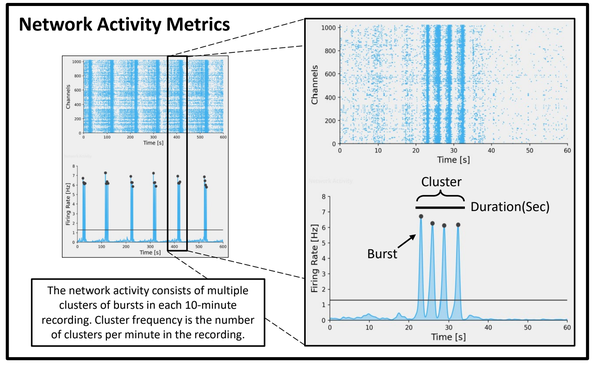
Methods

Neural Organoid Production



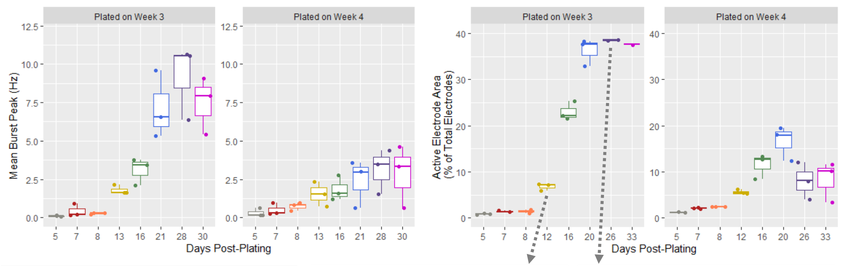


High-Density Microelectrode Array (hdMEA) Functional Assay

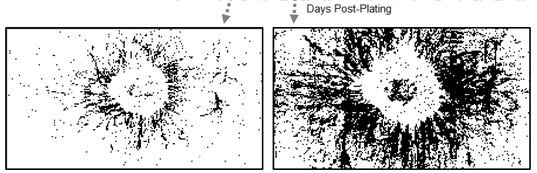


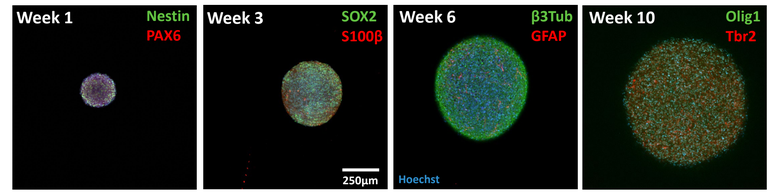
1. Activity Scan
2. Baseline Network Recording
3. Treatment (Mixed in media)
4. 10-minute Recordings every 15 minutes

Time-course analysis of the organoids follows neurodevelopmental ontogeny and shows formation of a complex network.



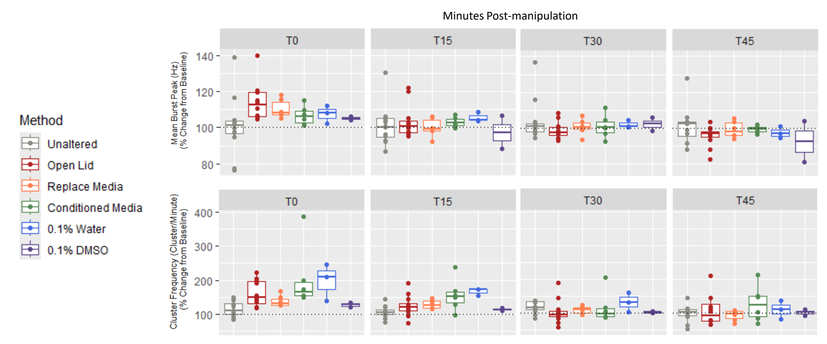
An Active Electrode is an electrode that has a firing rate larger than 0.1 Hz and a spike amplitude greater than 20 µV. Active Electrode Area is the percent of active electrodes of all 26,400 electrodes in each well.



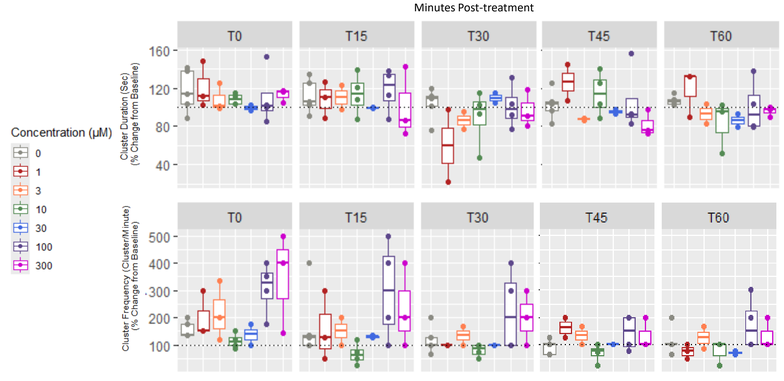


Immunocytochemistry and high-content imaging of the organoids showed a decrease in proliferation and neural progenitor markers (Nestin, PAX6, SOX2) and an increase in neuronal (β3Tub, Tbr2), astrocyte (S100β, GFAP), and oligodendrocyte (Olig1) markers during differentiation (weeks 0-10), indicating that the organoids progress along a neurodevelopmental ontogeny.

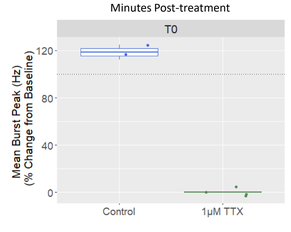
Physical manipulations cause minor changes in network activity, primarily at early timepoints.



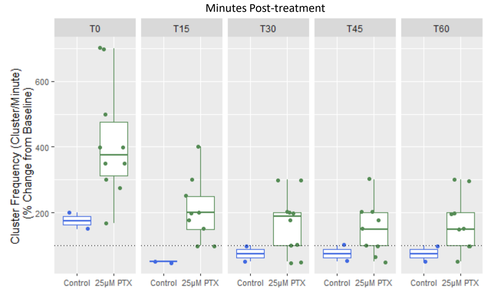
High concentrations of NIC appear to increase cluster frequency.



TTX immediately and completely inhibits network activity.



PTX increases network activity.



Conclusion & Future Work

- The neural organoids demonstrate complex network formation and maturation during development, as indicated by changes in the expression of developmental markers and complex spontaneous network activity.
- The organoids exhibit expected responses from well-characterized pharmacological agents: PTX (blocks $GABA_A$ receptors) and TTX (inhibits voltage-gated sodium channels).
- Further experimental replicates are needed to verify the activation of nicotinic acetylcholine receptor (NIC) response.
- Future studies will finalize an exposure and data analysis protocol and assess effects of environmentally relevant compounds, including neonicotinoid insecticides and per- and polyfluoroalkyl substances (PFAS).

Pamies D, Barreras P, Block K, et al. A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity ALTEX 2017; 34(3): 362-376 doi: 10.14573/altex.1609122