COMPARISON OF A HIGH-DENSITY MICROELECTRODE ARRAY (MEA) ASSAY FOR NEUROTOXICITY SCREENING USING A 3D HUMAN IPSC-DERIVED BRAIN ORGANOID MODEL VERSUS A 2D RAT CORTICAL CELL MODEL

Kelly Carstens, Elena Gronskaya, David Jaeckel, Timothy Shafer, Marie-Gabrielle Zurich, David Pamies

ABSTRACT

The relationship between environmental chemical exposure and a spectrum of neurological diseases is well-established. However, unraveling the underlying mechanism between human exposure and neurotoxicity presents significant challenges. Traditional in vivo neurotoxicity testing is resource intensive and faces uncertainties regarding biological relevance to human health outcomes; therefore, there is a need to develop efficient, human-relevant in vitro new approach methodologies (NAMs). Recording of neural network activity using microelectrode array (MEA) technology has been identified as a reliable and reproducible method to evaluate neurotoxicity. BrainSpheres is a promising functional human induced pluripotent stem cell (iPSC)-derived 3D brain model comprising neurons, astrocytes, and oligodendrocytes which provides a human-relevant alternative to the standard 2D rat primary cortical cell model commonly used with MEA. In this study, we demonstrate reproducible spontaneous neural firing and network bursting parameters from 8-week-old BrainSpheres using high-density MEA technology. The application of the BrainSpheres model as a human-relevant NAM was evaluated by conducting a multi-concentration, 12-day exposure study of a set of ten chemicals, including two assay positive controls (Loperamide, Domoic Acid), one assay negative control (Acetaminophen), and seven evaluation chemicals. Concentration-response modeling was performed on features of neuronal activity such as mean firing rate and network connectivity, and results were compared to screening data from a MEA model using a 2D-rat model. Similar to the 2D-rat model, Loperamide and Domoic Acid demonstrated bioactivity at non-cytotoxic concentrations in the BrainSpheres model, while Acetaminophen was inactive. For chemicals that demonstrated bioactivity in both models, minimum potencies estimated by each model fell within 0.5 log10-µM for three chemicals while four chemicals were more potent in the rat model by more than 0.5 log10-µM, suggesting that the 2D-rat model may be more sensitive for detecting changes in neural activity than the BrainSpheres. Only Loperamide demonstrated cytotoxicity in the BrainSpheres, while four chemicals, including Loperamide, were cytotoxic in the 2D-rat model. These results indicate that differences in experimental design and biological relevance between models should be considered in the fit-for-purpose implementation of NAMs for human neurotoxicity assessment.

(This abstract does not reflect US Environmental Protection Agency policy).

METHOD

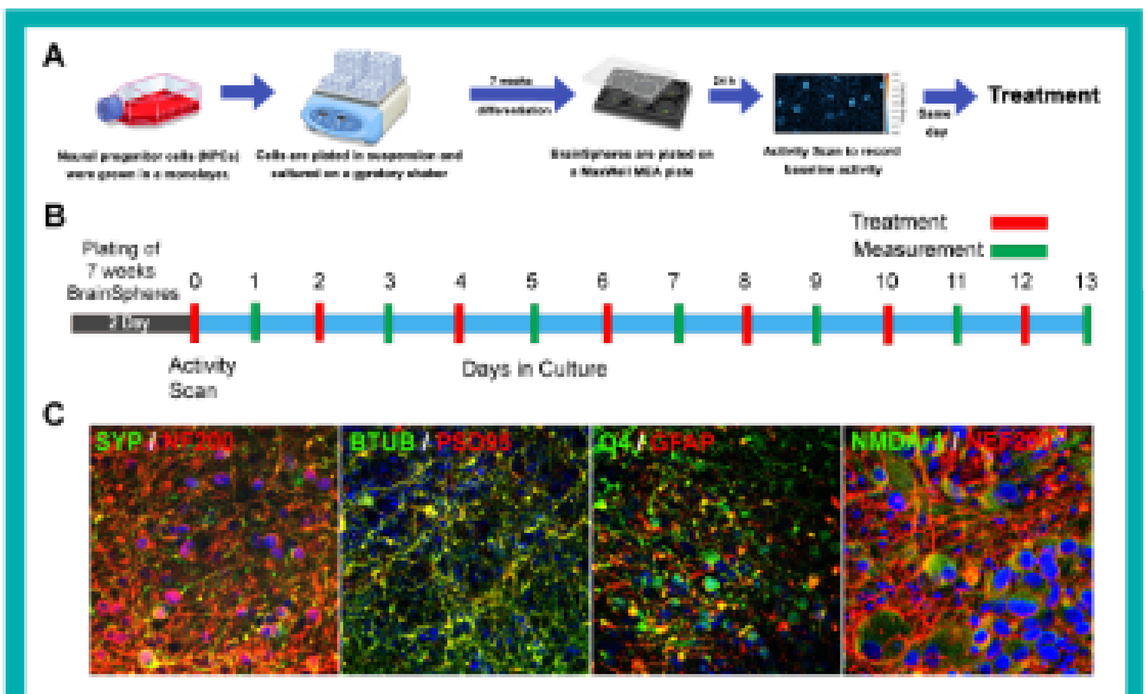
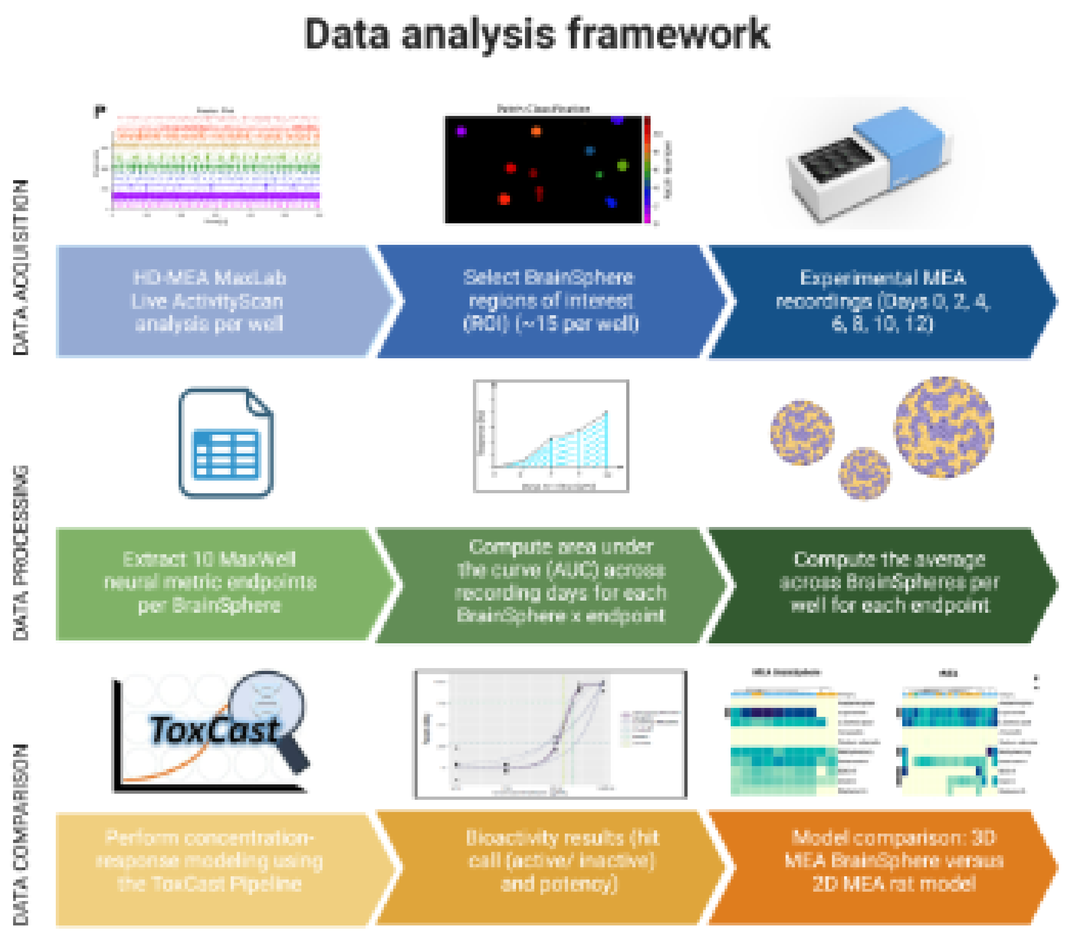


Figure 1. Experimental procedure and BrainSpheres characterization.
The BrainSpheres underwent 7-week differentiation process before being attached to HD MEAs. 48 after attachment cells were treated with the different compounds. BrainSpheres were also fixed at 8 weeks after differentiation for characterization by immunohistochemistry. A) Shows a diagram of the experimental procedure. B) Shows immunohistochemical confocal images of synaptic markers (SYP), postsynaptic markers (PSD95), neuronal markers (NF200 and BTUB), oligodendrocyte marker (O4), and astrocyte marker (GFAP). Bars represent 50 µm. C) Shows a diagram of the electrical activity measurements and treatment.



RESULTS

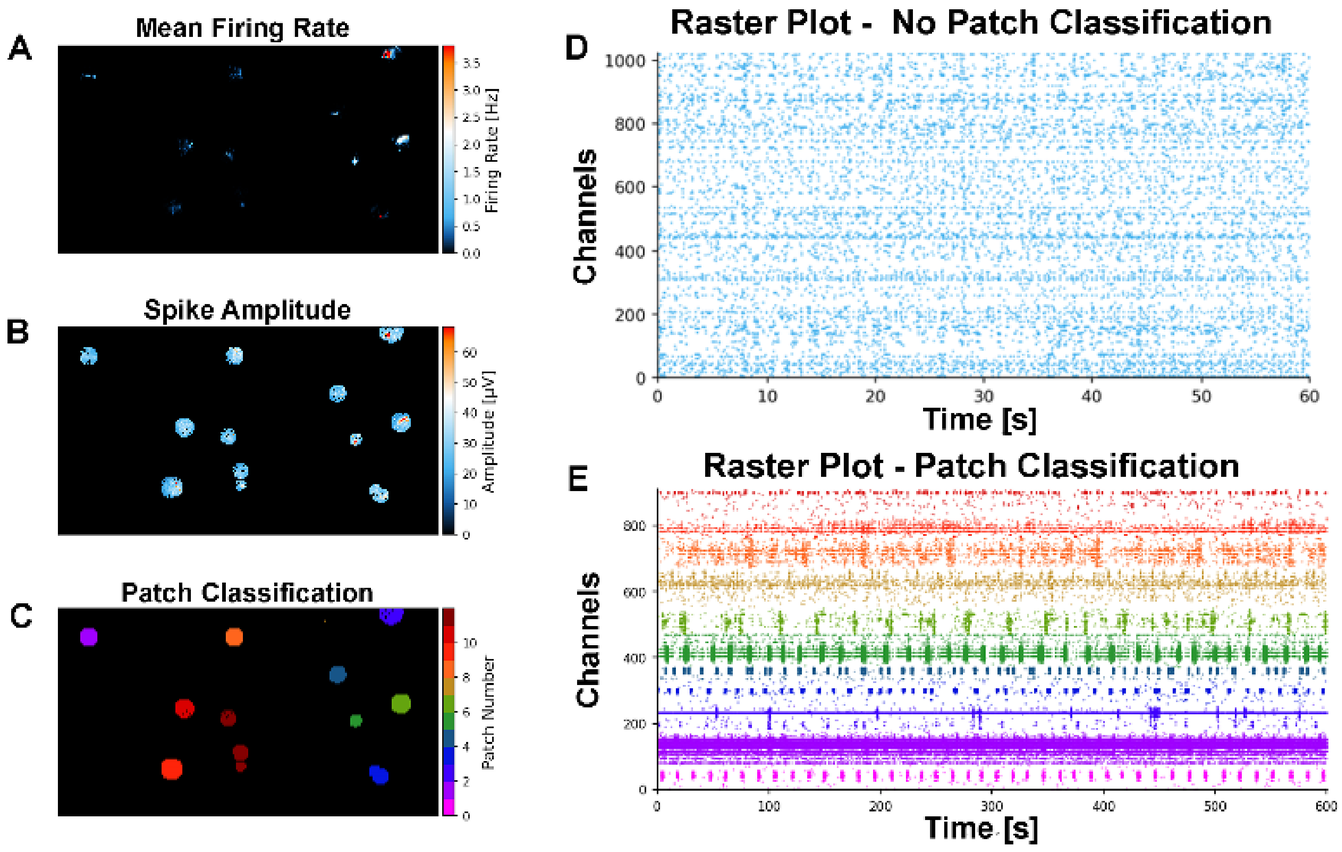


Figure 2. Spontaneous electrical activity data analysis (no treatment)
A) Activity Map of one MaxTwo HD-MEA well showing the firing rate and B) spike amplitude values of plated BrainSpheres. Each pixel corresponds to the mean firing rate or the 90th percentile spike amplitude value, respectively, of an individual electrode on the HD-MEA recorded in a Network assay. C) Distance-based classification of electrodes into 'patches' enabled grouping of electrodes into circular regions corresponding to activity of individual BrainSpheres. D) Raster plot showing detected events on all recording channels without patch classification. E) Raster plots of individual 'patches' of electrodes, stacked on top of each other, reveal distinct bursting dynamics of the individual BrainSpheres.

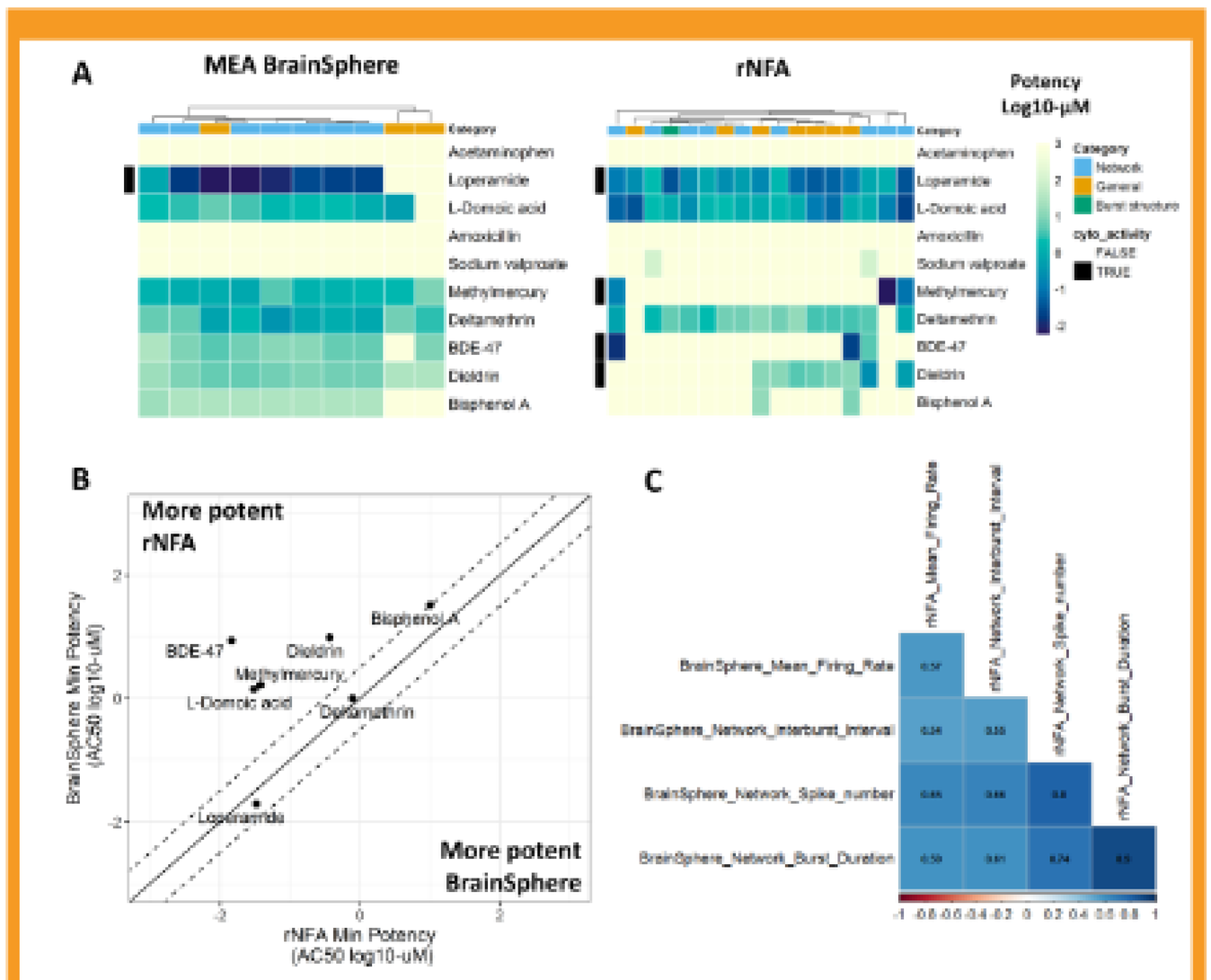


Figure 4. Comparison of MEA BrainSphere assay and rNFA concentration-response modeling results
A. Heatmap showing potency results across the MEA endpoints (columns) for the ten tested chemicals (rows) in the MEA BrainSphere (left) and the rNFA (right). The MEA BrainSphere comprised of 10 endpoints measuring general and network activity (Categories) and the rNFA comprised of 17 endpoints measuring general, network, and burst structure (Categories). Color key values indicate the potency (AC50 log10-µM) for each active curve with dark blue indicating more potent values and lighter green/yellow indicating less potent values. Inactive curves are colored yellow. B. Comparison of minimum potency (5th percentile across active hits) between the two assays. Solid line indicates the line of unity and dotted lines indicate +/- 0.5 log10-µM from the unity line. Upper left quadrant indicates chemicals that were more potent in the rNFA. C. Correlation matrix of bioactivity hit call determinations for four endpoints that were measured in both assays.

CONCLUSIONS

1. The HD-MEA BrainSphere assay effectively detects neural activity changes, showcasing its potential for advanced neurotoxicity screening.

2. Comparative studies show the 3D BrainSphere model may offer more human relevant and ethical models.

3. Technological advancements in HD-MEA and custom software enhance neural activity analysis in 3D cultures, and allow single sphere analysis.

4. The study supports the shift towards human-relevant methodologies for more ethical, accurate, and efficient toxicological evaluations.

scaht Swiss Centre for Applied Human Toxicology

NOVARTIS

maxwell BIOSYSTEMS

UNIL | Université de Lausanne

This poster does not reflect US EPA policy.