

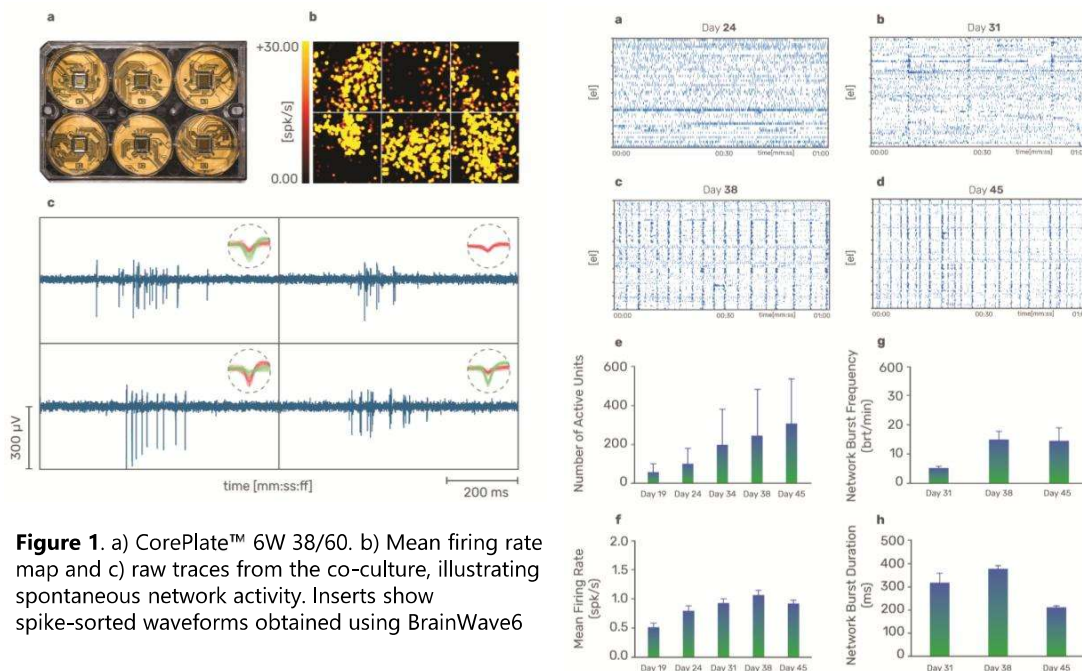
# Functional profiling of human iPSC-derived neuronal networks with 3Brain's CorePlate™ HD-MEA technology

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## Abstract

Functional neuronal networks derived from human iPSC-based cell types provide a powerful platform for studying synaptic development, network maturation, and pharmacological responses. This study presents the electrophysiological profiling of axoCells™ cortical excitatory neurons co-cultured with axoCells™ astrocytes, recorded on 3Brain's CorePlate™ HD-MEA system. Leveraging high-resolution sensing and integrated BrainWave6 analysis, we highlight robust, reproducible maturation and clear responses to excitatory and inhibitory modulators. Together, these results demonstrate a sensitive, reproducible, and assay-ready *in vitro* model ideally suited for neuroscience research and drug screening.



**Figure 1.** a) CorePlate™ 6W 38/60. b) Mean firing rate map and c) raw traces from the co-culture, illustrating spontaneous network activity. Inserts show spike-sorted waveforms obtained using BrainWave6

**Figure 2.** a-d) Raster plots of the co-culture at different maturation stages, showing the emergence of network bursts around Day 31 and their consolidation over time. e-h) Quantification of maturation metrics: active units, mean firing rate, network burst frequency, and duration.

## Methods

### Cell Culture

ax0015 Neural Stem Cells were cultured on Poly-D-lysine and Axol SureBond-XF-coated CorePlate™ devices. At Day 19, ax0665 human iPSC-derived astrocytes were added at an 8:1 neurons-to-astrocytes ratio to support network development.

### Electrophysiological Recordings

Electrophysiological activity was recorded using the 3Brain HyperCAM Alpha platform with CorePlate 6W 38/60, featuring 13,824 simultaneously active electrodes (2,304 per well) arranged within a  $2.9 \times 2.9 \text{ mm}^2$  active area ( $21 \times 21 \mu\text{m}$  electrodes,  $60 \mu\text{m}$  pitch). Signals were sampled at 10 kHz with a 100 Hz high-pass filter and acquired longitudinally from Day 19 to track network maturation.

### Pharmacological Assay

$5 \mu\text{M}$  AMPA +  $5 \mu\text{M}$  NMDA or  $10 \mu\text{M}$  NBQX +  $50 \mu\text{M}$  AP5 were used to modulate electrical activity.

### Analysis Software

Data were analyzed in BrainWave6, extracting spikes and network burst metrics, raster plots, connectivity maps, and CAT analysis within a unified, fully integrated workflow.

## Results

Longitudinal recordings revealed a clear progression of network development from Day 24 to Day 45, with early sparse spikes giving way to coordinated network bursts by Day 31 and further consolidation at Days 38 and 45 (Figure 2a-d). Quantitative metrics as number of active units, mean firing rate, network burst frequency, and network burst duration confirmed the maturation (Figure 2e-h). Mean firing rate map and raw-trace snapshots from Day 45 (Figure 1b-c) illustrate the spontaneous activity of fully developed networks. At this mature stage, cultures exhibited reproducible baseline firing patterns (Figure 3a). AMPA+NMDA increased excitability, enhancing spiking and network burst rates and reducing inter-spike intervals (Figure 3b-e), accompanied by broader, less clustered connectivity (Figure 4a-b). Conversely, NBQX+AP5 suppressed activity, eliminating synchronicity (Figure 3b-e) and increasing local clustering (Figure 4a-b). CAT trajectories mirrored these effects, with activation broadening propagation and blockade abolishing it (Figure 4c-d), demonstrating robust, predictable responses suitable for mechanistic and pharmacological evaluation.

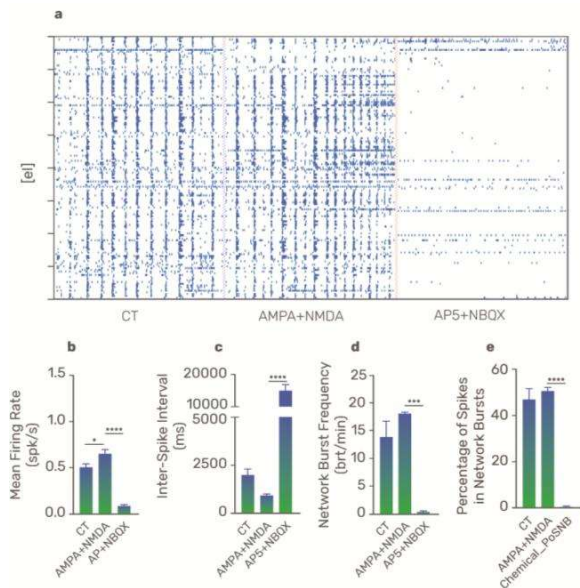
## Conclusion

The findings from this study highlight the complementary strengths of Axol's human iPSC-derived neuronal biology and 3Brain's CorePlate-enabled HD-MEA technology in resolving the functional properties of cortical excitatory networks. axoCells™ Cortical Excitatory Neurons co-cultured with axoCells™ Astrocytes showed clear and reproducible maturation signatures, developing stable, synchronized activity and predictable responses to excitatory modulation, demonstrating their suitability for mechanistic studies and pharmacological evaluation.

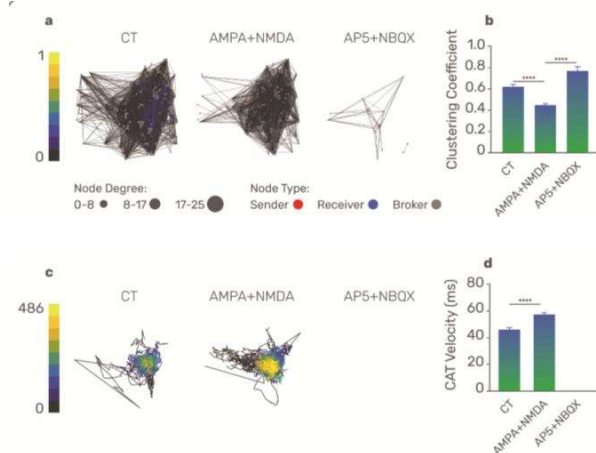
By leveraging 3Brain's CMOS-based HD-MEA platform, 13,824 simultaneously recording electrodes across the six-well format, we achieved high-resolution mapping of network behavior, capturing both large-scale population dynamics and subtle changes arising during maturation or after pharmacological intervention. This spatial resolution supported detailed assessment of connectivity, network propagation, and activity distribution.

The integration with BrainWave 6, a unified environment for acquisition, visualization, and advanced analysis, ensured a coherent and reproducible workflow, enabling complex metrics and spatiotemporal insights without external tools.

Together, the Axol-3Brain system provides a sensitive, high-resolution, and efficient platform for neuropharmacology, toxicity testing, and the study of human neuronal circuit function.



**Figure 3.** a) Raster plots of the co-culture at Day45 under control conditions, after AMPA+NMDA, and after NBQX+AP5 treatment. b-e) Mean firing rate, inter-spike interval, network burst frequency, and percentage of spikes in network bursts across conditions.



**Figure 4.** a) Cross-correlation maps showing connectivity changes under excitatory modulation. b) Clustering coefficient across conditions, with activation reducing clustering and blockade increasing it. c) Center of Activity Trajectories (CATs) before and after treatment. d) CAT velocity, increased by activation and reduced by blockade.