Application Note

Assessing Brain Organoid Network Activity Using the BioCAM DupleX and CorePlate™ 1W 38/60

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Abstract

Brain organoids have emerged as advanced *in vitro* models for investigating brain development, neurodegenerative diseases and neuropharmacology. These three-dimensional, self-organizing structures replicate key features of human brain architecture and functionality, offering a valuable platform for research areas such as developmental biology and disease modelling. This application note explores the acute response of a brain organoid to chemical (Bicuculline) and electrical stimulation, both independently, and in combination. Using the BioCAM DupleX & CorePlate™ 1W 38/60, we demonstrate that Bicuculline reduces inhibition within the organoid, enabling electrical stimulation to induce prolonged and regular network responses. In contrast, electrical stimulation alone produces shorter and less consistent activity. These findings display the effectiveness, sensitivity and accuracy of the BioCAM DupleX & CorePlate™ 1W 38/60 in providing a deeper understanding of how external stimuli influence brain organoid activity.

Introduction

Brain organoids have emerged as powerful *in vitro* models for studying brain development, neurodegenerative diseases, and neuropharmacological responses. These three-dimensional, self-organizing structures recapitulate key aspects of human brain architecture and functionality, enabling insights into vital research areas such as development, and disease-in-a-dish modelling with iPSCs.

One particularly useful application of brain organoids is the assessment of acute responses to chemical and electrical stimuli. Chemical and electrical stimulation can individually provide valuable insights into excitability, synaptic activity, receptor function and network connectivity. When combined they enable even more comprehensive investigations.

In order to study such phenotypic responses, electrophysiological changes can be characterized utilizing high density – micro electrode arrays (HD-MEA). The BioCAM DupleX together with CorePlate™ is a state-of-the-art HD-MEA platform featuring 4,096 bidirectional electrodes, a 20 kHz sampling frequency, and integrated temperature control. The BioCAM DupleX enables data acquisition from a variety of CorePlate™-powered HD-MEAs, allowing you to capture vast amounts of high-precision data from complex biological samples such as brain organoids, facilitating deeper insights and advancing research.

This application note highlights the accuracy and sensitivity of the BioCAM DupleX and CorePlate™ 1W 38/60 (21 µm x 21 µm electrodes, 60 µm pitch, 3.8 mm^2 recording area), in measuring the acute responses of a brain organoid to chemical (Bicuculline, a GABA-A receptor antagonist) and electrical stimulation, both individually and in combination.

By utilizing the BioCAM DupleX and CorePlate™ 1W 38/60, we demonstrate how electrophysiological data from an organoid was recorded and analyzed using Brainwave software. Enabling a deeper understanding of neural activity patterns and the effects of external stimuli, paving the way for advancements in drug discovery, neuropharmacology, and disease modeling.

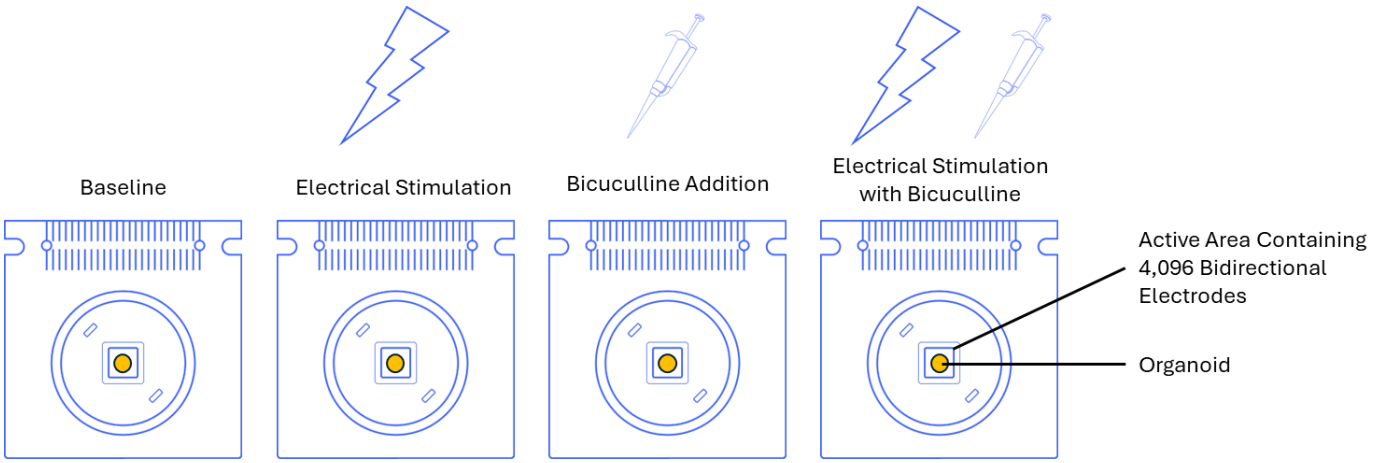

Figure 1. Assay Protocol.

Materials & Methods

Dosing & Stimulation Protocol

Organoids underwent the following procedure (as shown in Fig. 1), and were recorded for 4 minutes at each stage:

1. Baseline recording.
2. Electrical stimulation was applied for 10 repetitions at 0.05 Hz (biphasic pulse ±25 µA, 30 µs per phase).
3. Organoids were dosed with 15 µM Bicuculline (Sigma).
4. Electrical stimulation was applied for 10 repetitions at 0.05 Hz (biphasic pulse ±25 µA, 30 µs per phase) in the presence of Bicuculline.

Cells, Culture Method & Reagents

Organoids were cultured according to the Pasca protocol (1), and allowed to mature for 90 days. Media was then switched to BrainPhys™ (STEMCELL Technologies) for 15 days, bringing the total time in culture to 105 DIV.

HD-MEA

The BioCAM DupleX and CorePlate™ 38/60 were utilized to record extracellular electrophysiological activity from the organoid. BrainWave 5 was used to analyse the recorded data.

Results

Organoids show spontaneous spiking activity.

As neuronal cells and brain organoids mature, they begin to show spontaneous activity. In this case, after 15 days in BrainPhys™ media the spontaneous activity was visualized as a spiking map (spikes/second) over all of the 4,096 simultaneously recording electrodes. As can be seen in Fig. 2 organoids displayed widespread spiking activity (yellow indicating high levels of spiking) against the low background levels (black indicating little/no spiking). This shows the heterogenous neuronal activity within the organoid.

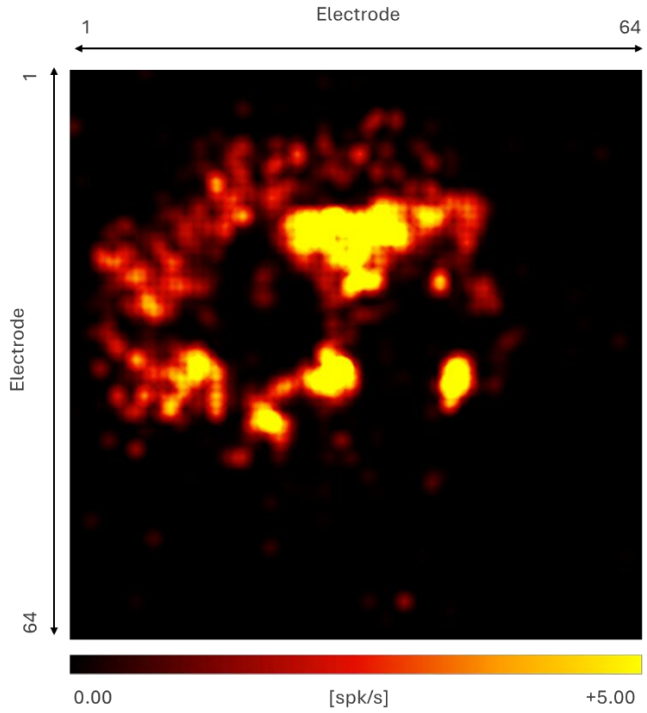

Figure 2. Activity Map showing spontaneous spiking activity within the organoid.

Organoids display induced network bursting activity in response to electrical and chemical modulation.

Although spontaneous activity and individual neuronal bursts may arise from single cells, strong synaptic connectivity within neuronal cultures/organoids can lead to coordinated activity. When one neuron fires, it can influence surrounding neurons, resulting in groups of interconnected cells bursting together and triggering a network burst, as illustrated in Fig 3.

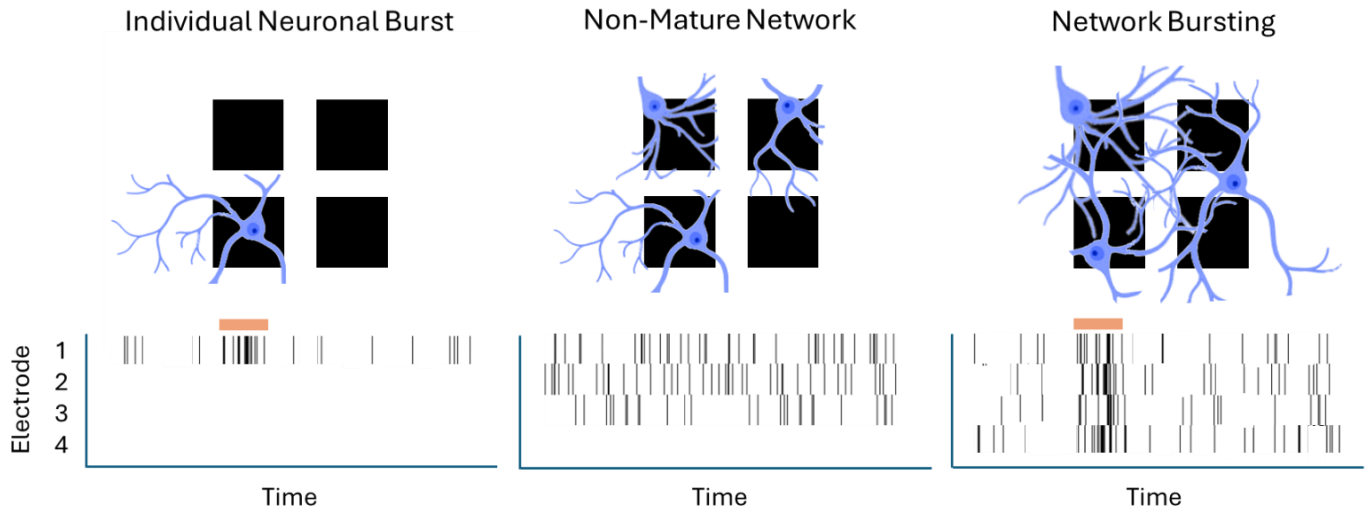

Figure 3. Diagram displaying connectivity within neuronal networks and example raster plots showing spiking & bursting activity. Vertical black lines indicate spiking, horizontal orange bars indicate network bursting.

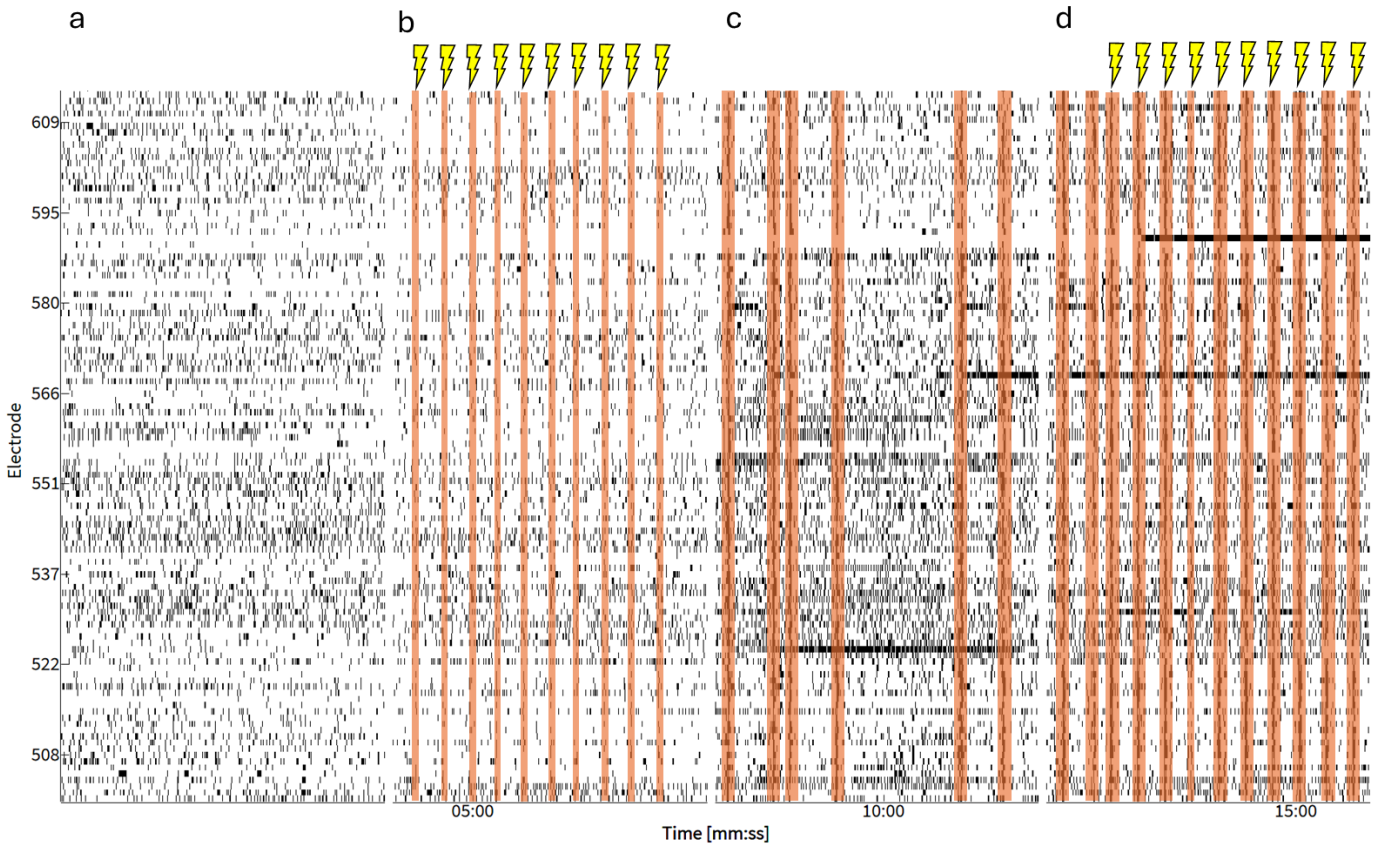

Figure 4. Raster Plot of selected example electrodes displaying network bursting activity within the different phases of the experiment. Baseline (a), Electrical Stimulation Only (b), Bicuculline (c), Electrical Stimulation in the presence of Bicuculline (d). Orange bars represent network bursts.

In Fig. 4, the raster plot displays the spiking of the active units (MFR > 0.5 spks/s) in the organoid at baseline (a), with electrical stimulation (b), with Bicuculline addition (c), and with electrical stimulation in the presence of Bicuculline (d). The raster plot illustrates network bursts (highlighted) which emerge with electrical stimulation (b), increase in duration with Bicuculline addition (c), and retain the larger duration and increase in regularity when Bicuculline is present alongside electrical stimulation (d). This is reflected by the increase in the number of spikes per network burst (either induced by electrical stimulation or spontaneous) as shown in Fig. 5.

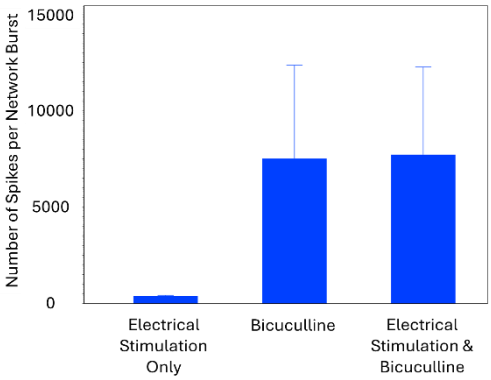

Figure 5. Average Chart indicating the Number of Spikes per Network Burst.

Network characteristics are altered by electrical and chemical modulation.

Network activity is a key function within *in vivo* neuronal systems and can also be used to assess the maturity of brain organoids. The ability of an organoid to perform network functions can also be altered by electrical and chemical modulation. In this case at baseline, no network bursts are detected, only the spontaneous activity of individual neurons are observed (Fig. 4a). When electrical stimulation was applied, network bursts emerge in response to the perturbation, demonstrating the excitability of a connected network (Fig. 4b).

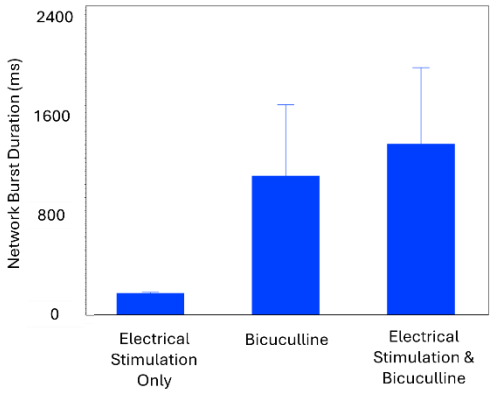

Figure 6. Average Chart indicating the Network Burst Duration.

With the addition of Bicuculline, spontaneous network bursts emerge and the duration of network bursts increases (Fig. 4c). Electrical stimulation in the presence of Bicuculline induced frequent, prolonged network bursts (Fig. 4d). This removal of the organoids innate inhibitory capability by Bicuculline, and the resulting increase in network burst duration can be seen in Fig. 6. Interestingly, Bicuculline also affected the Inter Spike Interval (ISI) within network bursts as shown in Fig. 7. The increase in ISI following Bicuculline addition, compared to electrical stimulation alone is likely due to the removal of inhibition, the extended network burst duration, and slowing down of spiking at the tail end of the bursting phase.

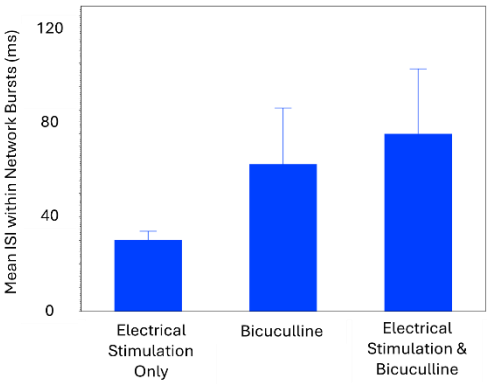

Figure 7. Average Chart indicating the Mean Inter Spike Interval within network bursts.

Network connectivity is altered by electrical and chemical modulation.

The organoids network capabilities can also be easily investigated utilizing BrainWave to perform spike cross correlation analysis with the data obtained from the 4,096 simultaneous recording electrodes. Here we can investigate the degree of connectivity within all 4 conditions. When this is visualized as a connectivity map (Fig. 8) it can be seen that there is little difference between the baseline (Fig. 8a) & the electrical stimulation only (Fig. 8b). However the degree of connectivity appears to increase with the addition of Bicuculline (Fig. 8c), and remains high in the presence of electrical stimulation and Bicuculline. This increase is likely due to the reduced inhibition and is reflected when the number of links is quantified (Fig. 9).

Centre of activity trajectory is altered by electrical and chemical modulation.

Centre of activity trajectory (CAT) represents the dynamic movement of network bursts over time, capturing how neural activation propagates spatially across the sample (2). CAT analysis revealed changes following the addition of Bicuculline. These changes are visible when plotted at a CAT MAP (Fig. 10), and upon further analysis, an increase in CAT duration was observed (Fig. 11). The prolonged CAT is likely due to the reduction in inhibition caused by Bicuculline, with CAT duration increasing slightly further when electrically stimulated in the presence of Bicuculline.

Discussion

In vitro models of the brain, such as brain organoids, are powerful tools for studying development, disease models, network function, and pharmacological responses to compounds. In order to make the most of these models, the

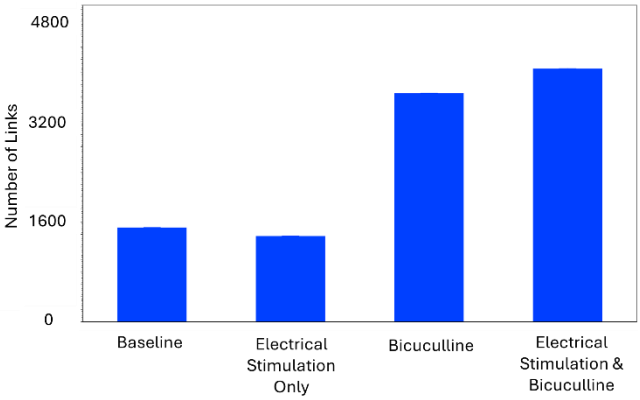

Figure 9. Average Chart indicating the Number of Links.

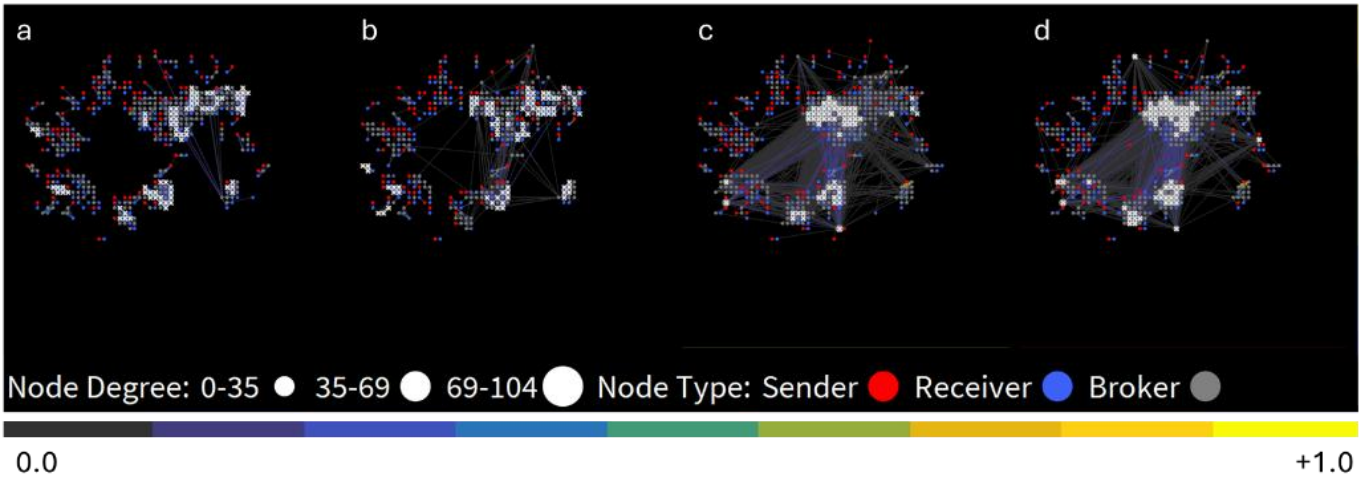

Figure 8. Network Connectivity Map. a. Baseline, b Electrical Stimulation Only, c. Bicuculline, d. Electrical Stimulation in the presence of Bicuculline.

HD-MEA must enable precise measurements of neuronal activity. In this application note, we demonstrate that the BioCAM DupleX paired with CorePlate™ 38/60 provides the ideal platform for investigating the function of neural organoids, particularly their network properties. Here we showcase a select few of the hundreds of available metrics in BrainWave that facilitate the analysis of organoid activity.

The 4,096 simultanously recording electrodes from CorePlate™ 38/60 generated a large and precise dataset from the total area covered by the organoid. Approximately 70% of the electrodes covered by the organoid detected activity (~1,280 electrodes covered by the organoid, of which activity was detected in 898 electrodes (MFR >0.1 spks/s)), enabling accurate and robust data analysis to be performed. Additionally, its bidirectional electrode capabilities allowed for electrical stimulation to be performed alongside pharmacological manipulation.

Electrical stimulation was found to induce short duration network bursts, and the addition of Bicuculline to block GABA-A mediated inhibition led to longer duration, spontaneous network bursts. Finally, in the presence of Bicuculline, electrical stimulation induced regular, long duration network bursting, highlighting the excitability of the organoid under reduced inhibitory control.

The degree of network connectivity can be easily assessed using BrainWave. The physical properties of CorePlate™ 38/60 enable the acquisition of extensive datasets, capturing spiking information at the single-cell level across a large array of thousands of electrodes, thus ensuring high accuracy for assessing the degree of network connectivity with spike cross-correlaton analysis. Within this organoid, the reduction in intrinsic inhibition from Bicuculline increased the degree of connectivity compared to electrical stimulation alone as shown in Fig. 8 & 9.

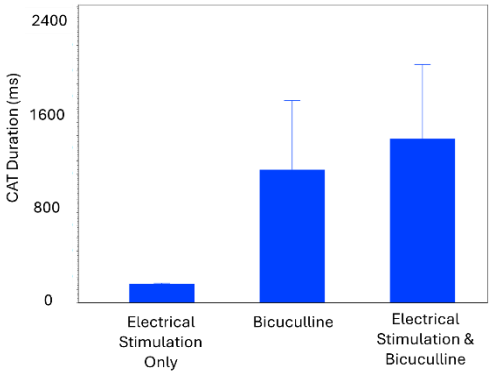

Figure 11. Average Chart indicating CAT duration.

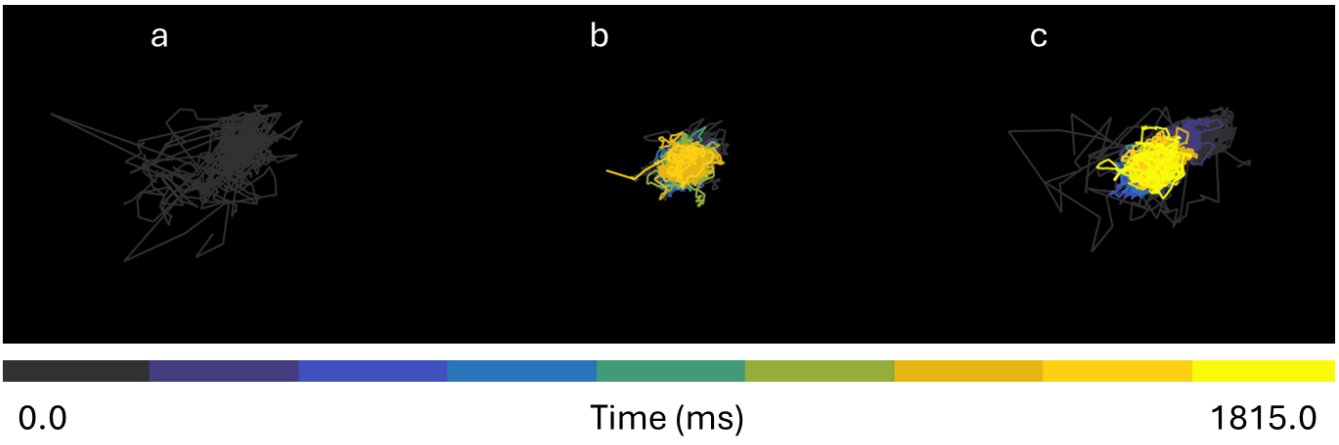

Figure 10. CAT Map displaying trajectory in a. electrical stimulation, b. Bicuculline and c. electrical stimulation and Bicuculline.

Finally, changes in CAT were also observed. An increase in CAT duration was observed following Bicuculline addition (Fig. 10 & 11) indicating a more excitable network with less inhibition, allowing activity to spread across the network for longer periods of time.

In summary, this study demonstrates the effectiveness of the BioCAM DupleX and CorePlate™ 38/60 to accurately characterize organoid network function, and identify electrophysiological changes induced by electrical stimulation and pharmacological modulation. These capabilities make BioCAM DupleX and CorePlate™ 38/60 the ideal platform for advancing organoid research and exploring network function.

References

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