Application Note

Characterizing ACROBiosystems Human iPSC Derived Cerebral Organoid with the BioCAM Duplex and CorePlate™ 1W 38/60

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In partnership with

Abstract

High density micro electrode arrays (HD-MEA) are a vital tool for characterizing the functional properties of cerebral organoids. As a leading innovator in this field, 3Brain develops high-quality HD-MEAs, enabling precise, high resolution electrophysiological recordings. ACROBiosystems is a global biotechnology company which can produce and deliver both ready-to-use and cryopreserved human iPSC-derived cerebral organoids for scientific applications and drug development. These organoids mimic key aspects of human cerebral cortex architecture and functionality making them valuable *in vitro* research models. This application note evaluates the activity modulation of ACROBiosystems' human iPSC-derived cerebral organoids due to chemical stimulation with Bicuculline, 4-AP, and TTX. Using the BioCAM DupleX and CorePlate™ 1W 38/60, we characterize the organoids' functional response to these known pharmacological agents.

Introduction

Human iPSC cerebral organoids serve as a powerful *in vitro* model for studying cerebral development, disease modelling and neuropharmacological responses. These three-dimensional, self-organizing structures replicate key aspects of human cerebral cortex architecture and functionality, enabling insights into vital research areas such as development and neuropharmacology.

One of the most valuable applications of brain organoids is the assessment of acute electrophysiological responses to pharmacological stimulation. This approach offers insights into a range of neuronal properties, including excitability, receptor function and network activity. Additionally, it allows researchers to evaluate how closely organoid behavior mirrors that of the *in vivo* brain, offering a promising new platform for investigating novel neuropharmacological compounds.

Electrophysiological responses can be characterized utilizing high density – micro electrode arrays (HD-MEA). The BioCAM DupleX is a state-of-the-art HD-MEA platform featuring integrated temperature control, a 20 kHz sampling frequency, and 4,096 bidirectional recording electrodes. It enables data acquisition from a variety of CorePlate™ HD-MEAs: 3Brain's advanced technology, for high-resolution, label-free electrophysiological recordings with unparalleled precision. This technology enables you to capture vast amounts of data from complex biological samples, such as cerebral organoids, facilitating deeper insights and advancing research.

ACROBiosystems is a global biotechnology company which can produce and deliver both ready to use and cryopreseved human iPSC cerebral organoids for research purposes. These organoids have been histologically and immunohistochemically characterized by ACROBiosystems confirming the presence of typical markers of neuronal and glial cells, verified by immunostaining and scRNA-seq (Fig. 1). This application note compliments this by characterizing their electrophysiological response with the BioCAM DupleX & CorePlate™ 1W 38/60 (21 µm x 21 µm electrodes, 60 µm pitch, 3.8mm^2 recording area) to known neuropharmacological agents: 4-Aminopyridine (4-AP) a K^+ channel blocker, Bicuculine (Bic) a GABA-A receptor antagonist and Tetrodotoxin (TTX) a voltage-gated Na^+ channel blocker. We demonstrate how high-resolution electrophysiological data was used to help characterize ACROBiosystems human iPSC cerebral organoids, further enabling researchers

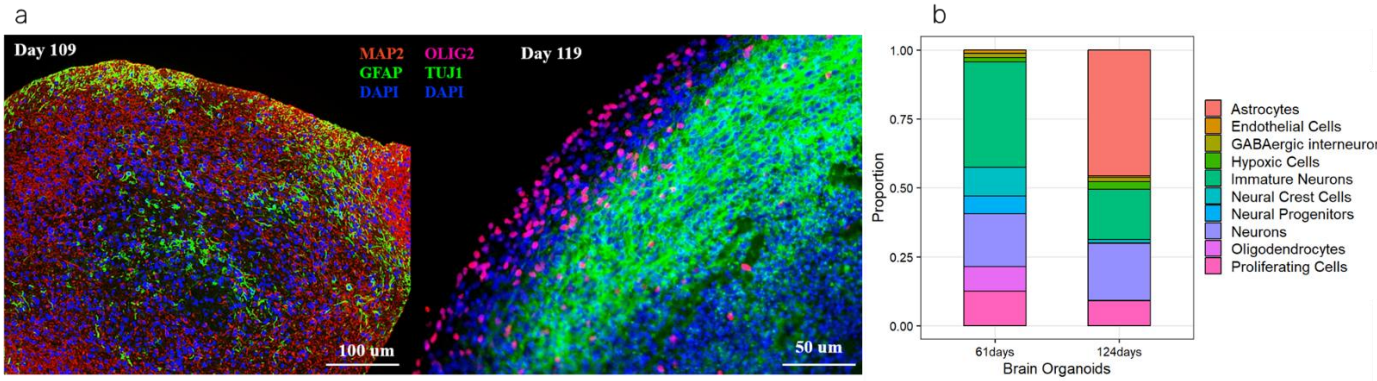

Figure 1. Immunostaining (a) and scRNA-seq analysis (b) showing that cerebral organoids highly express mature neurons and glia after 100+ days (CIPO-BWL002K, ACROBiosystems), compared to 60+ day organoids (CIPO-BWL001K, ACROBiosystems).

to streamline experimental workflows, advance neuropharmacology and drug discovery.

Methods

Cells, Culture Method & Reagents

Organoids were cultured by ACROBiosystems according to their in house protocol (1) using ACROBiosystems RIPO-BWM001K kit for iPSC-derived cerebral organoid differentiation and ACROBiosystems RIPO-BWM003 for cerebral organoid maintenance and maturation, whereby the organoids were allowed to mature for 100 days (CIPO-BWL002K, ACROBiosystems) before being shipped (2) to 3Brain. Following this, organoids were then cultured in BrainPhys™ (StemCell) for 15 days before being recorded.

Dosing & Stimulation Protocol

Organoids underwent the following procedure (as shown in Fig. 2):

1. A 3-minute baseline recording was conducted.
2. Organoids were then dosed with 100µM 4-AP and 20µM Bic (Sigma), and recorded for another 3 minutes.
3. Following this, organoids were dosed with 10µM TTX (Sigma), and recorded for an additional 3 minutes.

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

Spiking activity is altered by 4-AP, Bic and TTX

The application of 4-AP, bicuculline, and TTX induced changes in spiking activity as shown in the raster plot in Fig. 3. Following the addition of

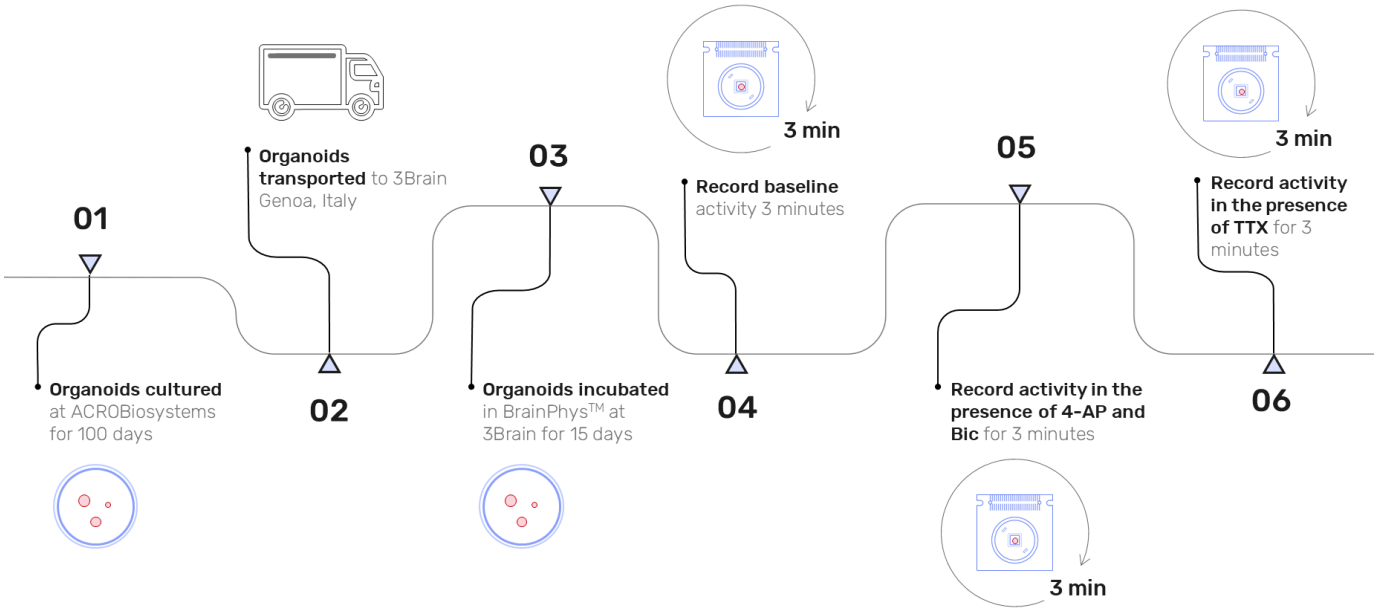

Figure 2. Experimental timeline illustrating the culture period, organoid delivery, organoid incubation in BrainPhys™ and three recording stages: baseline activity, activity after addition of 100µM 4-AP + 20µM Bic, activity after addition of 10µM TTX.

4-AP and bicuculline, spontaneous individual neuronal activity decreased, while synchronized network activity increased, reflecting reduced inhibition of the network. In contrast, the introduction of TTX led to a marked reduction in activity, indicating suppressed neuronal excitability.

Network characteristics are altered by 4-AP, Bic and TTX

Network burst frequency increased following the addition of 4-AP and Bic, indicating enhanced excitability and reduced network inhibition. This effect was completely abolished upon the introduction of TTX. Network burst duration significantly decreased with 4-AP and bicuculline, and was eliminated with TTX. Additionally, the inter-network burst interval was reduced following 4-AP and bicuculline application, suggesting more frequent bursting events. Similarly, the inter-spike interval within network bursts significantly decreased with 4-AP and bicuculline, further reflecting heightened excitability, and was abolished with TTX (Fig. 4).

Network connectivity is altered by 4-AP and Bic

The degree of network connectivity was altered following the addition of 4-AP and Bic, as reflected by a significant increase in correlation values and node degree, indicating that the compounds influenced network integrity potentially due to reduced inhibition (Fig. 5). The connectivity map which overlays the organoid in Fig. 6 further visualizes these changes, displaying the increased number of links, their distribution, and correlation strength.

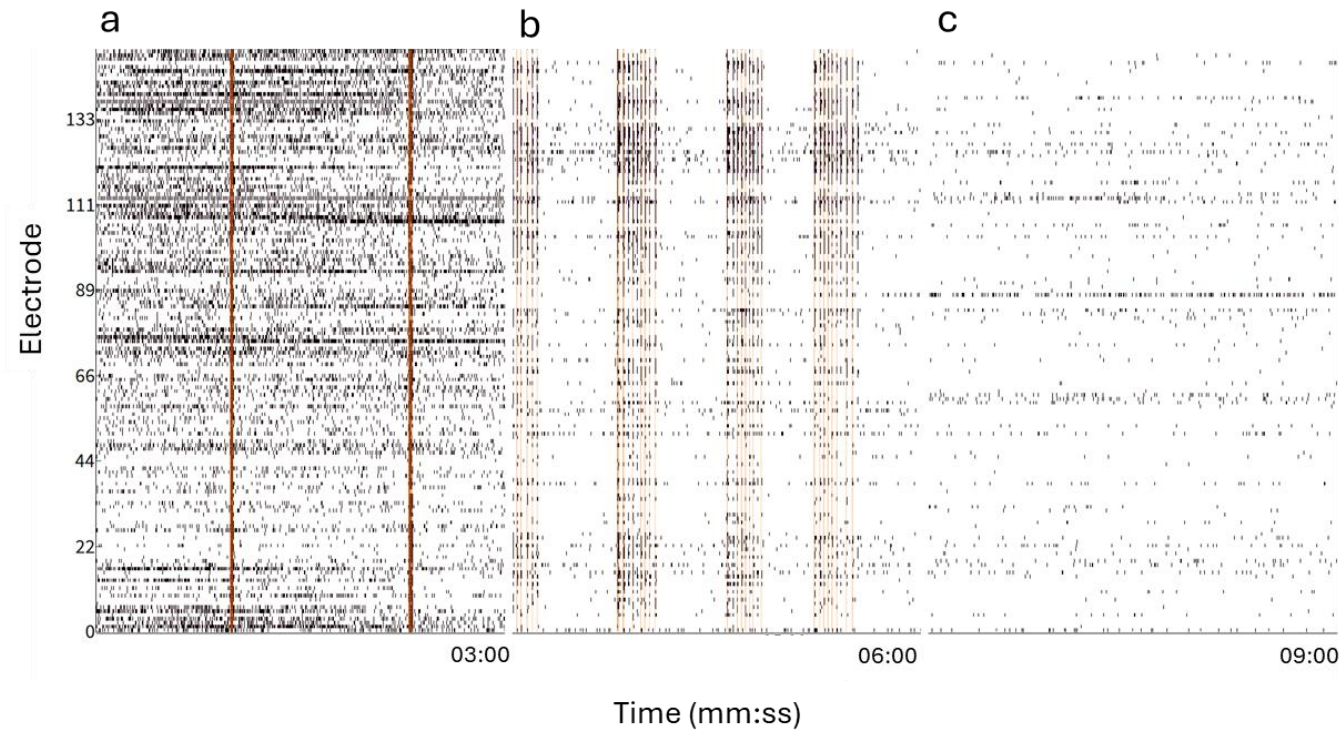

Figure 3. Raster Plot displaying the spiking activity of the organoid from a subset of example electrodes at baseline (a), 100μM 4-AP + 20μM Bic (b), 10μM TTX (c). Orange bars indicate network burst detection.

Centre of activity trajectory is modulated by 4-AP and Bic

Centre of activity trajectory (CAT) represents the dynamic movement of network bursts over time, capturing how neural activation propagates spatially across the sample (3). CAT analysis showed significantly increased velocity, and reduced duration following the addition of 4-AP and Bic (Fig. 7), likely due to heightened neuronal excitability from K^+ channel blockade and reduced GABAergic inhibition. These changes are further visualized in the CAT map (Fig. 8), where the expanded trajectory distribution across the organoid is evident.

Discussion

In vitro models of the cerebral cortex are crucial for advancing our understanding of cerebral development, function and advancing drug discovery and neuropharmacological research.

ACROBiosystems produces both ready-to use and cryopreserved human iPSC-derived cerebral organoids for research and drug development purposes which can be shipped globally and delivered directly to laboratories (2) to provide a standardized and consistent *in vitro* cerebral organoid model for vital neuroscientific studies and drug response evaluations.

In this application note, the BioCAM DupleX and CorePlate™ 1W 38/60 were utilized to obtain high-resolution electrophysiological data to characterize an ACROBiosystems human iPSC-derived cerebral organoid.

The electrophysiological recordings revealed key insights into the organoid's neural network dynamics. The observed shift in spiking activity following bicuculline application suggests that the organoids contain functional inhibitory neuronal cells that actively regulate network activity. By reducing inhibition, bicuculline, a GABA-A receptor antagonist, promoted synchronized network-wide activity. The simultaneous application of 4-AP, a K^+ channel blocker, may have further enhanced network synchrony by increasing excitability. In contrast, TTX, a Na^+ channel blocker, reduced the network activity.

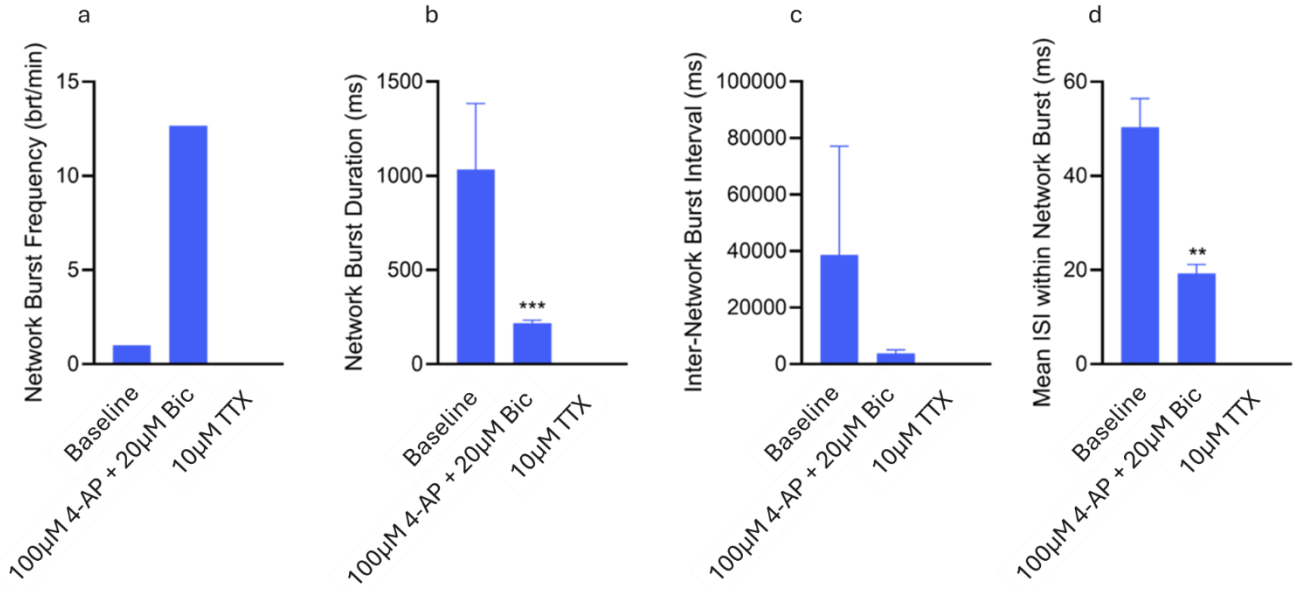

Figure 4. Network Burst metrics displaying the average Network Burst Frequency (a), Network Burst Duration (b), Inter Network Burst Interval (c) and Inter-Spike Interval within Network Bursts (d). Significant differences in Network Burst Duration and Mean ISI within Network bursts were found between Baseline & 100µM 4-AP + 20µM Bic (p=0.0008 & p=0.0019 respectively) (Kolmogorov-Smirnov test).

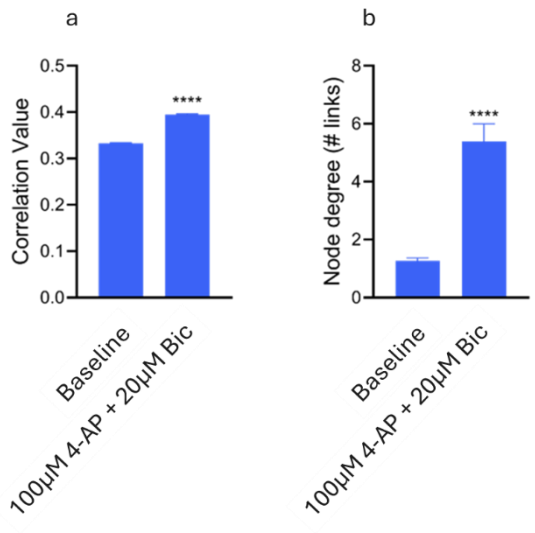

Figure 5. Connectivity metrics displaying the correlation value (a) and node degree (# links) (b). Significant differences in correlation value and node degree were found between Baseline & 100µM 4-AP + 20µM Bic ($p<0.0001$) (Kolmogorov-Smirnov test).

The changes in the network bursting properties following bicuculline, 4-AP, and TTX treatment further support the presence of a functional network within the organoids. The increased burst frequency and reduced inter-burst intervals observed with 4-AP and bicuculline likely result from disinhibition and heightened excitability. Additionally, the reduction in burst duration and inter-spike intervals within bursts suggests a shift toward shorter, more frequent network events, characteristic of reduced inhibition and a hyperexcitable state. This heightened activity was effectively suppressed by TTX.

Utilizing Brainwave software, the degree of network connectivity within the cerebral organoids was also easily analyzed. The increased correlation and number of links following 4-AP and bicuculline addition further highlight the impact of reduced inhibition and heightened excitability on the organoid network. Visualization of this connectivity map overlaying the organoid (Fig. 6) reflected this change

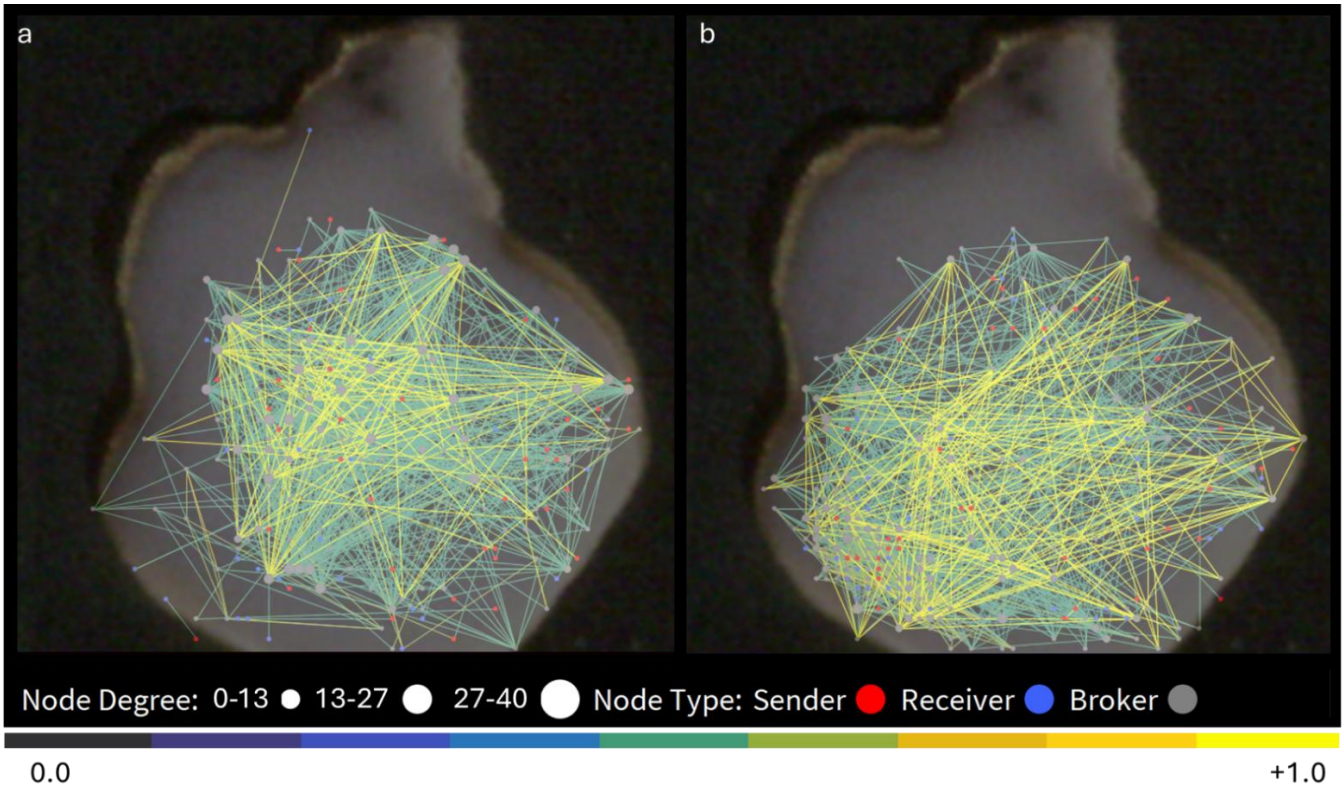

Figure 6. Network connectivity map overlayed on the recorded organoid in baseline condition (a), and after application of 4-AP and Bic (b).

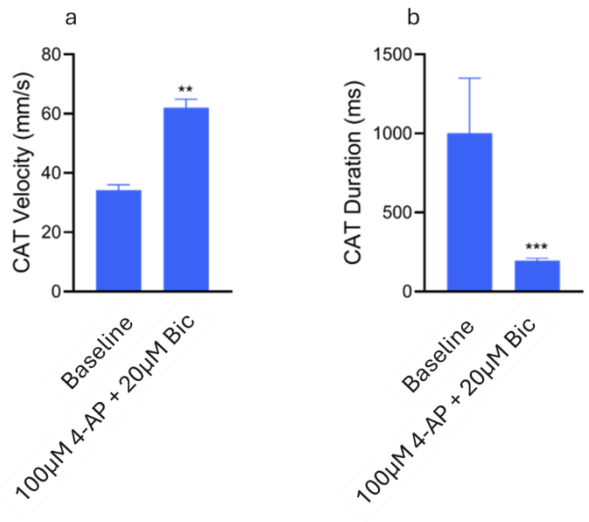

Figure 7. CAT metrics displaying the CAT velocity (a) and CAT duration (b). Significant differences in CAT velocity and CAT duration were found between Baseline & 100µM 4-AP + 20µM Bic (p=0.0049 & p=0.0008 respectively) (Kolmogorov-Smirnov test).

revealing the increased number of links and broader distribution of nodes.

Finally, changes in CAT were also observed. An increase in CAT velocity, and a reduction duration were observed following 4-AP and bicuculline treatment (Fig. 7) indicating rapid shifts in network activity and reflecting a more excitable network with less inhibition. When displayed as an overlay on the organoid, the expanded CAT trajectory distribution was visualized (Fig. 8), suggesting that reduced inhibition facilitates more widespread activity.

Overall, these findings demonstrate that pharmacological modulation with 4-AP, bicuculline, and TTX alters neuronal activity and network characteristics within the human iPSC-derived cerebral organoid (CIPO-BWL002K, ACROBiosystems) in an expected manner after an overseas shipment of live organoids. The BioCAM DupleX and CorePlate™ 38/60 (3Brain) enabled precise, high-resolution analysis of the surface activity of these brain organoids and offered insights into the neuronal functionality and network properties of the organoids. Although surface recordings offer valuable insight into organoid activity, this study could have been enhanced by capturing signals from within the organoid using the "gold-standard" for assessing brain organoid activity - CorePlate™ 3D in its single- or multi-well format.

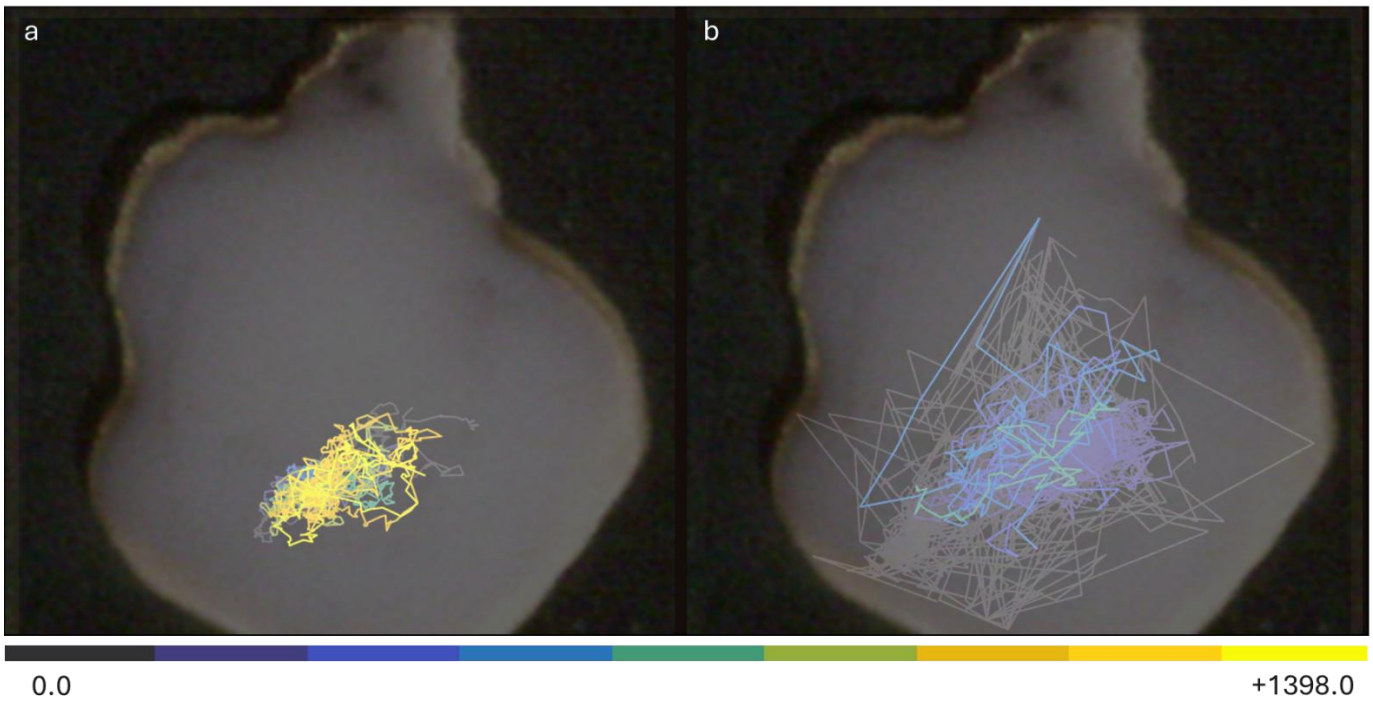

Figure 8. CAT Map overlayed on the recorded organoid displaying trajectory in baseline condition (a), and after application of 4-AP and Bic (b).

Internal recordings provide a more comprehensive view of organoid function and can reveal activity patterns not detectable from the surface alone.

In summary, this application note highlights the potential of 3Brain's CorePlate™ and ACROBiosystems in advancing research on the characterization of *in vitro* cerebral organoid models.

References

(1) https://www.acrobiosystems.com/P7373-Human-iPSC-Derived-Cerebral-Organoid-Differentiation-Kit.html
(2) https://www.acrobiosystems.com/P7372-Ready-to-use-Human-iPSC-Derived-Cerebral-Organoids.html
(3) Gandolfo M, Maccione A, Tedesco M, Martinoia S, Berdondini L. Tracking burst patterns in hippocampal cultures with high-density CMOS-MEAs. J Neural Eng. 2010 Oct;7(5):056001. doi: 10.1088/1741-2560/7/5/056001. Epub 2010 Aug 18. PMID: 20720282.

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