Application Note

Zonal Analysis of a Brain Assembloid Treated with Bicuculline on CorePlate™ 1W-3D 38/60/90

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Abstract

Human brain assembloids provide an opportunity to model the complex inter-regional communication of the human brain, however, capturing the extensive functionality of these remains a challenge. In this application note, we demonstrate how CorePlate™ 3D enables high-resolution functional analysis of assembloids. CorePlate™ 3D is a high-density microelectrode array (HD-MEA) featuring thousands of penetrating micro-electrodes, offering superior signal acquisition compared to standard planar 2D arrays. We monitor the activity of an assembloid in response to Bicuculline (Bic) and observed the expected increase in neuronal activity. Leveraging the new Zonal Analysis feature in BrainWave 6, we showcase how researchers can precisely segment an assembloid into distinct functional regions to quantify and visualize these localized activity patterns. Together, CorePlate™ 3D and BrainWave 6 provide an ideal platform for investigating advanced 3D *in vitro* models which may possess region dependent activity such as assembloids.

Introduction

The emergence of human-derived brain assembloids formed by the fusion of organoids of the same or different regions represents a significant leap in modelling complex inter-organoid and inter-regional connectivity and neural circuit dynamics *in vitro*. However, traditional planar microelectrode arrays (MEAs) and the associated analysis software often fail to capture the full complexity of activity across different regions of large, 3D *in vitro* structures when relying only on surface recordings.

To overcome these limitations, 3Brain developed CorePlate™ 3D, a high-density microelectrode array (HD-MEA) featuring 4,096 3D electrodes. These electrodes penetrate into the 3D culture, providing unprecedented signal quality and access to the activity and internal network dynamics of 3D cultures such as spheroids, organoids, assembloids and brain slices. Further to this, CorePlate™ 3D incorporates microchannels beneath the electrode pillars which enhance microfluidic flow around the sample. This improves oxygen and nutrient delivery to the base of the tissue and promotes more uniform compound diffusion across the entire sample.

Combined with the advanced zonal analysis capabilities of BrainWave 6, researchers can now dissect the functional contributions of individual regions of interest (zones) within the sample of interest. Users can define specific zones of interest over an activity map, or an image of the sample superimposed on the activity map, allowing for independent analysis of the different "zones" (e.g., Zone 1 vs. Zone 2 of an assembloid). This allows a full analysis of individual zones for features such as network connectivity and CAT mapping which typically require whole chip analysis, enabling accurate assessment of overall activity and network responses to pharmacological modulation.

Within this application note we utilize CorePlate™ 3D and Brainwave 6 to investigate an assembloid consisting of multiple fused cortical organoids, and its response to Bicuculline (Bic). We visualize the activity of the assembloid as a whole, and divided into individual zones, showing that although the trend of the assembloid follows a general pattern of increased activity, each individual zone (each individual organoid of the fused assembloid) does not follow the same pattern of activity.

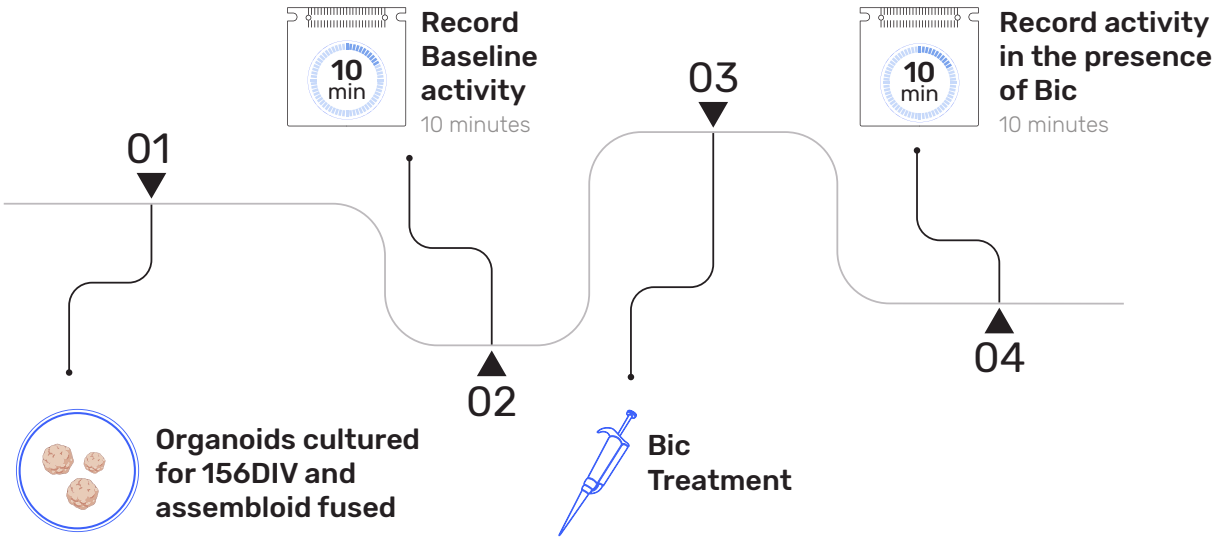

Figure 1. Experimental timeline illustrating the culture timeline, the recording periods and the dosing of the assembloid.

Methods

In order to functionally characterize the assembloid in response to Bic, the procedure shown in Fig. 1 and described below was followed.

Assembloid Culture: Human iPSC-derived brain organoids were fused to create a functional assembloid and recorded at 156 DIV.

Recording Protocol: The assembloid was positioned on CorePlate™ 1W-3D 38/60/90 and recorded according to the following timeline:

1. **Baseline:** 10 minutes of spontaneous activity was recorded.
2. **Bicuculline Treatment:** 25 µM Bic (a GABA_A receptor antagonist) was added to the media to induce disinhibition and activity was recorded for a further 10 minutes.

Analysis: BrainWave 6 was used to segment the assembloid into distinct zones based on the morphology and activity of the fused organoids.

Results

Zone Designation

To accurately characterize the functional activity of the assembloid, we first assessed whether the distinct morphological regions formed by the organoids fusion exhibited different activity patterns. As shown in Fig. 2, four main architectural regions can be seen within the structure, each displaying distinct activity levels that emerged and propagated at different time points, consistent with the underlying architecture.

To capture and analyse more accurately the activity differences within these regions, the BrainWave 6 zonal tool was used to subdivide the assembloid into four defined zones (shown as red, blue, green and yellow highlights in Fig. 2) based on both architectural features and activity patterns, enabling more precise regional analysis.

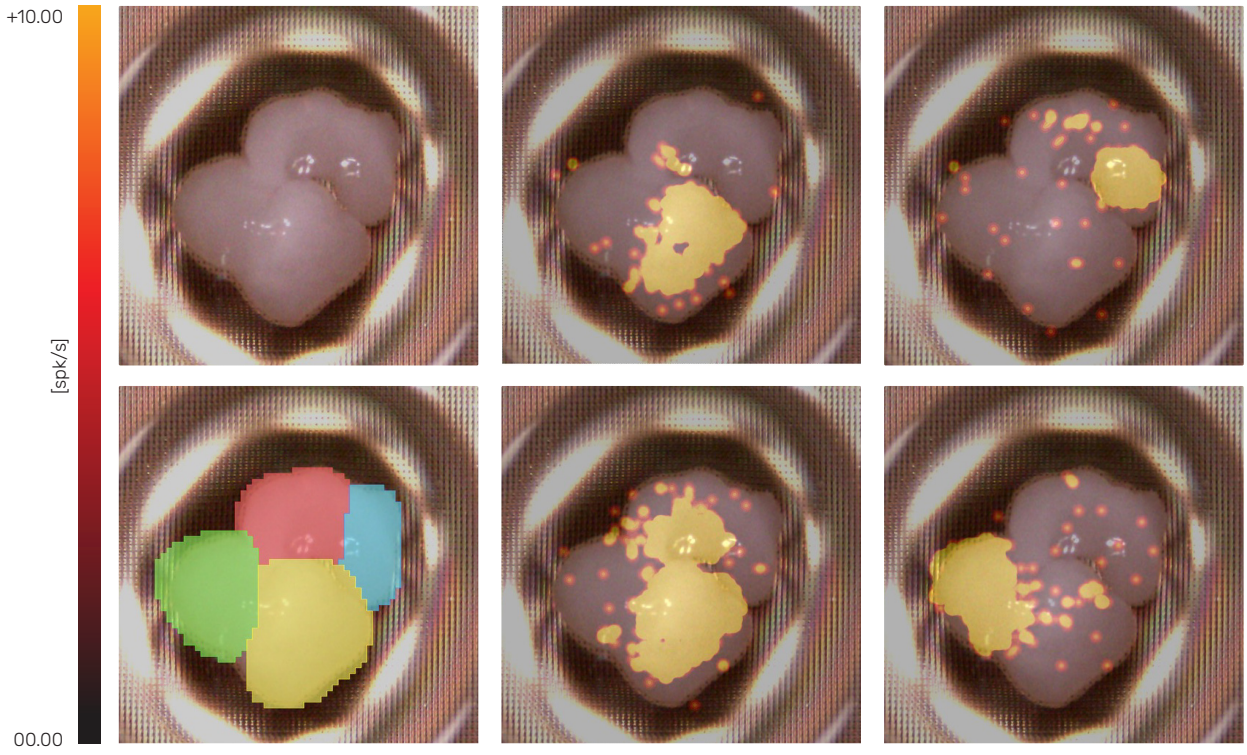

Figure 2. Visualization of activity patterns across various time points within the assembloid, and the designated zones (red, blue, yellow, green) based on the architectural features and functional activity.

Zonal Vs Whole Assembloid Activity

When the activity of the whole assembloid is visualized as a raster plot, the overall pattern appears to be coordinated (Fig. 3a). However, when examined as individual zones, clear differences in the activity can be seen both before and after Bic application (Fig. 3b).

When analysed at the whole assembloid level, firing-rate analysis showed the expected increase following bicuculline treatment, consistent with widespread disinhibition (Fig. 4a). When activity is instead analyzed within each defined zone, differences in the magnitude of these responses become evident (Fig. 4d). Some regions exhibit a pronounced elevation in firing rate, indicative of strong GABAergic modulation, whereas others show only modest changes. These variations suggest that local circuits within each zone of the assembloid retain distinct inhibitory sensitivities and baseline excitability states.

At the whole-assembloid level, neuronal burst frequency and duration increased in an expected manner with Bic-induced disinhibition (Fig. 4c, e). However, zone-specific analysis revealed variability in this bursting behaviour. Some zones exhibited large increases in burst frequency and duration, whereas others showed only subtle changes (Fig. 4d, f). These differences indicate that inhibitory control over burst initiation and termination is not uniform across the fused organoid structure. Instead, they likely reflect regional differences with local microcircuits maintaining distinct inhibitory and excitability profiles.

Zonal Vs Whole Assembloid Network Properties

When measured across the entire assembloid, network burst frequency showed a small increase following bicuculline treatment, accompanied by an increase in network burst duration (Fig. 5a, b). When analyzed at the individual zonal level, some zones displayed pronounced increases in burst frequency or duration, whereas others showed only minimal shifts (Fig. 5c, d). These differences highlight the importance of analysing activity at the regional level, as whole-assembloid measurements may mask the heterogeneity of responses.

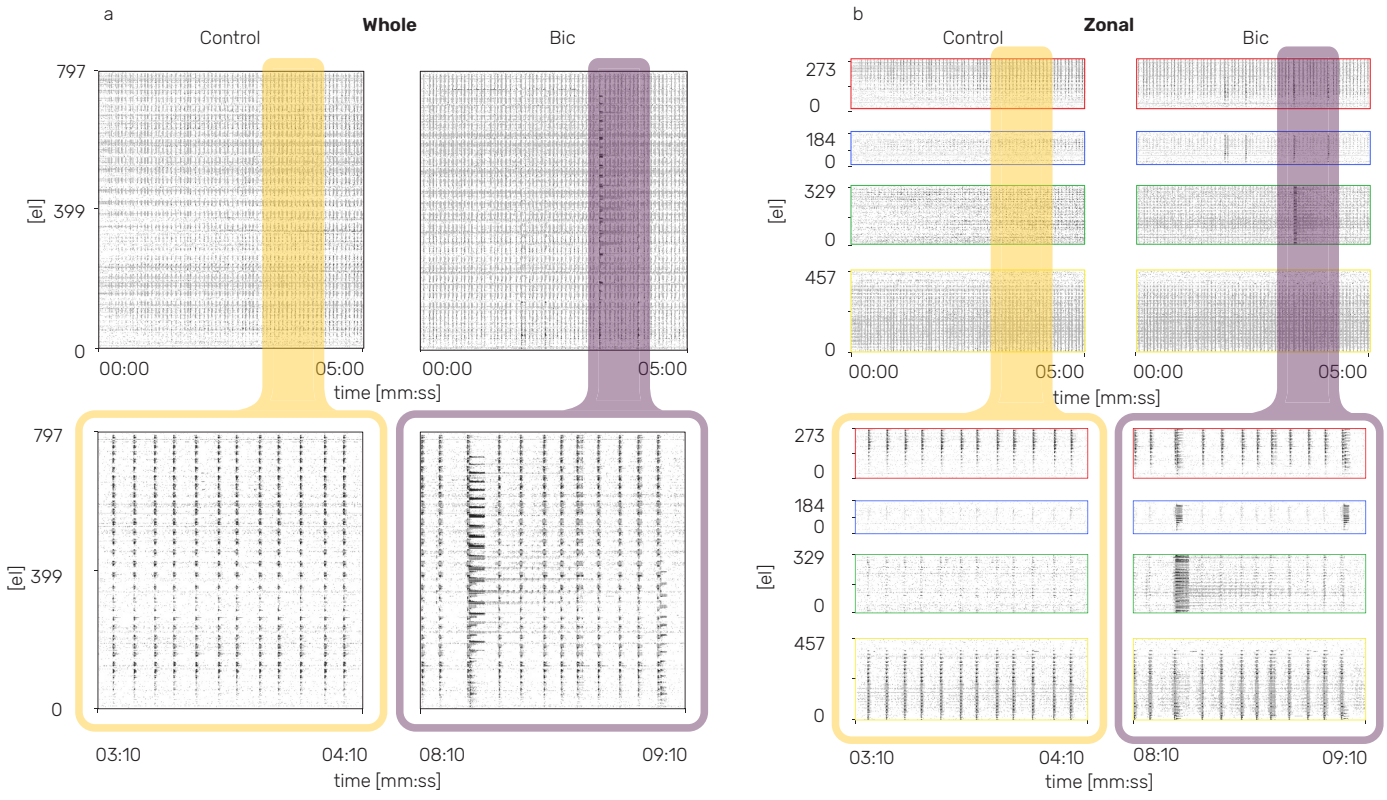

Figure 3. Raster plots showing baseline and Bic activity when investigating the assembloid as a whole (a) and in zones colour coded from Fig. 1 (b). A zoom in 1-minute window (baseline in orange, bicuculline in purple) highlights the differences between the whole assembloid activity and region-specific (zonal) activity.

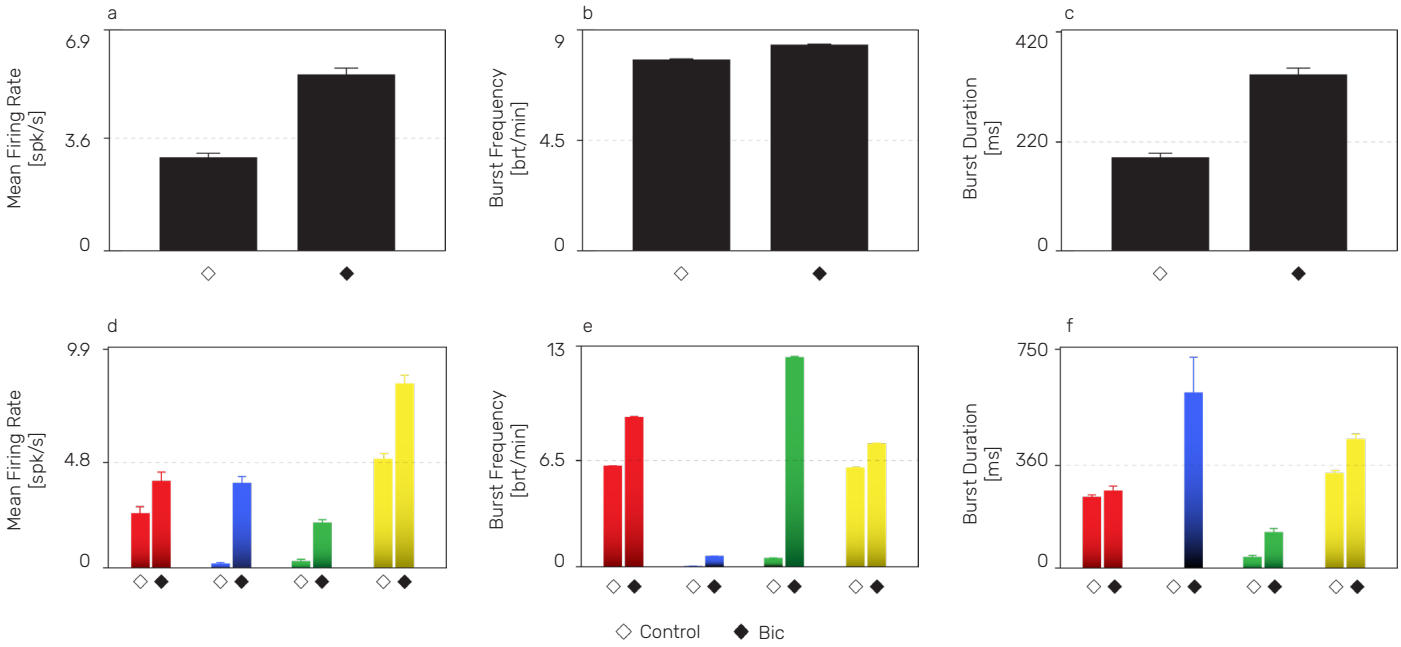

Figure 4. Metrics illustrating the differences in activity changes when analysing the assembloid as a whole (black bars) vs individual zones (coloured bars corresponding to each zone from Fig. 1). Mean firing rate (a, d). Burst frequency (b, e). Burst duration (c, f).

This highlights that individual circuits within the assembloid differ markedly in their sensitivity to inhibitory blockade and in their ability to sustain prolonged network-level events. Connectivity mapping across the entire assembloid revealed changes in the spatial distribution of connections (Fig. 6a) following Bic treatment. However, individual zonal analysis showed that certain regions underwent more substantial changes in the distribution of connections compared to others. When assessed as a whole assembloid, an overall rise in the number of links was found (Fig. 6b), whereas the zone level investigation demonstrates that these changes are not uniform across the assembloid (Fig. 6c).

Instead, specific regions appear to have had much larger network-level reorganizations than others. This pattern indicates that even though the overall connectivity of the assembloid may be influenced by inhibitory compounds such as Bic, each individual zone of the assembloid is actually shaped by a subset of highly responsive local networks associated within each fused organoid of the assembloid.

Zonal Vs Asembloid CAT Properties

Center-of-Activity Trajectory (CAT) analysis revealed changes in both trajectory patterns (Fig. 7a) and duration (Fig. 7b, c) across the assembloid following Bic treatment. However, when examined at the zone level, these effects were not uniform. While whole-assembloid CAT maps showed a more distributed and extended activity trajectory after Bic addition, zone-specific analysis uncovered substantial regional variability. Some zones developed more dispersed trajectories with markedly longer durations, whereas others exhibited smaller alterations. This heterogeneity suggests that although the whole structure is one large assembloid, the individual regions still differ in their underlying circuit architecture, leading to variable responsiveness to disinhibition despite exposure to the same compound.

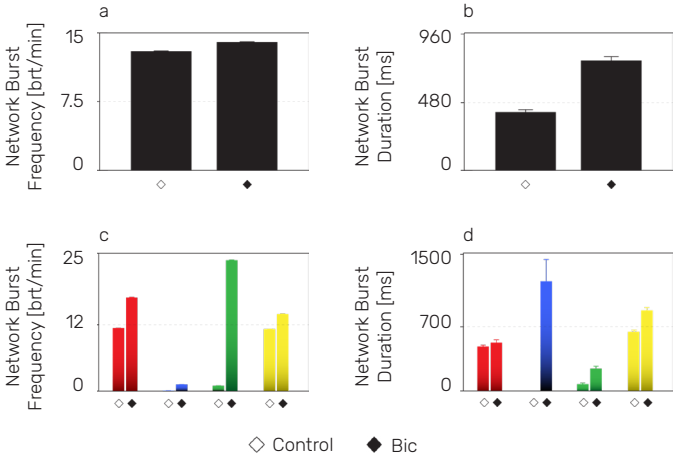

Figure 5. Metrics illustrating the differences in activity changes when analysing the assembloid as a whole (black bars) vs individual zones (coloured bars corresponding to each zone from Fig. 1). Network burst frequency (a, c). Network burst duration (b, d).

Discussion

Zonal-based analysis is a new feature in BrainWave 6 that enables precise investigation of individual regions within a sample, and enables analysis that typically requires whole-chip analyses such as network connectivity and CAT analysis to be conducted in these individual zones.

This provides a major advantage for researchers working with neuronal tissues composed of distinct subpopulations such as assembloids and brain slices where different regions may respond uniquely to the same stimulus. The tool is equally useful for wells containing multiple spheroids or organoids, as each structure can be analyzed independently on a single chip with full access to network and CAT-based readouts.

Importantly, the zonal tool is not restricted to single-well formats and can be applied across multi-well devices as well, further increasing the throughput of CorePlate™. In this application note, we utilized the Zonal Tool of BrainWave 6 to investigate an acute recording of an assembloid composed of multiple fused cortical organoids on CorePlate™ 3D, a HD-MEA featuring 4,096 3D electrodes. These electrodes penetrate into the tissue, providing enhanced signal quality and access to internal network dynamics, while the underlying microchannel architecture improves oxygenation, nutrient delivery, and compound diffusion throughout the sample (Mapelli et al.).

Application of bicuculline produced the expected disinhibitory effects at the whole-assembloid level; however, subdividing the dataset into spatially defined zones revealed a far richer and more biologically informative picture. Whole-assembloid analysis detected global increases in firing rates, bursting dynamics, network properties, connectivity, and CAT duration, but these whole measures obscured regional differences. Zone-level analysis showed that although each region generally followed the same trend, the extent of the response varied considerably across all assessed features including firing rate, burst frequency and duration, network properties, number of links, and CAT duration.

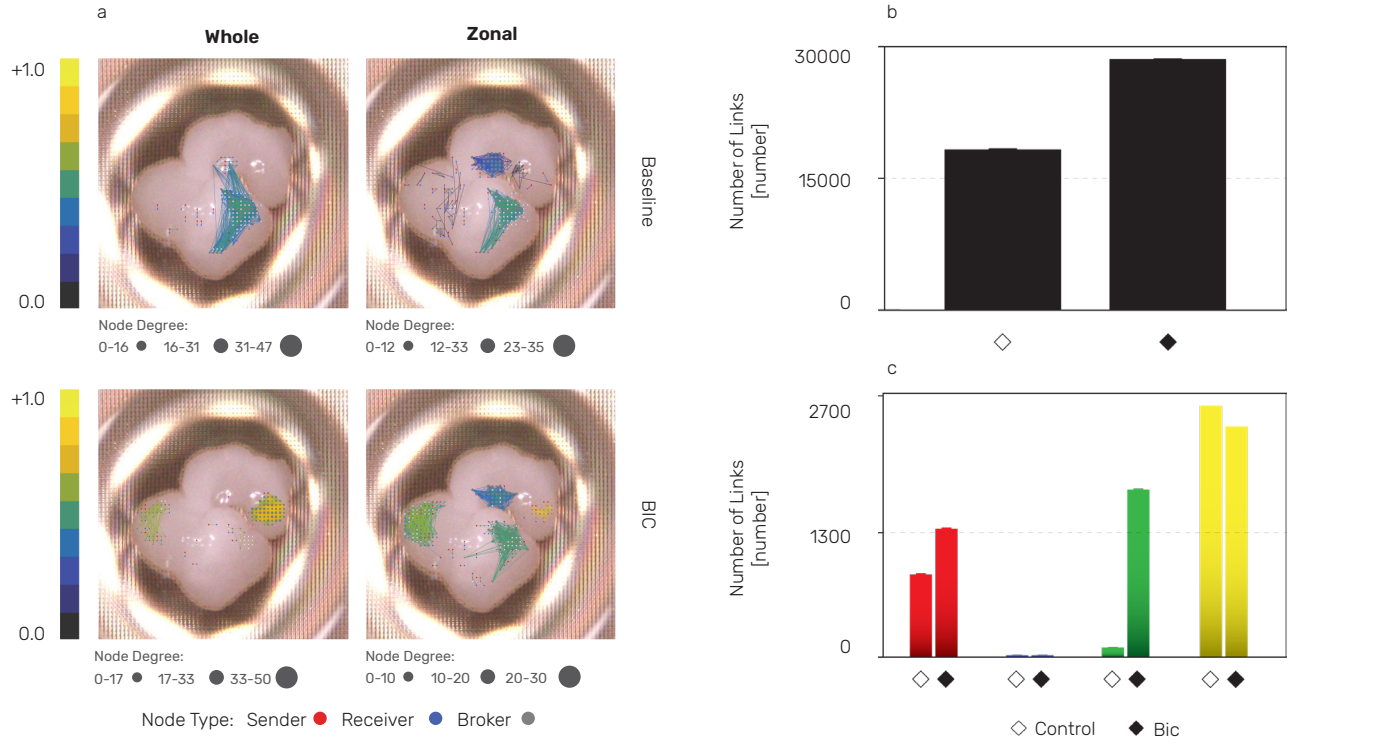

Figure 6. Network connectivity map showing the differences in the connectivity when analysing the assembloid as a whole vs individual zones (a). Metrics illustrating the differences in the number of links when analysing the assembloid as a whole (black bars) (b) vs individual zones (coloured bars corresponding to each zone from Fig. 1) (c).

These findings demonstrate that, in this system, fusing multiple organoids into a single assembloid does not eliminate their regional individuality. Instead, each zone retains distinct physiological properties potentially shaped by its cell composition, intrinsic connectivity, and maturation state differences. A feature which become apparent only when using spatially resolved zonal analysis.

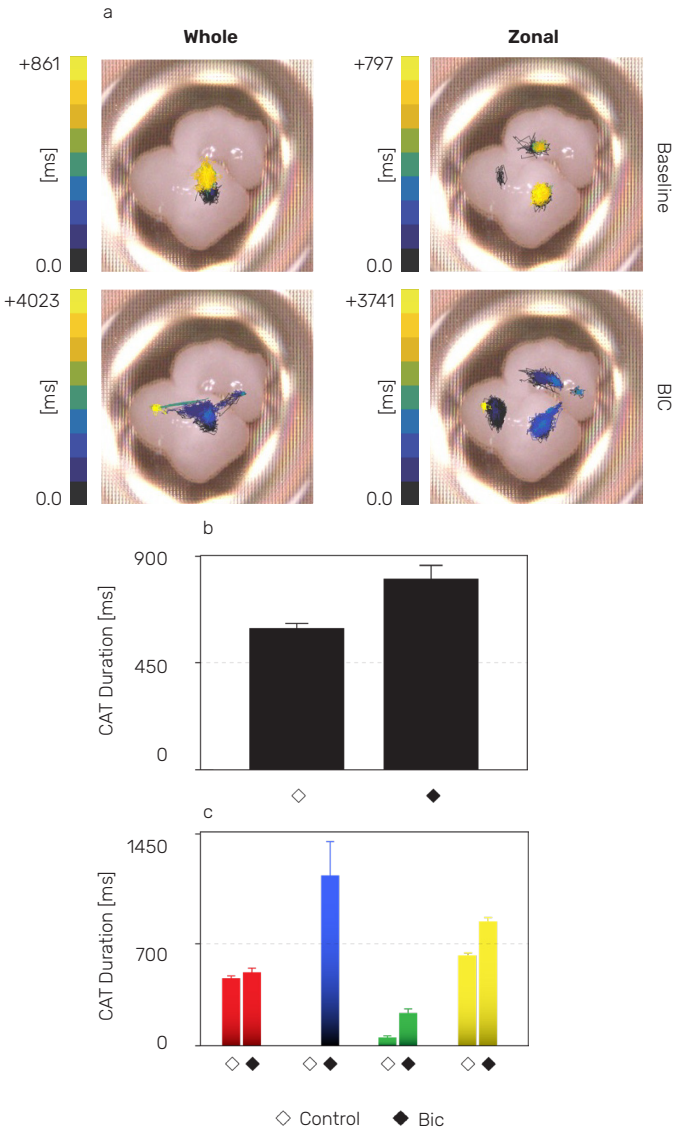

Figure 7. CAT connectivity map showing the difference in the CAT map when analysing the assembloid as a whole vs indi-vidual zones (a). Metrics illustrating the differences in the CAT map when analysing the assembloid as a whole (black bars) (b) vs individual zones (coloured bars corresponding to each zone from Fig. 1) (c).

Conclusion

Within this application note we utilized CorePlate™ 3D and Brainwave 6 to investigate an assembloid consisting of multiple fused cortical organoids, and their response to Bicuculline. CorePlate™ 3D's 4,096 penetrating electrodes and optimized microfluidics to allowed for optimal recording to be conducted from the assembloid. Complementing this hardware, the BrainWave 6 Zonal Analysis tool enabled the precise quantification of spatially distinct regions. While whole-assembloid measurements offered a useful overall view of the assembloids responses to Bic, the zonal investigation uncovered the underly-ing regional diversity in firing, bursting, network and CAT. Together, they enable accurate investigations into complex neural systems such as assembloids, making this integrated approach the ideal platform for studying complex neuronal samples such as as-sembloids, brain slices, and multi-organoid cultures.

References

- Mapelli L, Di Domenico D, Sciacca G, Mainardi F, Ottaviani A, Monteverdi A, Tedesco M, Batta-glia CR, Tritto S, Gandolfo M, Imfeld K, Kiderlen S, Krainer L, Cervetto C, Marcoli M, Sing A, Ander-sen J, Birey F, Sloan SA, Maccione A, D'Angelo E. Enhanced electrophysiological recordings in acute brain slices, spheroids, and organoids using 3D high-density multielectrode arrays. PLoS One. 2025 Sep 4;20(9):e0328903. doi: 10.1371/journal.pone.0328903. PMID: 40906696; PMCID: PMC12410755.

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