Application Note

3 Brain

Assessing Primary Cortical & Hippocampal Neurons with the HyperCAM Alpha & CorePlate™ 6W 38/60

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Abstract

High-density microelectrode arrays (HD-MEAs) are a valuable tool for capturing the complex spatio-temporal dynamics of neuronal networks with single cell resolution. As a leader in HD-MEA technology, 3Brain provides high-resolution electrophysiological platforms that allow for the simultaneous recording of thousands of electrodes in single and multi-well format. This application note demonstrates higher-throughput functional screening for primary cortical and hippocampal cultures using the HyperCAM Alpha and CorePlate™ 6W. We characterize spontaneous baseline and network-wide activity, subsequently evaluating their modulation in response to the pro-convulsant agent Cyclothiazide (CTZ). Our results demonstrate that the HyperCAM Alpha provides a precise and scalable solution for safety pharmacology and drug discovery, with increased throughput further possible with the HyperCAM Delta, which is compatible with CorePlate™ 24W and 96W.

Introduction

Primary cortical and hippocampal neurons serve as a powerful *in vitro* model. These primary cultures develop complex, two-dimensional functional networks that replicate the fundamental signalling mechanisms found in the respective regions of the mammalian brain, providing a strong platform for assessing neuronal activity, network plasticity, and functional responses to neuropharmacological agents. A critical application of these neuronal models is the assessment of acute electrophysiological responses to pharmacological stimulation. This approach offers more in depth investigations into neuronal function, and network-effects. By utilizing these primary cells, researchers can evaluate the effect of a known, or novel drug on mature synaptic connections, offering a reliable standard for investigating neuropharmacological compounds and improving safety pharmacology.

Electrophysiological monitoring provides critical insights into the function and maturity of neuronal cultures. High-Density Microelectrode Arrays (HD-MEAs), such as CorePlate™, represent the gold standard for this analysis, offering label-free recordings that combine single-cell resolution with network-wide accuracy. The HyperCAM Alpha is a state-of-the-art, 6-well HD-MEA platform specifically engineered for higher-throughput applications. Compatible with CorePlate™ 6W, the system leverages 2,304 simultaneously recording bidirectional electrodes per well, ensuring highly accurate investigations of electrogenic biological samples. The high density properties allow for large datasets to be collected from the intricate spike waveforms of thousands of individual neurons, allowing the coordinated spatio-temporal dynamics of the network to be assessed.

By providing unparalleled precision across multiple wells simultaneously, this technology facilitates deeper insights into the functional states of primary neuronal cultures, making it an ideal tool for complex disease modelling and pharmacological screening.

This application note demonstrates how CorePlate™ 6W and the HyperCAM Alpha facilitate the evaluation of the functional activity of primary hippocampal and cortical neurons and their response to Cyclothiazide (CTZ), a positive allosteric modulator of AMPA receptors, and a GABA-A antagonist. We characterize the overall activity and network dynamics in baseline conditions and in response to CTZ, demonstrating that while CTZ successfully induces an epileptiform phenotype in both cell lines, the resulting network reorganizations are fundamentally distinct. Furthermore this application note highlights how the multi-well capabilities of CorePlate™ 6W and the HyperCAM Alpha can help to streamline experimental workflows, accelerate neuropharmacology studies and drug discovery. These capabilities can be further enhanced utilizing the HyperCAM Delta and CorePlate™ 24W & 96W.

Methods

Cells, Culture Method & Reagents

CorePlate™ 6W was coated with 100 µg/mL poly-ornithine. Following this, primary rat postnatal hippocampal and cortical cells were suspended in neurobasal medium at a density of 1000 cells/µL. 80 µL was then seeded directly onto the active area of each well. When the cells were adhered, 500 µL of neurobasal was added. After 24 hours, the culture volume was brought up to 2 mL by adding additional neurobasal medium. At DIV5, neurobasal was completely

replaced with BrainPhys™ and recordings performed at DIV14.

HD-MEA

The HyperCAM Alpha and CorePlate™ 6W 38/60 were utilized to record extracellular electrophysiological activity from the primary neuronal cultures. BrainWave 6 was the software used to record and analyse the data.

Experimental Overview

To functionally characterize the primary cultures, the following procedure was followed (as shown in Fig. 1):

1. Cell Seeding and Maturation: Primary hippocampal neurons were seeded into the wells of Row A, while primary cortical neurons were seeded into Row B of CorePlate™ 6W. The cultures were maintained in an incubator for 14 days, allowing for the development of mature synaptic connections and robust network-wide synchronized bursting.
2. Baseline Activity: Following the maturation period, CorePlate™ 6W was placed into the HyperCAM Alpha. A 5-minute baseline recording was performed across all wells simultaneously to establish the baseline activity and network characteristics of the cultures.
3. Pharmacological Modulation: The middle well (A2 & B2) of both cultures were then acutely dosed with 3 μM Cyclothiazide (CTZ) to induce hyperexcitability.
4. Activity in the presence and absence of CTZ: Immediately following treatment, a subsequent 5-minute recording was captured to quantify the drug-induced modulation of neuronal activity and network synchrony.

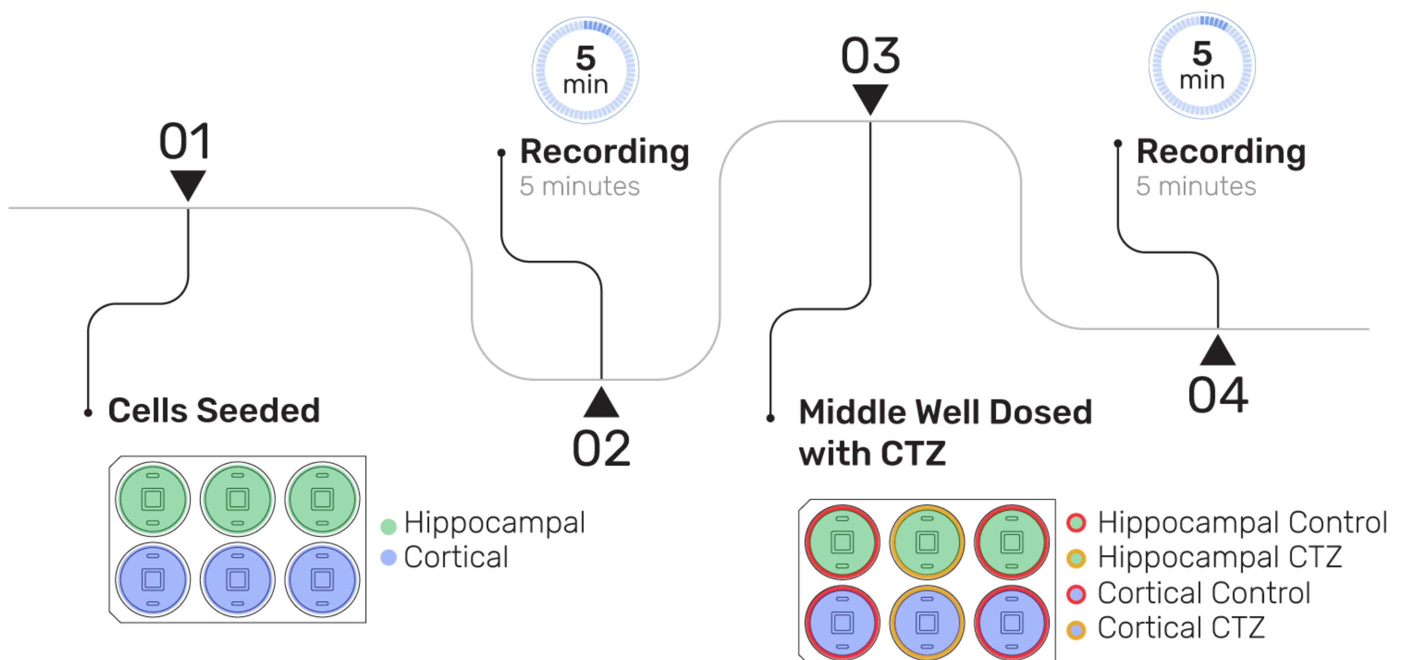

Figure 1. Experimental timeline illustrating the spatial arrangement of the hippocampal and cortical cultures within CorePlate™ 6W, the scheduled recording intervals, and the administration of CTZ.

Results

Hippocampal and cortical neurons display different baseline activity profiles

Hippocampal and cortical neuronal cultures exhibited distinct baseline firing profiles, as illustrated in the raster plots (Fig. 2). While the hippocampal cultures were characterized by highly synchronized, intermittent network bursts at regular intervals, the cortical cultures displayed a more continuous, stochastic firing pattern with more random burst periods. In the zoomed-in panel, the differences between the network bursts (highlighted in red) can be visualized more easily. When quantified, it is apparent that the different neuronal cultures display different baseline activity properties, with a higher MFR found in hippocampal neurons (Fig. 3a), and a larger, more distributed inter-spike-interval coefficient of variation (ISI-CoV) (Fig. 3b) as a result of the stronger changes in firing rate associated with the bursting & quiet periods of activity.

Hippocampal neurons also exhibited a higher burst frequency (Fig. 3c) and longer burst durations (Fig. 3d) compared to cortical neurons.

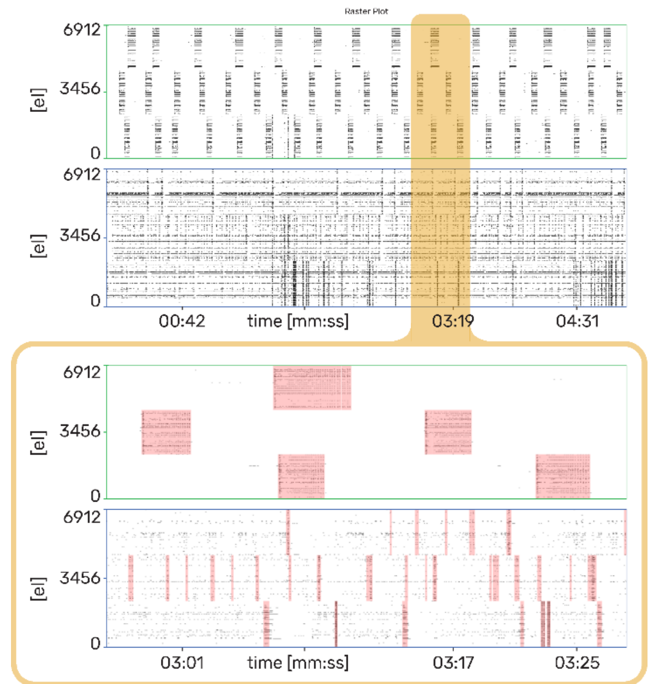

Figure 2. Raster plot showing the baseline activity of hippocampal (green) and cortical (blue) cells. A zoom-in panel (orange) highlighting a 30-second period containing neuronal network bursts (red).

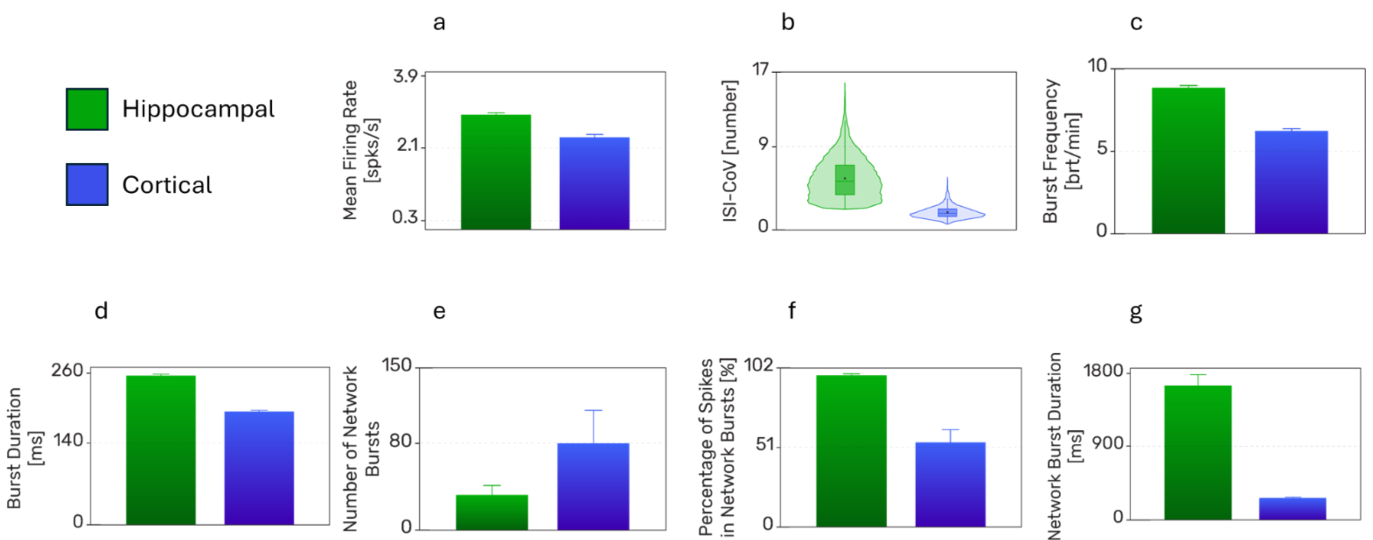

Figure 3. Firing metrics of the baseline activity of hippocampal (green) and cortical (blue) cultures. Mean firing rate (a), inter-spike-interval coefficient of variation (b), burst frequency (c), burst duration (d), number of network bursts (e), percentage of spikes in network bursts (f), network burst duration (g).

Conversely, the total number of network bursts was lower in the hippocampal cultures than in the cortical cultures (Fig. 3e). This lower number of network bursts was offset by a much higher higher percentage of spikes participating in network bursts (Fig. 3f), and an increased network burst duration (Fig. 3g). These dynamics are reflected in the raster plots shown in Fig. 2, which illustrate that hippocampal network bursts, while less frequent, are more sustained and recruitment-heavy than the shorter, more frequent bursts seen in cortical lines.

CTZ alters the network activity of both hippocampal and cortical neuronal cultures

Following the addition of CTZ to the central wells, disruption of the regular baseline activity is evident in the raster plots of both cell lines as shown in Fig. 4. The zoom in of Fig. 4 shows that CTZ affected the regular network activity with what appears to be more frequent and shorter duration network bursts (highlighted in red) in both hippocampal and cortical cultures compared to the surrounding control wells. Analysis of the network bursting properties revealed a large increase in the number of network bursts in the hippocampal culture (Fig. 5a, green), with a relatively similar % of spikes in network bursts (Fig. 5b, green) and a much shorter duration (Fig. 5c, green).

This is likely due to the change from long duration network bursting typical of hippocampal neuronal cultures to a more epileptiform phenotype with increased short frequency network bursts.

When looking at the Cortical cultures, we can see a slightly different trend, with an increase (albeit less strong) in the number of network bursts (Fig. 5a, blue), an increase in the

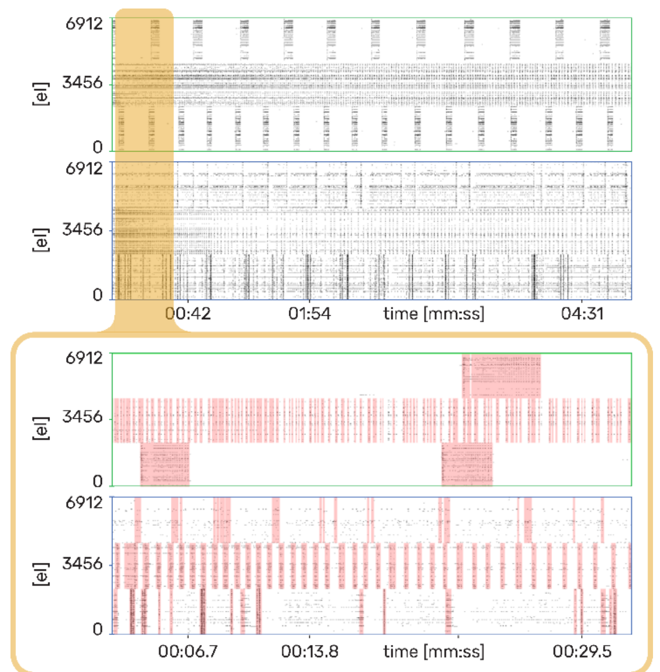

Figure 4. Raster plot showing the activity of hippocampal (green) and cortical (blue) cells in which the central well has been treated with CTZ. A zoom in panel (orange) highlighting a 30 second period containing neuronal network bursts (red).

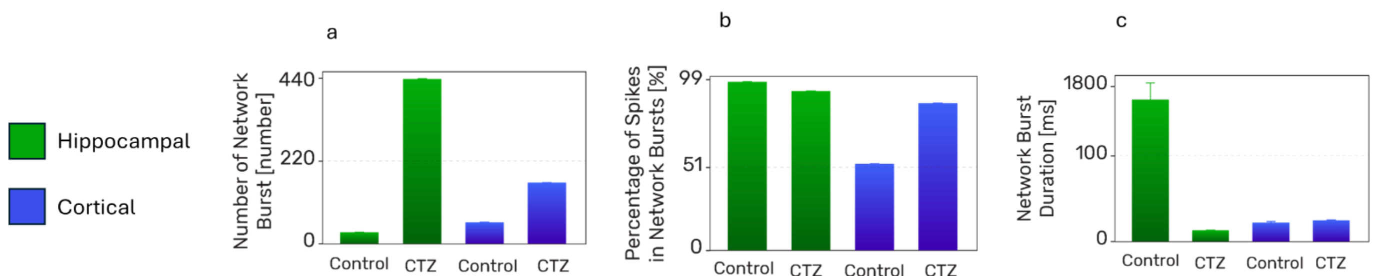

Figure 5. Network activity of hippocampal (green) and cortical (blue) cultures in the presence & absence of CTZ. Number of network bursts (a), percentage of spikes in network bursts (b), network burst duration (c).

percentage of spikes in network bursts (Fig. 5b, blue) and little to no change in the duration of network bursts (Fig. 5c, blue). As with the hippocampal culture, an increase in the number of network bursts was found, however, since cortical cultures naturally exhibit short frequency network bursts, it is not surprising to see less of a change in the duration of these network bursts with CTZ applied. Interestingly the increase in the number and regularity of the network bursts was associated with an increase in the % of spikes in network bursts, indicating that CTZ enforced coordinated activity.

Network connectivity is altered with the addition of CTZ

The network properties of both cell lines were altered with the addition of CTZ, albeit in different ways.

Hippocampal networks exhibited a strong reorganization of functional connectivity upon CTZ application, characteristic of an induced epileptiform phenotype. The correlation map (Fig. 6a, b) showed an expansion in the functional interconnectivity of the culture, evidenced in the analysis by an increase in the total number of links (Fig. 6c). Interestingly, this was accompanied by a decrease in average spike cross-correlation (Fig. 6d), suggesting that although there were more links, the overall correlation decreased. This was accompanied by an increase in the rich club effect (Fig. 6e). This

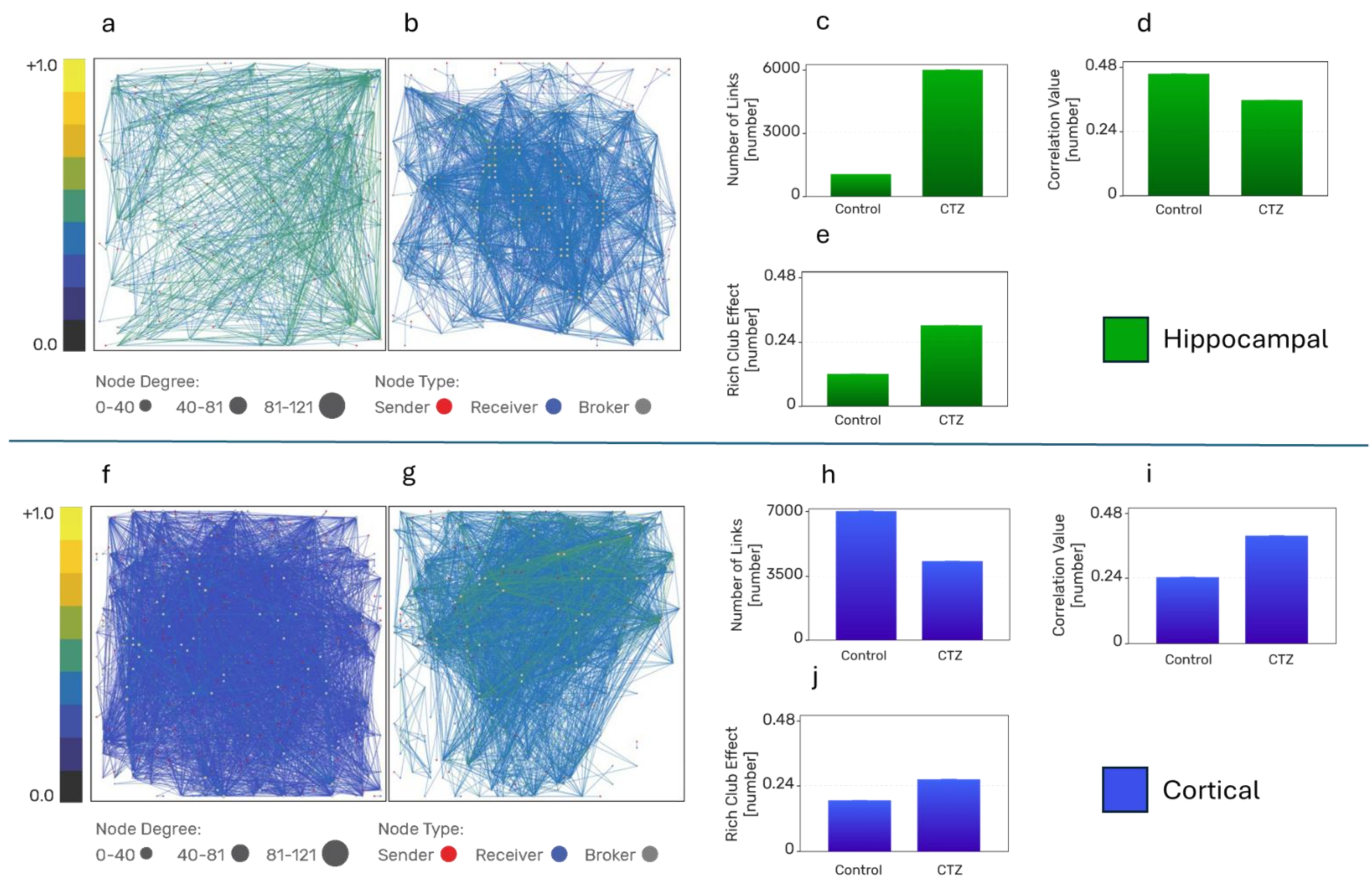

Figure 6. Network connectivity metrics of hippocampal (green) and cortical (blue) cultures in the presence & absence of CTZ. Hippocampal cells: Network connectivity map under control conditions (a) and CTZ (b), number of links (c), correlation value (d), rich club effect (e). Cortical cells: Network connectivity map under control conditions (f) and CTZ (g), number of links (h), correlation value (i), rich club effect (j).

increased rich-club effect indicates that a select sub-population of highly connected nodes has taken control of network dynamics, driving the higher frequency bursts.

Interestingly cortical networks demonstrated a different transition toward an epileptiform phenotype upon CTZ application compared to hippocampal cells as shown in the correlation map (Fig. 6f, g), the network underwent a large decrease in the number of functional connections, marked by a large decrease in the number of links (Fig. 6h). However, the remaining connections exhibited an increase in correlation (Fig. 6i), suggesting that the network has shifted from a distributed state into a collection of more synchronized clusters indicated by the increase in the rich club effect (Fig. 6j).

Centre of Activity Trajectory is altered with the addition of CTZ

The Centre of Activity Trajectory (CAT) revealed distinct spatio-temporal reorganizations in both cell lines following CTZ application.

In hippocampal cultures, a strong reduction in CAT duration was observed (Fig. 7c) which is reflected in the CAT maps (Fig. 7a, b). This indicates the induction of an epileptiform phenotype where CTZ has caused AMPA receptor desensitization and GABAergic inhibition, inducing short network bursts as seen in Fig. 4. Consequently, the trajectory no longer takes its normal, longer propagation of activity, instead, it turns into a short trajectory that mirrors the reduced duration of these network bursts.

In contrast, cortical cells displayed a much smaller change in CAT with CTZ application (Fig. 7d, e, f). This small shift reflects the intrinsic properties of cortical circuits, which intrinsically possess shorter, more frequent network bursts compared to their hippocampal counterparts.

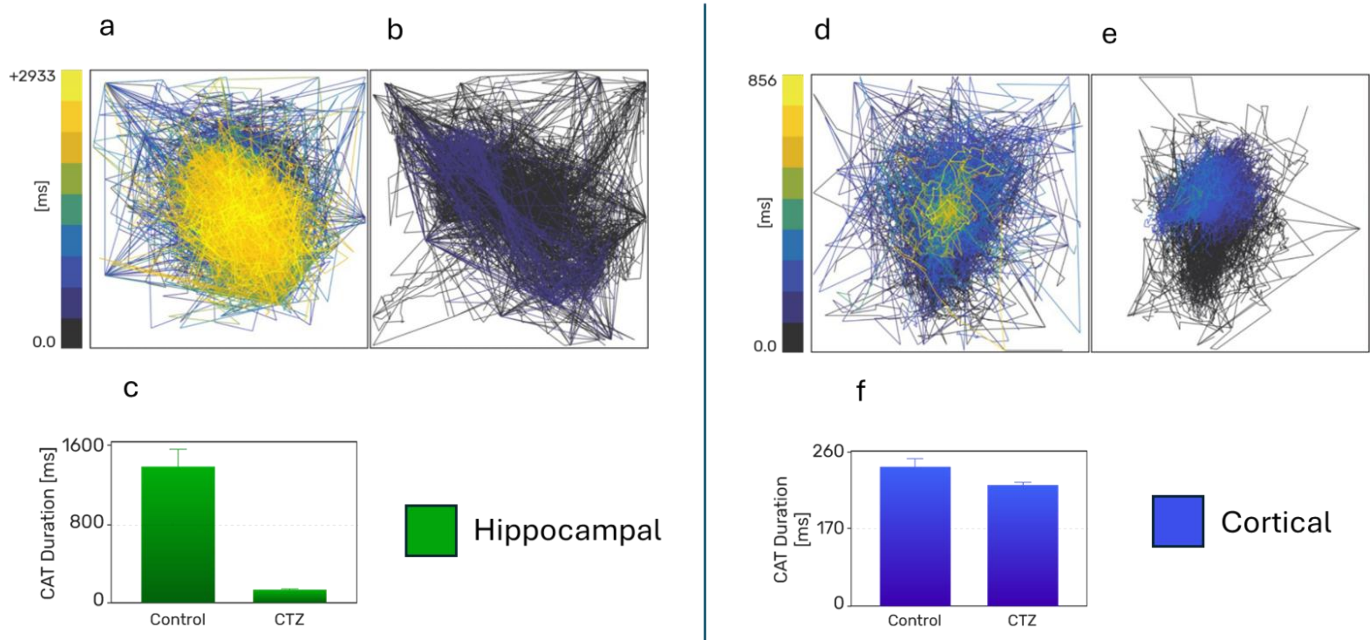

Figure 7. CAT metrics of hippocampal (green) and cortical (blue) cultures in the presence & absence of CTZ. Hippocampal cells: CAT map under control conditions (a) and CTZ (b), CAT duration (c). Cortical cells: CAT map under control conditions (d) and CTZ (e), CAT duration (f).

Because of these shorter network bursts, the time window for propagation is already small, consequently, the application of CTZ and induction of an epileptiform phenotype causes a smaller reduction in CAT duration compared to hippocampal CATs.

Discussion

The functional differences between different neuronal cultures such as hippocampal and cortical neuronal cultures as shown here can be readily and accurately studied utilizing high-density microelectrode arrays such as CorePlate™. The thousands of simultaneously recording electrodes along with the size and spacing of the electrodes are optimized for single cell recording and network wide analysis of neuronal cultures. Allowing accurate analysis of individual cells and network wide activity to contribute to characterization of different cultures and their responses to neuroactive compounds.

The differing responses of hippocampal and cortical cultures to Cyclothiazide (CTZ) reveal that a network's reaction to neuroactive compounds is dictated by its baseline activity. By using the CorePlate™ 6W HD-MEA, we were able to move beyond simple firing rates and further investigate how hippocampal and cortical neurons are induced into an epileptic state on a network basis.

In hippocampal cultures, the induced epileptiform state is characterized by a global transition to higher frequency network bursts. The application of CTZ, by preventing AMPA receptor desensitization and blocking GABA-A inhibition, intensifies the regularity of the naturally recurrent network bursts and reduces the duration. This resulted in an expansion of the functional connectivity, evidenced by an increase in the number of links and accompanied by a decrease in overall correlation alongside the formation of a dominant rich club architecture. The faster recruitment of the entire network also lead to a reduction in the CAT duration as the signal propagation changed from a gradual trajectory to a shorter duration trajectory.

Conversely, as the cortical cultures inherently possess short, irregular network bursts the response to CTZ was less dramatic than that of the hippocampal cells. Characterised by a smaller increase in the number of network bursts and little change in the duration, and interestingly higher recruitment into the network bursts, with a higher % of spikes in network bursts. This was reflected in the network connectivity whereby the cortical network underwent a reduction in the total number of links with an increase in the correlation and rich club effect suggesting CTZ induces stronger, more regular synchrony. Furthermore, the CAT duration in cortical cultures exhibited different responses to CTZ, since these networks possess naturally shorter network bursts, the induction of an epileptiform phenotype did not reduce the CAT properties as drastically as it did for the hippocampal cell line.

Conclusion

In conclusion, this study demonstrates that CorePlate™ 6W delivers the spatio-temporal resolution necessary to distinguish between specific brain region neuronal models such as hippocampal and cortical cultures, and their specific responses to neuropharmacological agents. While CTZ induces epileptiform activity in both cultures, CorePlate™ is able to accurately elaborate on the differing network changes induced by CTZ. Furthermore, the 6-well format enabled the simultaneous characterization of both cell lines and their responses, streamlining experimental workflows. This scalable approach to HD-MEA recording provides a powerful foundation for high-throughput

neuropharmacology, with the capacity for further expansion utilizing CorePlate™ 24W and 96W formats, compatible with HyperCAM Delta.

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