Application Note

3Brain

Functional Screening of Primary Rat Hippocampal Neurons in Response to NBQX and AP5 with the HyperCAM Delta and CorePlate™ 24W

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Abstract

High-Density Microelectrode Arrays (HD-MEAs) represent the gold standard for capturing neuronal network activity with single-cell resolution in a label-free format. Here, we demonstrate the functional screening of primary rat hippocampal neurons with the HyperCAM Delta in combination with the multi-well HD-MEA, CorePlate™ 24W. We characterized baseline activity and assessed pharmacological responses to increasing concentrations of the glutamate receptor antagonists NBQX and AP5. Our results demonstrate the ability of the HyperCAM Delta and CorePlate™ 24W to deliver robust, scalable, and high-throughput electrophysiological screening with simultaneous recording across all wells and electrodes, establishing a powerful platform for safety pharmacology and drug discovery applications.

Introduction

Primary rat hippocampal neurons serve as a robust *in vitro* model for investigating neuronal function, network synchrony, and pharmacological modulation. When cultured, these neurons develop complex, interconnected networks that exhibit synchronized bursting activity primarily driven by glutamatergic signalling. A key application of this in vitro model is the potential for screening compounds and obtaining translatable functional data regarding their responses.

High-Density Microelectrode Arrays (HD-MEAs) are a valuable tool for functional neuronal screening, enabling label-free electrophysiological analysis with unparalleled spatial and temporal resolution. Advanced HD-MEA platforms such as the HyperCAM Delta, together with multiwell HD-MEA formats like CorePlate™ 24W enable accurate and efficient high-throughput functional screening of neuronal cells. With 1,024 electrodes per well and simultaneous acquisition across the full array, this system enables rapid and accurate electrophysiological recording with single cell resolution and network-level analysis in every well. This combination makes the platform ideally suited for high-content neuropharmacology screening and complex dose-response studies, with CorePlate™ 96W providing the next step in throughput.

In this application note, we demonstrate how CorePlate™ 24W recordings enable functional quantification of primary rat hippocampal cultures in response to cumulative dosing with NBQX and AP5. By inhibing glutamatergic signalling, we show that CorePlate™ 24W enables an accurate assessment of the reduction in neuronal activity and network dynamics induced by these antagonists.

Methods

Cell Culture:

CorePlate™ 24W wells were coated with Poly-L-Ornithine 100µg/ml (Sigma-Aldrich, A-004-C).

Primary rat hippocampal neurons were dissociated and seeded at a density of 2,500 cells/mm^2 (25,000 cells in a 25 µl droplet) directly onto the active area of each well. Cultures were maintained in B-27™ Plus Neuronal Culture System (Thermo Fisher, A3653401). Recordings were performed at DIV17.

HD-MEA Recording:

Extracellular activity was recorded using the HyperCAM Delta platform with CorePlate™ 24W. Simultaneous acquisition from all wells and electrodes allowed for rapid monitoring of pharmacological conditions. Data were recorded and analyzed using BrainWave 6 software.

Experimental Design:

A dose-response paradigm was implemented across the 24-well plate.

Baseline recording: A 5-minute recording was acquired to establish spontaneous activity, including firing rate and network bursting.

Compound application & recording: Wells were treated with increasing concentrations of a combination of NBQX (AMPA receptor inhibition) & AP5 (NMDA receptor inhibition) and recorded for 5 minutes with each concentration:

- 30 nM NBQX & 3 µM AP5
- 100 nM NBQX & 10 µM AP5
- 300 nM NBQX & 30 µM AP5
- 1 µM NBQX & 50 µM AP5
- 13 µM NBQX & 100 µM AP5

Results

Baseline activity

Across all 24,576 electrodes and the whole 24 well plate, the hippocampal cultures displayed spiking activity with periods of stronger bursting (Fig. 1a).

When taking a closer look at a single well (1,024 electrodes), the denser activity showing the bursting periods can be more clearly seen (Fig. 1b).

The individual spikes and bursts can be more clearly seen on a further zoom of a select few electrodes of the raster plot (Fig. 2).

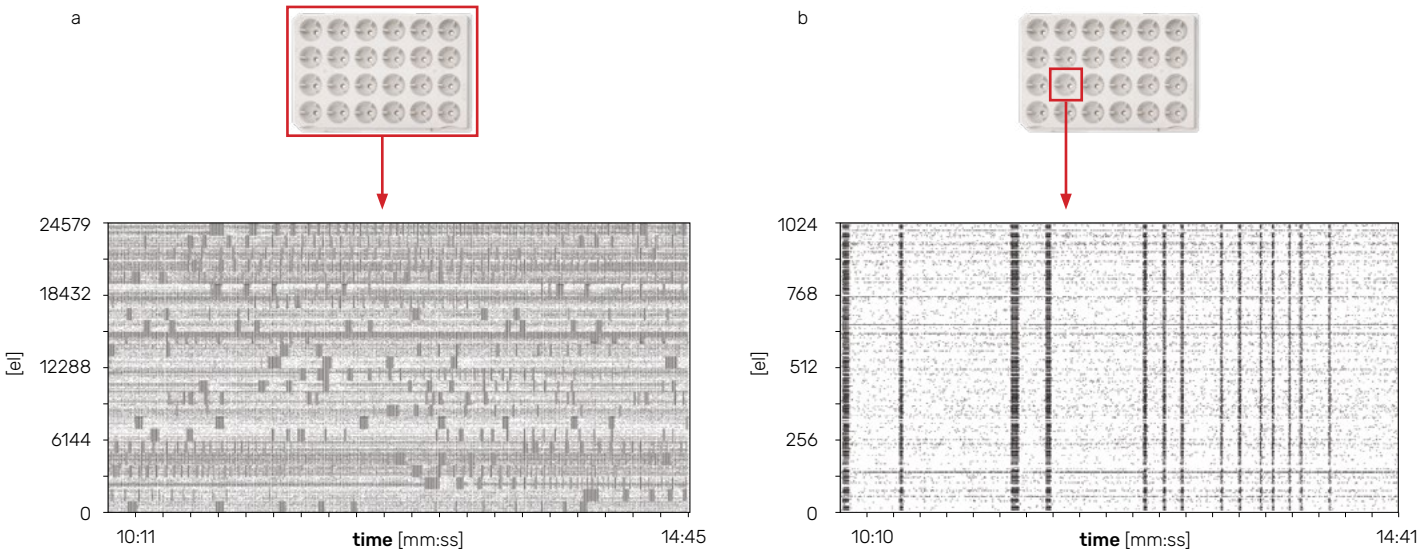

Figure 1: Overview of baseline activity shown as raster plots from a 5-minute recording of the full 24-well plate (a) and an individual well (b), illustrating the spontaneous activity of primary hippocampal neurons.

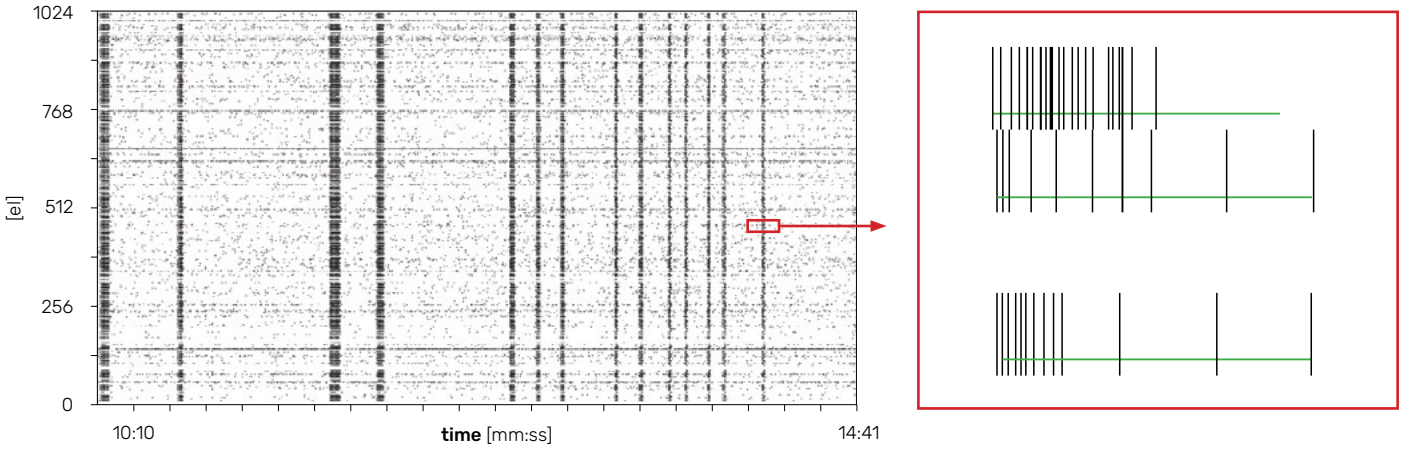

Figure 2: Zoom in of the single well raster plot showing individual neuronal spiking and bursting activity of primary hippocampal neurons.

Dose response of firing metrics and bursts

Following baseline characterization, a clear dose dependent modulation of activity was observed across the entire plate upon combined application of NBQX and AP5, with a progressive reduction in spiking activity, as illustrated in the activity maps (Fig. 3a - f). When quantified, the increasing concentrations caused a progressive reduction in the mean firing rate (Fig. 3g), accompanied by a decrease in burst frequency (Fig. 3h) and the percentage of spikes participating in bursts (Fig. 3i).

This gradual transition from highly active, synchronized bursts to quieter neuronal states reflects the critical role of combined AMPA- and NMDA-receptor–mediated transmission in sustaining hippocampal network excitability, as excitatory synaptic signalling is increasingly suppressed, the result leads to a reduced state of activity.

Importantly, the large number of simultaneously recording electrodes enabled highly consistent measurements with minimal variability across wells, highlighting the power of simultaneous 24-well HD-MEA recordings to resolve dose-response effects with high precision at the single-cell level.

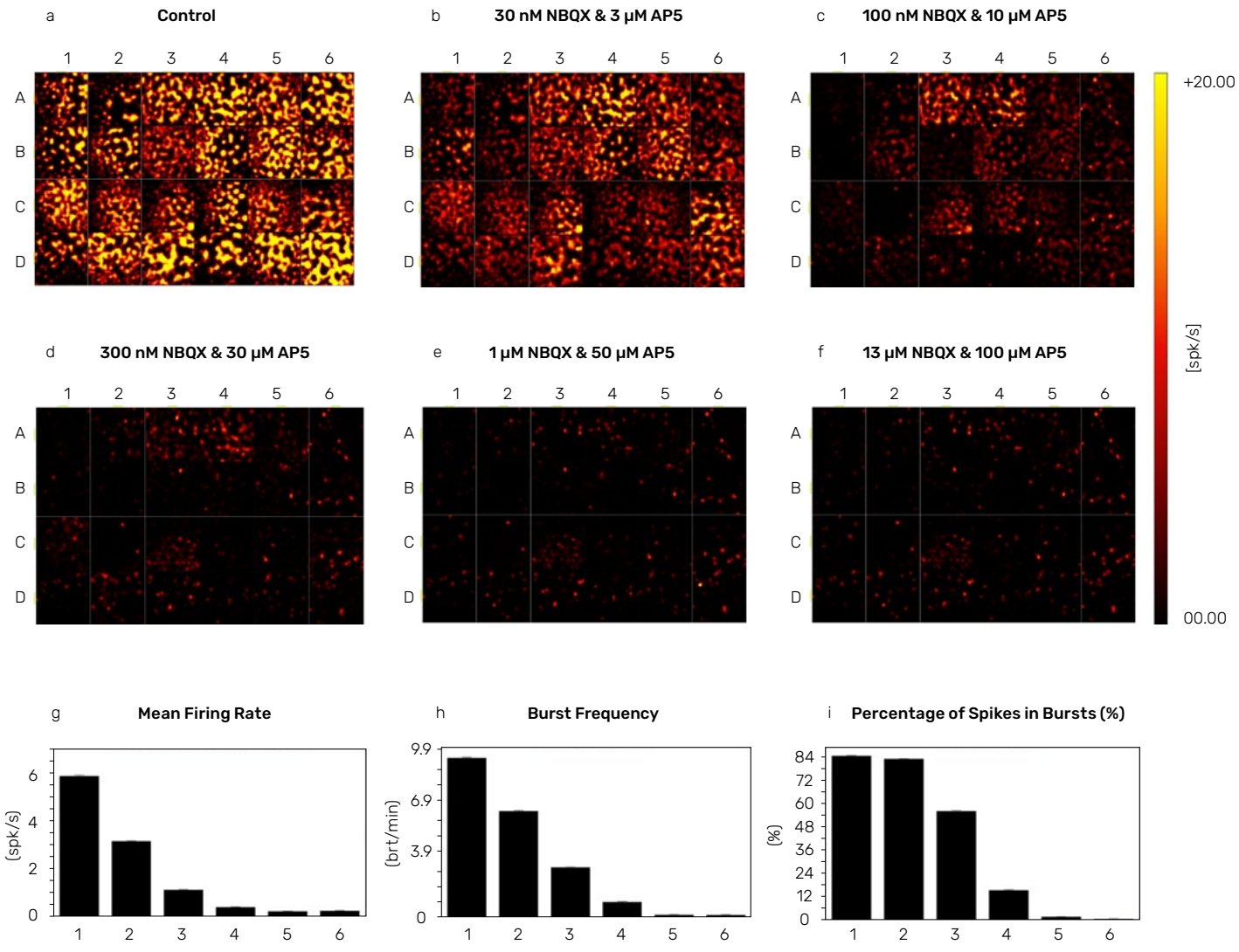

Figure 3: Dose-dependent modulation of neuronal activity across the full plate following combined application of NBQX and AP5. Activity maps (a–f) show a progressive reduction in spiking activity with increasing concentrations. Quantification reveals a corresponding decrease in mean firing rate (g), burst frequency (h), and the percentage of spikes participating in bursts (i).

Dose response of network metrics and bursts

Network bursting is a defining feature of hippocampal activity, serving as a key indicator of functional connectivity and maturity within the *in vitro* hippocampal cultures. As such, it provides a highly informative readout of how neuroactive compounds can impact network behavior.

Network bursts were detected across the full 24-well plate and all 24,576 electrodes (highlighted in red) (Fig. 4a). When taking a closer look at a single well (1,024 electrodes), these bursts could be observed in greater detail (Fig. 4b). This highlights the ability of CorePlate™ 24W to reliably detect network bursts across the whole plate, enabled by the HD-MEA integrated in each well.

The changes in network bursting in response to NBQX and AP5 can be clearly seen across the whole plate (Fig. 5a) as both the frequency and duration of network bursts decrease in the raster plots. This effect is confirmed when quantified, with a reduction in network burst frequency (Fig. 5b), network burst duration (Fig. 5c) and number of spikes per network burst (Fig. 5d).

This reduction of network activity reflects the combined blockade of AMPA and NMDA receptors, which disrupts excitatory synaptic transmission. As a result, the ability of the network to initiate, sustain, and propagate synchronized bursting is impaired.

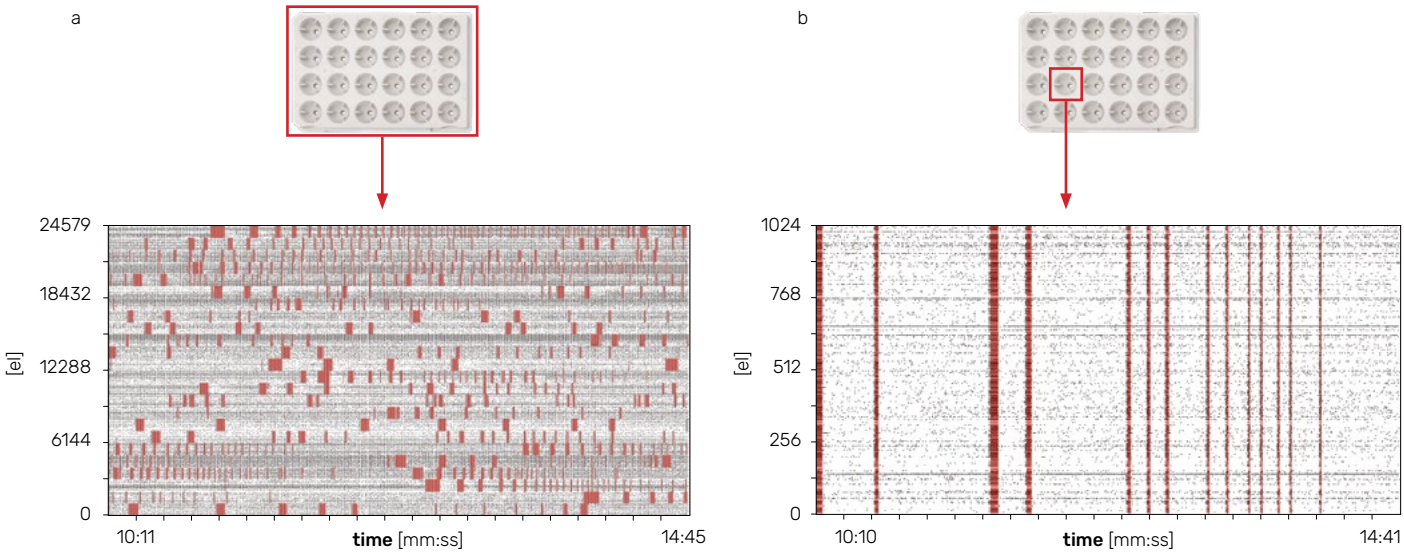

Figure 4: Overview of baseline network bursts, highlighted in red, over raster plots from a 5-minute recording of the full 24-well plate (a) and a representative single well (b), illustrating spontaneous network bursting activity in the primary hippocampal cultures.

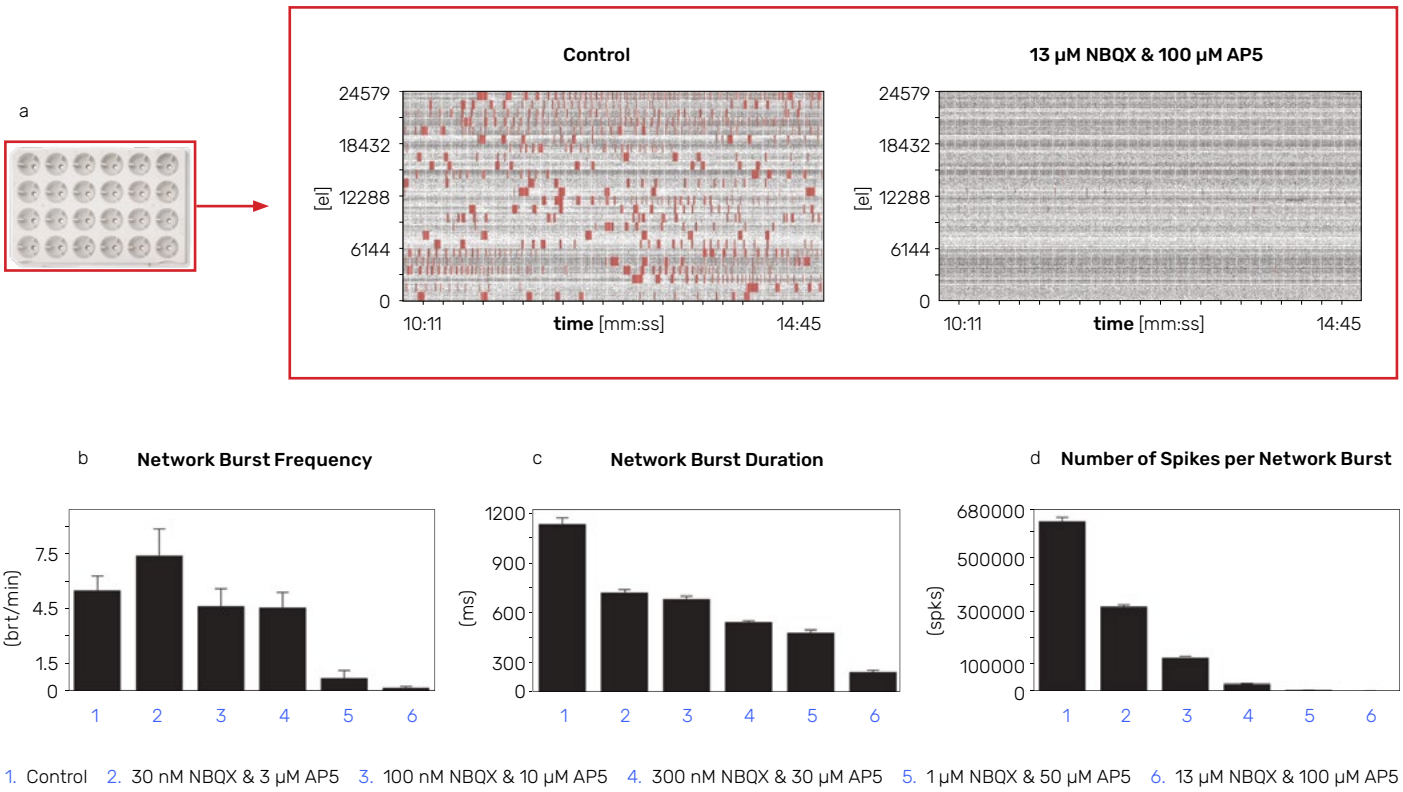

Figure 5: Dose-dependent modulation of network bursting activity across the full plate following combined application of NBQX and AP5. Raster plots (a) show the decrease in the network bursting in baseline compared to the highest concentration of NBQX & AP5. Quantitative analysis of the reduced network burst frequency (b), network burst duration (c), and the number of spikes per network burst (d).

Network connectivity is a powerful feature enabled by HD-MEA technology, providing a detailed view of how neurons communicate across a culture. It serves as a strong indicator of network maturity and offers a sensitive readout of how neuroactive compounds affect functional connectivity.

By leveraging the HD-MEA embedded in every well, connectivity can be assessed across the entire plate. Under baseline conditions, strong and well-distributed connectivity can be observed in every well (Fig. 6a). As the concentration of NBQX and AP5 increases, a progressive breakdown in connectivity becomes apparent (Fig. 6b), culminating in a near-total loss of functional links at the highest concentration (Fig. 6c).

Quantitative analysis of the number of links reveals a pronounced drop between 100 nM NBQX / 10 µM AP5 and 300 nM NBQX / 30 µM AP5. Notably, this shift occurs slightly later than the initial reduction in single-cell firing rates seen in Fig. 4b, suggesting that the network may be able to compensate slightly before the concentration becomes too high, and the effects of NBQX & AP5 too strong.

This progressive loss of connectivity reflects the combined blockade of AMPA and NMDA receptors, which disrupts excitatory synaptic transmission. As a result, the ability of neurons to synchronize and maintain functional connections is impaired, leading to a transition from highly interconnected networks to fragmented and eventually disconnected states.

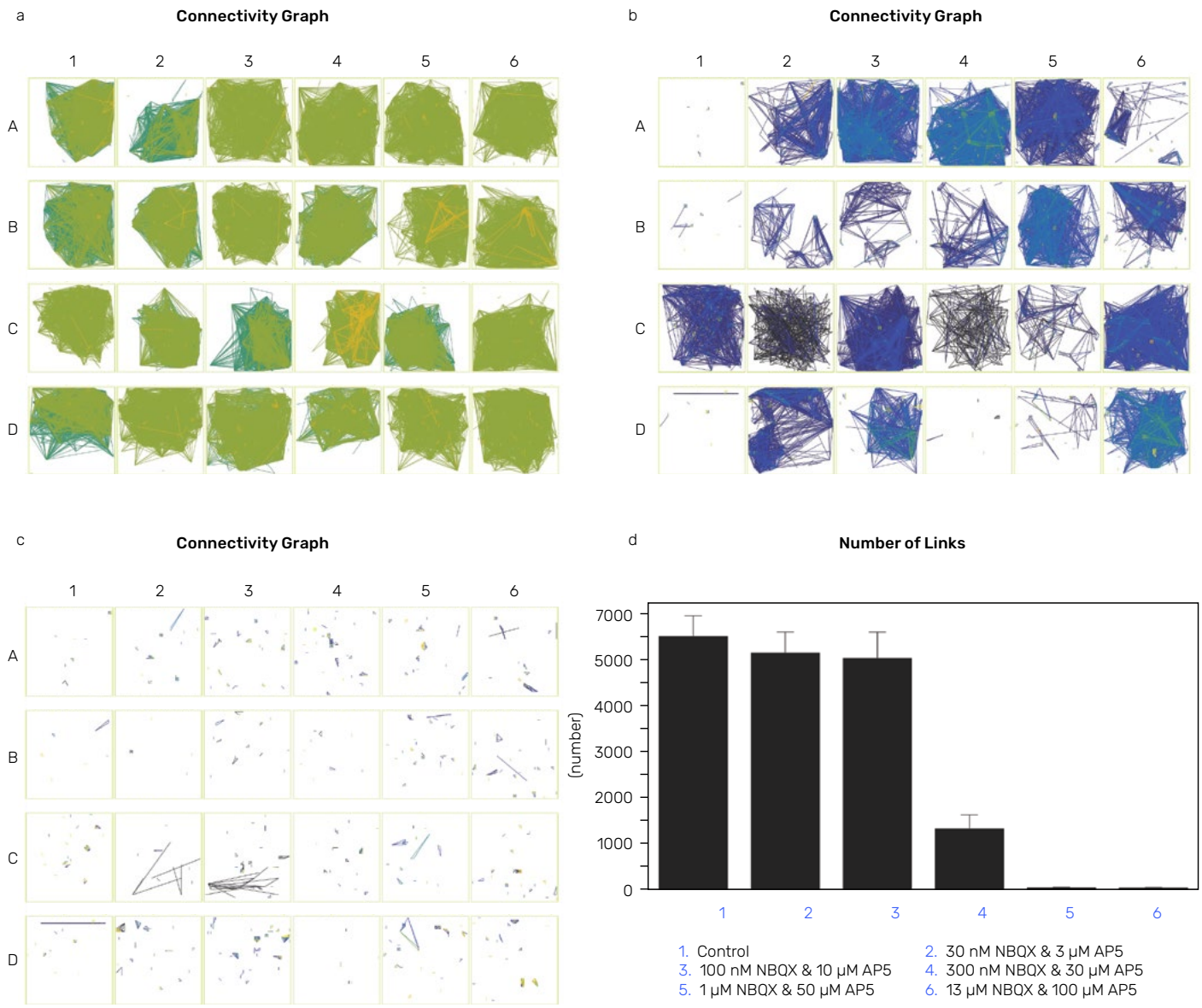

Figure 6: Dose-dependent modulation of network connectivity across the full plate following combined application of NBQX and AP5. Connectivity maps show the decrease in the network connectivity in baseline (a) compared to 300 nM NBQX & 30 µM AP5 (b) and 13 µM NBQX & 100 µM AP5 (c). Quantitative analysis of the reduced number of links (d).

Discussion

The functional properties of primary rat hippocampal networks, and their response to synaptic modulation can be accurately captured by CorePlate™ 24W. By utilizing the 1,024 simultaneously recording electrodes per well, this platform bridges the gap between single-neuron spiking activity and network-level investigations and allows for a comprehensive view of neuronal function and its response to pharmacological compounds across all 24 wells.

Leveraging the high spatial and temporal resolution of CorePlate™ 24W across all wells, we were able to reveal clear, dose-dependent effects on the activity, reflected in changes to spiking activity and bursting behaviour. Alongside this CorePlate™ 24W enabled us to move beyond simple firing rate analysis to capture how progressive glutamatergic blockade reshapes network function and connectivity. NBQX and AP5 treatment resulted in a concentration-dependent suppression of network properties such as network bursting and network connectivity, as the concentration increased, coordinated network activity also transitioned from

synchronized bursting & high connectivity to near complete silence. These findings underscore the essential role of AMPA and NMDA receptor mediated signalling in hippocampal culture activity and network dynamics.

Crucially, the ability to record simultaneously across all 24 wells and all electrodes enabled efficient and highly reproducible dose-response experiments to be conducted within a single experiment. The large electrode count combined with this simultaneous acquisition not only minimized variability, but enabled rapid network level investigations to be conducted. This capability is particularly advantageous for pharmacological screening, where quick and direct comparison across multiple concentrations and compound combinations is essential for accurate characterization.

Overall, these results demonstrate how CorePlate™ 24W enabled a detailed, high-content functional analysis of neuronal cultures such as primary hippocampal neuronal cultures as shown here, providing powerful insights into the screening of compounds on individual neuronal activity and network wide properties.

Conclusion

This study demonstrates that CorePlate™ 24W, combined with HyperCAM Delta, provides the resolution, scalability, and throughput required for advanced neuropharmacology applications.
Using primary rat hippocampal neurons, we show that the HD-MEA integrated into every well of CorePlate™ 24W enables precise, dose-dependent characterization of the effects of NBQX and AP5 neuronal activity, capturing changes from single-cell spiking to coordinated network activity.

Furthermore, the simultaneous recording abilities of CorePlate™ 24W across all 24 wells increases the efficiency of screening, streamlining workflows and enhancing reproducibility.

Overall, CorePlate™ 24W and the HyperCAM Delta offer a scalable HD-MEA platform and a powerful tool for drug discovery and screening, with clear potential for further throughput expansion using CorePlate™ 96W, alongside the automation-ready capabilities of the HyperCAM Delta.

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