NeuCyte
Translatable Neuroscience

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Application Note

Long-term functional characterization of SynFire® iNs and astrocytes co-cultured on MaxTwo Multi-Well High-Density Microelectrode Arrays

Key findings

- SynFire iNs co-cultured with astrocytes develop mature spontaneous and synchronous network activity on MaxTwo 6-Well Plates.
- SynFire iNs can be cultured for more than two months while maintaining healthy spontaneous and synchronous network activity.
- SynFire iNs develop mature axons and demonstrate stabilized axonal electrophysiological parameters.
- SynFire iNs exhibit increased synchronous network activity following chemically induced long-term potentiation.
- SynFire iNs cultured on the MaxTwo Multi-Well HD-MEA System can be functionally characterized for phenotypic assessment.

Introduction

The brain is a complex system composed of multiple cell types working synergistically to develop stable network activity[1]. Progress in understanding complex network electrophysiology phenotypes as well as how they are altered in disease states has been facilitated by the development of neuron-astrocyte co-cultures. Complementary to neurons, astrocytes provide support for neuronal outgrowth and are essential for synapse formation and stabilization[2]. To best utilize neuron-astrocyte co-cultures for phenotypic assessment, it is imperative to understand their developmental trajectory, which will enable optimized sampling windows for assay development.

NeuCyte manufactures highly pure, ready-to-use human induced pluripotent stem cells (iPSC)-induced Glutamatergic and GABAergic neurons, the SynFire® iNs (induced neurons). SynFire iNs are generated using a patented procedure for direct reprogramming and exhibit many salient characteristics of human neurons, such as expression of typical pan-neuronal markers and complex electrophysiology, including spontaneous and evoked action potentials and synchronized network activity. These human iPSC-derived neurons are valuable because of a lack of a natural source of human neurons for use in research. These neurons are an essential tool for in vitro disease modeling, compound efficacy assessment, and drug discovery. These human neurons can also be used for preclinical safety assessment and chemical neurotoxicity evaluation.

To functionally characterize the SynFire iNs, the MaxWell Biosystems MaxTwo Multi-Well High-Density Microelectrode Array (HD-MEA) platform offers high quality and high-resolution functional readouts of electrical activity and network dynamics. With 26,400 electrodes (at a density greater than 3,150 electrodes per mm^2) in every well[3], MaxTwo Multi-Well HD-MEA System allows for simultaneous electrophysiological recordings across six wells in an automated and non-invasive manner. These features ensure that the biologically relevant electrical activity is recorded across virtually all cells on the HD-MEA surface, from network to sub-cellular levels. Complemented with a user-friendly graphical interface of the MaxLab Live software, the MaxTwo Multi-Well HD-MEA System extracts meaningful metrics related to neuronal and network maturation as well as axonal morphology and characteristics.

The work presented summarizes the findings from SynFire iNs cultured on MaxTwo 6-Well Plates across multiple recording days over two months. The SynFire iNs developed and maintained mature neuronal network activity. Moreover, these mature networks were sensitive to and potentiated by chemical treatments. Together, the assessment of SynFire iNs on the MaxTwo Multi-Well HD-MEA System is shown to be a valuable assay for phenotypic screening, disease modeling and drug discovery.

Results

SynFire iNs co-cultured with astrocytes develop mature spontaneous and synchronous activity on MaxTwo 6-Well Plates

Understanding the developmental timeline for network activity in iPSC models is essential in determining time points for subsequent experiments and assay robustness. To investigate the developmental timeline of co-cultures consisting of SynFire iNs and astrocytes, Glutamatergic and GABAergic iNs were cultured together with human primary astrocytes on MaxTwo 6-Well Plates and monitored across multiple days. Activity Scan and Network recordings were taken every seven days to assess the spontaneous firing activity (Figure 1A-C) and the synchronous network formation and maturation (Figure 1D-F), respectively. The presence of spontaneous neuronal activity at the individual cell level was detected early in the culture, at 14 days in-vitro (DIV). In the subsequent weeks as the culture developed further (particularly from DIV 28 onwards), increased spontaneous activity

was observed along with regular network bursting, and synchronous activity across neurons. The ability to culture cells long-term allows for greater flexibility in assay design for phenotype extraction influenced by developmental trajectories as well as long-term effects of compounds. To demonstrate such capabilities, these cultures were maintained for more than two months. At DIV 63, the cultures continued to exhibit robust spontaneous activity as well as regular network bursting (insert). This data shows that SynFire® iNs can survive and remain functional on MaxTwo 6-Well Plates long-term, and that the MaxWell Biosystems HD-MEA platform offers a high-resolution, robust, and sensitive electrophysiological solution for examining neuronal culture activity and maturation.

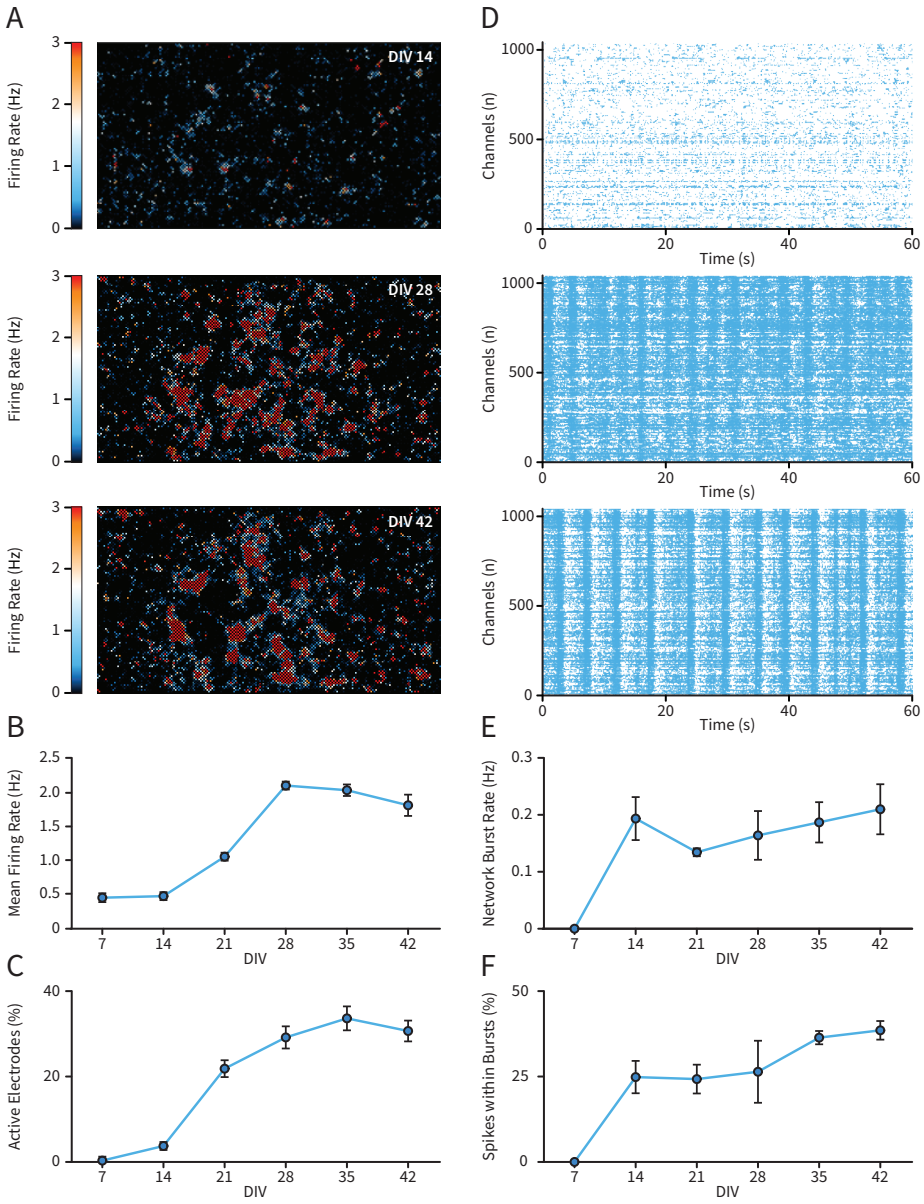
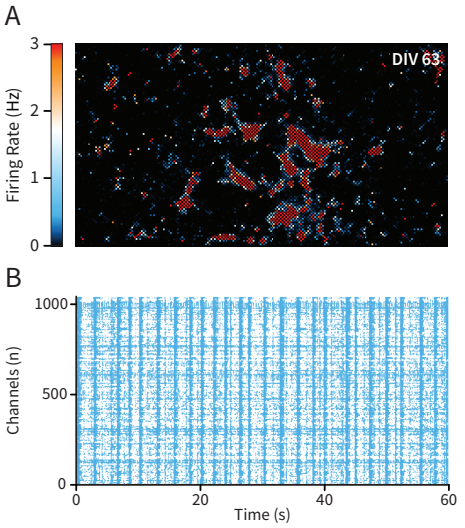


Figure 1. Co-culture of NeuCyte SynFire iNs (glutamatergic and GABAergic neurons) and primary astrocytes develop mature spontaneous and synchronous network activity. (A) Heatmap depicting the firing rate of the co-culture at DIV 14, DIV 28, and DIV 42. (B) Quantification of the mean firing rate and (C) quantification of the percentage of active electrodes over time. (D) Raster plots highlighting the synchronous network activity at DIV 14, DIV 21, and DIV 42. (E) Quantification of the network burst frequency and (F) quantification of the percentage of spikes within bursts longitudinally. Data represents mean ± standard error of the mean.



Insert. Co-culture of NeuCyte SynFire iNs and primary astrocytes show robust spontaneous and synchronous network activity after two months. (A) Heatmap depicting the firing rate of the co-culture at DIV 63. (B) Raster plot highlighting the synchronous network bursts.

Human iPSC-derived neurons develop mature axons in-vitro

The functional characterization of the spontaneous and synchronous network activity showed that the SynFire® iNs co-cultured with astrocytes progressively developed and maintained mature neuronal networks for up to two months in culture. To further assess whether the cells were mature at the cellular as well as axonal levels, the Axon Tracking Assay was performed.

Taking advantage of the high spatio-temporal resolution of the MaxWell Biosystems HD MEA platform, axonal branches were tracked and investigated. At DIV 14 (Figure 2A-B), axonal features could be extracted for a subset of sampled cells while the branches remained limited in length and bifurcation. On the other hand, at DIV 35 (Figure 2C-D), axonal features were extracted from most of the sampled cells and the branches were more extensive. Likewise, the quantification of axonal features such as the spike amplitude at the axon initiation site (Figure 2E), branch length (Figure 2F), and conduction velocity (Figure 2G), showed the same trend.

These data suggest that individual cells as well as their axons were immature at DIV 14, but mature in the subsequent weeks, and stabilize by DIV 35. Furthermore, the data indicate that the Axon Tracking Assay is sensitive to and capable of detecting longitudinal developmental changes in the SynFire iN co-culture.

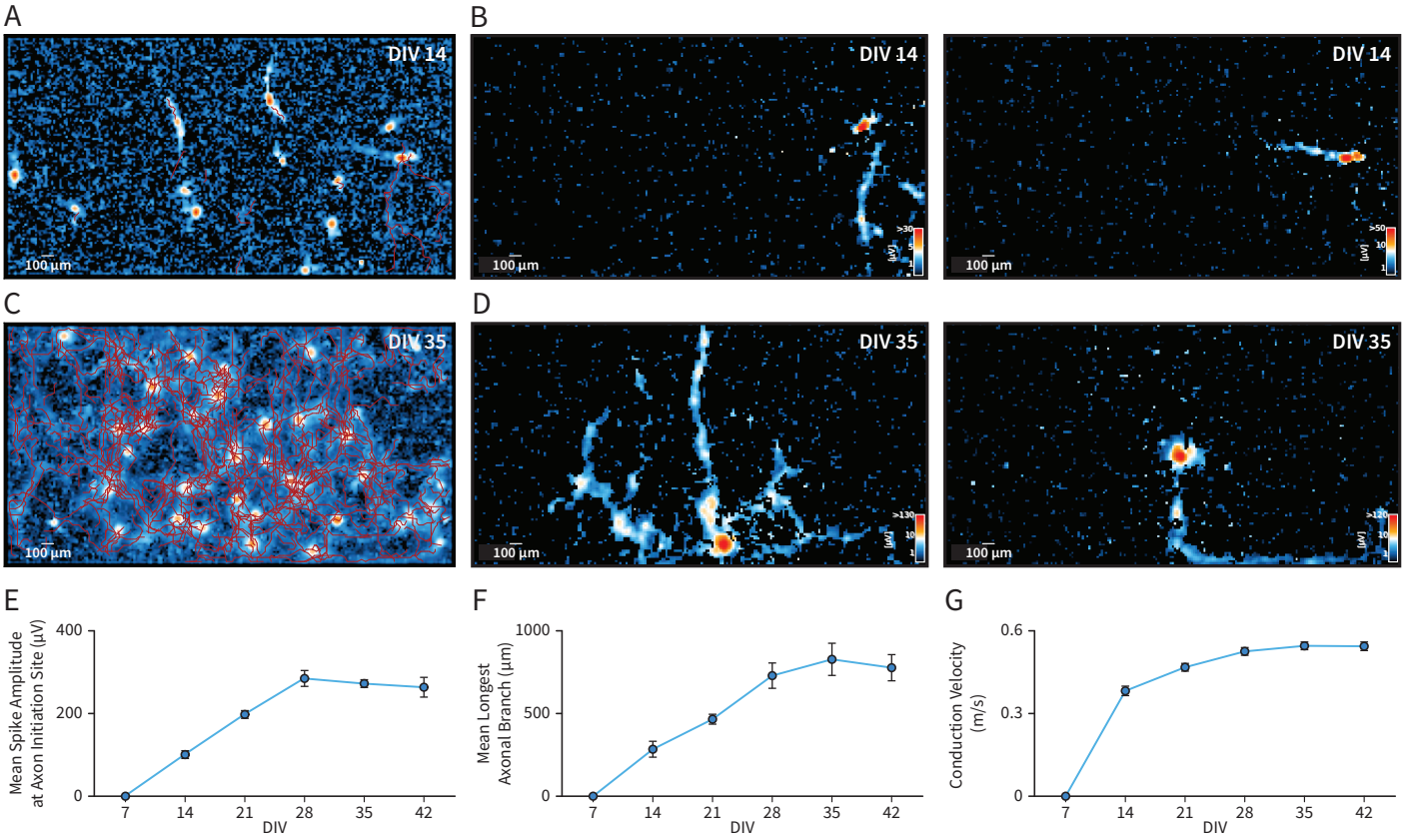


Figure 2. Ontogeny of axon development and maturation (A) Map of a whole well with sampled neurons and their axonal branches and (B) maps of representative neurons and their axonal branches at DIV 14. (C) Map of a whole well with sampled neurons and their axonal branches at DIV 35 and (D) maps of representative neurons and their axonal branches at DIV 35. (E) Quantification of mean spike amplitude at the axon initiation site, (F) mean longest axonal branch, and (G) axonal conduction velocity over time. Axon tracking is highlighted in red. Data represents mean ± standard error of the mean.

Chemical treatment evokes long-term potentiation in SynFire® iNs and astrocyte co-culture

Long-term potentiation (LTP) is a persistent increase in synaptic strength following high-frequency stimulation, either by chemical or electrical means[4]. LTP leads to a long-lasting increase in signal transmission between neurons and is associated with the cellular model of learning and memory formation[5].

To assess whether the SynFire iNs and astrocyte co-culture can be a viable cellular model for assessing learning and memory, the co-culture was chemically treated with Forskolin and Rolipram to chemically induce LTP (cLTP) in-vitro[6]. Results in Figures 1 and 2 indicated that both the network activity and axon development stabilized by DIV 35, therefore, this timepoint was selected.

Following baseline measurements, either the chemical cocktail to induce cLTP or the vehicle (DMSO) was applied, and subsequent recordings were collected (Figure 3A-B). In contrast to the vehicle treated culture, which displayed no observable change in synchronous network activity, the culture that was chemically treated to induce LTP exhibited an increased network burst rate as well as a larger proportion of spikes within bursts (Figure 3C-D). Interestingly, both metrics returned to baseline levels 48 hours after treatment. These data demonstrate the feasibility of using the co-culture to study cLTP in-vitro.

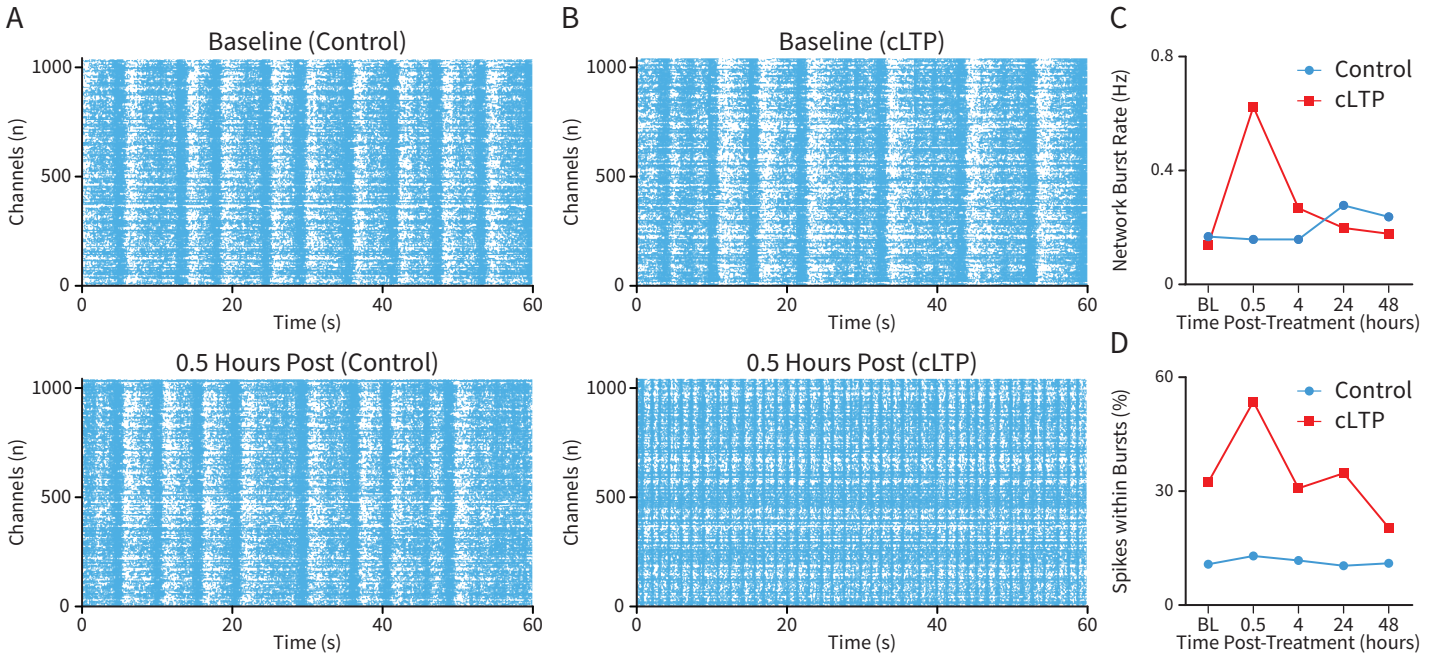


Figure 3. SynFire iNs show increased network bursts after cLTP treatment. (A) Raster plots of control cultures during baseline and 30 minutes following DMSO treatment. (B) Raster plots of cLTP treated cultures during baseline and 30 minutes following cLTP treatment. (C) Quantification of the network burst rate. (D) Quantification of percentage of spikes within bursts. Data represents mean ± standard error of the mean.

Discussion

In this application note, the utility of the NeuCyte SynFire® iNs and astrocyte co-culture was demonstrated on the MaxWell Biosystems MaxTwo Multi-Well HD-MEA System. By regularly monitoring the activity of the SynFire iN co-culture at high spatio-temporal resolution, across network, cellular, and sub-cellular levels, the developmental timeline was assessed, allowing for the optimized selection of assay-specific time points for future studies. The datasets here highlight the developmental progression of spontaneous firing activity, synchronous network bursts, and axonal features over time. Such robust spontaneous spiking and synchronous network activity were maintained for more than 2 months. The potential to chemically induce LTP provides a possible approach to investigate synaptic plasticity using this model. Furthermore, the unprecedented resolution and high signal quality of the MaxTwo Multi-Well HD-MEA System allowed for reliable, non-invasive electrophysiological assesment of neuronal activity, network dynamics and axonal physiology of neuron-glia co-cultures longitudinally. Taken together, these findings serve as a proof-of-concept for the application of this approach to disease modeling, studying developmental biology, and compound testing, particularly for neurological diseases.

References

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NeuCyte is an innovative biotechnology company focused on CNS drug discovery. Based on its proprietary SynFire® technology for generating human iPSC-derived neurons, NeuCyte has developed a highly functional in vitro platform for target identification and validation, compound efficacy testing, neurotoxicity assessment, and disease modeling. The team is actively pursuing drug discovery programs on Alzheimer's disease, ALS, Epilepsy, Fragile X Syndrome and more.

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MaxWell Biosystems has engineered an advanced high-resolution functional imaging platform for electrical recordings of in-vitro brain models for basic research, disease modelling and drug discovery. With high-density micoelectrode arrays (HD-MEAs) one can discover the fuction of neurons at the network, cellular and subcellular level.

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