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BrainPhys™ Neuronal Medium Supports the Electrical Activities of Neurons Derived from Human Pluripotent Stem Cells and Primary CNS Tissues in Long-Term Cultures

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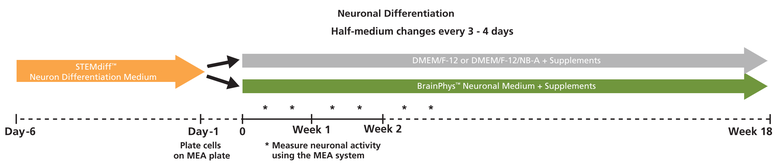
Abstract

Action potential firing and synaptic activity are fundamental properties of neurons in the brain. Bardy et al. (PNAS, 2015) have recently reported that Neurobasal® Medium and DMEM/F-12 support neuron survival but suppress their synaptic activities in culture. To solve this problem, we developed BrainPhys™ Neuronal Medium (BrainPhys™), based on the formulation published by Bardy et al., to support growth and synaptic function of neurons in long-term cultures. Here we describe the effect of BrainPhys™ and DMEM/F-12 based media on neuronal electrical activity of human pluripotent stem cell (hPSC)-derived and primary E18 rat cortical neurons in 18 and 6 weeks cultures, respectively. For hPSC cultures, neural progenitor cells derived from induced pluripotent stem cells (iPSCs) were differentiated in BrainPhys™ or DMEM/F-12 (control) with supplements, and cultured for 18 weeks. We performed half-medium changes every 3 - 4 days and measured the neuronal electrical activity twice a week using the multielectrode array (MEA) system. Our data showed that the mean firing rate of iPSC-derived neurons (n = 1; 128 electrodes) in BrainPhys™ increased from < 0.17 Hz at week 10, to 1.60 Hz by week 18. In contrast, the mean firing rate of neurons in DMEM/F-12 remained low (≤ 0.15Hz) over the same 18-week period. For experiments using primary tissues, E18 rat cortical cells were plated in Neurobasal® Medium with NeuroCult™ SM1 Neuronal Supplement (SM1). After 5 days, cultures were either transitioned to BrainPhys™ with SM1 or maintained in the Neurobasal® control by performing half-medium changes every 3 - 4 days for 6 weeks. Electrical activities were measured twice a week throughout the culture period. Our data showed that the mean firing rate of neurons in BrainPhys™ medium increased over time, from 0.03 Hz at week 2, to 1.4 Hz by week 6 (n = 1; 128 electrodes). The percentage of active electrodes (> 0.005Hz) also increased from 24% at week 2 to 69% by week 3, and then remained stable at 60 - 70% from weeks 3 - 6. In contrast, the mean firing rate remained low (< 0.1 Hz) in the control, with < 5% of electrodes being active over a 6-week period. These data confirm that BrainPhys™ Neuronal Medium with appropriate supplements supports the synaptic function of hPSC-derived and primary neurons by providing a physiological in vitro condition that closely mimics the environment of the brain.

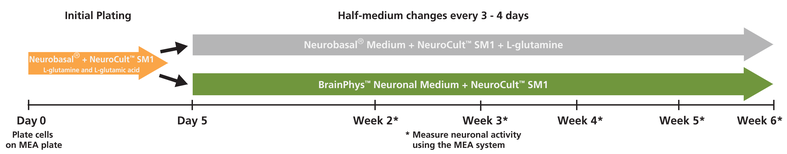
Methods

(A) Culture of hPSC-derived Neurons



Neural progenitor cells derived from hPSCs (XCL1-NPC) were cultured in STEMdiff™ Neuron Differentiation Medium on poly-L-ornithine (PLO)/laminin-coated 6-well plate for 5 days. On day 5, neural progenitor cells were dissociated and single cells were re-plated onto a PLO/laminin-coated MEA plate at 30,000 cells/cm^2 in STEMdiff™ Neuron Differentiation Medium. After one day, half of the medium was replaced with differentiation media, (DMEM/F-12, DMEM/F-12/NB-A [DMEM/F-12 and Neurobasal-A mixed in a 1:1 ratio], or BrainPhys ™ Neuronal Medium + supplements: 1% N2 Supplement-A, 2% NeuroCult™ SM1 Neuronal Supplement, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP and 200 nM Ascorbic Acid). Half-medium changes were performed every 3 - 4 days throughout the culture period.

(B) Culture of Primary E18 Rat Cortical Neurons



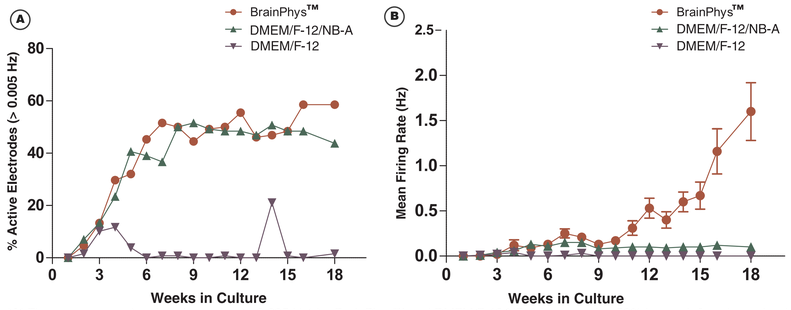
E18 rat cortices were dissociated into single cells and plated onto PLO/laminin-coated MEA plate at 200,000 cells/cm^2 in Neurobasal® Medium (Neurobasal® Medium with 2% NeuroCult™ SM1 Neuronal Supplement, 0.5 mM L-glutamine and 25 µM L-glutamic acid). After 5 days, half of the cultures were transitioned to BrainPhys™ Neuronal Medium with 2% NeuroCult™ SM1, while the remaining cultures maintained in Neurobasal® Medium with 2% NeuroCult™ SM1 and 0.5 mM L-glutamine. Half-medium changes were performed every 3 - 4 days for up to 6 weeks.

(C) Multi-Electrode Array (MEA) Recording and Analysis

Cells in each culture condition were plated in duplicate wells of a 12-well plate (Axion Biosystems; M768-GL1- 30Pt200), with 64 recording electrodes in each well. Spontaneous neuronal activity was acquired at 37°C under a 5% CO_2 atmosphere using an MEA system (Maestro, Axion Biosystems) at a sampling rate of 12.5 kHz/channel. For all recordings, a Butterworth band-pass filter (200 Hz - 3000 Hz) was applied and the adaptive threshold spike detector was set at 6x standard deviation. A 15-minute recording was taken twice a week for hPSC culture and once a week for primary neuronal cultures. Only the last 10 minutes of each recordings were exported for analyses using the AxIS (2.3.3) analysis software.

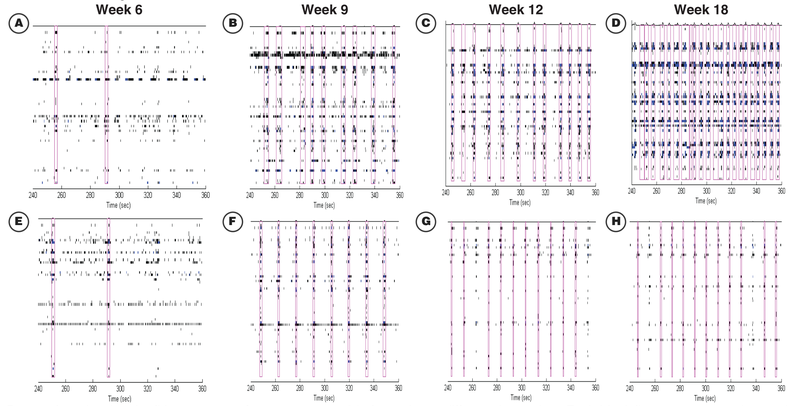
hPSC-derived Neurons in BrainPhys™

Figure 1. hPSC-derived neurons cultured in BrainPhys™ Neuronal Medium showed improved mean firing rates



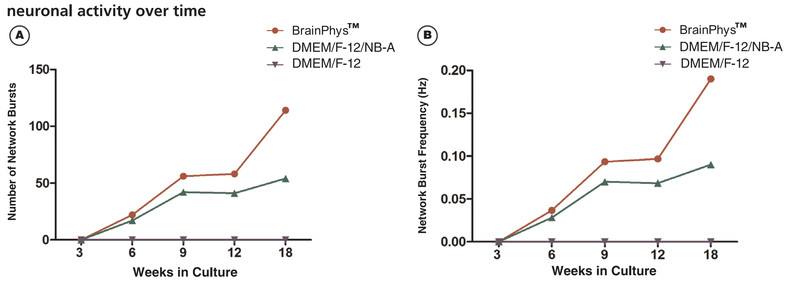
(A) The percentage of active electrodes (> 0.005 Hz) for BrainPhys™ and DMEM/F-12/NB-A increased to 50% by week 8 and remained stable at 40 - 60% thereafter until the end of the culture. In contrast, < 20% of electrodes were active in DMEM/F-12 for the same 18-week period. **(B)** For all culture conditions, the mean firing rate (MFR) was low (≤ 0.2Hz) for the first 10 weeks in culture. However, there was a gradual increase in MFR for neurons cultured in BrainPhys™ over time, from 0.17 ± 0.04 Hz at week 10 to 1.60 ± 0.32 Hz at week 18 (n = 1; mean ± SEM, 128 electrodes). In contrast, the MFR of neurons cultured in DMEM/F-12/NB-A and DMEM/F-12 remained low (≤ 0.15Hz) throughout the same culture period. To measure neuronal activity, MFR was calculated based on the activity recorded from 128 electrodes for each condition (cultures were set up in duplicate wells, 64 electrodes per well).

Figure 2. hPSC-derived neurons cultured in BrainPhys™ Neuronal Medium showed improved neuronal network activity



Raster plots showing the firing patterns of neurons across 64 electrodes at different time points. Each black line represents a detected spike. Blue lines represent single channel burst - a collection of at least 5 spikes, each separated by an inter-spike interval (ISI) of no more than 100 ms. Network bursts are marked with purple boxes and are defined as a collection of at least 10 spikes from a minimum of 25% of participating electrodes across the entire well, each separated by an ISI of no more than 100 ms. **(A-D)** Neurons cultured in BrainPhys™ demonstrated improved electrical activity as shown by the increased number of spikes (neuronal activity) over time. In addition, there was an increase in network bursting frequency and duration in the BrainPhys™ conditions, which suggests that neuronal firing was gradually organized into a synchronized network burst as they matured. **(E-H)** In contrast, the number of spikes and network bursts in DMEM/F-12/NB-A remained relatively low throughout the culture, showing that DMEM/F-12/NB-A is suboptimal in supporting network level activities in vitro. No activity was detected in DMEM/F-12, showing that neuronal activity was impaired in the DMEM/F-12 condition (raster plots not shown).

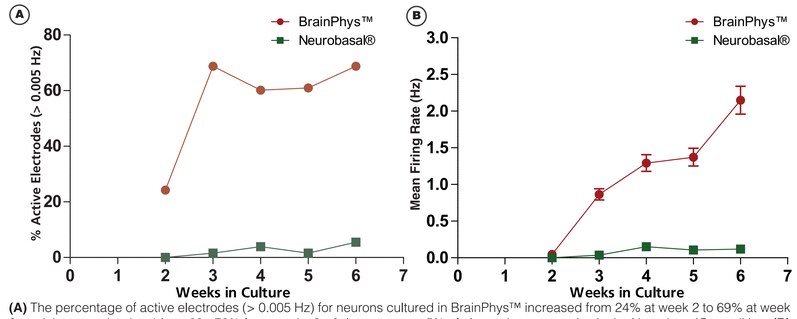
Figure 3. hPSC-derived neurons cultured in BrainPhys™ Neuronal Medium developed strong synchronous neuronal activity over time



(A) Network bursts were first detected at week 6 in BrainPhys™ and DMEM/F-12/NB-A conditions. The number of network bursts increased gradually over time, suggesting that both cultures became more synchronous as they matured. After 18 weeks in culture, the number of network bursts detected in a 10-minute recording in BrainPhys™ and DMEM/F-12/NB-A were 114 and 54, respectively, indicating that a synchronous neuronal network was developed more efficiently in BrainPhys™. **(B)** The network bursts also occurred more frequently as the neurons matured over time. In contrast, no network burst activity was detected in DMEM/F-12 over the same culture period.

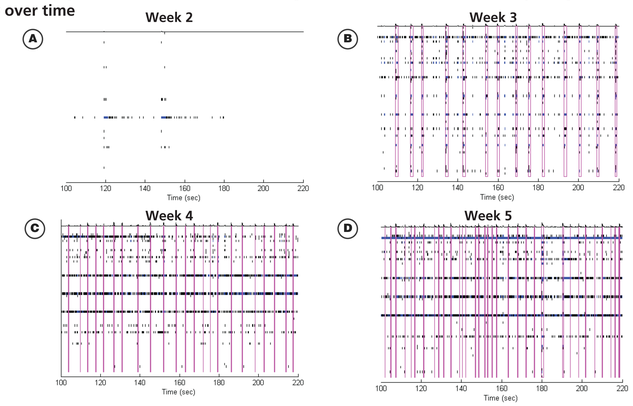
Primary E18 Rat Cortical Neurons in BrainPhys™

Figure 4. Primary neurons cultured in BrainPhys™ Neuronal Medium showed improved electrical activity



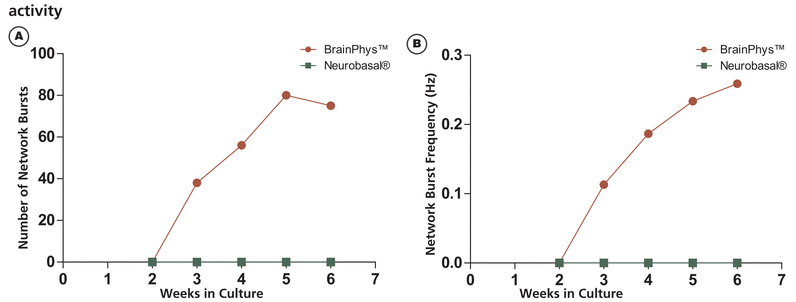
(A) The percentage of active electrodes (> 0.005 Hz) for neurons cultured in BrainPhys™ increased from 24% at week 2 to 69% at week 3, and then remained stable at 60 - 70% from weeks 3 - 6. In contrast, < 5% of electrodes were active in the Neurobasal® condition. **(B)** The MFR of neurons cultured in BrainPhys™ increased over time, from 0.047 ± 0.004 Hz at week 2, to 2.1 ± 0.2 Hz by week 6 (n = 1, mean ± SEM, 128 electrodes), whereas the MFR of neurons cultured in Neurobasal® remained low (< 0.1 Hz) for the same 6-week period.

Figure 5. Primary neurons cultured in BrainPhys™ Neuronal Medium developed synchronous neuronal activity over time



Raster plots showing the change in firing patterns for primary neurons that were cultured in BrainPhys™. Each black line represents a spike detected across 64 electrodes. Blue lines represent single channel burst - a collection of at least 5 spikes, each separated by an inter-spike interval (ISI) of no more than 100 ms. Network bursts are marked with purple boxes, and are defined as a collection of at least 10 spikes from a minimum of 25% of participating electrodes across the entire well, each separated by an ISI of no more than 100 ms. **(A-B)** There was a significant increase in the number of spikes (neuronal activity) detected from week 2 to week 3, with a network burst first appearing at week 3 in culture. **(B-D)** As the neurons matured from week 3 to week 5, there was an increase in network burst frequency, showing that a synchronous neuronal activity was developed over time. In contrast, no activity was detected in the Neurobasal® conditions at all recording time points (raster plots not shown).

Figure 6. Primary neurons cultured in BrainPhys™ Neuronal Medium showed improved neuronal network activity



(A) Network bursts were first detected at week 3 in the BrainPhys™ culture. The number of network bursts detected in a 5-minute recording increased from 38 to 75 from week 3 to week 6, showing that a synchronous neuronal network was developed in the BrainPhys™ culture over time. **(B)** The network bursts also occured more frequently as the neurons matured in BrainPhys™. In contrast, neurons cultured in Neurobasal® did not show any network burst activity for the same 6-week period.

Summary

BrainPhys™ Neuronal Medium:

- BrainPhys™ Neuronal Medium supports the maturation of hPSC-derived and primary neurons.
- Neurons cultured in BrainPhys™ Neuronal Medium exhibit improved electrical activity and develop synchronous network activity over time based on MEA data.

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