Determining the Metabolic Requirements of Electrically Active Rodent Primary Neurons in Long-Term Culture

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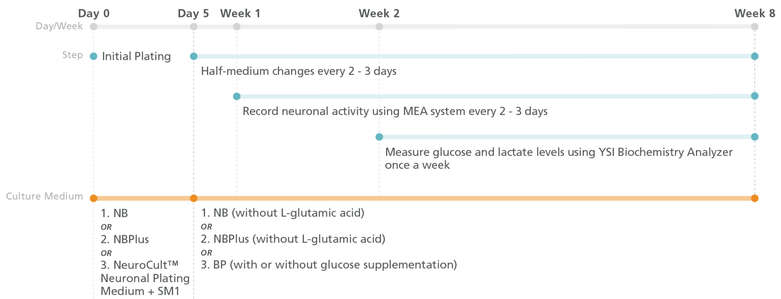
INTRODUCTION

Neurons have a high metabolic demand, with the brain accounting for 20% of total body energy consumption. Most neuronal cell culture media contain energetic substrates in excess to ensure this demand is met. We investigated whether media with physiologic (e.g. BrainPhys™) or supraphysiologic (e.g. NeurobasalTM) glucose levels supported the metabolic requirements and sustained activity of primary neurons in high-density cultures. E18 rat cortices were dissociated into single-cell suspensions and plated in NeuroCult™ Neuronal Plating Medium supplemented with NeuroCult™ SM1 Neuronal Supplement (SM1) in 96-well microelectrode array (MEA) plates. After 5 days of incubation, cultures were transitioned to BrainPhys™ + SM1 (BP) by performing half-medium changes every 2 - 3 days for up to 8 weeks. In separate cultures, increasing levels of glucose were added to BP, ranging from 2.5 to 25 mM final concentration. Cells were also cultured independently in high-glucose media (Neurobasal™ + B-27™ [NB] or Neurobasal™ Plus + B27™ Plus [NBPlus]) following the supplier's protocols. Before each half-medium change, media were collected for glucose and lactate measurements. Spontaneous neuronal activity was recorded three times weekly using the Axion Biosystems MEA system. Our data demonstrate that BrainPhys™ is not only optimized for energetic substrates but its performance can be further enhanced by supplementation with glucose to meet higher metabolic demands of electrically active neurons in vitro.

METHODS

Culture of Rodent Primary Neurons: E18 rat cortices were dissociated to single cells and plated at 50,000 cells per well in a poly(ethyleneimine) (PEI)-coated MEA plate. Cells were plated in three medium formulations: (1) Neurobasal™ + 0.2% B-27™ (NB), (2) Neurobasal™ Plus + 2% B27™ Plus (NBPlus), or (3) NeuroCult™ Neuronal Plating Medium + 2% SM1. All three plating media were supplemented with 0.5 mM L-glutamine and 25 µM L-glutamic acid. After 5 days, half-medium changes were performed on NB and NBPlus, both in the absence of L-glutamic acid. In condition (3), cultures were transitioned to BrainPhys™ + 2% SM1 (BP) by performing half-medium changes every 2 - 3 days for up to 8 weeks. Increasing concentrations of glucose, ranging from 2.5 to 25 mM, were also added to BP. Cells in each condition were plated in four replicate wells in a 96-well MEA plate, with 8 electrodes in each well (n = 3).



*NB = Neurobasal™ + B-27™; NBPlus = Neurobasal™ Plus + B-27™ Plus; BP = BrainPhys™ Neuronal Medium + SM1

FIGURE 1. Workflow for the Culture of Primary E18 Rat Cortical Neurons

Glucose and Lactate Measurements and MEA Analysis: For glucose and lactate measurements, spent media were pooled from replicate wells of the same conditions and analyzed weekly using a YSI Biochemistry Analyzer. Data were reported as mean ± standard error (n = 3). Spontaneous neuronal activity was acquired at 37°C and 5% CO_2 using an MEA system at a sampling rate of 12.5 kHz/channel. For all recordings, a band-pass filter (200 Hz - 3000 Hz) was applied and the adaptive threshold spike detector was set at 6X standard deviation. The MEA plate was allowed to equilibrate on the MEA system for 5 minutes before taking a 10-minute recording every 2 - 3 days for the duration of the culture period. Data were exported for analysis using AxIS (2.3.3) analysis software. Mean firing rate is defined as the total number of spikes divided by the duration of the recording, in Hz, whereas an active electrode refers to an electrode with activity greater than a minimum spike rate of 6.7 spikes/min. Network bursts are defined as a collection of at least 50 spikes from a minimum of 35% of participating electrodes across each well, each separated by an inter-spike interval of no more than 100 ms. Synchrony index is a measure of synchrony between 0 and 1 (Paiva et al.). Values closer to 1 indicate higher synchrony. All data were reported as mean ± standard error (n = 3).

RESULTS

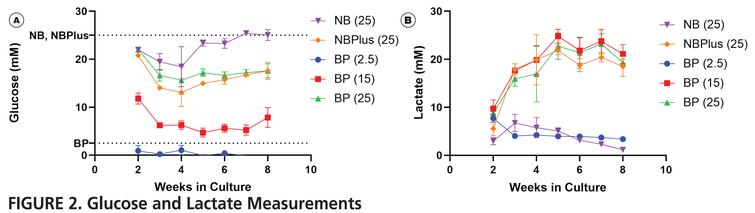


FIGURE 2. Glucose and Lactate Measurements

(A) BP has a level of glucose (2.5 mM) which matches the glucose concentration in human cerebrospinal fluid. The dotted lines in the graph indicate the concentration of glucose in NB, NBPlus, and BP. Numbers in ( ) represent starting glucose concentration in each medium, in mM. Glucose measurements indicated nearly complete depletion of glucose in BP by week 2 of culture. When glucose was added to BP, at final concentrations of 15 mM (red line) and 25 mM (green line), glucose availability was maintained throughout the 8-week culture period. In contrast, both NB and NBPlus maintained > 13 mM glucose with a decrease in glucose consumption over time. **(B)** Without glucose supplementation (2.5 mM), a low level of lactate was detected in BP throughout the culture period. For NB-cultured neurons (purple line), lactate levels steadily declined after week 3; this decline coincided with the decrease in glucose consumption in NB. In contrast, lactate production increased in the first 5 weeks for NBPlus and glucose-supplemented BP conditions. Lactate levels remained constantly high (> 18 mM) in these conditions after week 5.

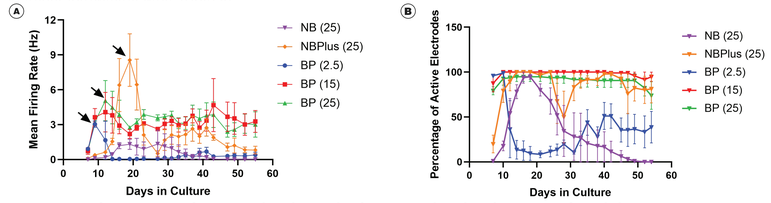


FIGURE 3. Glucose Supplementation in BrainPhys™ Maintained Neuronal Activity Over 8 Weeks in Culture

(A) Neuronal activity was detected at day 9 in all BP conditions, whereas activity was not detected until day 14 in both NB and NBPlus cultures. For NB-cultured neurons, mean firing rate (MFR) remained low throughout culture (purple line). In contrast, a "peak-drop" activity pattern was observed in all other conditions, where MFR increased rapidly within 2 days followed by a drop in activity in the next 2 - 4 days, as indicated by arrows in the graph. BP with 15 mM (red line) and 25 mM (green line) glucose maintained the highest level of activity throughout the 8-week culture period. **(B)** BP-cultured neurons reached 100% active electrodes (> 6.7 spikes per min) at day 9. Without glucose supplementation (2.5 mM; blue line), active electrodes dropped on day 21 but slowly recovered up to day 54. NB-cultured neurons reached nearly 100% active electrodes at day 16 but this steadily declined to 0% by day 52 (purple line). For NBPlus-cultured neurons, around 90% of active electrodes were recorded over time with a transient drop on day 29 that we could not explain. In contrast, glucose-supplemented BP (15 and 25 mM; red and green lines) were the only conditions that maintained nearly 100% active electrodes over the 8-week culture period.

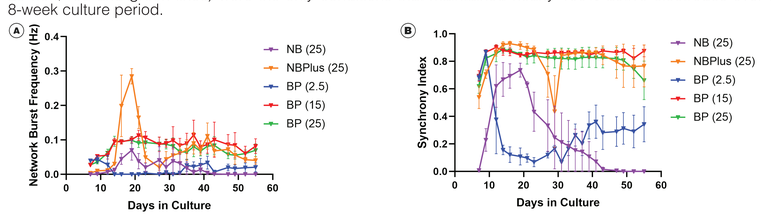


FIGURE 4. Primary Neurons Cultured in Glucose-Supplemented BrainPhys™ Developed Strong Synchronous Network Activity Over 8 Weeks in Culture

(A) Network burst frequency was low in NB (purple line) and in BP with 2.5 mM glucose (blue line) for most of the 8 weeks in culture. For NBPlus, network burst frequency peaked at day 19 then dropped significantly to a level below 0.1 Hz for the remainder of the culture period (orange line). In contrast, glucose-supplemented BP (15 and 25 mM; red and green lines) maintained a constant network burst frequency around 0.1 Hz from day 14 until day 54. **(B)** Synchrony in BP with 2.5 mM glucose dropped dramatically after day 9, corresponding to the drop in MFR in Figure 3A, but after day 35 the synchrony index increased until the end of culture (blue line). Activity of NB-cultured neurons was most synchronous on day 18 but this declined steadily after day 21 (purple line), corresponding to the gradual drop in MFR in Figure 3A. Neurons in NBPlus were highly synchronous for most of the culture, but there was a drop in synchrony around day 29, corresponding to the drop in percentage of active electrodes seen in Figure 3B. Neurons cultured in BP (15 and 25 mM) were the only conditions that were highly synchronous throughout the 8 weeks in culture.

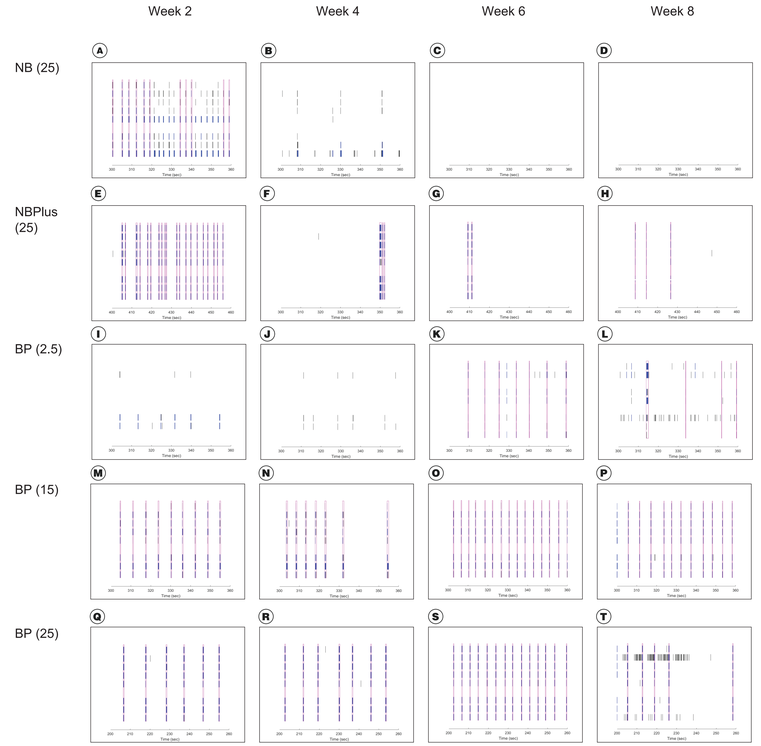


FIGURE 5. Primary Neurons Matured in Glucose-Supplemented BrainPhys™ Showed Improved Neuronal Activity and More Consistent Network Bursting in Long-Term Culture

(A-T) Raster plots showing the firing patterns of neurons across 8 electrodes at weeks 2, 4, 6, and 8. Each black line represents a detected spike. Blue lines represent single channel bursts—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 magenta boxes and are defined as a collection of at least 50 spikes from a minimum of 35% of participating electrodes across each well, each separated by an ISI of no more than 100 ms. **(A-D)** Neurons cultured in NB exhibited network bursting in week 2 but no spiking activity was detected in subsequent timepoints. **(E-H)** For NBPlus-cultured neurons, a high number of spikes and regular network bursting were detected at week 2. However, a decreased number of spikes and inconsistent network bursting were observed in later timepoints, corresponding to the drop in MFR seen in Figure 3A. **(I-L)** Without glucose, individual spiking was observed at weeks 2 and 4 in BP but network bursting was detected in weeks 6 and 8, corresponding to the recovery of active electrodes in Figure 3B. **(M-T)** In contrast, neurons cultured in BP (15 and 25 mM glucose) demonstrated strong spiking activity and consistent network bursting at all timepoints.

Summary

- Glucose is required to support neuronal activity in high-density cultures; depletion of glucose results in a drop in neuronal activity
- BrainPhys™ Neuronal Medium requires glucose supplementation at a higher (but not supraphysiologic) level to support the metabolic demand of neurons in high-density cultures
- Glucose is not the only metabolic substrate required for neuronal activity; traditional media with high glucose concentrations failed to support neuronal activity in long-term cultures
- BrainPhys™ Neuronal Medium is uniquely formulated to support the metabolic demands and electrical function of neurons in vitro

References

Paiva ARC, Park I, & Príncipe JCA. (2010) Neural Comput & Applic 19: 405–19.