

A New Culture Medium that Reduces Phototoxicity and Autofluorescence While Supporting Activity in Long-Term Primary Tissue- and hPSC-Derived Neurons

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INTRODUCTION

The rapid development of imaging technologies coupled with fluorescent sensors has dramatically increased the number of live-cell imaging applications for cell analyses and detection in vitro. However, autofluorescence of cell culture media reduces the signal-to-noise ratio, and repeated light exposure can result in accumulation of toxic byproducts, especially in long-term neuronal cultures. We have developed BrainPhys™ Imaging Optimized Medium (BrainPhys™ IO), a new medium based on the original BrainPhys™ formulation (Bardy et al. 2015) that overcomes these technical issues while maintaining neuronal survival and functional activity. Here we describe the advantages of culturing primary- and human pluripotent stem cell (hPSC)-derived neurons in BrainPhys™ IO.

METHODS

Culture of hPSC-Derived Neurons and Multi-electrode Array (MEA) Analysis: On Day -6, hPSC-derived neural progenitor cells (XCL-1) were thawed and plated on a poly-L-ornithine (PLO)/laminin-coated 6-well plate and cultured in STEMdiff™ Neuron Differentiation Medium for 5 days. On Day -1, the resulting neuron precursor cells were dissociated to single cells and seeded onto a PLO/laminin-coated 48-well MEA plate at 30,000 cells/cm² in fresh differentiation medium. On Day 0, half of the medium was replaced with BrainPhys™ + Supplements (1% N2 Supplement-A, 2% NeuroCult™ SM1 Neuronal Supplement [SM1], 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 for 8 weeks. Subsequently, half the wells were switched from BrainPhys™ to BrainPhys™ IO using medium changes for 3 weeks. Afterwards, the medium in all wells was transitioned back to BrainPhys™ with half-medium changes until week 14. Recordings were taken twice weekly from week 1 to week 14. Recordings were taken twice weekly using a Maestro MEA system (Axion Biosystems) at a sampling rate of 12.5 kHz/channel. For recording, a Butterworth band-pass filter (200 Hz - 3000 Hz) was applied and the adaptive threshold spike detector was set at 6X standard deviation.

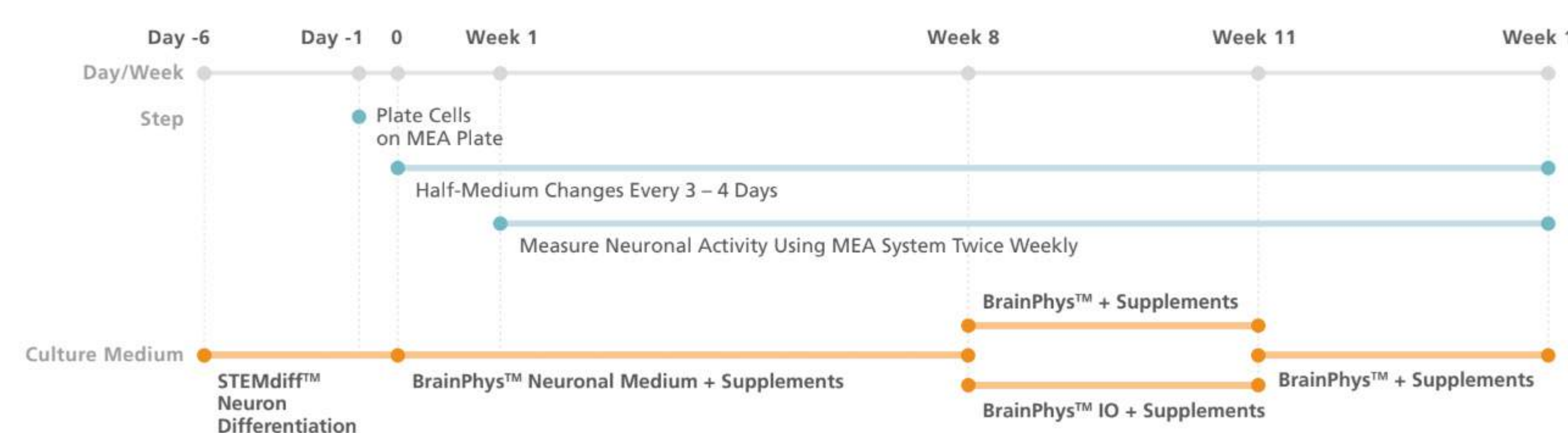


FIGURE 1. Workflow for Neuronal Differentiation and Maturation of hPSC-derived Neurons

Primary E18 Rat Cortical Neurons: On Day 0, E18 rat cortices were dissociated into single-cell suspensions by enzymatic dissociation and plated at 60,000 cells/well onto poly-D-lysine-coated 24-well plates in NeuroCult™ Neuronal Plating Medium supplemented with NeuroCult™ SM1. After 5 days, cultures were transitioned using half-medium changes to BrainPhys™ or BrainPhys™ IO (both supplemented with SM1) for up to 21 days. Half-medium changes were performed every 3 - 4 days for the duration of the culture period. Neurons (βIII tubulin-positive) were quantified in 25 random fields per well, in triplicate.

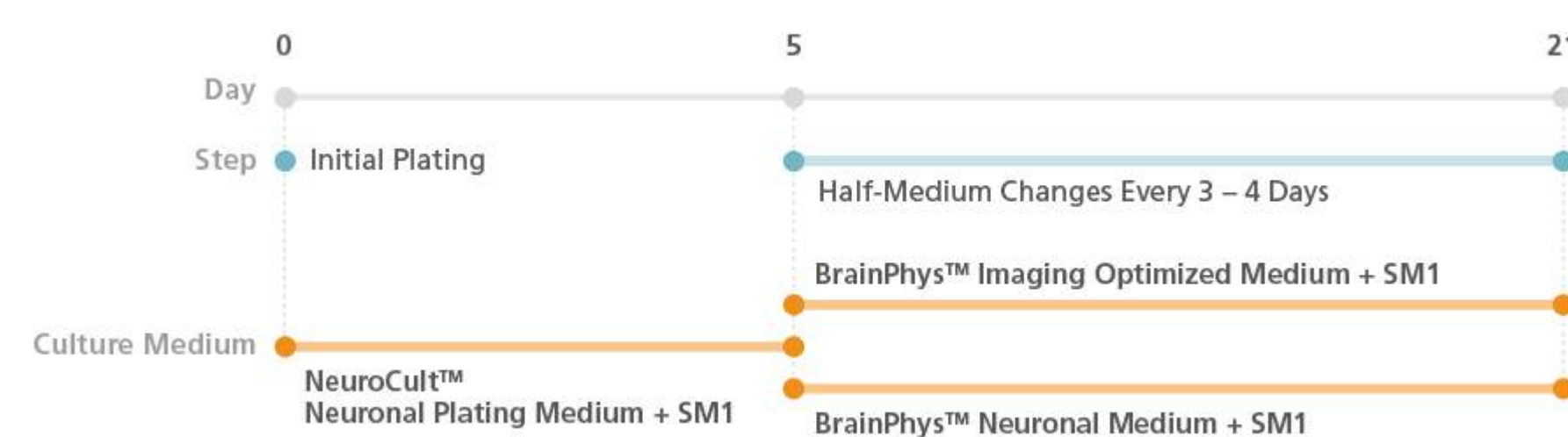


FIGURE 2. Workflow for Neuronal Differentiation and Maturation of Primary Tissue-derived Neurons

Phototoxicity and Autofluorescence Assays: To assess phototoxicity, a string of waterproof blue LED lights (470 nm excitation wavelength) were wrapped around a tissue culture plate to expose the entire culture surface to light for two separate 6-hour periods. At the end of the assay the number of surviving neurons was counted. To assess autofluorescence of the media (BrainPhys™ or BrainPhys™ IO), equal volumes of control (BrainPhys™) and test (BrainPhys™ IO) media were aliquoted to triplicate wells per condition in a 24-well plate. One image per well was taken with a Zeiss Axio Observer Z1 microscope using a 450 - 490 nm excitation filter. Mean emission at 525 nm from each image was recorded.

RESULTS

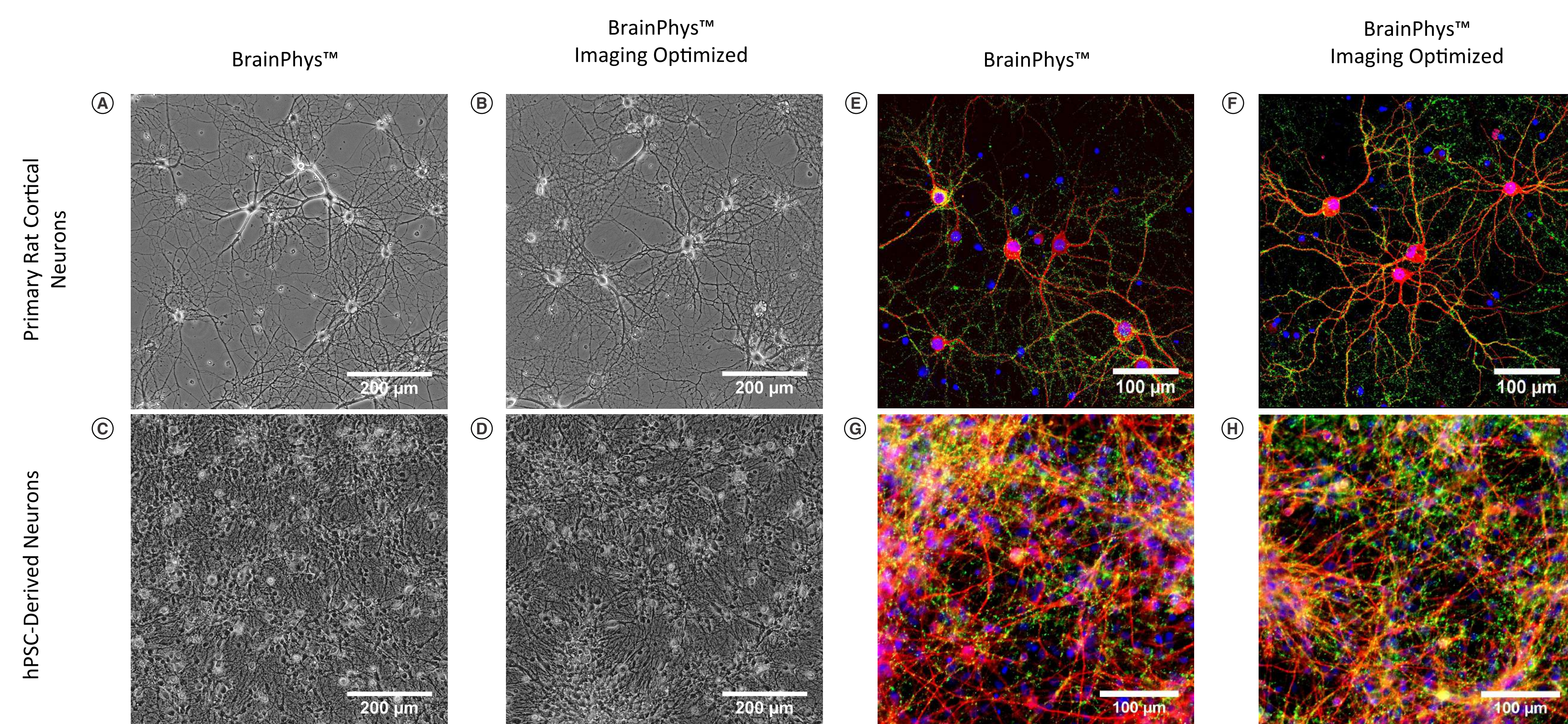


FIGURE 3. Primary Tissue and hPSC-Derived Neurons Cultured in BrainPhys™ IO for 21 Days Express Mature Neuron Markers

(A-D) Representative phase contrast images showing 21 days-in-vitro (DIV) primary rat cortical neurons and XCL1-derived neurons matured in BrainPhys™ and BrainPhys™ IO exhibit healthy neuronal morphology and distinct neuronal networks. (E-H) Representative immunocytochemistry images showing 21 DIV primary rat cortical neurons and H14-derived neurons characterized by MAP2 expression (red) matured in BrainPhys™ or BrainPhys™ IO also display appropriate expression of synaptic marker Synapsin1 (green).

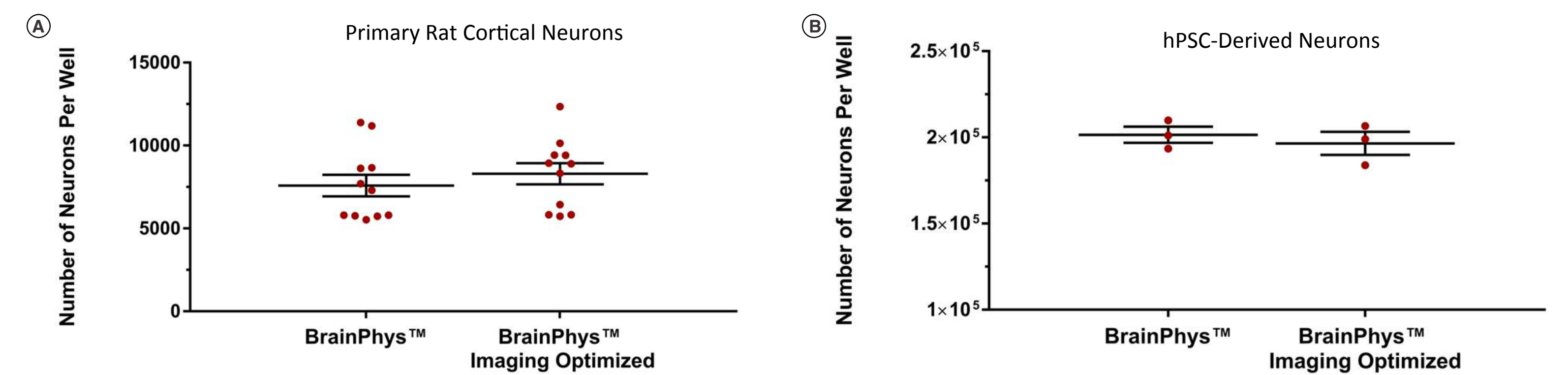


FIGURE 4. BrainPhys™ IO Supports Survival of Primary Tissue and hPSC-Derived Neurons for 21 Days in Culture (A-B) Quantification of the number of neurons per well at day 21 shows that BrainPhys™ IO supports equivalent neuronal survival relative to BrainPhys™ for both primary rat cortical (mean ± SE; n = 11) and hPSC-derived neurons (mean ± SE; n = 3; XCL1, H14, and STiPS-M001).

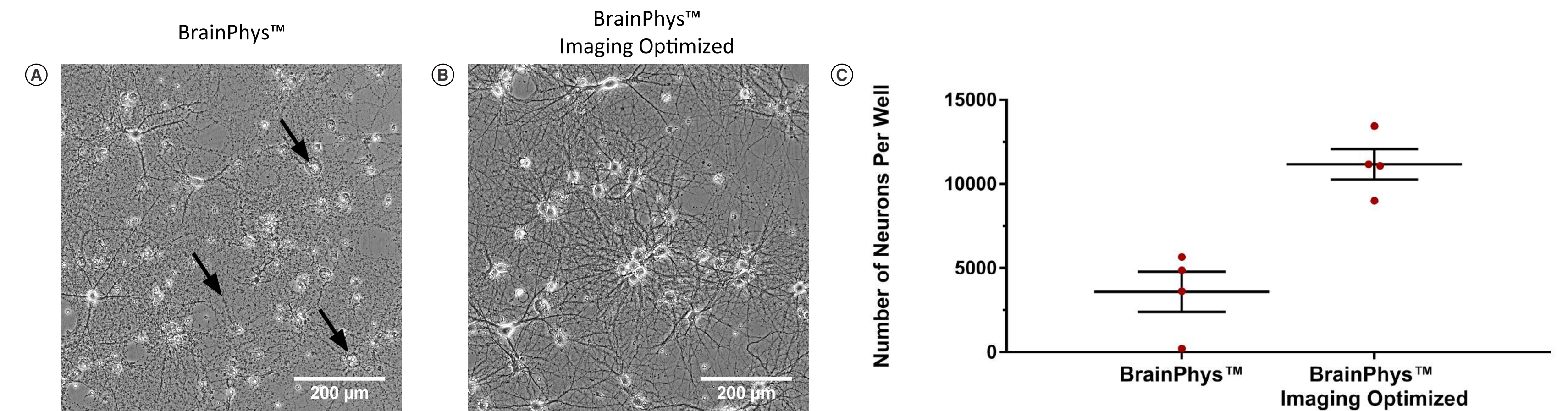


FIGURE 5. BrainPhys™ IO Reduces Phototoxicity After Blue Light Exposure

(A-B) Representative phase contrast images of primary rat cortical neurons cultured in BrainPhys™ and BrainPhys™ IO after exposure to blue LED light for 12 hours. Post-light exposure neurons in BrainPhys™ have disintegrated cell bodies and neurites (black arrows) while BrainPhys™ IO-cultured neurons maintain a healthy morphology. (C) Quantification of the number of neurons per well surviving post-light exposure. Phototoxicity reduces neuron survival in BrainPhys™ cultures to 30.2 ± 9.6% (mean ± SE; n = 4) of that in BrainPhys™ IO.

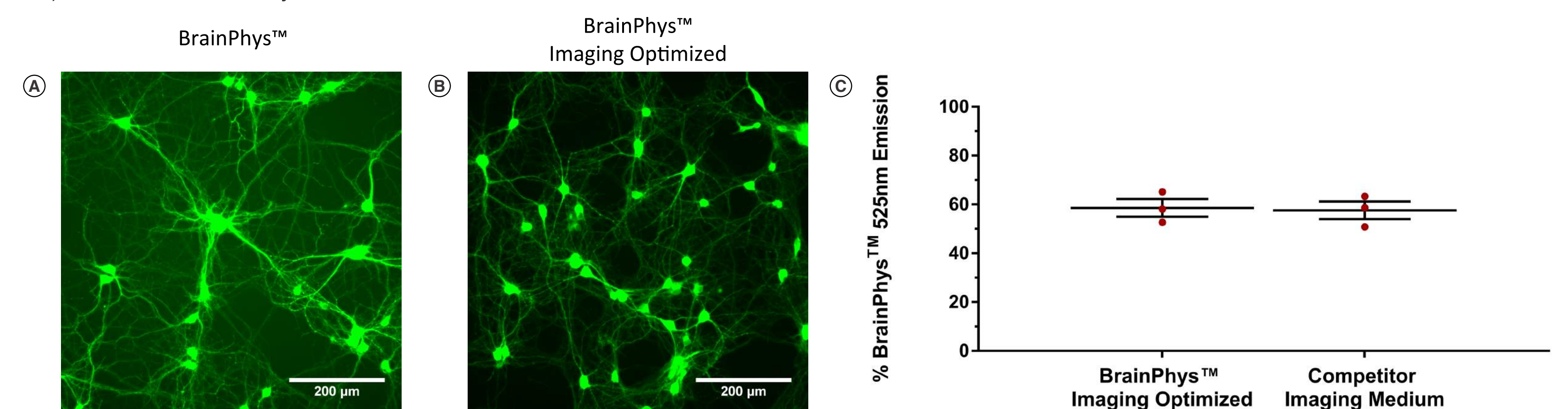


FIGURE 6. BrainPhys™ IO Reduces Autofluorescence When Exposed to Light at 450 - 490 nm

(A-B) Representative images of primary rat cortical neurons labeled with live neuron dye NeuroFluor™ NeuO (as per the Product Information Sheet, available at www.stemcell.com) imaged using 450 - 490 nm light in either BrainPhys™ or BrainPhys™ IO. BrainPhys™ IO showed reduced background fluorescence at a mean emission of 525 nm, resulting in improved image contrast. (C) Quantification of the background fluorescence at a mean emission of 525 nm from BrainPhys™, BrainPhys™ IO, and a competitor imaging medium across three fields of view in the absence of cells. Autofluorescence of BrainPhys™ IO was reduced by 41.1% ± 3.6% (mean ± SE; n = 3) relative to BrainPhys™ and was comparable to the competitor imaging medium.

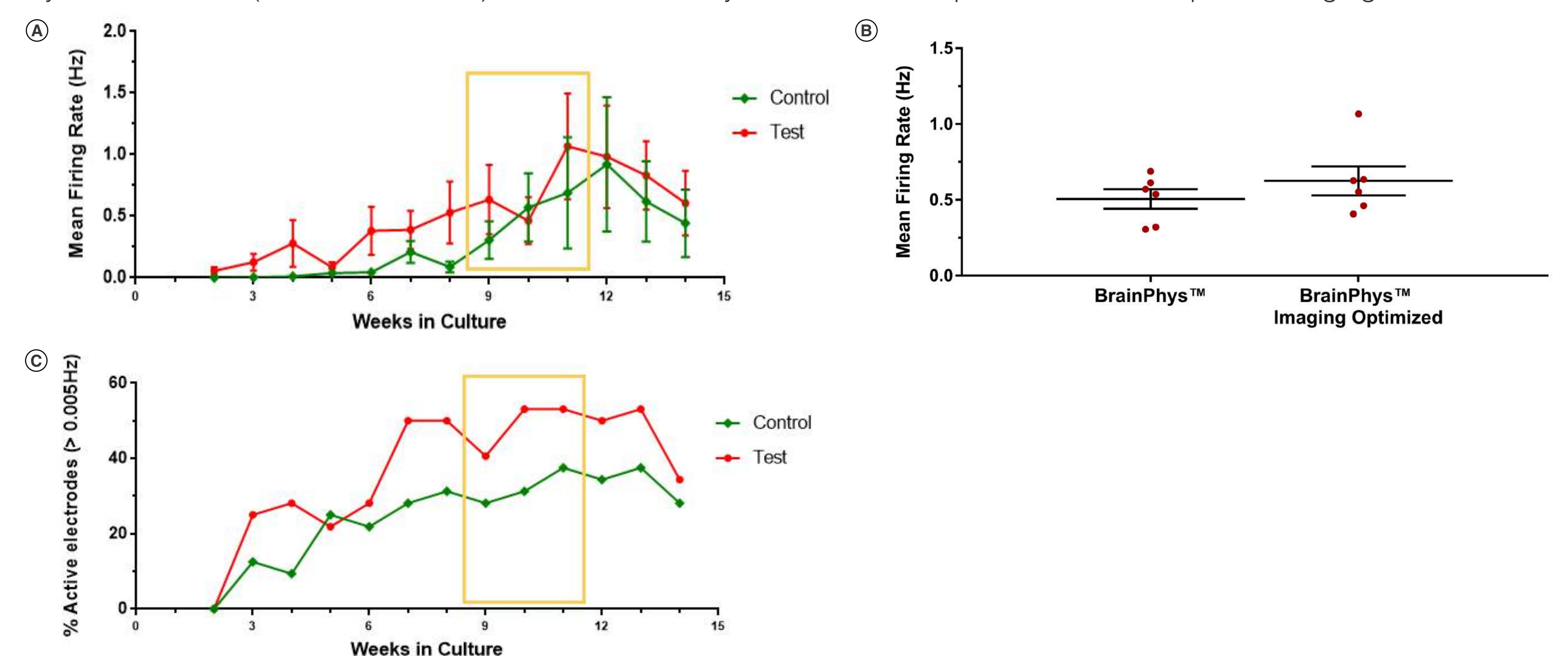


FIGURE 7. Neurons Cultured in BrainPhys™ IO Maintain the Same Level of Activity as Those Cultured in BrainPhys™ Neuronal Medium

Representative MEA recordings from XCL1-derived neurons cultured in BrainPhys™ for 14 weeks (control) or switched to BrainPhys™ IO from weeks 8 - 11 (test, BrainPhys™ IO culture period boxed in yellow). (A) No significant difference in mean firing rates were observed when medium was switched from BrainPhys™ to BrainPhys™ IO for 3 weeks. (B) During those 3 weeks, the average mean firing rate of neurons cultured in BrainPhys™ IO was 0.63 ± 0.10 Hz, which was similar to that of neurons cultured in BrainPhys™ (0.51 ± 0.06 Hz, mean ± SE; from 6 recordings; n=1). (C) The percentage of active electrodes was also maintained when medium was switched from BrainPhys™ to BrainPhys™ IO.

Summary

BrainPhys™ Imaging Optimized Medium:

- Reduces the phototoxic effects of blue light on neurons
- Improves imaging resolution by reducing autofluorescence
- Supports the high survival rates of primary tissue- and hPSC-derived neurons
- Maintains the same level of functional synaptic activity as BrainPhys™ Neuronal Medium