

Improving Functional Activity of Human Pluripotent Stem Cell-Derived Neural Organoids with BrainPhys™ Neuronal Medium

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INTRODUCTION

Neural organoids are a powerful technology to model neurodevelopmental processes, brain cytoarchitecture, and disease mechanisms. A key feature of neural organoids is their ability to form functional neural networks with spontaneous electrophysiological activity. Here, we optimized the use of BrainPhys™ Neuronal Medium to better promote functional activity in neural organoids generated using the STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit and the STEMdiff™ Cerebral Organoid Kit. Functional activity was assayed using a high-density multielectrode array (HD-MEA) from 3Brain. Organoids were cultured in Organoid Maturation Medium (OMM) and then switched to BrainPhys™ (supplemented with 10 mM glucose). Organoids cultured in BrainPhys™ exhibited enhanced activity compared to controls. Both dorsal forebrain organoids and cerebral organoids at day 150 showed improved activity in BrainPhys™ compared to control medium. To evaluate neural organoids as a platform for studying neuromodulatory drugs, we applied 100 μM 4-aminopyridine (4-AP) to induce an epileptic phenotype, followed by 1 mM valproic acid. Acute 4-AP treatment caused a 10- to 15-fold increase in the mean firing rate, while valproic acid reduced the firing rate by 3- to 5-fold relative to baseline. These findings suggest that neural organoids generated using STEMdiff™ kits exhibit robust neural activity enhanced by BrainPhys™ Neuronal Medium, providing a reliable platform for neuronal disease modeling and drug discovery.

METHODS

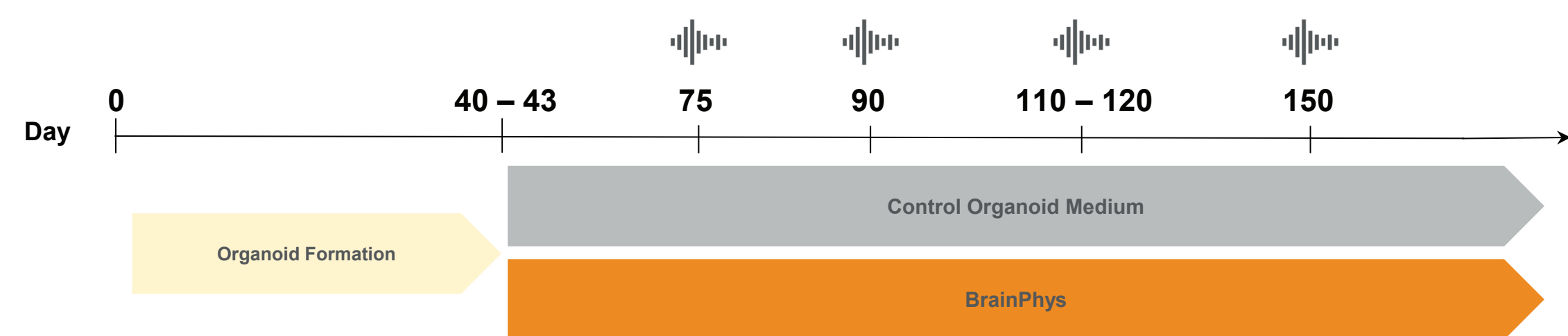


FIGURE 1. Overview of Experimental Method

Dorsal Forebrain Organoid Differentiation: hPSCs derived from the induced pluripotent cell line, SCTi003-A, or human embryonic cell line H1, were dissociated into single cells and seeded into an AggreWell™800 plate using Seeding Medium. The following day, 75% of spent medium was replaced with Organoid Formation Medium, and organoids were fed daily until day 6. On day 6, organoids were transferred to an ultra-low attachment (ULA) plate and cultured on an orbital shaker set at 70 RPM in Dorsal Forebrain Organoid Expansion Medium until day 25, then switched to Dorsal Forebrain Organoid Differentiation Medium until day 43. From day 43 onwards, organoids were cultured in either Neural Organoid Maintenance Medium (Control Medium) or BrainPhys + Supplements (1X SM1, 1X N2-A, 20 ng/mL BDNF, 20 ng/mL GDNF, 1 mM dibutyryl-cAMP, 200 nM ascorbic acid, 7.5 mM glucose) to promote further maturation. Full-medium changes were performed every 2 - 3 days throughout the culture period.

Cerebral Organoid Differentiation: STi003-A-derived hPSCs were dissociated into single cells and seeded into an AggreWell™800 plate using Seeding Medium. The following day, 75% of spent medium was replaced with Formation Medium, and organoids were fed daily until day 5. On day 5, organoids were transferred to Induction Medium for 2 days. On day 7, organoids were embedded in Matrigel® and transferred to a 6-well ULA plate, with 12 - 16 organoids per well. On Day 10, organoids were switched to Maturation Medium and cultured on an orbital shaker set at 70 RPM. Full-medium changes were performed every 2 - 3 days for the remaining culture period. From day 40 onwards, organoids were cultured in either Neural Organoid Maintenance Medium (Control Medium) or BrainPhys + Supplements (1X SM1, 1X N2-A, 20 ng/mL BDNF, 20 ng/mL GDNF, 1 mM dibutyryl-cAMP, 200 nM ascorbic acid, 7.5 mM glucose) to promote further maturation. Full-medium changes were performed every 2 - 3 days throughout the culture period.

MEA Recording and Drug Treatment: Neural Organoid functional activity was assessed on 3Brain 3D HD-MEA chip (4096 electrodes with 90 μm pillars, ~12 x 12 μm²; 3Brain AG) on day 60, 75, 90, 110/120, 150. The organoid was acclimated to BP medium for 90 minutes at 37°C, followed by baseline recordings using the BioCAM DupleX system (3Brain AG). For measuring neuroactive compounds, day 150 cerebral organoids were transferred to a 3D HD-MEA chip in 1.5 mL of protein-free BrainPhys™ medium (BP medium = BrainPhys™ Neuronal Medium with 1 mM cAMP, 200 nM ascorbic acid, 10 mM D-glucose, 100 μg/mL BDNF, and 100 μg/mL GDNF). The organoid was acclimated to BP medium for 90 minutes at 37°C, followed by baseline recordings. Afterward, the medium was replaced with BP medium containing 100 μM 4-AP and acclimated for an additional 90 minutes before a second recording was taken. A subsequent full-medium change was performed, replacing the medium with BP medium containing both 100 μM 4-AP and 1 mM valproic acid. The final recording was performed after a further 90-minute incubation. All data were acquired and analyzed using the BrainWave 5 software (3Brain AG).

RESULTS

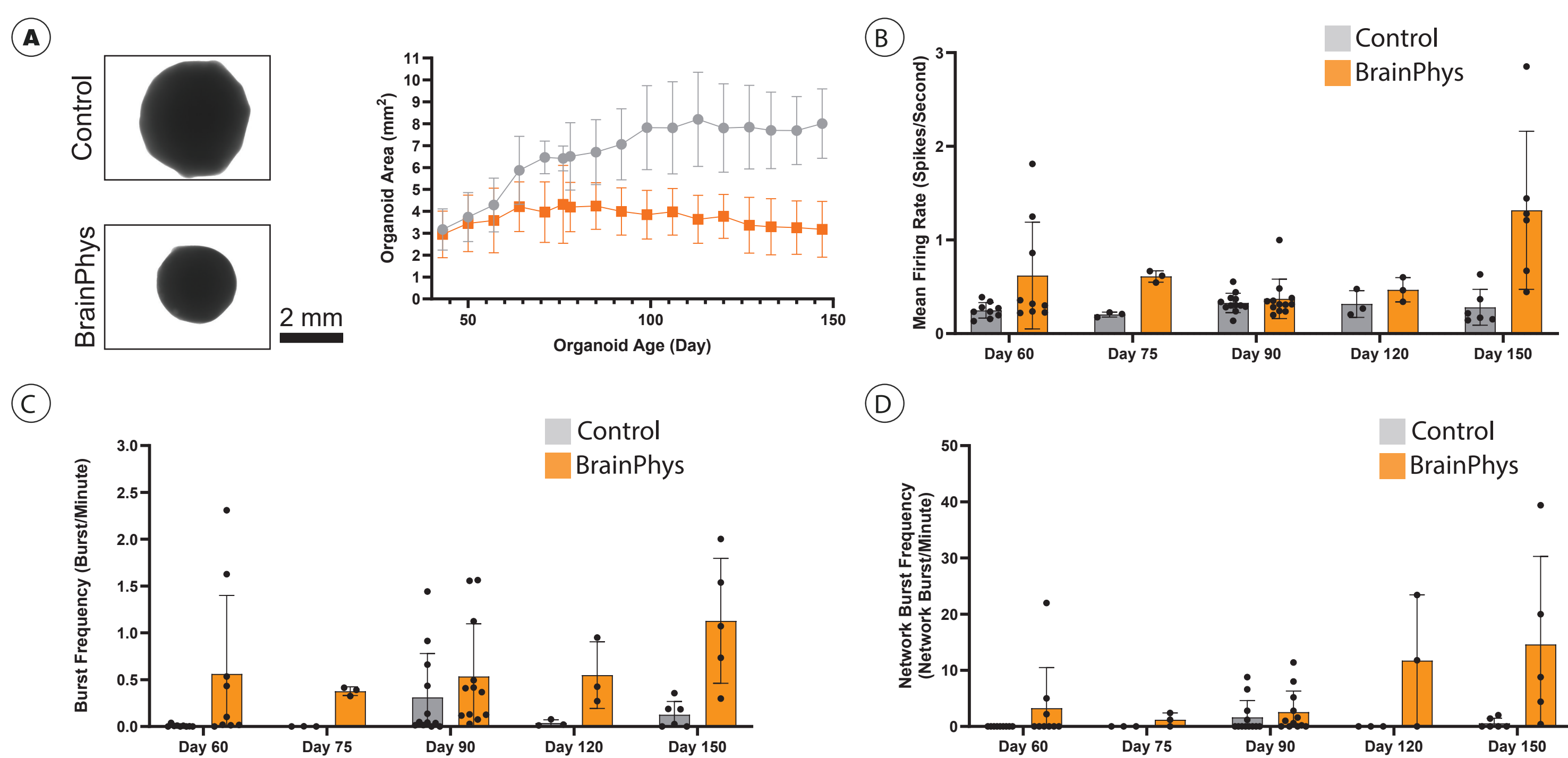


FIGURE 2. Long-Term BrainPhys Culture of Dorsal Forebrain Organoids Improves Functional Activity

(A) Representative brightfield images of dorsal forebrain organoids maintained in maintenance media or BrainPhys media. Organoids maintained in control media were significantly larger than organoids cultured in BrainPhys. (Average Mean Area +/- SEM from Day 43 - 150; Control 6.65 +/- 0.378 mm vs. BrainPhys 3.72 +/- 0.420 mm. $p < 0.0001$ using paired t-test) (B) Dorsal forebrain organoids maintained in BrainPhys media display an increase in mean firing rate (MFR) by day 150 compared to control media cultured organoids. (Average MFR +/- SEM at day 150; Control: 0.281 +/- 0.078 Hz, BrainPhys: 1.317 +/- 0.344 Hz. $n = 2$ cell lines, 3 - 9 organoids per time point. $p < 0.0001$ at day 150 by paired t-test). (C) Dorsal forebrain organoids maintained in BrainPhys media display an increase in burst frequency (BF) by day 150 compared to control media cultured organoids. (Average BF +/- SEM at day 150; Control: 0.125 +/- 0.058 Hz, BrainPhys: 1.129 +/- 0.298 Hz. $n = 2$ cell lines, 3 - 9 organoids per time point. $p < 0.01$ at day 150 by paired t-test). (D) Dorsal forebrain organoids maintained in BrainPhys media display an increase in network burst frequency (NBF) by day 150 compared to control media cultured organoids. (Average NBF +/- SEM at day 150; Maintenance: 0.567 +/- 0.367 Hz, BrainPhys: 14.60 +/- 7.013 Hz. $n = 2$ cell lines, 3 - 9 organoids per time point. $p < 0.01$ at day 150 by paired t-test).

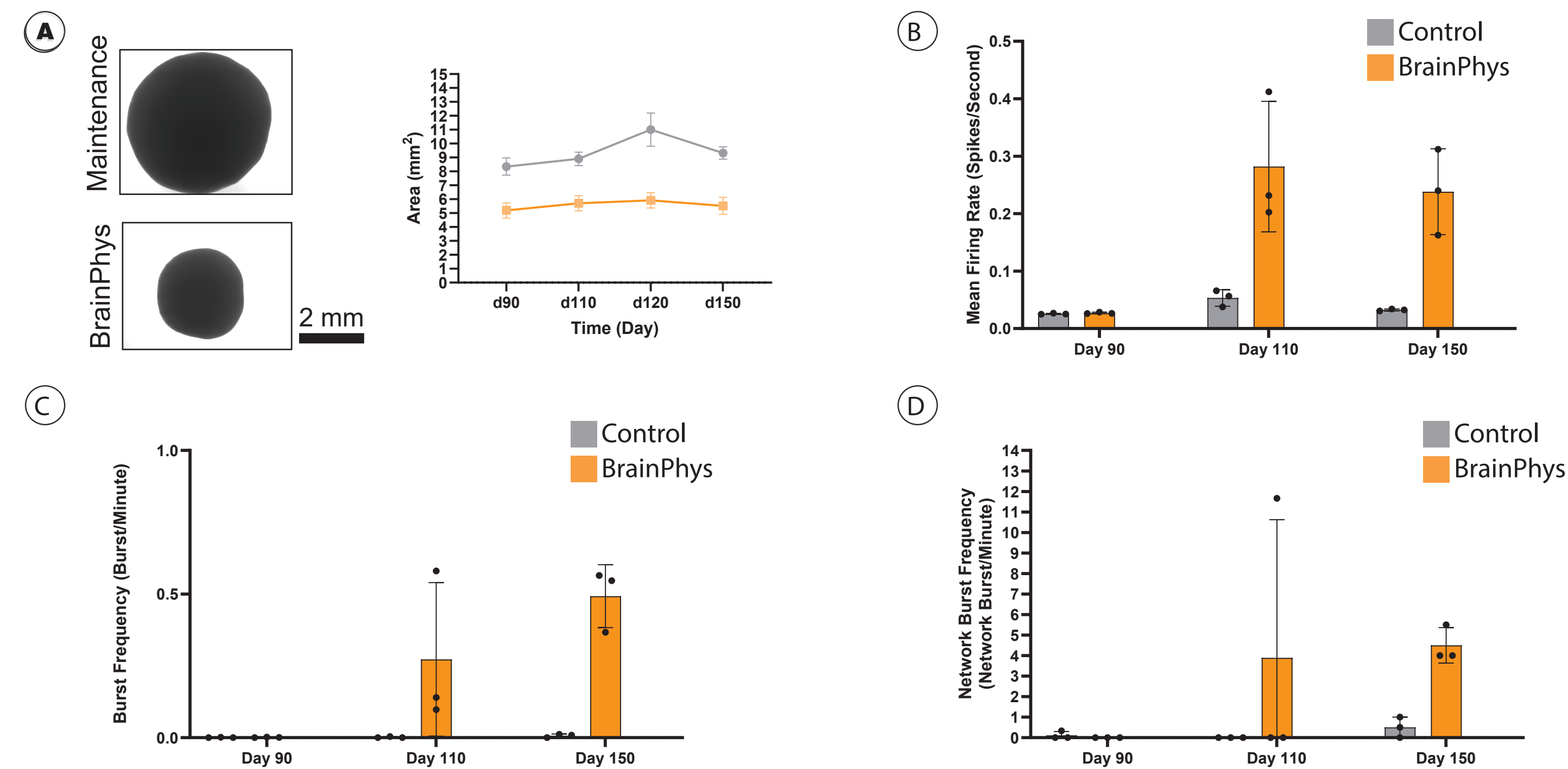


FIGURE 3. Long-Term BrainPhys Culture of Cerebral Organoids Improves Functional Activity

(A) Representative brightfield images of cerebral organoids maintained in control media or BrainPhys media. Organoids maintained in control media were significantly larger than organoids cultured in BrainPhys. (Average Mean Area +/- SEM from Day 90 - 150; Control 9.39 +/- 1.14 mm vs. BrainPhys 5.59 +/- 0.31 mm. $p < 0.0001$ using paired t-test) (B) Cerebral organoids maintained in BrainPhys media display an increase in mean firing rate (MFR) by day 150 compared to control media cultured organoids. (Average MFR +/- SEM at day 150; Control: 0.032 +/- 0.001 Hz, BrainPhys: 0.238 +/- 0.043 Hz. $n = 1$ cell line, 3 organoids per time point. $p < 0.01$ at day 150 by paired t-test). (C) Cerebral organoids maintained in BrainPhys media display an increase in burst frequency (BF) by day 150 compared to control media cultured organoids. (Average BF +/- SEM at day 150; Maintenance: 0.0069 +/- 0.0036 Hz, BrainPhys: 0.493 +/- 0.063 Hz. $n = 1$ cell line, 3 organoids per time point. $p < 0.01$ at day 150 by paired t-test). (D) Cerebral organoids maintained in BrainPhys media display an increase in network burst frequency (NBF) by day 150 compared to control media cultured organoids. (Average NBF +/- SEM at day 150; Control: 0.500 +/- 0.289 Hz, BrainPhys: 4.500 +/- 0.500 Hz. $n = 1$ cell line, 3 organoids per time point. $p < 0.01$ at day 150 by paired t-test).

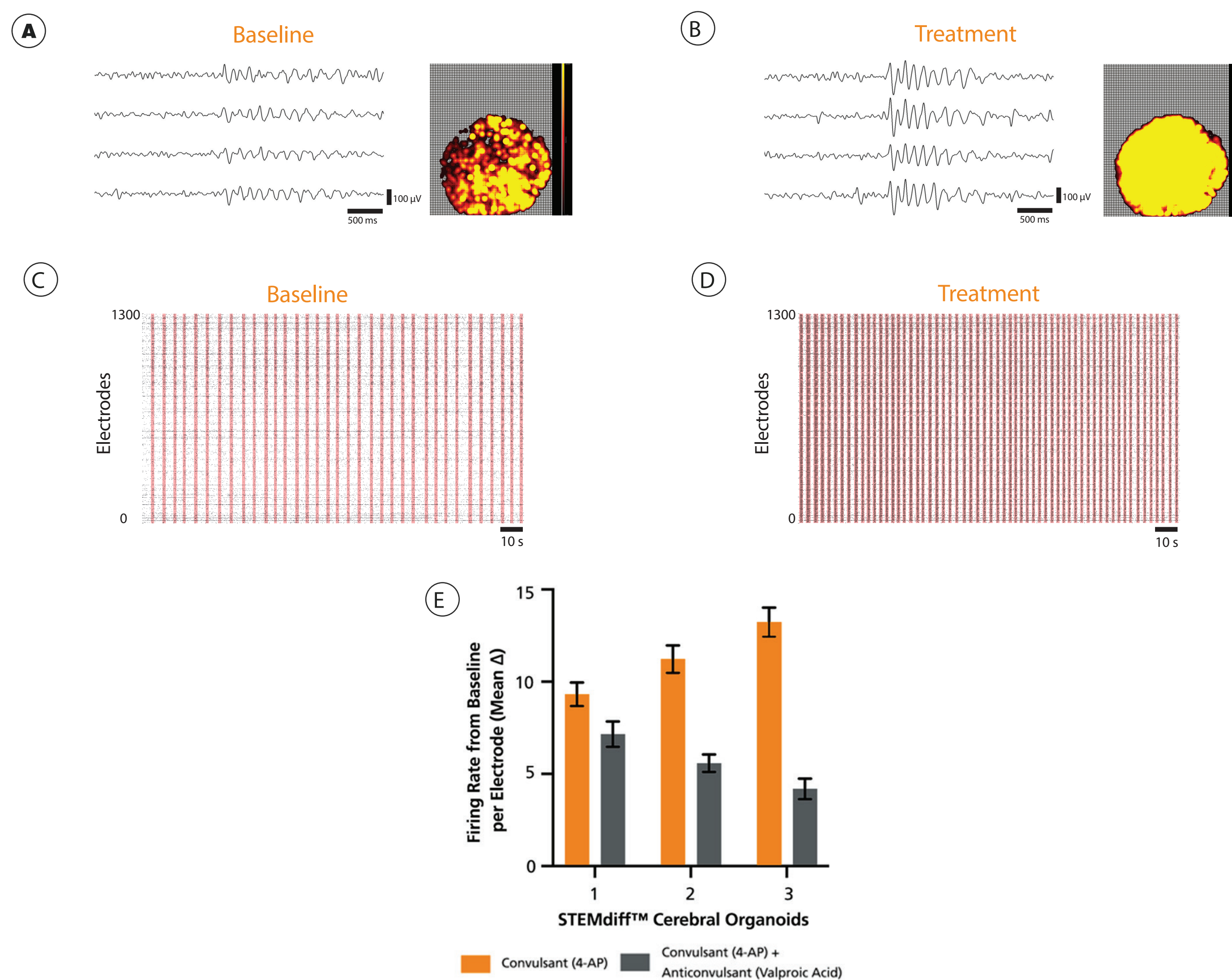


FIGURE 4. STEMdiff™-Derived Cerebral Organoids Were Responsive to Both Convulsant and Anti-Seizure Drugs

Field potentials (FPs) were recorded before and after the application of a chemical convulsant (100 μM 4-AP). (A) Baseline FPs measured by 4 electrodes (left) and an activity map of the entire organoid (right). (B) FP trace and activity map demonstrating the increase in FP amplitude after 4-AP treatment. (C) Representative raster plot showing the mean baseline FP firing rate of ≥ 1 FP/min, with network activity highlighted in red. (D) Raster plot showing an increase in FP firing rate after 4-AP treatment. (E) The change in FP firing rate (Hz) was measured in three ~170-day-old STEMdiff™-derived mature cerebral organoids (Organoids 1, 2, and 3) after 4-AP treatment. Paired electrode analysis was performed, with data represented as mean \pm 95% confidence interval (CI). All organoids exhibited an increase in FP firing rate following 4-AP treatment (orange). When the same organoids were treated with 4-AP in conjunction with the clinically-used anti-seizure medication, valproic acid (gray), the increase in FP firing rate was reduced compared to 4-AP treatment alone. Data generated in collaboration with a:head bio AG and 3Brain AG.

Summary

- Dorsal forebrain and cerebral organoids matured in BrainPhys™ Neuronal Medium are smaller than organoids grown in control medium
- Dorsal forebrain and cerebral organoids matured in BrainPhys™ Neuronal Medium displayed improved functional activity compared to control media.
- STEMdiff™-derived neural organoids provide a reliable platform for neuronal disease modeling and drug discovery.