Long-term live cell visualization and quantification of spontaneous neuronal activity and pharmacological response from human induced pluripotent stem cell-derived networks

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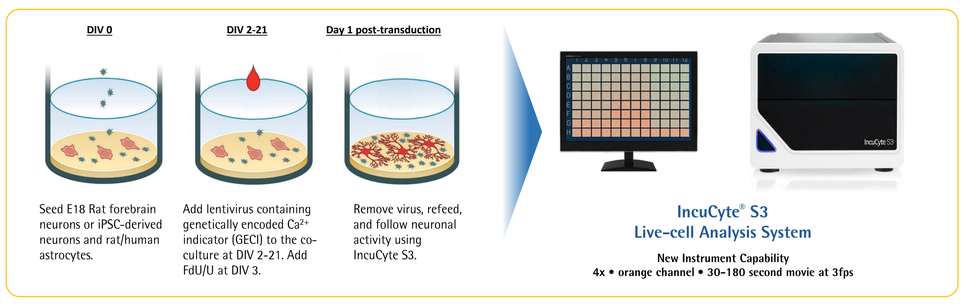
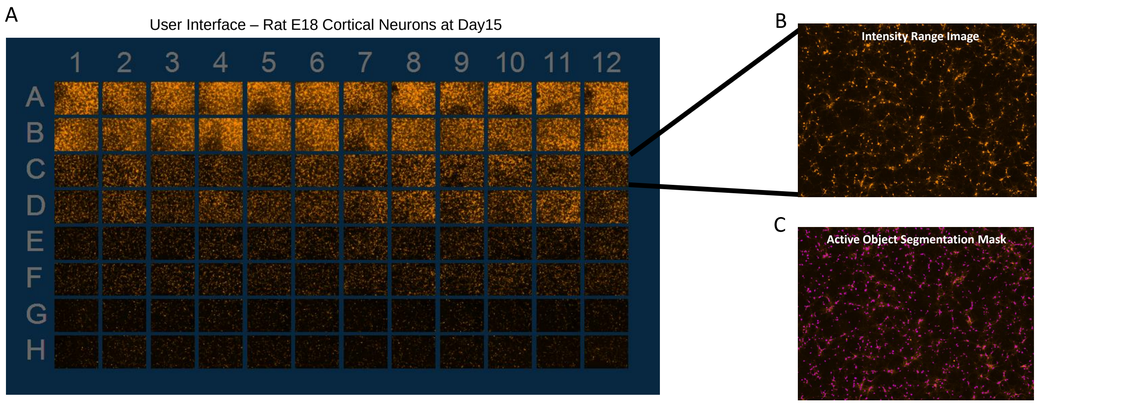
Summary & Impact

- A major impediment to studying human diseases affecting the nervous system is the ability to monitor, analyze, and quantify the activity of neuronal cells that accurately represent human phenotypes.
- The use of human induced pluripotent stem cell (hiPSC)-derived neurons has provided a new approach aimed at monitoring neurological disorders.
- Limitations of current instrumentation and biological protocols has revealed a clear need for more sophisticated methods designed to measure the functional activity and connectivity of iPSC-derived neurons over time with minimal perturbation.
- Here we present validation data using the IncuCyte S3® live cell analysis system to characterize spontaneous neuronal activity and connectivity from a variety of hiPSC-derived neurons and rat primary neurons following transduction of a genetically-encoded calcium indicator (GECI).
- Various metrics of neuronal activity were analyzed, including active object #, mean intensity, strength, rate, and duration, as well as network correlation (connectivity) over days/weeks in culture using the automated live cell neuronal activity analysis software of the IncuCyte S3®.

Neuronal Activity Workflow



Automated Neuronal Activity Analysis and Metrics



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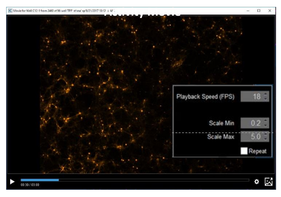
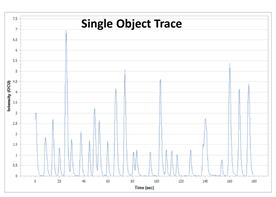
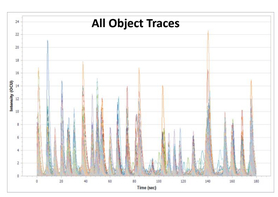
Fully Automated Analysis Metrics

Neuronal Activity
- Active Object #
- Mean Intensity
- Mean Burst Strength
- Mean Burst Rate
- Mean Burst Duration

Network Connectivity
- Mean Correlation







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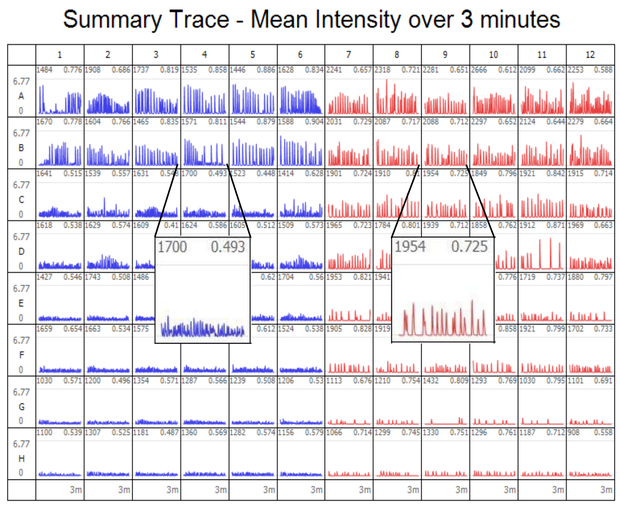

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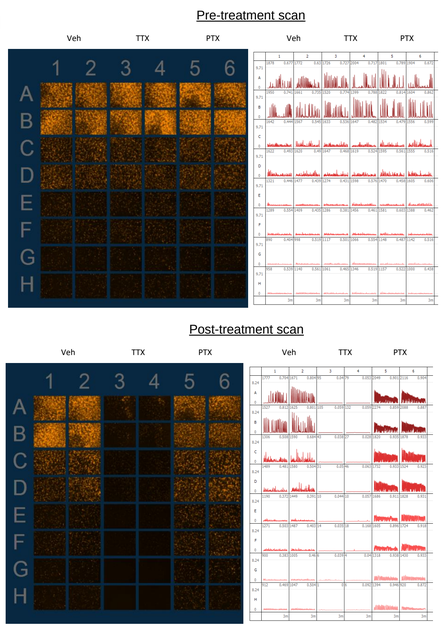
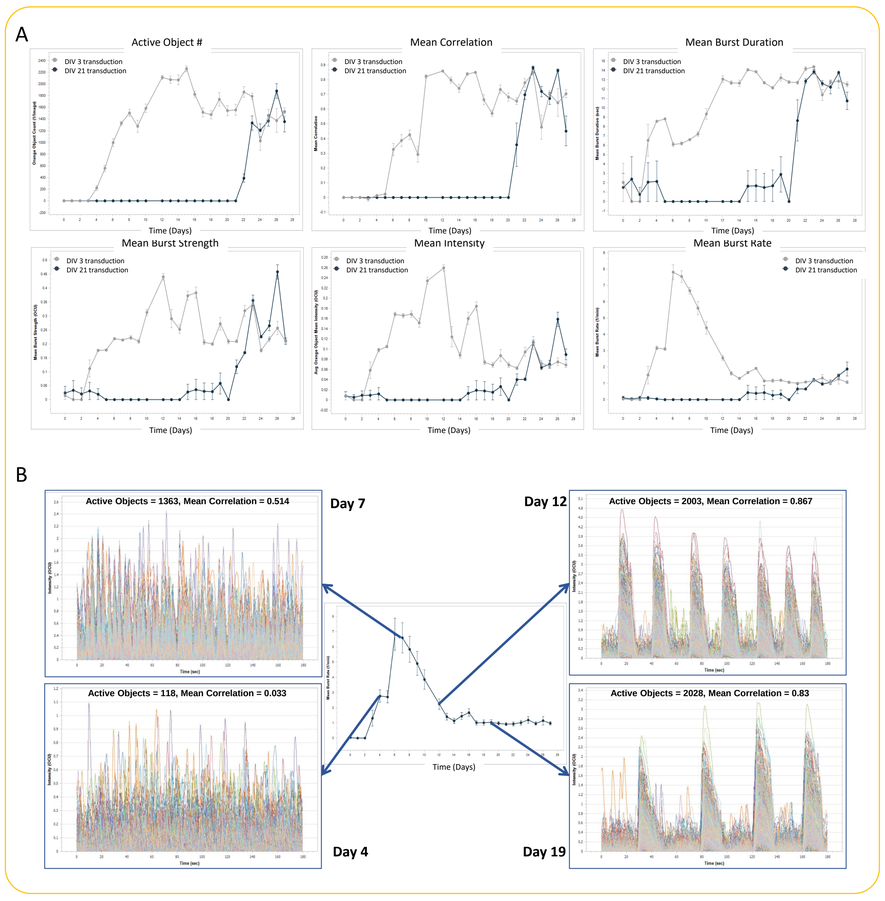


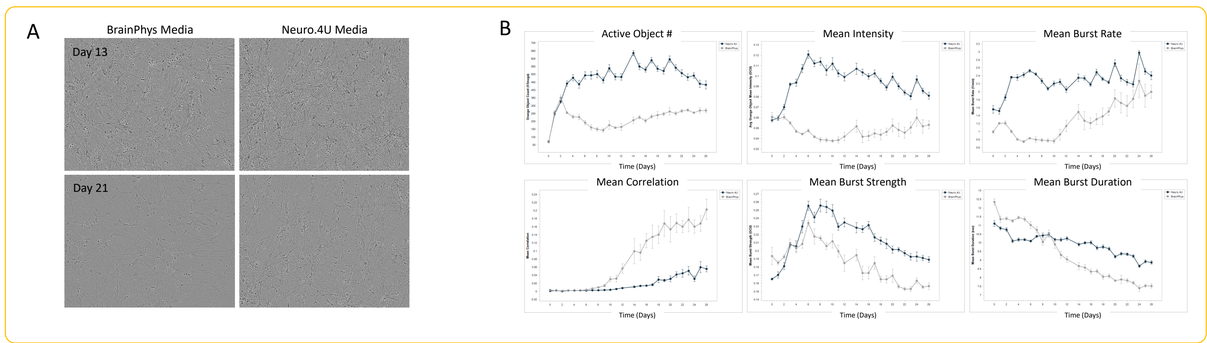
- User interface designed to visualize data from a 96 well plate at each scan of the experiment (A)
- Fluorescent image represents activity range over complete 30-180 second scan (B)
- Automated segmentation defines active objects (C)
- Built in analytical tools facilitate data analysis of neuronal activity with metrics that define neuronal activity (active object #, mean intensity, burst strength, burst duration, burst rate) and connectivity (mean correlation) (D)
- Movies can be generated for each well with viewing and export tools (E) and allows display of single object temporal traces (F) or overlays of temporal traces of all active objects within an image (G)
- Summary traces of whole well mean intensity provide complete plate view of data along with active object # and mean correlation metrics for well to well comparison (H)
- Sample data with endpoint pharmacology of tetrodotoxin (TTX, 1 µM) or picrotoxin (PTX,100 µM) show qualitative effects on neuronal activity, with PTX treatment increasing bursting rate and correlation (connectivity) and TTX treatment significantly decreasing all measured metrics, indicating that activity measured is synaptically driven (I).

iCell GlutaNeuron Spontaneous Activity Kinetic Analysis



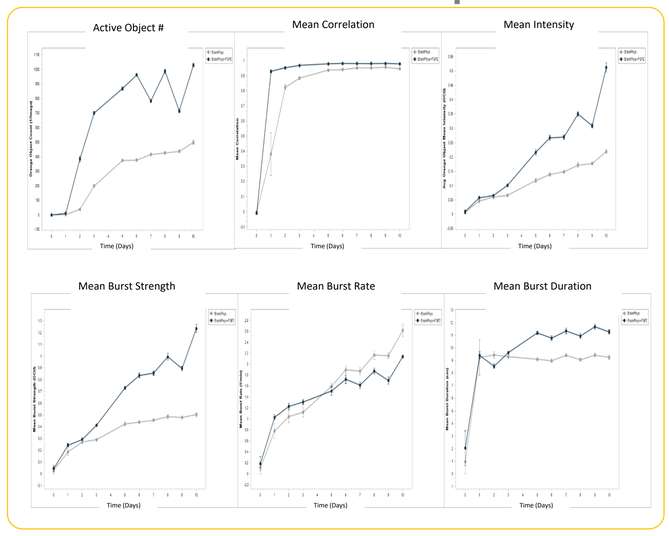
- iCell GlutaNeurons from Cellular Dynamics International were seeded at 30K cells/well with a co-culture of rat astrocytes seeded at 15K cells/well on PEI/laminin coated 96-well culture plates.
- Neurons were transduced with GECI reagent at DIV 3 or DIV 21; spontaneous neuronal activity was monitored and analyzed over a period of 27 days beginning at DIV 3.
- Automated analysis was used to quantify: active object #, mean correlation, mean intensity, mean burst strength, mean burst rate and mean burst duration **(A)** (n 3 wells for each condition). No differences in metrics were found between DIV 3 and DIV 21 GECI transduction at later time points.
- Example trace overlays of all active objects from a single well at Days 4, 7, 12 and 19 illustrate the change in the pattern of neuronal activity during the experiment with DIV 3 GECI transduction (**B**). Mean burst rate kinetic graph included to highlight activity (trace) differences over time.

Peri.4U Morphology + Spontaneous Activity Analysis



- Peri.4U Neurons from Ncardia were seeded at 25K cells/well with a co-culture of rat astrocytes seeded at 15K cells/well on PEI/laminin coating.
- Neurons were transduced with GECI reagent at DIV 2 and cultured in either BrainPhys media or Neuro.4U media.
- Morphology of the neurons was not affected by media differences at Day 13 or Day 21 of scanning (A).
- Metrics of spontaneous activity were quantified beginning at DIV 3 (n 3 wells).
- All metrics analyzed were increased in Neuro.4U media, with the exception of mean correlation, demonstrating the effect of culture media on neuronal activity (B).

Modulation of iNeuron Spontaneous Activity via FGF2 Treatment



- Induced iPSC-derived neurons (iNeurons) from Michael Uhler's lab at the University of Michigan were seeded at 10K cells/well with a co-culture of rat astrocytes seeded at 15K cells/well on Matrigel coating.
- Cells were cultured +/- FGF2 (20 ng/ml). iNeurons were transduced with GECI reagent at DIV 21, and automated analysis was performed to quantify spontaneous neuronal activity for a period of 10 days after GECI transduction (n 3 wells).
- FGF2 treatment increased active object #, mean intensity, mean burst strength and mean burst duration. FGF2 had no effect on mean correlation, while mean burst rate was decreased.

Conclusions

- The IncuCyte S3® spontaneous neuronal activity and analysis system has been validated using both rat primary neurons and multiple human iPSC-derived neuronal models (see summary table).
- Culture conditions (e.g. media/coating substrate) and growth factor addition can alter spontaneous neuronal activity over time.
- These data demonstrate the utility of the IncuCyte S3® spontaneous neuronal activity and analysis system as an effective tool to visualize, monitor and analyze live cell neuronal activity over time from hundreds to thousands of cells cultured in 96-well microplates with minimal perturbation.

Cells	Type	Setup	Vendor	Differentiation	Media Pref
Rat E18 Forebrain	Primary	Co-culture	Thermo/ Global Stem	NA	BrainPhys or NB
iCell GlutaNeurons	iPSC	Mono and Co	CDI	Unknown	BrainPhys
iCell GABANeurons	iPSC	Mono and Co	CDI	Unknown	BrainPhys
iCell DopaNeurons	iPSC	Co-culture	CDI	Unknown	BrainPhys
MyCell DopaNeurons (A53T)	iPSC	Co-culture	CDI	Unknown	BrainPhys
Peri.4U	iPSC	Co-culture	Ncardia	Unknown	Ncardia
CNS.4U	iPSC	Co-culture	Ncardia	Unknown	Ncardia
iNeurons	iPSC	Co-culture	Academic	Neurogenin2	BrainPhys