Application Note

The study of neurodegenerative diseases has produced a number of interesting lead compounds but very few that become approved drugs.

Over the years High Throughput Screening (HTS) has been used to discover new information on cellular processes in normal and diseased states.

Rodent neurons are widely used in these neuronal screening assays but if human cells could be used they would provide an improved model system to study disease biology and be more clinically relevant.

Human Induced Pluripotent Stem Cells are becoming a promising option for researchers creating in vitro models for the study of neurodegenerative diseases.

HUMAN INDUCED PLURIPOTENT NEURAL STEM CELLS

A CASE FOR USE IN NEURONAL SCREENING ASSAYS

The interest in Induced Pluripotent Stem Cells (iPSC) has intensified over the years and proves to be promising in its ability to impact human health.[1] The field is exploding with new methods for generating iPS cells and there are still questions about their performance in vitro compared to rodent neurons. What are the advantages to using iPS cells *vs.* rodent neurons? Can these neurons derived from iPS cells differentiate and function in vitro in the same ways that rodent neurons do? Can they be used in a HTS system to improve the success rate of drug discovery?

A novel system for differentiating iPSC to neurons was created by scientists at MTI-GlobalStem™ demonstrating that iPSC-derived neuronal progenitor cells could be differentiated into functional, mature neurons. Using Human iPSC-derived Neural Stem Cells (HIP™ NSC, Cat# GSC-4301) we showed that these cells are capable of being expanded providing a continuous supply of neurons and eliminating the process of deriving rodent neurons. Having the ability to expand human cells capable of differentiating into neurons provides us with new ways to study neurodegenerative diseases in a human context helping to improve the validity of drug screening data.

Once these cells have been expanded the big question is, can they be differentiated into functional neurons?

When grown in GlobalStem's PluriQ™ Human Cell Conditioned Medium (Cat# GSM-9200) on a special substrate, we were able to induce differentiation of HIP™ NSC's to produce cells containing neuron-like morphology. Once induced the cells were then passaged and plated into differentiation media NeuralQ™ (Cat# GSM-9420) and GS21™ Neural Supplement (Cat# GSM-3100) onto multi-well plates for approximately 5 weeks to confirm functionality.

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Characterization assays showed HIP™ iPS-Cell Derived Neurons (Cat# GSC-4312) over time displayed a neuron-like morphology staining positive for MAP-2, VGLUT 1, ChAT and GAD-67 shown in Figure 1. Figure 2 shows the HIP™ iPS-Cell Derived Neurons developed a spontaneous synchronized calcium flux. These cells were shown to be functional in Multi-Electrode Array assays in Figure 3 and also show pre-synaptic release and excitability comparable to primary neurons in Figure 4. All supporting the theory that HIP™ NSC's can be differentiated into functional neurons and used in drug discovery assays to provide an improved model system.

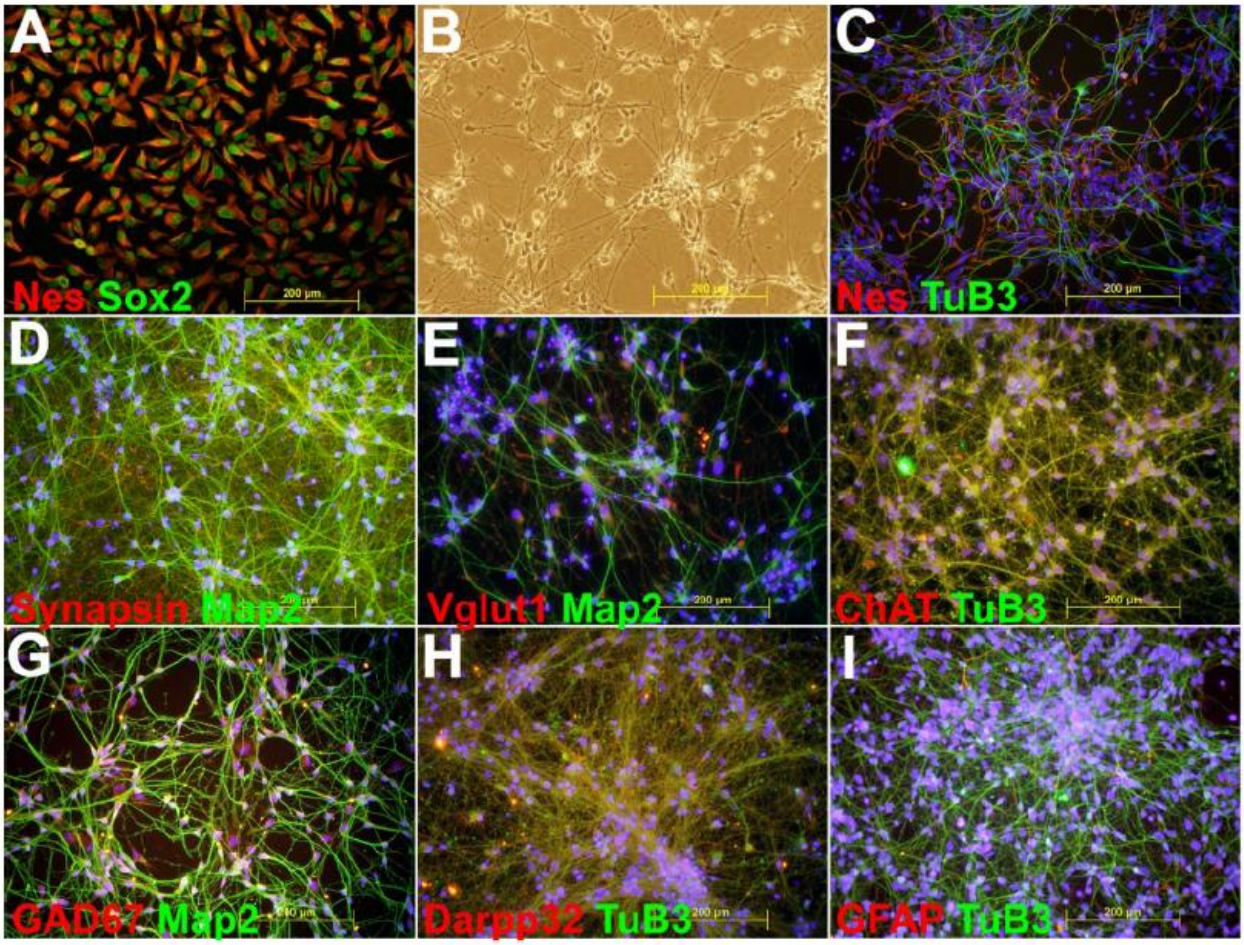

Figure 1: Neuronal differentiation of HIP™ Neural Stem Cells. (A) Proliferating Human iPSC-derived NSC express the neural markers nestin and sox1. (B) Four days in Differentiation Medium changes the cells to primarily elongated bipolar cells, consisting of TuB3-positive neurons and nestin-positive progenitors (C). After five weeks of differentiation, HIP™ NSCs produce (D) abundant Map2-positive neurons expressing the synaptic marker Synpasin; (E) Vglut1-positive astrocytes and glutamatergic neurons; (F) ChAT-positive cholinergic neurons; and (G) GABAergic neurons marked by GAD67 or (H) the inhibitory neuron-associated marker Darpp32. (I) Five week cultures also contained elongated astrocytes marked by GFAP. Blue in all images in Hoeschst 33342 nuclear stain. Scale bar = 200 µm

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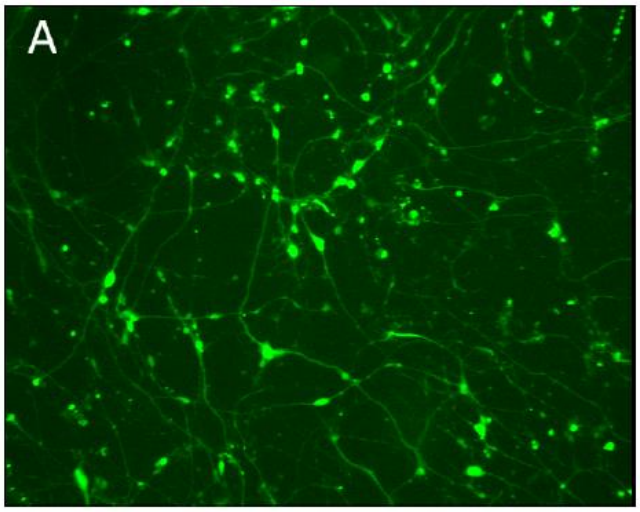


Figure 2: Human iPS-derived neurons develop spontaneous synchronized calcium flux. HIP™ Neurons were stained with Fluo-4 after six weeks of maturation and imaged n the BD Pathway kinetic imaging system produced (A). (B) Raw Fluo-4 fluorescence changes over a large number of neurons. The bracket indicates the frames depicted in Image A. (C) Raw Fluo-4 fluorescence traces of a grop of synchronously fluxing neurons shows the effect of Tetrodotoxin (TTX) added at the midpoint (arrow in C).

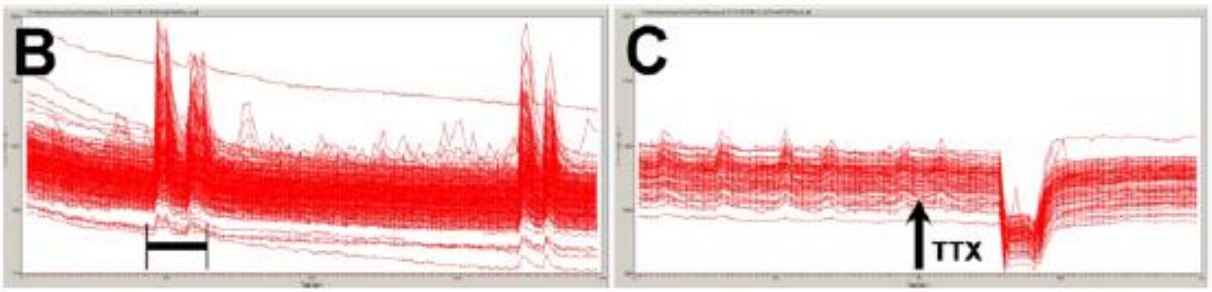
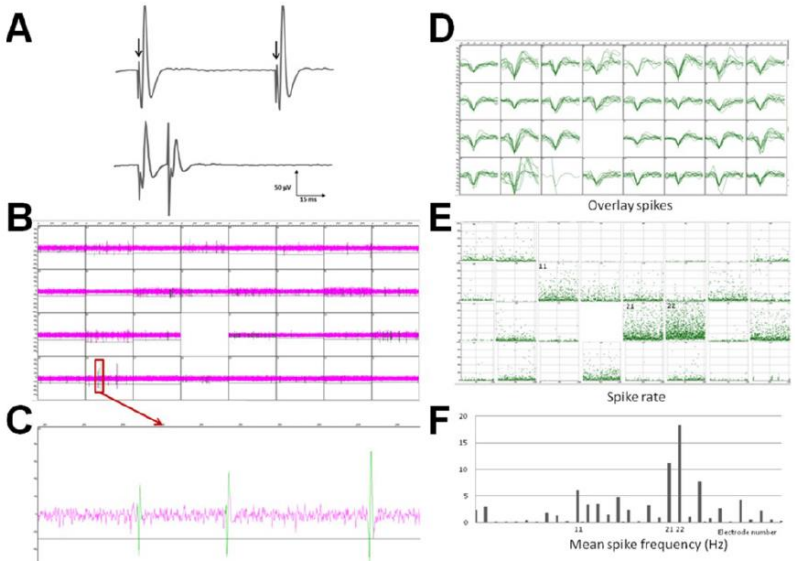


Figure 3: HIP™ iPS-derived neurons are functional in Multi-electrode Array assays. (A) Upper trace shows a paired-pulse stimulation with 70 ms interval inducing evoked field potentials (EFP). Stimulation artifacts are indicated by arrows. Lower trace illustrates that when the interval between two stimuli is set to 15 ms, we can observe a net diminution of the second EFP indicating a paired-pulse inhibition (PPI). Spontaneous activity from 31 electrodes is shown in (B). Many extracellular action potentials either individually or by burst could be recorded. An enlargement of three spikes is displayed in (C). (D) Overlay of spikes from the 31 electrodes (10 spike sweeps per electrode). (E) Spike rates (Hz) of each of the 31 electrodes are displayed during 100 sec. The mean frequency of the 31 electrodes is shown in (F). *Data Courtesy of Dr. Luc Stuppini, University of Applied Sciences Western Switzerland*



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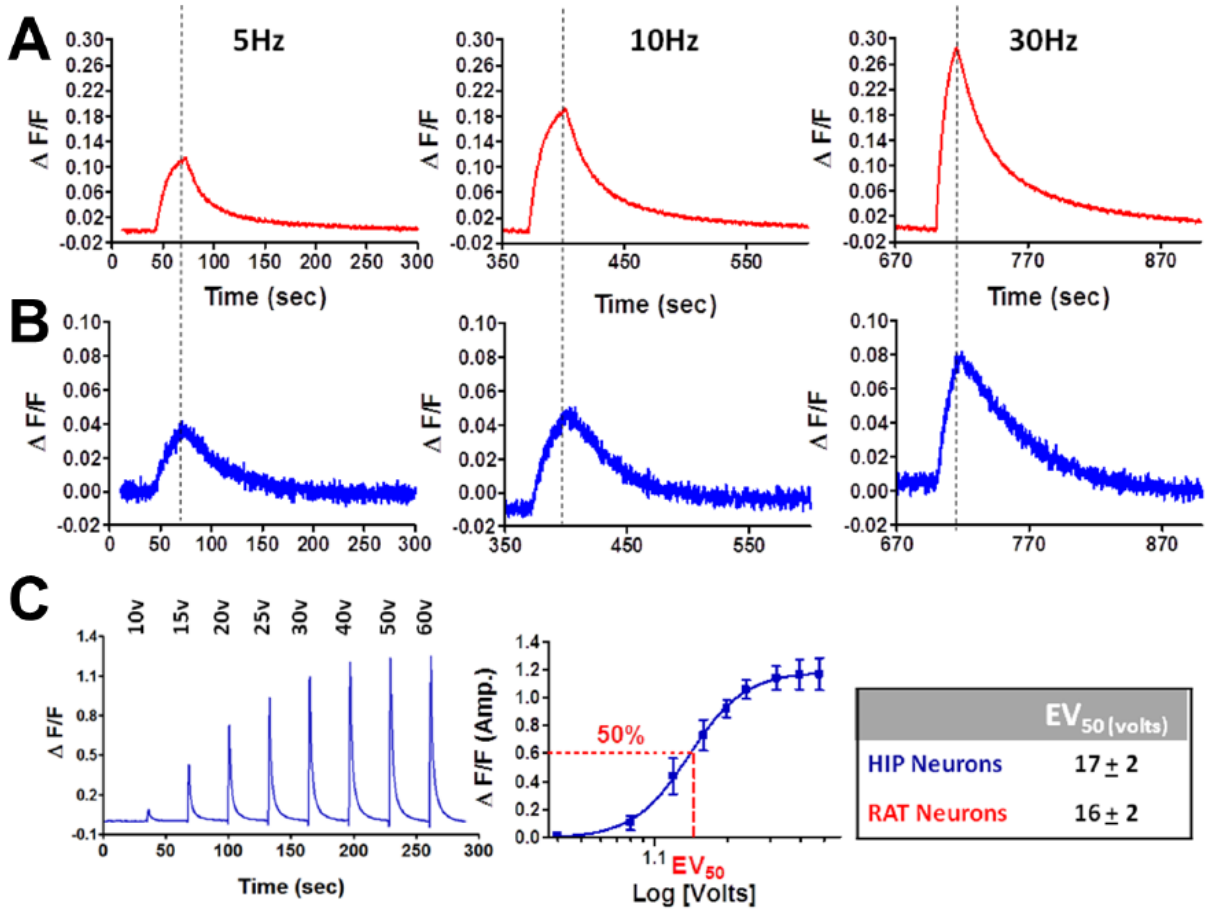

Figure 4: HIP™ iPS-derived neurons have presynaptic release and excitability comparable to primary rat neurons. (A, B) Cultures infected with a synaptophysin-pHluorin fusion fluorescent reporter construct (SypHy) were analyzed for vesicle presynaptic cycling activity using the Multiwell Automated NeuroTRansmission Assay (MANTRA) system (Galanea Corp.). Figures show functional evoked SypHy presynaptic responses at 5, 10 and 30 Hz. HIP™ Neurons (A) show similar frequency-dependent evoked waveforms to primary rat cortical neurons (B). HIP™ Neurons grown for five weeks were incubated with Fluo-4 and evoked Ca^{2+} transients were measured using the MANTRA system following increasing voltage stimulation intensities (C). Amplitudes (Amp.) were plotted against voltage to determine EV_{50} values. The EV_{50} of evoked Ca^{2+} transients in HIP™ Neurons are similar to those measured from rat neuronal cultures, indicating a similar action potential threshold.

To summarize, iPSCs can lead to exciting new advances in the neuroscience community. [2] These cells can be continuously expanded and stocked for future use, induced to differentiate into functional mature neurons, and used in drug discovery assays to study a host of neurodegenerative diseases, providing reproducible, clinically relevant data.

1. Methods Enzymol. 2012; 506: 331–360.
2. Science. 2007 Dec 21;318(5858):1917-20. Epub 2007 Nov 20.

Data Courtesy of GALENEA

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