Human Dopaminergic Neurons *In Vitro* Model Of Parkinson's Disease

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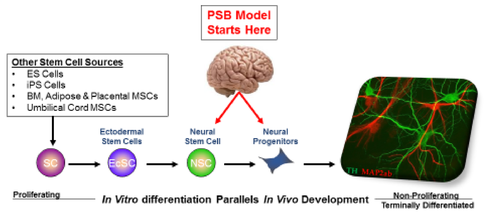
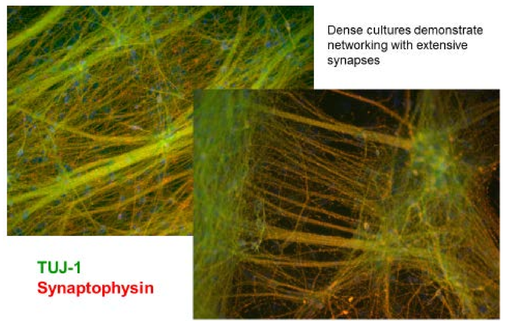
Stem Cell-Derived Neural Cultures



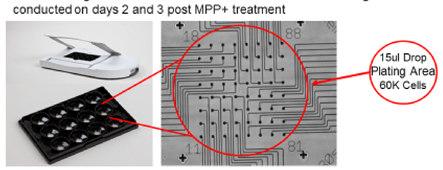
- Neural Stem Cells (NSCs) are already committed to neural lineages
- Growth conditions capable of a scale >10^9 NSCs
- NSCs differentiate into vary mature neurons with excitatory and inhibitory synapses in HTS formats (384 well)
- Neurons remain functional beyond 72 days *in vitro*

DA-H9 NSCs Differentiated 39 Days

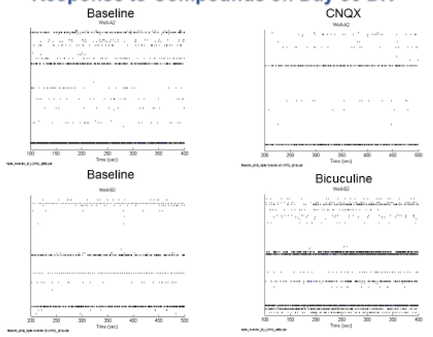


Neural Function Measured on MultiElectrode Arrays (MEAs)

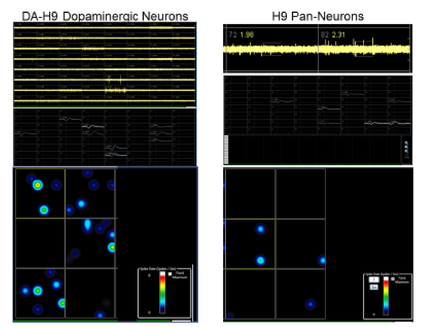
- Human DA-H9 NSCs were plated on PDL/Laminin coated MEAs from Axion Biosystems in Dopa Differentiation (DA-Diff) Media
- H9 NSCs were plated on PDL/Laminin coated MEAs in Neural Differentiation (N-Diff) Media
- Half feeds of DA-Diff or N-Diff were carried out every 4-7 days *in vitro* (DIV)
- MEA recordings were conducted randomly throughout the time in culture beginning on day 23 DIV and ending on day 72 DIV
- MPP+ w/wo growth factors was added to neurons and recordings were conducted on days 2 and 3 post MPP+ treatment



DA-H9 Dopaminergic Neurons Have Normal Response to Compounds on Day 36 DIV



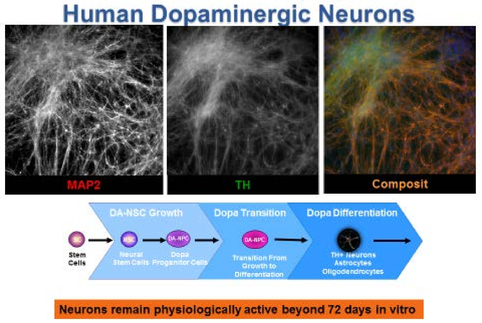
Spikes Detected in DA-H9 & H9 Neurons



Abstract

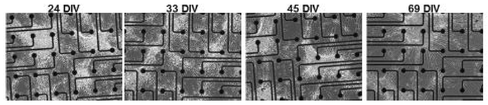
Parkinson's Disease (PD) is a chronic neurodegenerative disease, affecting approximately 1 million individuals in the US alone which is more than people diagnosed with multiple sclerosis, muscular dystrophy and Lou Gehrig's disease combined.. Parkinson's disease results in the loss of dopamine neurons (DA-neurons) that innervate the striatum. These dopamine neurons regulate the activity of a cortico-striatal-pallidal-thalamo-cortical (basal ganglia) circuit that controls the initiation and execution of motor and cognitive patterns. The loss of dopaminergic regulation of this circuit accounts for the development of the cardinal motor symptoms of the disease as well as many of the cognitive sequelae. Currently, a robust in vitro model of functional DA-neurons does not exist although many laboratories are trying to develop one. Therefore we set out to develop a robust *in vitro* Parkinson's model using stem cell-derived dopaminergic neurons at a scale that will be compatible with high throughput screens (HTS). Here we report development of a new PD model using human neural stem cells lineage committed to differentiate into a neural population where >60% of the neurons are TH+ mature functional DA-neurons that remain functional beyond 60 days in culture. Data reported will include standard MPP+ assays and neural network activity measured on multi-electrode arrays (MEAs).

Parkinson's Disease (PD) Model Human Dopaminergic Neurons

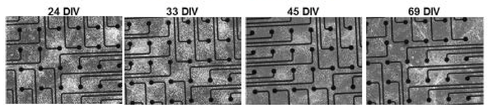


Neurons remain physiologically active beyond 72 days in vitro

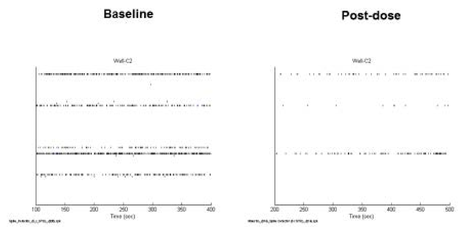
DA-H9 Dopaminergic Neurons



H9 Pan-Neurons



DA-H9 Dopaminergic Neurons Have Normal Response to LiCl on Day 36 DIV



DA vs. Pan Neural Results

- Dopaminergic neurons:
  - High percentage of dopaminergic neurons
  - Shows spontaneous firing of action potentials
  - Extremely high level of baseline activity—more than has been reported with other stem cell-derived dopaminergic neurons
  - Increased level of baseline activity compared to pan-neural, which is expected for this type of neuron
  - Responded as expected to compound treatment
  - Dose responce to MPP+ as expected
  - MPP+ treatment demonstrated a decrease in firing
- Pan-neural neurons:
  - Shows spontaneous firing of action potentials
  - Activity increased with DIV
  - Compounds treatments worked as expected

Materials and Methods

HIP-009 Neural stem cells (H9 NSCs) were isolated from human brain tissue obtained with the proper informed consent. H9 NSCs were expanded in Neural StemCell Growth media (PhoenixSongs 21001-250) with additional growth factors to direct developmental specification toward dopaminergic fate. The expanded dopaminergic H9 neural progenitor cells (DA-H9 NPCs) were then transitioned for differentiation for 48 hours in Neural Transition media (PhoenixSongs 21003-250) with additional factors to transition the DA-NPCs for differentiation into dopaminergic neurons. The transitioned DA-H9 NPCs were then dissociated with Trypsin (PhoenixSongs 41004-100) followed by inhibition of the trypsin with Soybean Trypsin Inhibitor (PhoenixSongs 41005-100). The dissociated transitioned DA-H9 NPCs were pelleted by centrifugation and resuspended in Dopaminergic Differentiation media (PhoenixSongs 21002-250) and the cells were then plated either on poly-d-lysine(PDL)/Laminin coated multi-electrode arrays (MEAs from Axion Biosystems) for functional analysis of the neural network or into PDL/Laminin coated 384-well plates for the MPP+ assay. The differentiated DA-H9 NPCs were maintained in Dopaminergic Differentiation media throughout the days *in vitro (DIV)* for out to 72 days.

- MPP+ Assay
  - Cells - DA-H9 transitioned NPCs differentiated into dopaminergic neurons
  - Allow *in vitro* Differentiation (IVD) to continue in complete DA-Differentiation media
  - MPP+ dose response curve (DRC)
    - 3 IVD time points
      - Dose MPP+ on 18d, 25d and 31d IVD neurons
        - (28d IVD required for mature neurons)
      - Fix neural populations on day 3 of MPP+ exposure
    - 2 Treatment (Trt) media
      - Trt-1 – without growth factors
      - Trt-2 – with high BDNF/GDNF
  - ICC: store fixed cells at each time-point and applied antibodies as one group
    - TH, MAP2 primary antibodies, Alexa 594, Alexa 488 and Hoeschst 33342
    - High content imaging/analysis (HCI/A) carried out at the Hamner Institutes
      - Capture images and analyze data
- Functional Analysis of dopaminergic neurons on MEAs
  - Measure spikes and burst activity in neural networks
    - Show spontaneous firing of action potentials
    - Compare baseline activity across days in vitro (DIV)
    - Show effects of compounds
      - 10μM CNQX – AMPA/Kinate Non-NMDA glutamate receptor antagonist
      - 20μM Bicuculline – Blocks Inhibitory action of GABA receptor
      - 10μM CNQX + 20μM Bicuculline
      - 50μM APV – NMDA receptor antagonist
    - Show effects of treatment with MPP+ +/- growth factors for PD model

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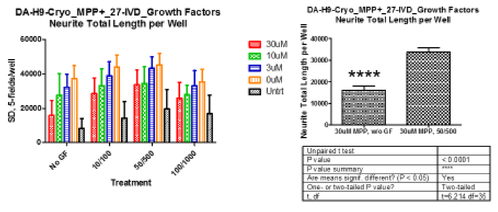
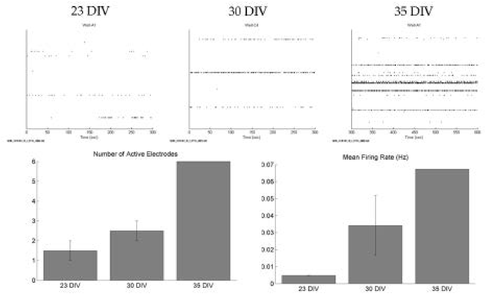
MPP+ Dose Response Assay High Content Data



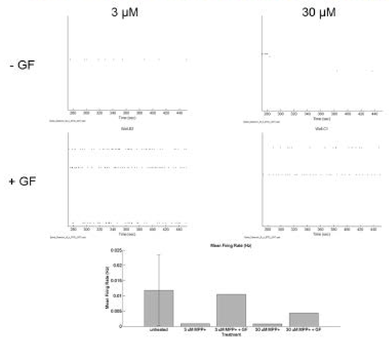
Unpaired t test	
P value	< 0.0001
P value summary	****
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=6.214, df=36

BDNF/GDNF Offer Neuroprotection in MPP+ Dose Response Assay

Dopaminergic Neurons (Untreated Wells) at Different Times in Culture



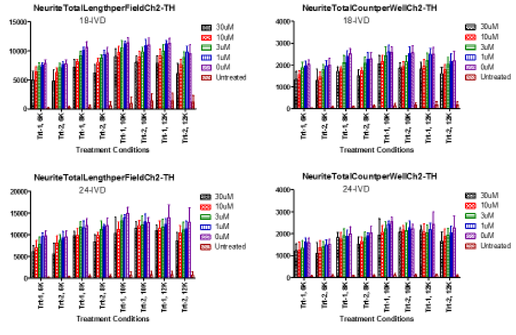
MPP+ +/-GF Effect on Mean Firing Rate



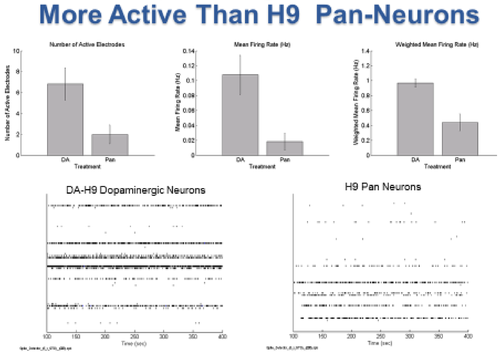
Contextual Disease Models

- Mature dopaminergic neurons for contextual disease models
- Disease pathology often involves interplay between different cell types and target biology dependent on cell environment.
- Need mature cells that represent human pathology and disease models.
  - Morphology
  - Electrophysiology
  - Neural network activity
  - Intracellular Signaling and enzyme activation
  - Receptor biology
  - Biomarkers
- Human Disease Models for functional screens
  - Dopaminergic neurons in a model for Parkinson's Disease (PD)

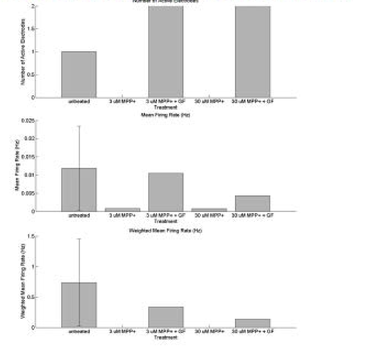
MPP+ Density, DIV & Dose Response



DA-H9 Dopaminergic Neurons Were More Active Than H9 Pan-Neurons



MPP+ Decreased Firing and was Rescued by GF Corresponding to MPP+ Concentration



PhoenixSongs' Competitive Advantage

- Our models validated against gold standard primary rodent neurons
- Cells & media products validated in house prior to release – buy with confidence
- Scalability of our NSCs due to our novel media formulations
- Robust differentiation into functional neural populations with excitatory and inhibitory synapses
- High percentage of dopaminergic neurons
- Neural cultures remain functional *in vitro* beyond 70 days post plating in differentiation media
- Reproducibility of data
- Affordable compared to primary rodent neurons
- Supports FDAs 3R initiative to replace animal models with physiologically relevant *in vitro* models