Advancements in the Use of iPS Cell-derived Cells for In Vitro Disease Modeling and Phenotypic Screening
HTS screening paradigms

Phenotypic screening

Target-based screening
Brief history of drug discovery strategies

Prior to 1980s

**Phenotypic screening**

- Only option
- Relied on biological model systems available

**Target-based screening**
Phenotypic screening
- difficult to identify target
- lack in advances of relevant human biology model systems

Target-based screening
+ molecular biology/genomics technology facilitates target ID
+ chemical/structural tools/informatics for lead optimization
Phenotypic screening

+ improved target ID by chemical proteomics
+ improved human biological model systems

Target-based screening

- Limited to targets under study
- Less efficient at discovering first-in-class medicines

2000-present
Phenotypic screening vs. Target-based screening

Figure 3 | **Cumulative distribution of new drugs by discovery strategy.**

**a** | First-in-class drugs. A lag is not strongly apparent in a comparison of the cumulative number of small-molecule new molecular entities (NMEs) that were discovered from the different approaches during the period analysed.

**b** | Follower drugs. For follower drugs, the ratio of small-molecule NMEs discovered through target-based screening to those discovered through phenotypic screening appears to increase in the second half of the time period.

David C. Swinney and Jason Anthony
“How were new medicines discovered?”
Phenotypic screening vs. Target-based screening

David C. Swinney and Jason Anthony
“How were new medicines discovered?”
Application of iCell® Neurons in Phenotypic Screening

SLAS January 2013
CDI Technology & Company Background

Application of iCell Products in Drug Development

• iCell Cardiomyocytes

• iCell Neurons

Case Study of phenotypic screen application of iCell Neurons
To revolutionize life-science research and medicine, mastery of all three technology platforms is required: *reprogramming, differentiation, and engineering*

Mastery of one or two is evolutionary, not revolutionary.
Cellular Dynamics International (CDI) is the world’s largest producer of human iPS cells and iPS cell-derived cell types

Headquartered in Madison, WI

Currently employs >100 total staff (mostly scientists)

~400 yrs human stem cell experience (in a field ~15 yrs old)

>700 patents (owned or licensed) to enable FTO

Core competencies

- Creation and culture of human iPS cells
  » Normal and disease phenotypes
- Genetic engineering of iPS cells
  » Lineage and pathway-specific markers can be introduced
- Development of new differentiation protocols
  » Differentiated cells from all three germ layers
- Manufacture of human iPS cell-derived cell types
  » Scalable production of highly purified cells

Partnership with iPS Academia Japan enables access and support for CDI’s products in Japan

The Wall Street Journal
Gold Winner: CDI
Technology Innovation Awards 2011
CDI Overview
Quality, Quantity, Purity

**Quality**
- Exhibit key cellular characteristics
- Recapitulate normal human biology
- Reproducible
- Known and relevant genotype

**Quantity**
- Sufficient to support HTP drug screening and safety testing
- Currently 1Bn iCell Cardiomyocytes/day

**Purity**

![Graph showing cell purity and days in culture](image)
**iCell® Products**

- **iCell Cardiomyocytes**
  
  *First commercial product*

- **iCell Endothelial Cells**
  
  *Launched Q3 2011*

- **iCell Neurons**
  
  *Launched Q4 2011*

- **iCell Hepatocytes**
  
  *“Pre-commercial” release*  
  (>40 Bn shipped since July, 2011)
iCell products are supplied as kits that contain the following:

- Cryopreserved human cells (>95% purity)
- Plating & maintenance media
- User’s Guide

iCell products are supported by:

- Team of application scientists
- Dedicated applications lab
- Application Notes & Protocols
- iCell Institute
  » Product & application webinars
  » iCertification courses
  » iCell Grant programs
  » User Group Meetings
Platforms & Partners

Cell-based Assays/Toxicity
High Content Imaging
Label-Free Cell Analysis
Multi-Electrode Array
Bioenergetics
Ca\(^{2+}\) Handling/Contractility
Manual/Automated Patch Clamp
Transfection
RNAi
Reporter Assays
3D Tissue Modeling
Etc.....
Astrocytes
Nociceptors
Dopaminergic Neurons
Motor Neurons
Hematopoietic CD34+ Cells
Cardiac Progenitor Cells
Skeletal Muscle Cells
Assay-Ready Cells
Etc…..
CDI Technology & Company Background

Application of iCell Products in Drug Development

- iCell Cardiomyocytes
- iCell Neurons

Case Study of phenotypic screen application of iCell Neurons
### Key Cardiomyocyte Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Adherent monolayer; striated myofibril network</td>
</tr>
<tr>
<td><strong>Molecular Markers</strong></td>
<td>α-actinin, cardiac troponin-T, connexin-43</td>
</tr>
</tbody>
</table>
| **Functional Characteristics** | - Electrically and mechanically active  
|                         | - Fully functional biochemical processes         |

### Purity and Morphology

![Image of purity and morphology analysis]

### Gene Expression

![Gene expression graph]


### Electrophysiology

![Electrophysiology graph]

iCell Cardiomyocytes
Assay Endpoints

Ion Channel and Action Potential

- **I\(_{\text{Kr}}\) Block**
  - APD prolongation

Mechanical Activity

- **A. Contraction**
  - Contraction IC\(_{50}\) = 87nM
  - Ca\(^{2+}\) Transient IC\(_{50}\) = 437nM

Mitochondrial Function

- **Measured O\(_2\) Consumption Rates (OCR)**
  - Min oxygen consumption = Rotenone
  - Max oxygen consumption = DNP
  - Basal oxygen consumption + Media

Cytotoxicity

- Healthy
- Unhealthy

GPCR Modulation

- Epinephrine
- FLIPR\(^\circledR\) Tetra

Proarrhythmia

- Control
- Cisapride

**xCELLigence RTCA Cardio**
<table>
<thead>
<tr>
<th>iCell Cardiomyocyte Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reynolds, et al. (2012)</td>
</tr>
<tr>
<td>Circulation Res*</td>
</tr>
<tr>
<td>HER-2 targeted liposomal doxorubicin displays enhanced anti-tumorigenic effects without associated cardiotoxicity</td>
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<tr>
<td>Lee, et al. (2012)</td>
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<tr>
<td>Circulation Res</td>
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<tr>
<td>Simultaneous voltage and calcium mapping of genetically purified human induced iPS cell-derived cardiac myocyte monolayers</td>
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<tr>
<td>Zhi, et al. (2012)</td>
</tr>
<tr>
<td>Frontiers in Genetics</td>
</tr>
<tr>
<td>Whole exome sequencing and an iPSC-derived cardiomyocyte model provides a powerful platform for gene discovery in left ventricular hypertrophy.</td>
</tr>
<tr>
<td>Puppala, D. et al. (2012)</td>
</tr>
<tr>
<td>Toxicol Sci</td>
</tr>
<tr>
<td>Comparative gene expression profiling in human induced pluripotent stem cell derived cardiomyocytes and human and cynomologus heart tissue.</td>
</tr>
<tr>
<td>Babiarz, et al. (2012)</td>
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<tr>
<td>Stem Cells and Dev</td>
</tr>
<tr>
<td>Determination of the human cardiomyocyte mRNA and miRNA differentiation network by fine-scale profiling.</td>
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<td>Rana, et al. (2012)</td>
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<td>Toxicol Sci</td>
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<td>Characterization of human induced pluripotent stem cell derived cardiomyocytes: bioenergetics and utilization in safety screening.</td>
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<tr>
<td>Guo, et al. (2011)</td>
</tr>
<tr>
<td>Cell Physiol Biochem</td>
</tr>
<tr>
<td>The electrophysiological effects of cardiac glycosides in human iPSC-derived cardiomyocytes and in guinea pig isolated hearts.</td>
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<tr>
<td>Cohen, et al. (2011)</td>
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<tr>
<td>Tox and App Pharm</td>
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<td>Use of human stem cell derived cardiomyocytes to examine sunitinib mediated cardiotoxicity and electrophysiological alterations.</td>
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<td>Estimating the risk of drug-induced proarhythmia using human induced pluripotent stem cell-derived cardiomyocytes.</td>
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<td>Sirenko, et al. (2012)</td>
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<tr>
<td>J Biomol Screening</td>
</tr>
<tr>
<td>Multiparameter in vitro assessment of compound effects on cardiomyocyte physiology using iPS cells</td>
</tr>
</tbody>
</table>

* Data generated with iCell Cardiomyocytes has been included in three separate IND filings by two separate companies to date.
Cardiac Hypertrophy: Increase in heart mass, due to increase in size of cardiomyocytes

Olson, E.N., et. al., Genes & Dev (2003); 17, 1937
Bernardo, B.C., et. al., Pharmacology & Therapeutics (2010); 128, 191
Multiple Readouts for Cardiac Hypertrophy

**iCell® Cardiomyocytes**

- Modeling cardiac hypertrophy *in vitro* using a workflow that has been shortened to a **5 day protocol** (now HTS-compatible)

- Hypertrophic response induced by ET-1

- Assays monitor the expression of BNP protein and levels of *NPPB* mRNA
Key Neuron Characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Bipolar or multi-polar neurite outgrowths</td>
</tr>
<tr>
<td><strong>Molecular Markers</strong></td>
<td>βIII-Tubulin, Map2, Synaptophysin, Gephyrin, PSD-95, vGAT (GABAergic), vGLUT (Glutamatergic), Tau, A-Beta</td>
</tr>
</tbody>
</table>
| **Functional Characteristics** | - Neurotransmission: receptor and channel activation  
                          | - Electrophysiology: patch clamp and MEA  
                          | - Neurite outgrowth/sprouting |

Molecular Markers

- GABA/Map2/Hoechst
- vGLUT/vGAT/Hoechst

iCell Neurons are a mixed population of primarily GABAergic and Glutamatergic neurons

Purity and Morphology

βIII Tubulin / Nestin / Hoechst  
Day 7 post-thaw

5 ml Pellet of Pure Neurons = ~4 Billion Neurons
- Panel of TaqMan Gene Expression Assays
- Forebrain identity (top)
- Primarily glutamatergic & GABAergic neuronal subtypes (middle)
- Express characteristic receptors (bottom)
iCell Neurons
Functional Endpoints

Electrophysiology

Neurite Outgrowth

Cytotoxicity

Receptor Signaling

Mitochondrial Health

Disease Modeling
(Tau & β-Amyloid Proteins)

Disease Modeling
(Data generated by Meso Scale Discovery)
iCell Neuron Publications

<table>
<thead>
<tr>
<th>Publication</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Haythornthwaite, et al. (2012) J. Biomolecular Screening</td>
<td>Characterizing human ion channels in induced pluripotent stem cell-derived neurons</td>
</tr>
</tbody>
</table>

iCell Neuron Presentations and Posters

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Description</th>
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</table>

iCell Neurons were plated directly on MEA plates followed by analysis for spontaneous electrical activity.
iCell Neurons secrete tau at levels that translate to the in vivo situation; Comparable to tau release occurring under normal physiological conditions in vivo.
Prevention of β-amyloid induced toxicity in human iPS cell-derived neurons by inhibition of Cyclin-dependent kinases and associated cell cycle events

In vitro modeling of Alzheimer’s Disease (AD)

• AD neuropathology is characterized by following cellular/histological indications
  • Amyloid plaques
  • Neurofibrillary tangles
  • Neuronal loss in cerebral cortex and hippocampus

• AD has been associated with ectopic cell cycle events in post-mitotic neurons which may be associated with neuronal apoptosis.

• Report investigates the role of cell cycle events in the mechanism of amyloid beta (Aβ)-mediated neurotoxicity.

From AHAF.org
Differentiation of forebrain region neurons from hiPS cells

A

<table>
<thead>
<tr>
<th>Day</th>
<th>Reseeding</th>
<th>Reseeding</th>
<th>Reseeding</th>
<th>For Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>-28</td>
<td>Day -14</td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Noggin + SB431542</td>
<td>bFGF + EGF + B</td>
<td>B + G + N</td>
<td>B + G + N + AA + cAMP</td>
<td>Neural induction</td>
</tr>
</tbody>
</table>

B

- Nestin
- SOX2
- DAPI
- Merge

C

- GFAP
- TuJ1
- DAPI
- Merge

- FOXG1
- SOX2
- DAPI
- Merge

- SOX2
- MAP2
- DAPI
- Merge

iCell Neurons
Characterization of hiPSC-derived forebrain neurons

D. Differentiation of hiPS-C4 cells
- TuJ1
- MAP2
- SOX2
- GFAP

E. Relative expression to GAPDH

F. Spontaneous
- 0 mV
- 10 mV
- 1 sec

G. Evoked
- 0 mV
- 20 mV
- 10 ms
Characterization of Aβ1-42-induced neurotoxicity

A

Relative cell viability (% control)

Aβ1-42 Concentration (μM)

B

Control

Aβ1-42

% BrdU+ neurons

C

***

% Colocalization

D

BrdU

TUNEL

DAPI

Merge

E

***P<0.001

BrdU+/TUNEL-

BrdU+/TUNEL+
Cell cycle modulation in iPS cell-derived neurons by Aβ1-42
Rescue of Aβ1-42-induced neurotoxicity

(A) GW8510

(B) Cdk2 inhibitor II

(C) TUNEL/TuJ1/DAPI

Control | Aβ1-42 | Aβ + GW8510 | Aβ + Cdk2 inhibitor II
Quantitation of Aβ1-42-induced neurotoxicity rescue

D

E

***P<0.001
Aβ1-42 neurotoxicity in iCell Neurons

A

TuJ1/Hoechst
MAP2/GABA/Hoechst
vGAT/vGLUT2/Hoechst

B

TuJ1

Nestin

C

Relative cell viability

% Control

Aβ1-42 Concentration (μM)

D

Aβ 0 μM
Aβ 0.625 μM
Aβ 1.25 μM

Aβ 2.5 μM
Aβ 5 μM
Aβ 10 μM

Average Neurite length

% Control

Aβ1-42 Concentration (μM)
Profiling Aβ1-42 toxicity in iCell Neurons

E

1,2,5

Roscovitine

OloMOUCINE

1,2,5

GW510

1,2,4

Cdk2 Inhibitor II

2

Cdk4 Inhibitor II

4,6

PD0332951

F

DMSO

Aβ1-42

Aβ + GW1μM

Aβ + GW0.3μM

Aβ + GW0.1μM

Aβ + GW0.03μM

G

Average neurite length

Relative fold change

***

Aβ1-42 - + + + +

GW8510 (μM) - - 1 0.3 0.1 0.03

* 

** 

***
Target knock-down reduces Aβ1-42-mediated neurotoxicity

*P<0.05
**P<0.01
***P<0.001
Cell cycle regulator modulation in Aβ1-42-treated iCell Neurons

Biochemical analysis required pure population of neurons provided by iCell Neurons.

Proliferative contaminating cells in lower purity cultures confound quantitation of cell cycle effects in target neuron cell population.

*P<0.05  
**P<0.01  
***P<0.001
Cell cycle regulator modulation in Aβ1-42-treated iCell Neurons

- **P<0.05
- **P<0.01
- ***P<0.001
Screening in iCell Neurons for AB1-42 protective compounds

- 384-well plate assay
- Cell viability measured by CellTiter Glo®
- Hundreds of compounds screened
- Hit defined as protective effect > 3*SD of untreated group
- 19 hits identified, one is a known CDK2 inhibitor
Conclusions

• iPSC-derived neurons can perform well in phenotypic screens to identify hits conferring protection from Aβ1-42-mediated neurotoxicity.

• Modulators of cell cycle activity can reduce Ab1-42-mediated neurotoxicity in hiPSC-derived neurons.

• The high level of neuronal purity in iCell Neurons enables a more straightforward analysis of the mechanism of action of cell cycle modulator compounds in the study.

• iCell Neurons are a readily available and easy to use human neuronal cell model platform which can accelerate phenotypic drug discovery.
  • Cryopreserved for easy use
  • Availably in unlimited quantities
  • Batch-to-batch consistency
  • Available in genetic background of choice
Acknowledgements

Collaborators

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Ming Kuei Jang
Selena Yang
Mu Sun

Cheryl Tay (GIS)
Chao Liu (NUS)

Gavin Dawe (NUS)
Eyleen Goh (NUS-Duke)
“Optimization Of Neuronal Cultures Derived From Human Inducible Pluripotent Stem Cells For High Throughput Assays Of Synaptic Function”

Pascal Laeng, PhD
Outline

• Brief Galenea overview
• Our approach to CNS drug discovery
• MANTRA system
• Application of MANTRA system to hiPSC-derived neurons
• Conclusions/perspectives
Our Company

- A small fully integrated CNS discovery organization (30 FTEs)
  - Assay development, HTS, electrophysiology, behavioral pharmacology, medicinal chemistry and computation all in-house

- Founded in 2004 by Nobel laureate Susumu Tonegawa

- Located initially in Cambridge, recently moved to Wakefield, MA

- Unique platform technology already developed and yielding pro-cognitive, HD and Alzheimer programs
  - Applicable to schizophrenia, autism spectrum disorders, epilepsy and other indications

- Tremendous support from NIMH over the past several years
  - R01, RC1, RC4 and 2 SBIR grants awarded
Dysfunctions in Synaptic Transmission are Central to Many CNS Disorders

Neuroligins and neurexins link synaptic function to cognitive disease
Thomas C. Südhof

Alzheimer's Disease Is a Synaptic Failure
Dennis J. Selkoe, et al.
Science 298, 789 (2002);
DOI: 10.1126/science.1074069

Synaptic Dysfunction in Neurodevelopmental Disorders Associated with Autism and Intellectual Disabilities
Huda Y. Zoghbi and Mark F. Bear
Cold Spring Harb Perspect Biol 2012; doi: 10.1101/cshperspect/a009086 originally published online January 18, 2012

Synapses and Alzheimer's Disease
Morgan Sheng, Bernardo L. Sabatini and Thomas C. Südhof
Cold Spring Harb Perspect Biol 2012; doi: 10.1101/cshperspect.a005777 originally published online April 4, 2012

Presynaptic function in health and disease
Clarissa L. Waites and Craig C. Garner

Developmental Neurobiology
Giovanni Esposito, Fernandes Ana Clara, Patrik Verstreken

Cold Spring Harbor Perspectives in Biology

Synaptic Vesicle Trafficking and Parkinson's Disease
Dysfunctions in Synaptic Transmission Play Key Role in Disease Pathogenesis

- Altered synaptic transmission (ST) is emerging as a key mechanism underlying many neurological and neuropsychiatric disorders, including Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, epilepsy, schizophrenia, bipolar disorder, migraine and autism.

- Galenea’s technology measures synaptic transmission at the neuronal and network levels.

- Our goal is to develop novel therapeutics which modulate synaptic transmission.
How were new medicines discovered?


**How Were New Medicines Discovered?**

Between 1999 & 2008:
- Phenotypic screening was the most successful approach for first-in-class drugs
- Target-based screening was the most successful for follower drugs
- The largest apparent benefit of functional screening for first-in-class drugs was in CNS

For First-in-Class Molecules, Functional/Phenotypic Screening is Far More Successful

**ANALYSIS**

Investment in drug research and development (R&D) has increased substantially in recent decades, but the annual number of truly innovative new medicines approved by the US Food and Drug Administration (FDA) has not increased accordingly, and attrition rates are very high. Indeed, in a recent analysis it was found that without a dramatic improvement in R&D productivity, the pharmaceutical industry cannot sustain sufficient inventors to replace the loss of revenue due to patent expiration and success failures.

The authors of this analysis also considered R&D productivity in two dimensions: efficiency and effectiveness. R&D efficiency represents the ability to translate inputs (such as ideas, investment and effort) into desired outputs (such as molecules that represent resolved uncertainties). Each R&D effectiveness can be considered the ability to produce outputs with various intended and desired qualities. A key efficiency variable for increased productivity is the probability of technical success. If the probability of technical success could be increased by reducing attrition for any given drug candidate or ideally for a portfolio of drug candidates, then productivity would increase accordingly. The authors also suggested that target selection may be one of the most important determinants of attrition and overall R&D productivity.

However, despite the power of these tools to identify potential drug candidates, R&D productivity remains a crucial challenge for the pharmaceutical industry, which raises questions about the possible limitations of target-centric approaches to drug discovery. Indeed, before the introduction of target-based approaches, drug discovery was driven primarily by phenotypic assays, often with limited knowledge of the molecular mechanisms of disease. Nevertheless, the pharmaceutical industry was successful in the discovery and development of new

**Disease area**

<table>
<thead>
<tr>
<th>Disease area</th>
<th>Target-based screening</th>
<th>Phenotypic screening</th>
<th>Biologics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious diseases</td>
<td>3</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Immune</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Metabolic</td>
<td>3</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Cardiovascular</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Gastrointestinal</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Rare diseases</td>
<td>0</td>
<td>2</td>
<td>5</td>
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</tbody>
</table>

**For First-in-Class Molecules, Functional/Phenotypic Screening is Far More Successful**
Physiological Measures of Synaptic Transmission In Neuronal Cultures

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Generation of Action Potentials</td>
</tr>
<tr>
<td>2</td>
<td>Opening Ca$^{2+}$ Channels</td>
</tr>
<tr>
<td>3</td>
<td>Fusion of vesicles with presynaptic membrane</td>
</tr>
<tr>
<td>4</td>
<td>Release of Neurotransmitters</td>
</tr>
<tr>
<td>5</td>
<td>Neurotransmitters bind with postsynaptic receptors</td>
</tr>
<tr>
<td>6</td>
<td>Ions (i.e. Na$^+$) flow into Postsynaptic neuron</td>
</tr>
<tr>
<td>7</td>
<td>Potential change: EPSP, IPSP</td>
</tr>
</tbody>
</table>

**Synaptic Connections**
- **MANTRA**
  - ✓
  - ✓

**HTS Capabilities**
- Manual Patch-clamp
- Autom. Patch-clamp
- MEA
  - ✓
  - x
  - ✓
  - x

*Modified from Huettel, Song and McCarthy, Functional MRI, 2009*
Integrated Synaptic Transmission Drug Discovery Platform

MANTRA™ → Rodent EEG → Human EEG → Novel Therapeutic Candidates

Synaptic modulators with new MOA
Tested in more predictive efficacy models
Utilizing innovative biomarkers to guide clinical development
Delivering break-through drugs
High-Throughput Assay to Identify Modulators of Synaptic Function

1. Primary neurons cultured in 96-well plates → 21 DIV

2. Made to express fluorescent reporter of synaptic function by virus infection

3. Compounds added to cultures prior to stimulation

4. Synaptic activity induced with physiologically relevant electrical stimulation trains in 96 wells in parallel via multi-electrode tip module

5. During stimulation, plates monitored with highly sensitive 96 mini-lens array & fluorescence data captured to provide dynamic read-out of synaptic vesicle cycle

Custom Data Analysis Suite
Assay Performed on Primary Neurons

- Culture system: rat embryonic forebrain neurons in 96-well plates
  - Assay performed at 21 days in vitro for full synaptic differentiation
  - Postsynaptic glutamatergic activity blocked by AMPA and NMDA receptors in assay buffer

- Deliver reporter constructs with adeno-associated virus (AAV)
  - Human synapsin promoter for neuron-specific expression
  - >90% expression rate in neurons
SynaptopHluorin Reporters Utilized to Track Synaptic Vesicle Cycling

- SynaptopHluorin reporter: pH-sensitive GFP fused to lumenal domain of synaptic vesicle protein (e.g. synaptophysin)

![SynaptopHluorin Waveform]

- Resting State: Reporter Off
- Exocytosis: Reporter ON
- Endocytosis & Re-acidification
MANTRA: Integrated and Automated System
Dynamic Assay Yields Multiple Parameters

**Amplitude**: measure of number of vesicles on the synaptic surface – read-out on balance between vesicle release and recovery.

**Stimulus period**: electrical stimulation activates neurons driving vesicle release and recovery.

**Stimulus Parameters**: Analysis performed on waveforms generated by stimulation trains of multiple different frequencies.

**Recovery phase**: uptake of synaptic vesicles after neuronal firing ends - the time constant for this decline is a measure of the rate of synaptic vesicle uptake.

---

**SynaptopHluorin Waveform**

![SynaptopHluorin Waveform](image)
**Bench-Top Assay Results Achieved with High Throughput Capacity**

- PMA (Phorbol 12-myristate 13-acetate): known positive modulator of presynaptic activity used as test compound

![PMA data: single chamber system](image1)

![PMA data: MANTRA system](image2)

![PMA concentration response curve: MANTRA system](image3)

- MANTRA throughput permits concentration response analysis to support lead optimization
  - Data generated in 1hr; would require weeks using single chamber system

Neuronal firing at different frequencies places differential demand on presynaptic activity.

Disease mechanisms can be specific for particular activity regimes:
- Disruption of high frequency activity in schizophrenia
- Excessive or uncontrolled high frequency activity in epilepsy

Essential to evaluate compound effects over a range of physiologically relevant neuronal activity:
- Ideal compound may specifically modulate a defined frequency range

Typical stimulus protocol employs 4 trains:
- 30 Hz pulse train to establish baseline
- 5 Hz train to assess at low frequency
- 10 Hz train to assess at intermediate frequency
- 30 Hz train to assess at high frequency

Graph: SynaptoPfluorin Waveform
Compound Effects on Synaptic Vesicle Cycling

**Vesicle Release Effects**
- Increased Vesicle Release
  - Vehicle
  - Phorbol Ester

- Reduced # Released Vesicles
  - Vehicle
  - Nuclear receptor modulator

- Reduced Rate Vesicle Release
  - Vehicle
  - GPCR agonist

**Vesicle Recovery Effects**
- Slowed Vesicle Endocytosis
  - Vehicle
  - Kinase Inhibitor 1

- Increased Bulk Endocytosis
  - Vehicle
  - Kinase Inhibitor 2

**Frequency-Specific Effects**
- Vehicle
  - PDE Inhibitor

**Graphs**
- 5Hz
- 30Hz
MANTRA Approaches to Discovery

**Therapeutics**

- Compounds with known target(s), or desired clinical and preclinical effects
- MANTRA screen
  - Class-based synaptic signature
  - Screen diverse libraries
    - For the same synaptic signature
- MANTRA screen
  - Identify novel chemical matter, which has the same basic characteristics, but which can now be optimized for synaptic modulation

**Synaptic Pathology**

- eg. AD, PD, HD, epilepsy, autism, schizophrenia, bipolar depression
- MANTRA screen
  - Identify key synaptic dysfunctions in disease
  - Screen diverse libraries for compounds which restore normal synaptic transmission in diseased neurons
  - MANTRA screen
  - A new class of synaptic modulators, which provide both symptomatic relief and - potentially – disease modification
MANTRA Approaches to Discovery: Cellular Models

**Therapeutics**
- Rat
  - Healthy donors
  - hiPSC
  - Hm Neurons
  - Rat Neurons
  - MANTRA™

**Synaptic Pathology**
- Mouse
  - KO Transgenic
  - hiPSC
  - Ms Neurons
  - Disease modeling/screening

**Use of Human iPSC-Neurons**
- Compound validation
- Disease modeling/screening
Key Requirements of Human iPSC-derived Neurons for Measuring Synaptic Activity on MANTRA

- Mature neurons with functional synapses
- High expression of SypHy reporter following AAV infection
- Long term survival
- Large quantity (high cell density)
- Homogenous cultures

Compare with synaptic activity in primary rat neuronal cultures
Evaluate responses of modulators of synaptic activity identified in rodent neuronal cultures
Human Neurons from iPS Cells

- Early access to human “iCell Neurons” derived from inducible pluripotent stem (iPS) cells through collaborative research agreement with Cellular Dynamics International (Madison, WI)
- Evaluating these neurons for degree of synaptic maturation and applicability for assays on the MANTRA system

![Synapsin I](image1.png)  ![MAP2](image2.png)

**Synapsin I**  **MAP2**
Characterization of iCell Neurons Transduced by hSynapsin-SypHy-AAV

Most neurons express hSypHy (70%) in cell body and axons

4 weeks

Co-expression of hSypHy and synapsin can be detected

2 weeks
Physiological Measures of Synaptic Transmission In Neuronal Cultures

- Generation of Action Potentials
- Opening Ca\(^{2+}\) Channels
- Fusion of vesicles with presynaptic membrane
- Release of Neurotransmitters
- Neurotransmitters bind with postsynaptic receptors
- Ions (i.e. Na\(^+\)) flow into Postsynaptic neuron
- Potential change: EPSP, IPSP
Evoked Ca\(^{+2}\) flux in Human iCell Neurons Measured by MANTRA

The EV\(_{50}\) of evoked Ca\(^{2+}\) transients in iCell Neurons (71 days) is similar to that measured from rat forebrain neuronal cultures (3 weeks) indicating a similar action potential threshold.
Physiological Measures of Synaptic Transmission In Neuronal Cultures

- Generation of Action Potentials
- Opening Ca\(^{2+}\) Channels
- Fusion of vesicles with presynaptic membrane
- Release of Neurotransmitters
- Neurotransmitters bind with postsynaptic receptors
- Ions (i.e. Na\(^{+}\)) flow into Postsynaptic neuron
- Potential change: EPSP, IPSP
Human neurons show measurable pre-synaptic activity with lower magnitude but similar overall waveform shapes and frequency dependence compared to rat neurons.

Compound A shows same effects on synaptic function in rat and human iPSC-derived neurons measured on MANTRA.

**p < 0.001; **p < 0.01
Evoked Presynaptic Response is Localized in Synapses of Human iCell Neurons (High Resolution Microscope)

The difference of MANTRA activity observed between rat and human neurons might reflect a difference in the total number of mature synapses/neurons present in the cultures.
Human Neurons Grown in Presence of Glia Show Larger Presynaptic Responses at All Frequencies
Presence of Glia Increases Neurite Outgrowth and Synapsin Expression (Puncta) in Human Neurons

- Glia

+ Rat Glia

Synapsin

GFP

Map2

- Glia

+ Glia
iCell Neurons on MANTRA

Preliminary Positive Results

✓ Homogenous differentiation
✓ Long term survival
✓ Good expression of SypHy reporter
✓ Comparable synaptic activities to rat neurons
✓ Compound validation (rat to human)
✓ Achieved POC to initiate assay development

Remaining Challenges

❖ Need to improve amplitudes of synaptic responses to levels closer to those of rat neurons
❖ Identify conditions to increase density of functional synapses
❖ Identify conditions to accelerate synaptic maturation
❖ Cell quantity: Development of fully functional neurons requires high cell seeding density (80,000/well)
Conclusions/Perspectives

- Achieved proof-of-concept that evoked presynaptic activity can be measured in mature cultures (>4 weeks) of human iPSC-derived neurons with the MANTRA system

- Enables application of human neurons to hit/lead compound validation in CNS drug discovery

- Further optimization required for HTS in human neurons

- **The high-throughput capacity of the MANTRA system provides a unique ability to test multiple conditions in parallel to generate human iPSCs-derived neurons with optimal synaptic functionality**

- Ultimately, the MANTRA system can be used to characterize synaptic abnormalities in neurons derived from patients and to screen for compounds to restore normal synaptic transmission
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• Rachel Llanas

NIMH
• RC4
• RC1
Synaptic Function Assays Drive Drug Discovery

Enabling identification of critical synaptic disease mechanisms and discovery of relevant targets and compound effects