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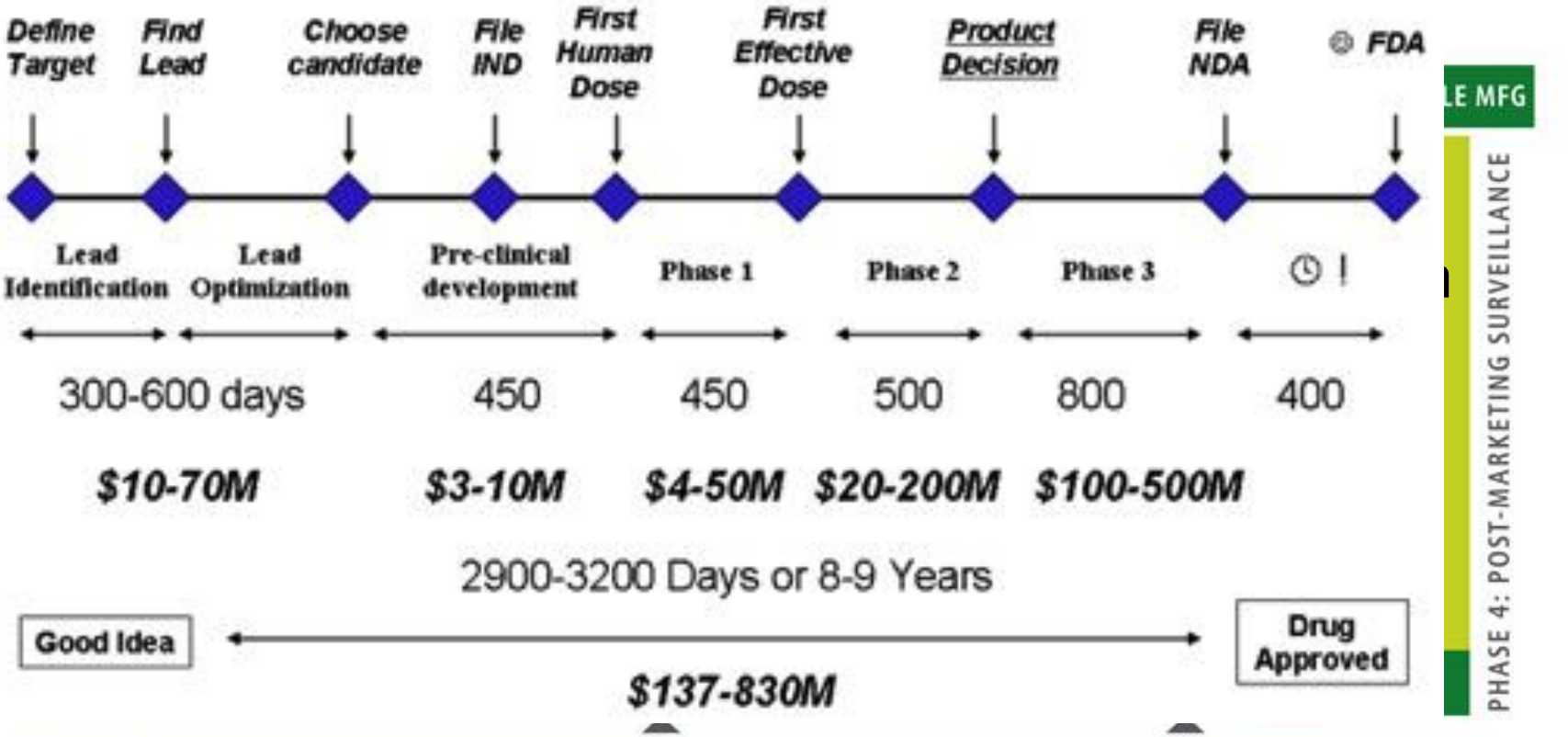
The use of iPSC-derived cells (& primary cells) as *in vitro* models for toxicity screening

15th March 2016
SOT
New Orleans
Booth #419

Drug Discovery & Development
" A long, risky road"

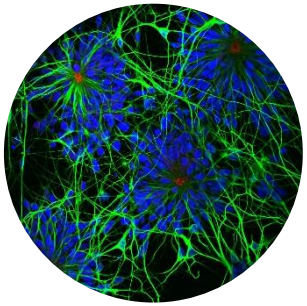
Need for early toxicity testing and improved prediction



Slide taken from the Pivotal Point Group, LLC

Source: Pharmaceutical Research and Manufacturers of America

Overview

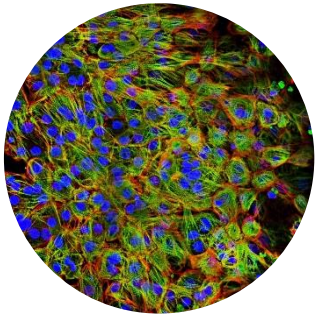


iPSC-Derived Neural Stem Cells

Neurotoxicity in drug safety testing

Functional Integrity

Gene Expression, Electrophysiology,
Multi-Electrode Array,
Effects of developmental neurotoxin



iPSC-Derived Cardiomyocytes

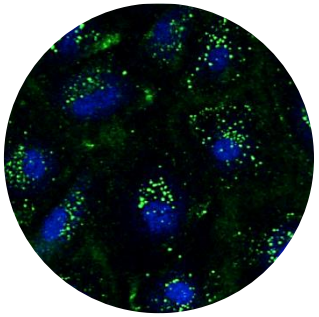
Cardiotoxicity in drug safety testing

Functional Integrity

Express major cardiac-selective markers
Beat spontaneously in culture, $Ca2^{+}$ imaging

Electrophysiology

Pharmacology



Hepatocytes

Hepatotoxicity in drug safety testing

Metabolism studies, Hepatotoxicity studies,
Genotoxicity micronucleus studies

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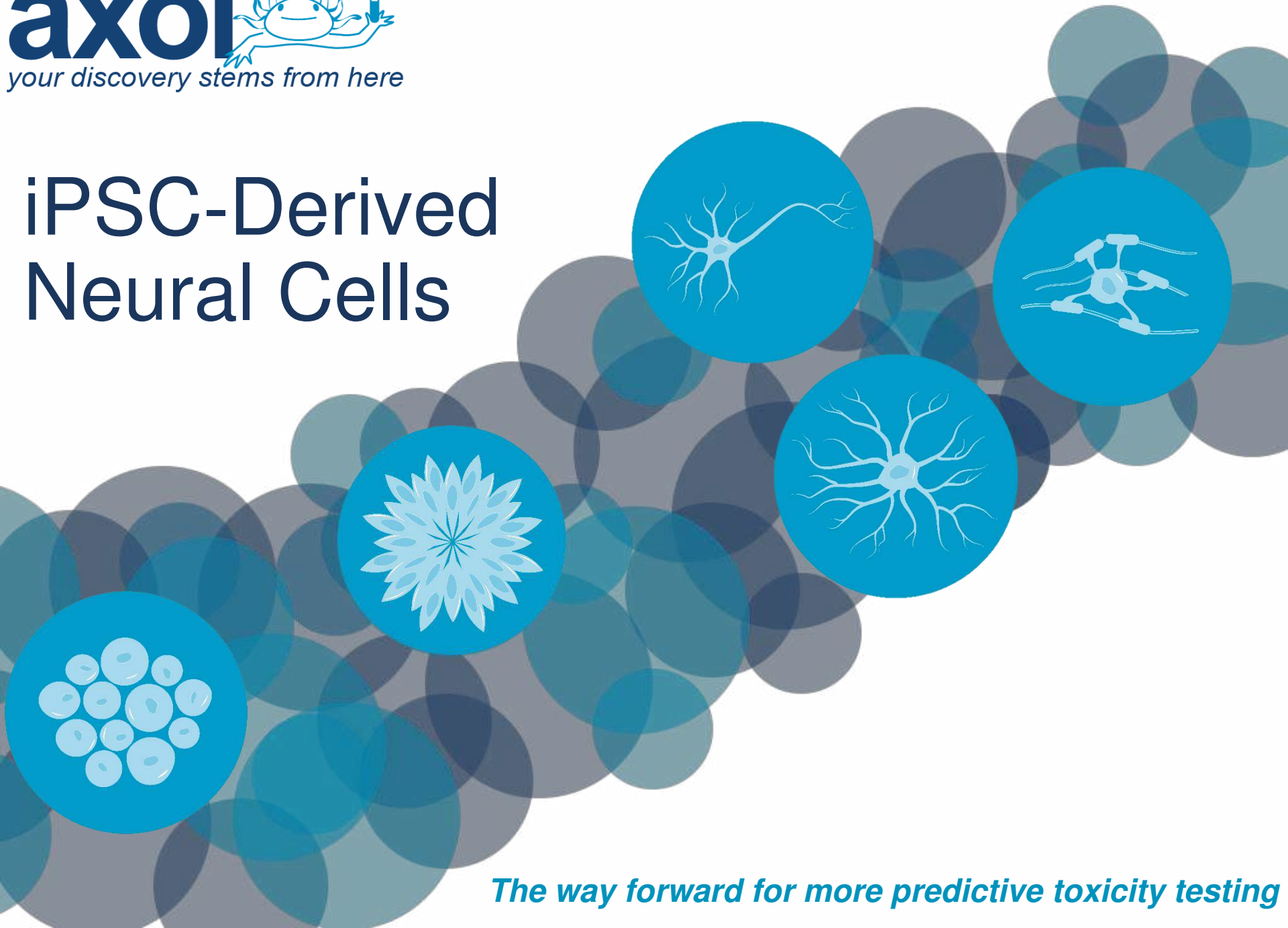
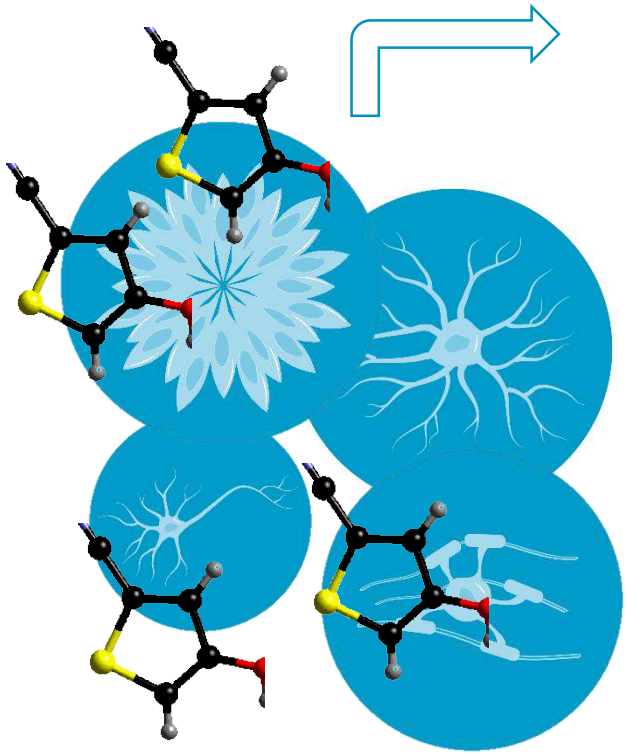
iPSC-Derived Neural Cells



The way forward for more predictive toxicity testing

Neurotoxicity in Drug Safety Testing



Functional Integrity

Gene Expression
Protein Expression
Electrophysiology
Multi-Electrode Array
Whole Cell Patch

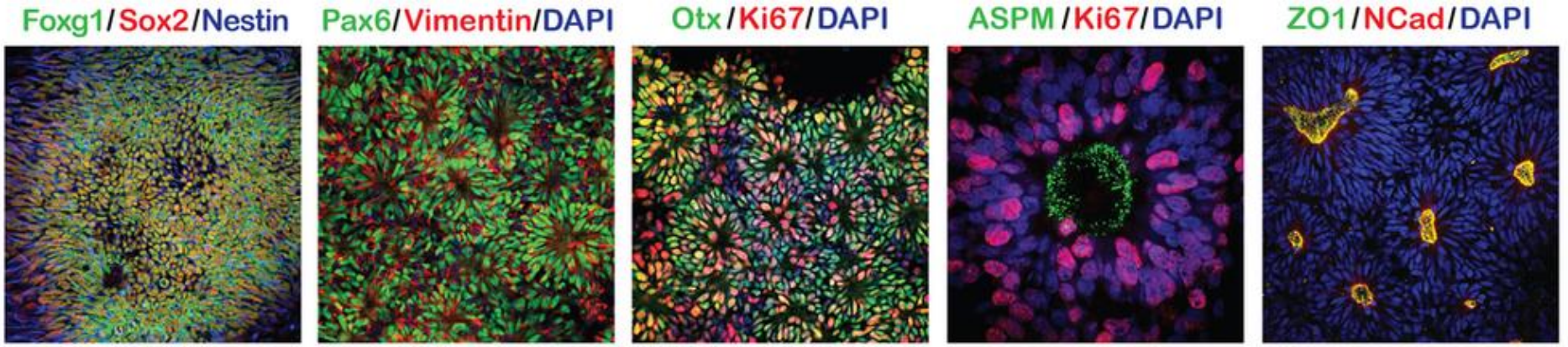
Disease Modeling

Responsive to drug treatments
Expression disease-relevant phenotypes

- Patch clamp
- Gene expression
- Biochemical analysis
- Multi-Electrode Array
- Neurite outgrowth

General Characterization of NSCs

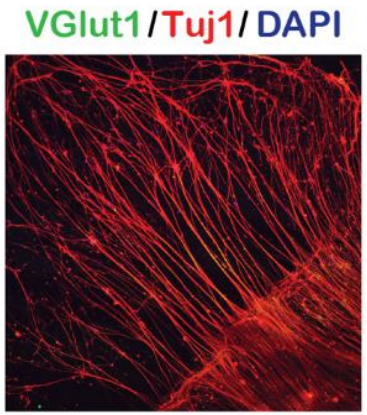
We confirmed expression of neural stem cell markers like SOX2, PAX6, Ki67 and ZO1

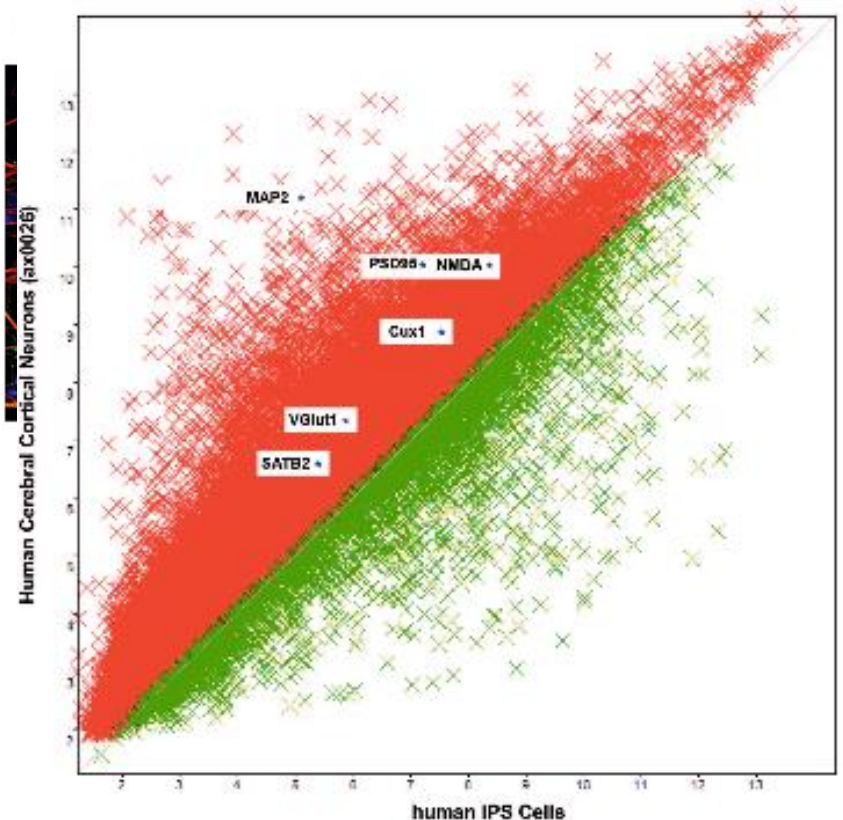


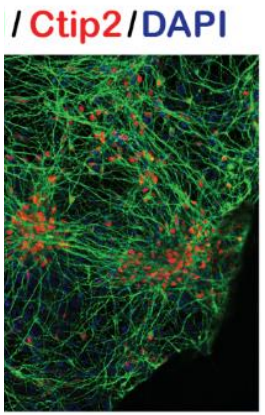
Characterization of Cortical Neurons

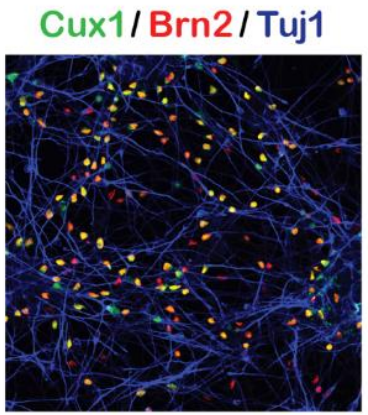
We looked at cerebral cortical neuronal markers like VGlut1, Tbr1, Cux1, Tujl by BCC

Our transcriptomic data confirmed down regulation of iPSCs markers and up regulation of cortical neuronal markers like MAP2, NMDA, VGlut1, Cux1 etc



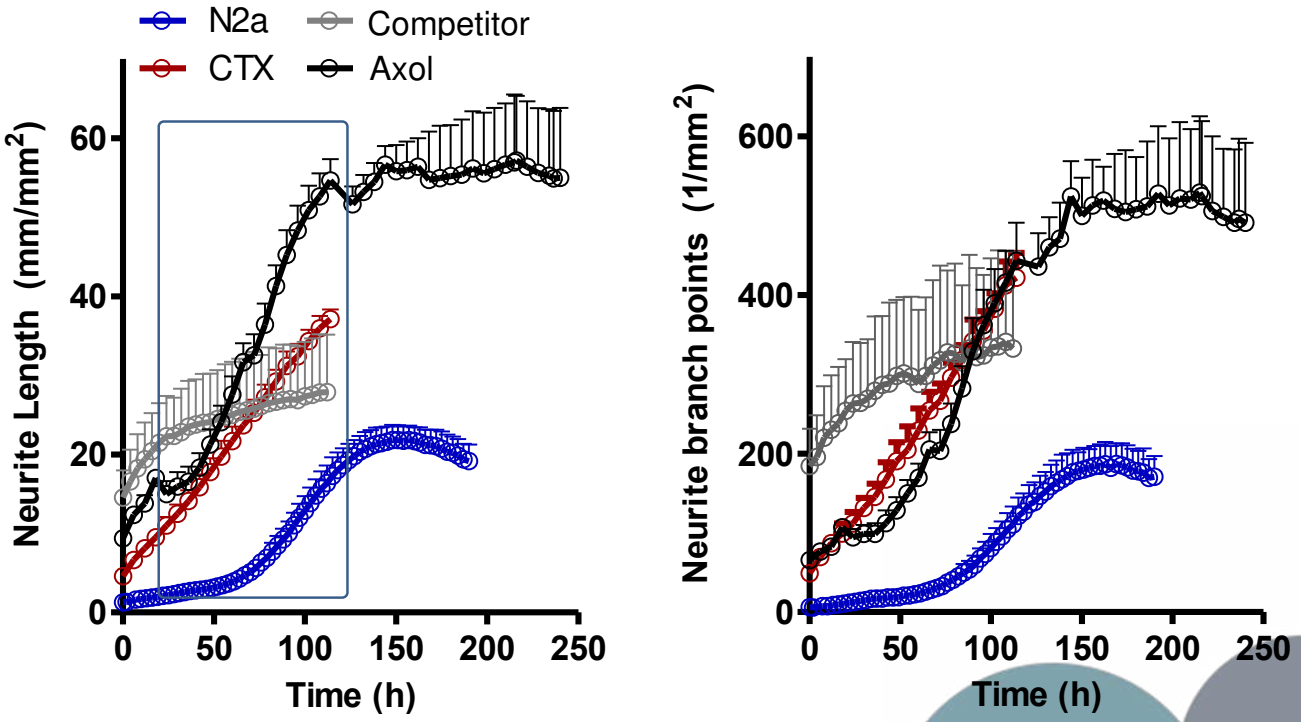






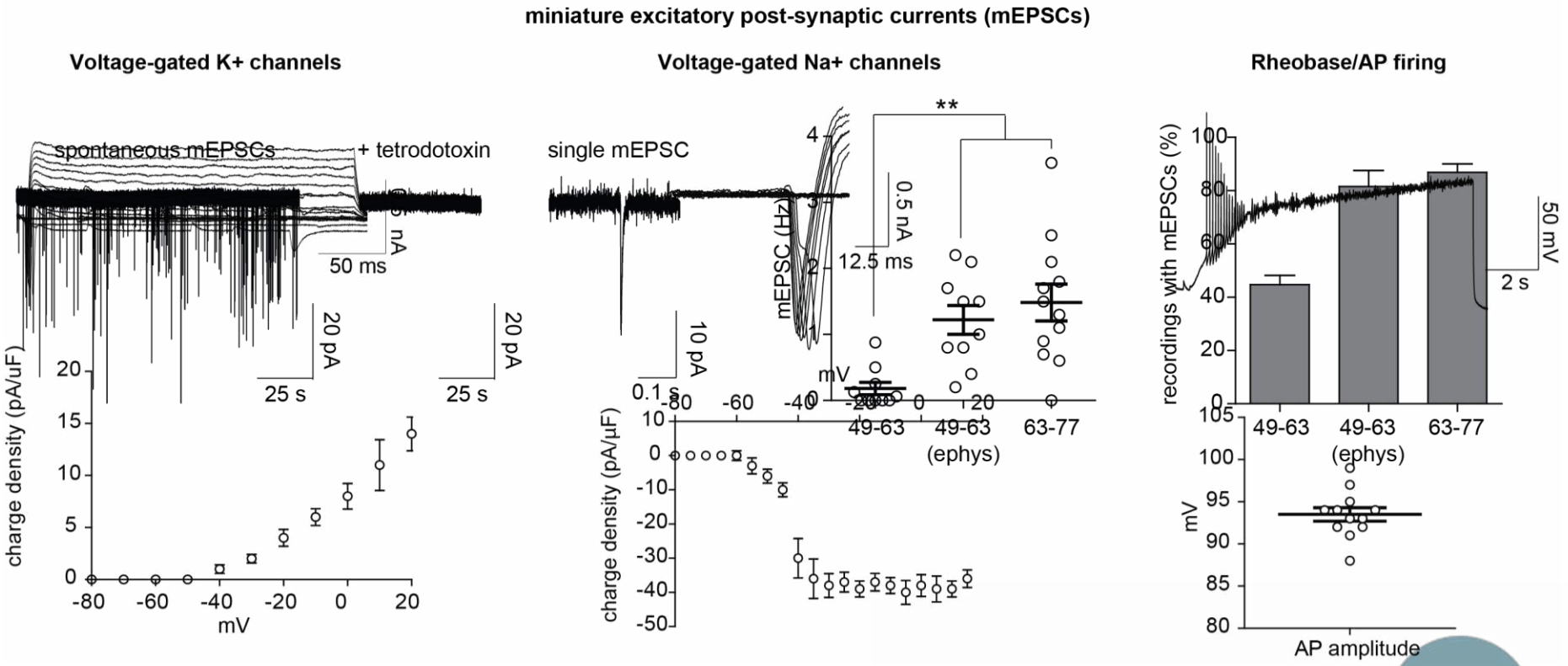
Functional Characterization

We confirmed the functional integrity by looking into neural networks with increased neurite length and branching in cortical neurons



Essen Bioscience Ltd using the IncuCyte NeuroTrack platform

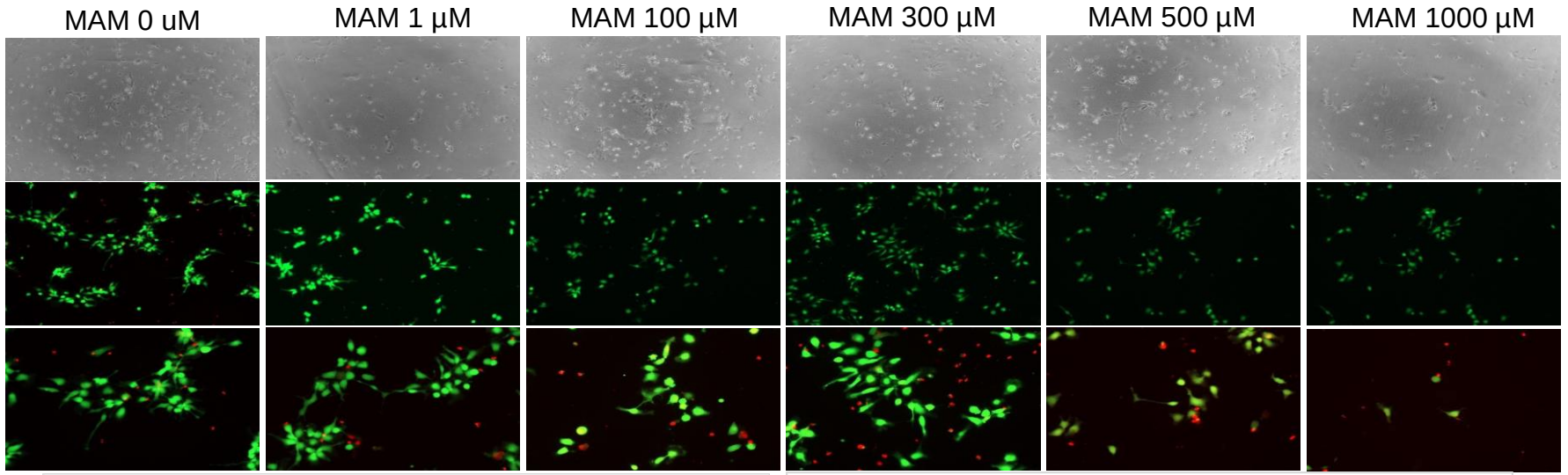
Electrophysiological Characterization

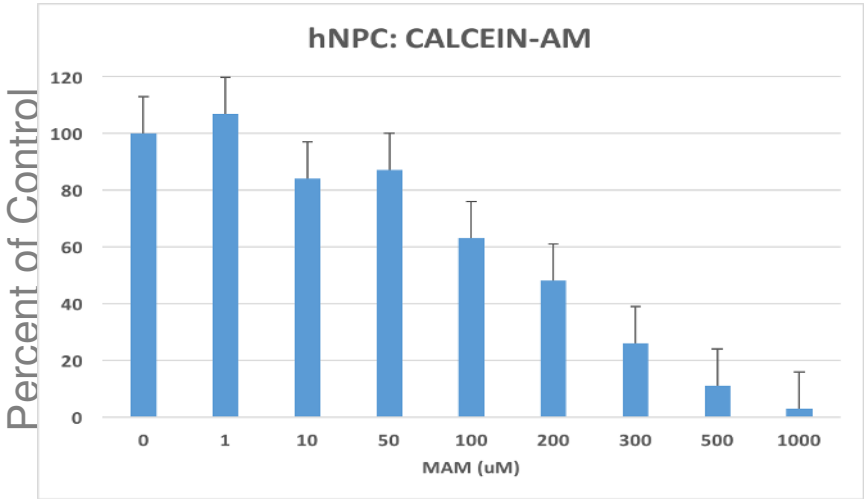


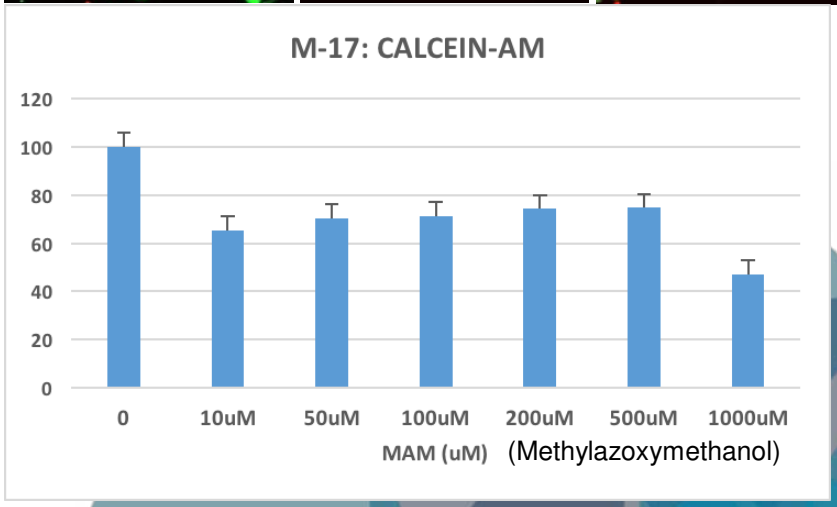
Spontaneous activity

Data from our collaborators

Neurotoxin Effects on iPSC-Derived Neural Stem Cells



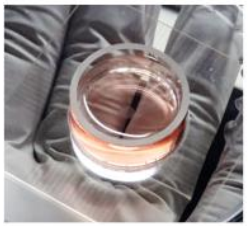




Data provided by Dr Kisby's lab by Michael Czulinski and Morgan Florek

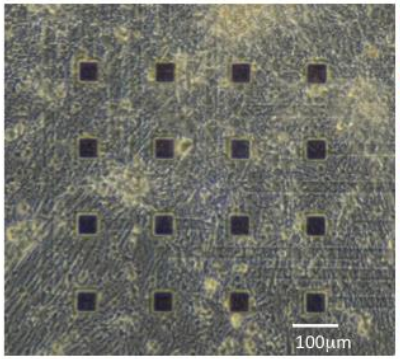
iPSC-Derived Cortical Neurons as *in-Vitro* Models for Drug Screening

Multi-electrode array chip

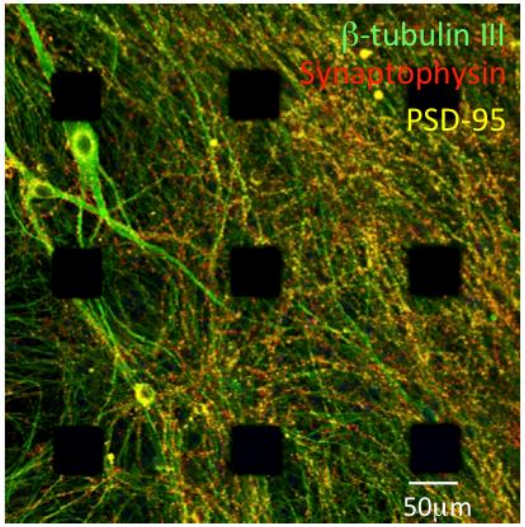


Alpha Med Scientific Inc

294 days culture on the MEA dish



300 days culture on the MEA dish



TOHOKU INSTITUTE OF TECHNOLOGY

iPSC-derived neural cells used to demonstrate LTP & LTD on an MEA platform

Biochemical and Biophysical Research Communications 469 (2016) 856–862

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/ybbrc

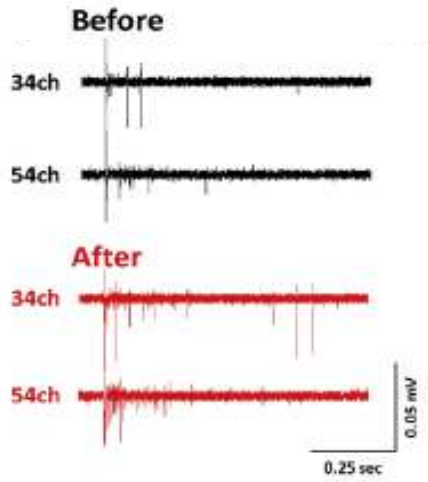
Induction of long-term potentiation and depression phenomena in human induced pluripotent stem cell-derived cortical neurons

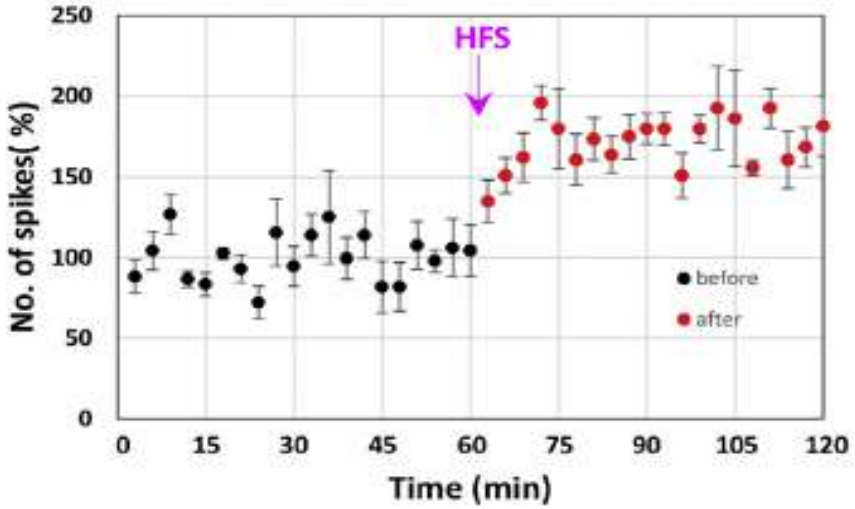
A. Odawara [a, c, 1], H. Katoh [a, 1], N. Matsuda [b], I. Suzuki [a, b, *]

iPSC-Derived Neurons Show Potential for Synaptic Plasticity

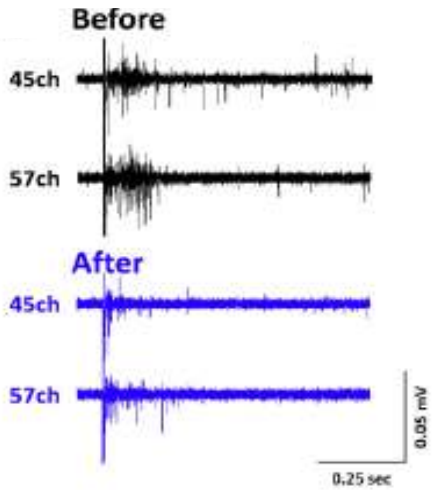
Induction of long-term potentiation (LTP) and long-term depression (LTD) by high-frequency stimulation (HFS) (112 DIV)

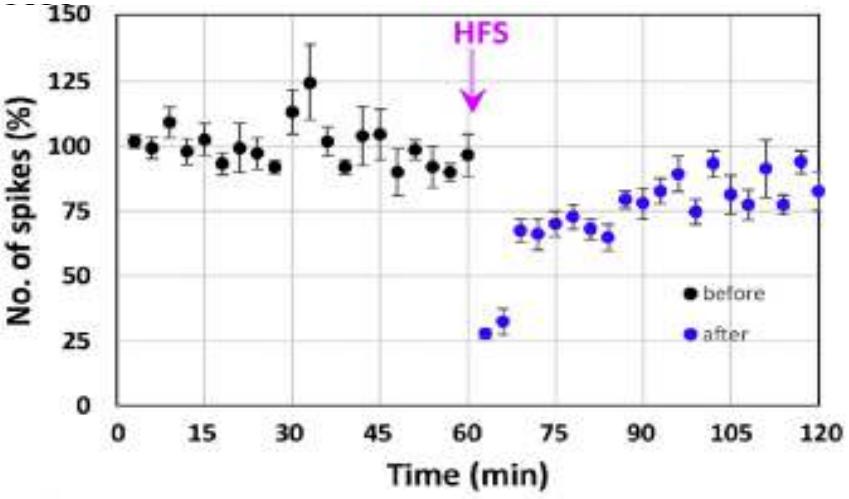
Long-Term Potentiation





Long-Term Depression

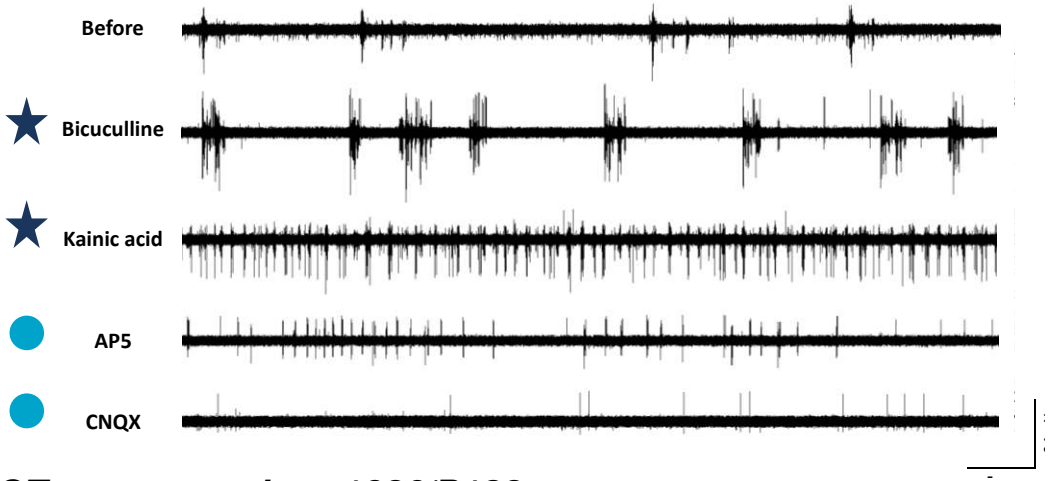


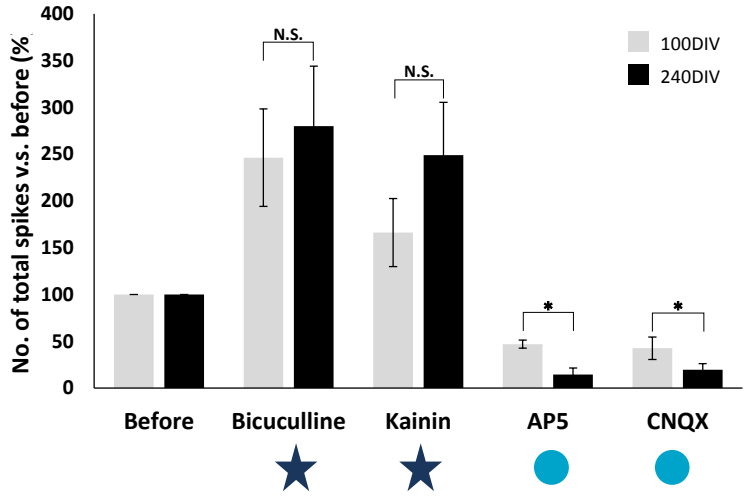


iPSC-Derived Neurons Respond to Drug Application

iPSC-derived neurons in response to drug application:

- ★ Synapse agonists (Bicuculline & Kainin acid)
  - Increase in firing
  - No change over days in culture
- ● Synaptic antagonists (CNQX & AP5)
  - Inhibit firing
  - Decrease with days in culture (100 v 240)



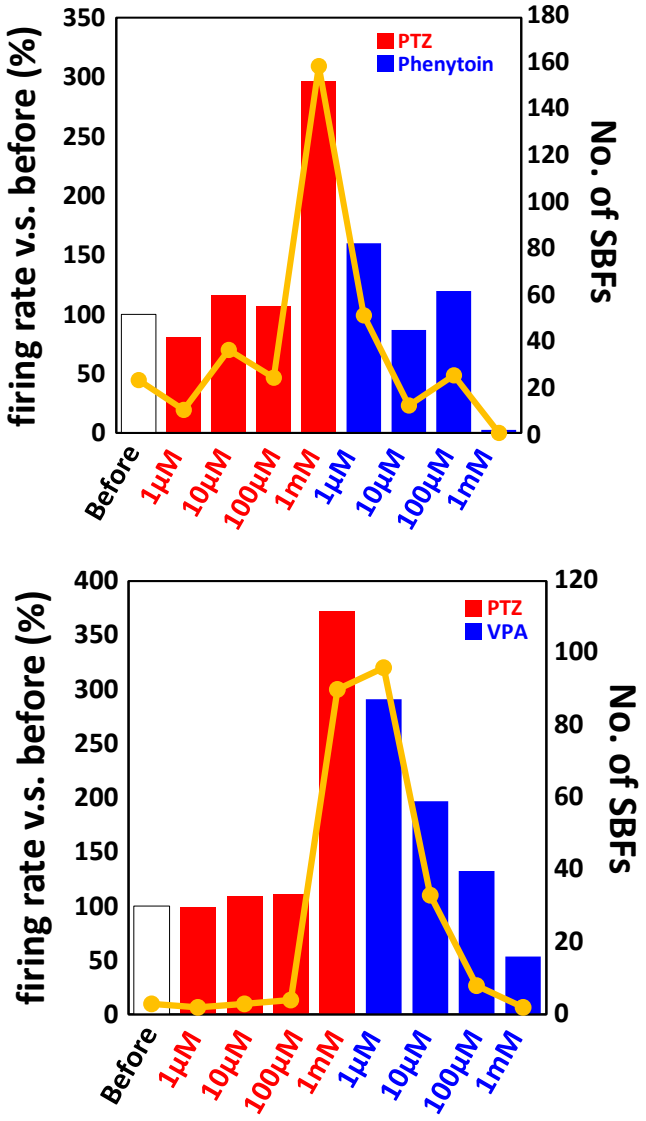


SOT poster number: 1826/P132
Copies available at booth #419

Induction of Epileptiform Activity & Effects of Anti-Epilepsy Drugs

- Induced epilepsy by adding PTZ (pentylentetrazole) (>1mM)
- Anti-epilepsy drugs, **phenytoin** & **sodium valproate (VPA)** were able to reverse the high frequency synchronized bursts evoked with PTZ

These results suggested that long-term electrophysiological measurements in iPSC-derived neurons using a MEA system may be beneficial for ***drug screening applications***



Neurotoxicity Summary

- iPSC-derived NSC
    - Express neural markers at gene & protein level
    - Excellent neurite outgrowth
    - Electrophysiologically functional
    - Capable of synaptic plasticity
- iPSC-derived NSCs are more sensitive to the developmental neurotoxin MAM & can replace routinely cell lines use for screening for neurotoxins
- Responsive to drug treatment
- Can be cultured long-term
- Physiologically relevant tool for drug discovery & toxicity studies

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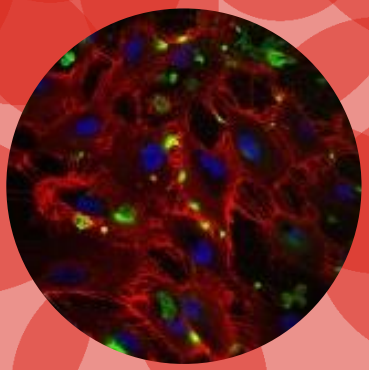
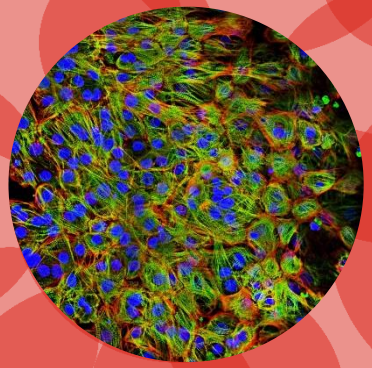
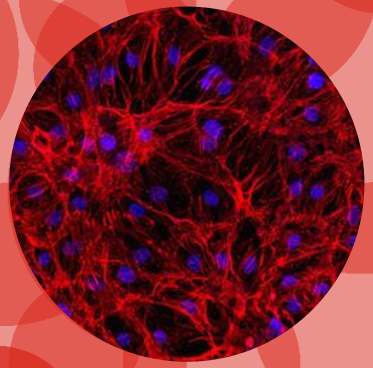
iPSC-Derived Cardiomyocytes

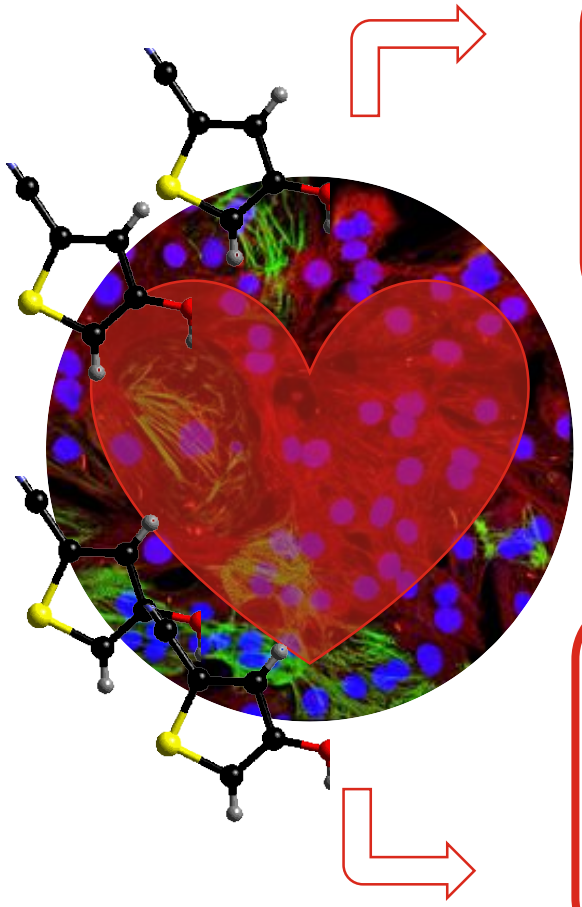






A way forward for more predictive toxicity testing

Cardiotoxicity in Drug Safety Testing



Electrophysiology
Contractility
QT prolongation
Na^+ & Ca^{2+} channels
Pharmacology

Functional Integrity
Ca^{2+} signaling
Morphology
Stress & toxic response markers

Patch clamp

Impedance

Biochemical analysis

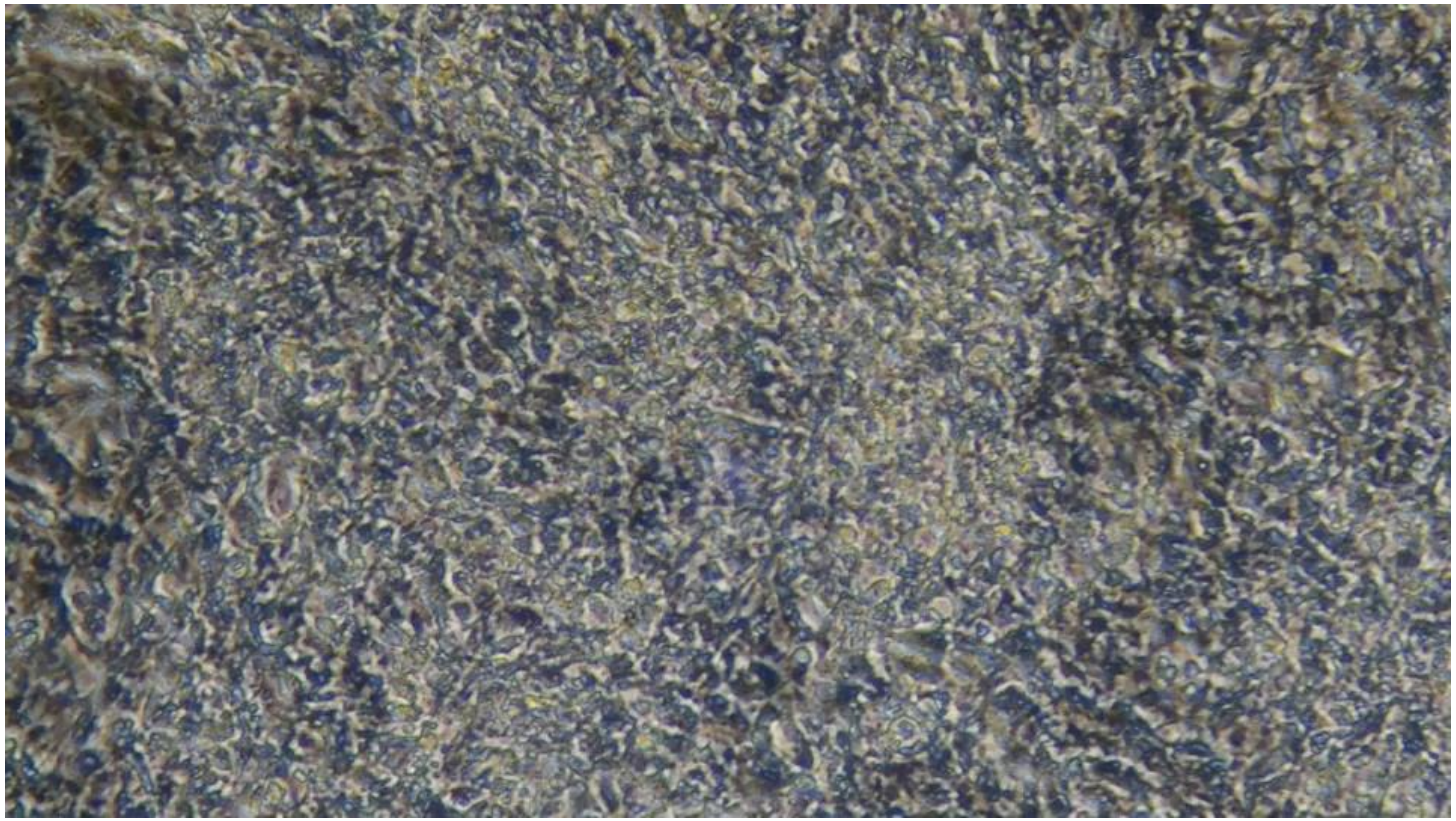
Immuno-cytochemistry

Multi-electrode Array

Why iPSC-Derived Cardiomyocytes?

- Benefits of a synchronously beating monolayer
  - React as a unit syncytium of cells, electrically coupled
- Robust & reproducible
- Large quantities available
- High purity
- Functional on xCelligence, for calcium imaging & for electrophysiology

iPSC-Derived Cardiomyocytes Showing Synchronized Beating

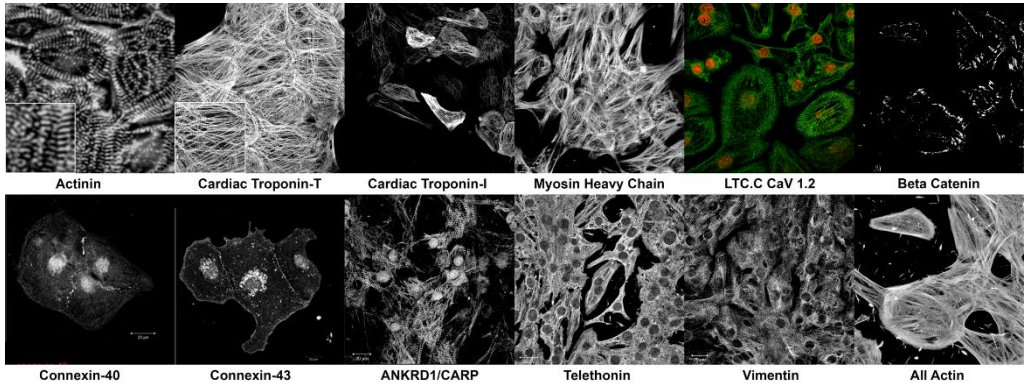


Benefits of a synchronously beating monolayer

- Electrically coupled
- Physiologically relevant to human heart

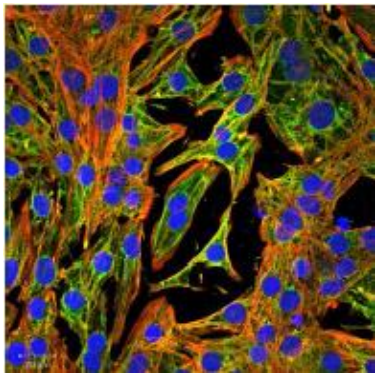
Functional iPSC-Derived Cardiomyocytes

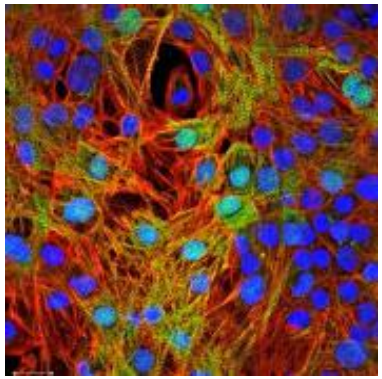
Protein Expression



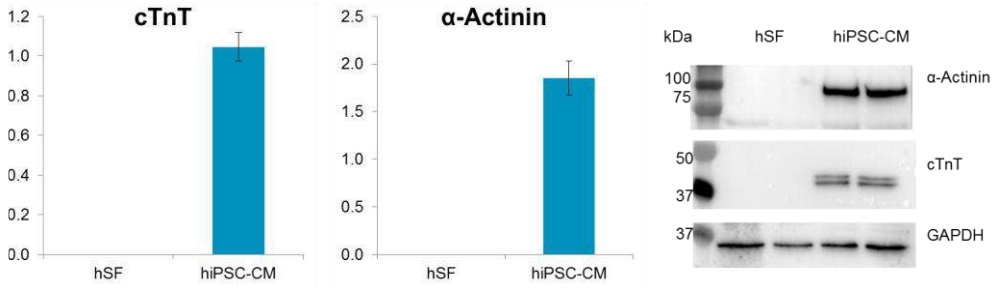
Data from Dr Christian Zuppinger

Signaling & Stress-Response





Data from Dr Christian Zuppinger



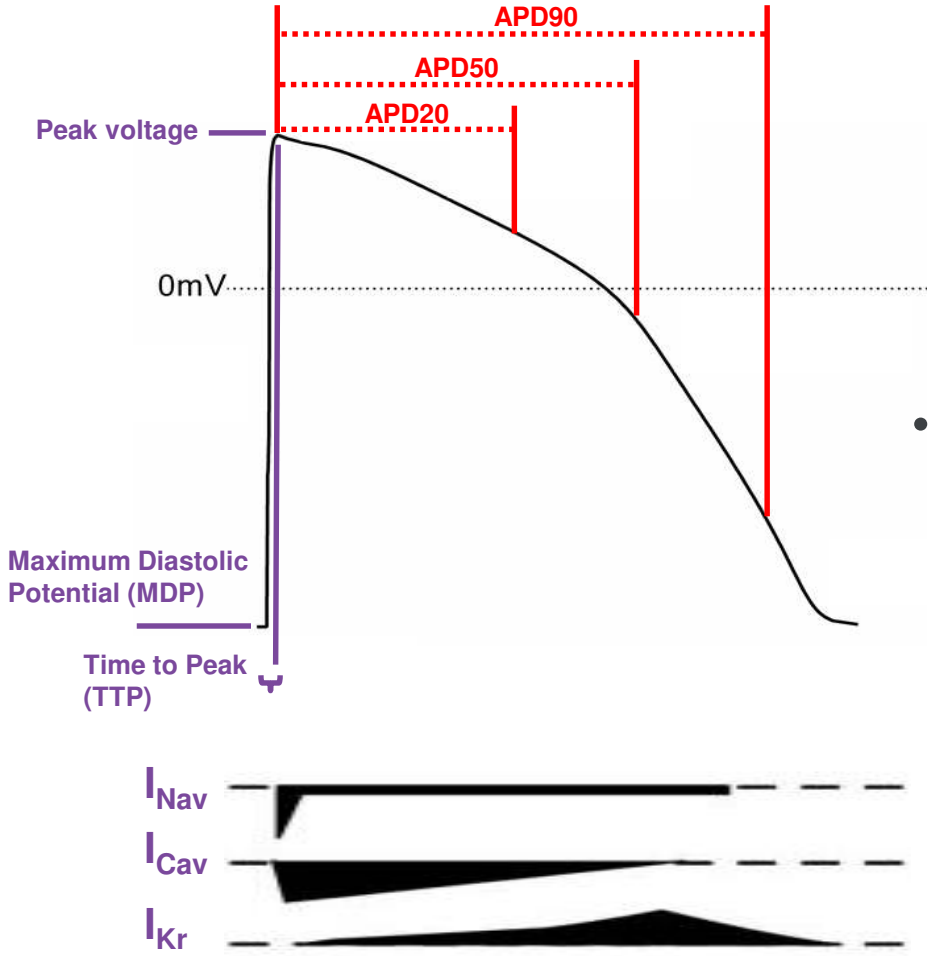
Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & **α-Actinin than human skin fibroblasts (hSFs)**

Data from Abigail Robertson from University of Manchester

Telethonin (green) suggested signalling & stress-response functions is present iPSC-CMs with a pattern of sarcomeric striation observed in patched inside some cells. (All actin, red)

Ankyrin repeat domain 1 (ANKRD1) (green) could be used a marker of toxic stress, showed similar expression to telethonin (All Actin, red)

Methods, Tools & Recording Parameters

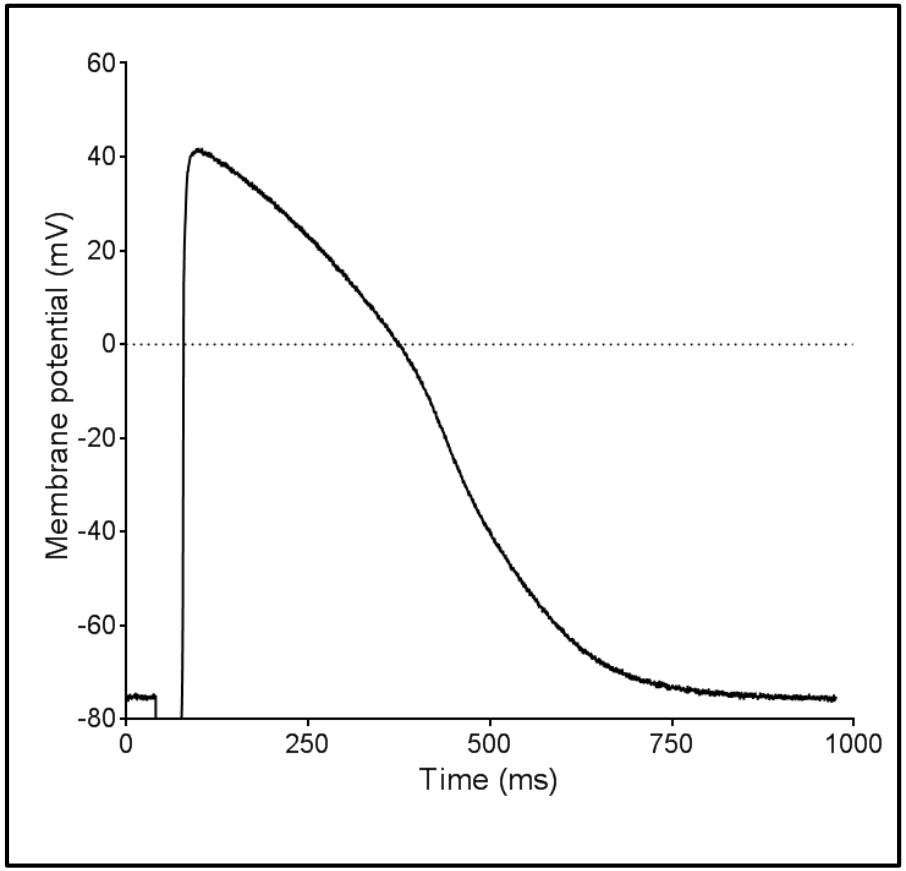


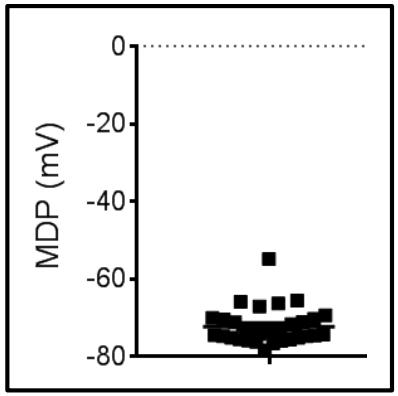
- Patched 7-14 days post seeding
  - Action potentials (AP) recorded from syncytial cells (field stimulation)
  - Perforated patch clamp (100 µg/ml gramicidin)

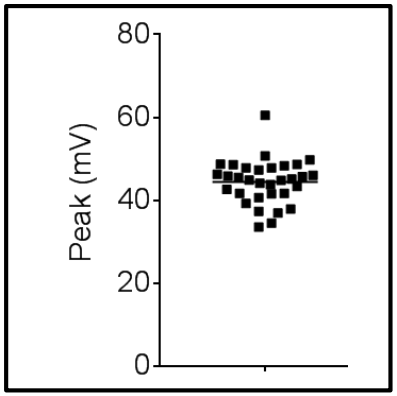
- Pharmacological tools:

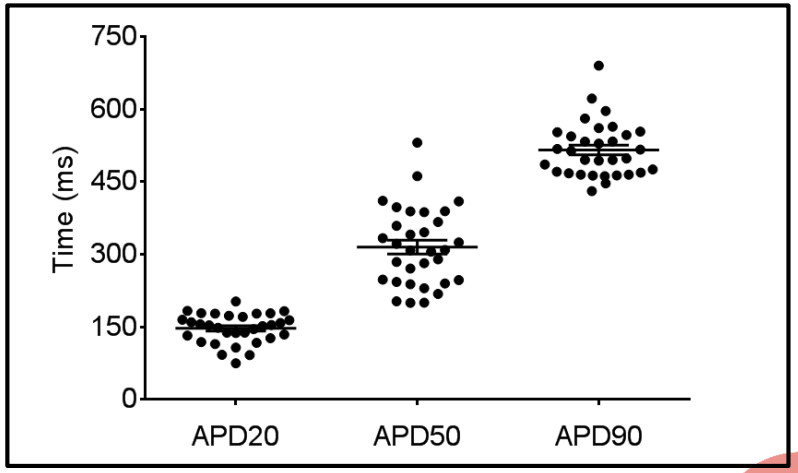
Compound	Ion channel
Carbachol	I_{KACh}
TTX	I_{Nav}
Mexiletine	I_{Nav}
Nifedipine	I_{Cav}
Verapamil	I_{Cav} & I_{Kr}
Dofetilide	I_{Kr}

AP Parameters





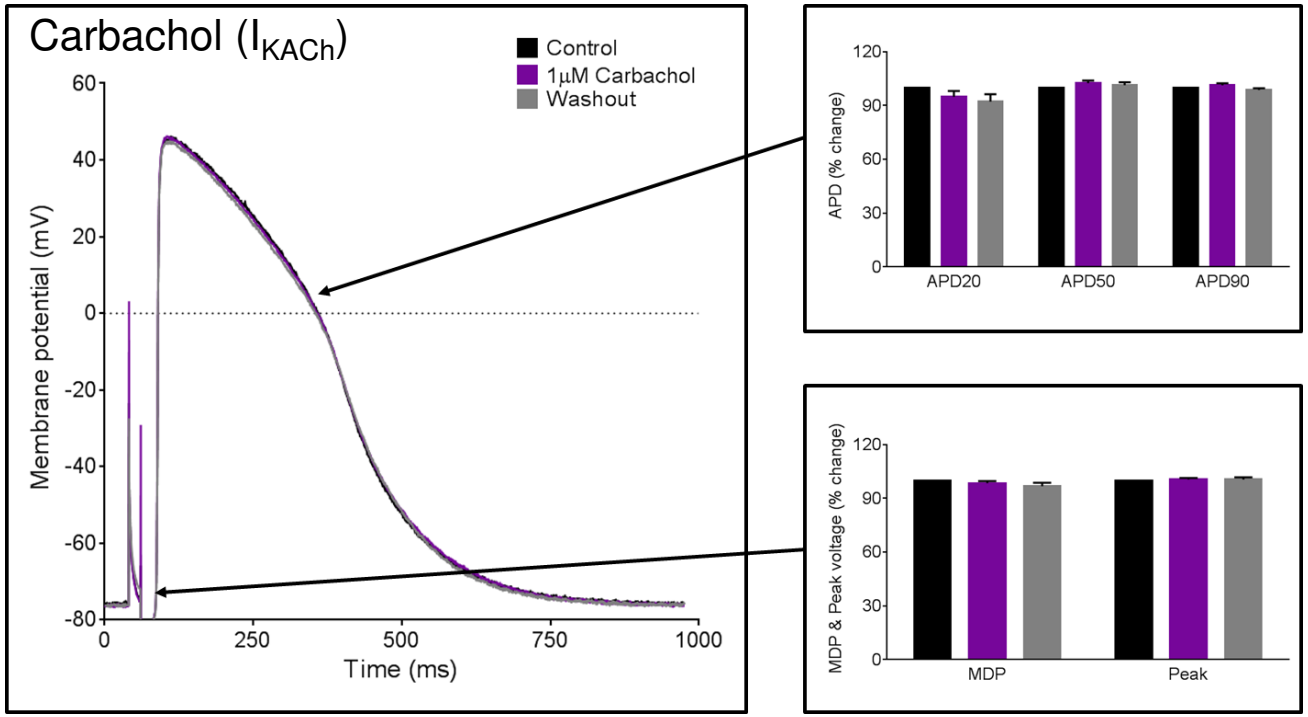


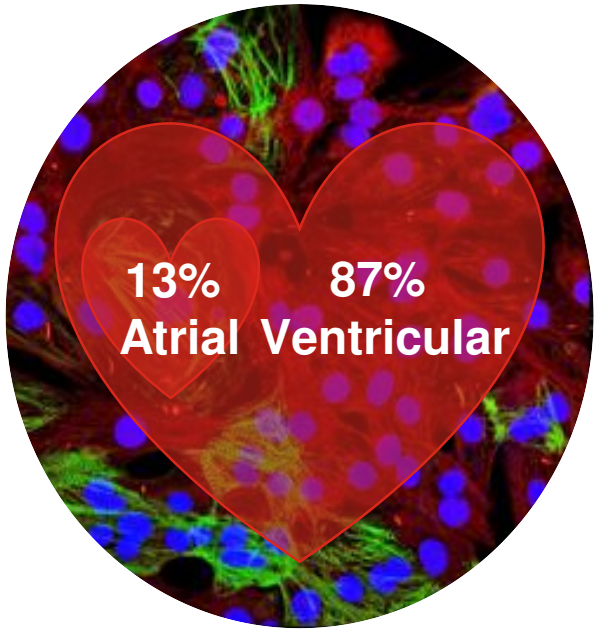


n = 32 control recordings

Cells paced at either 0.5 or 1Hz

Pure Population Ventricular Cardiomyocytes



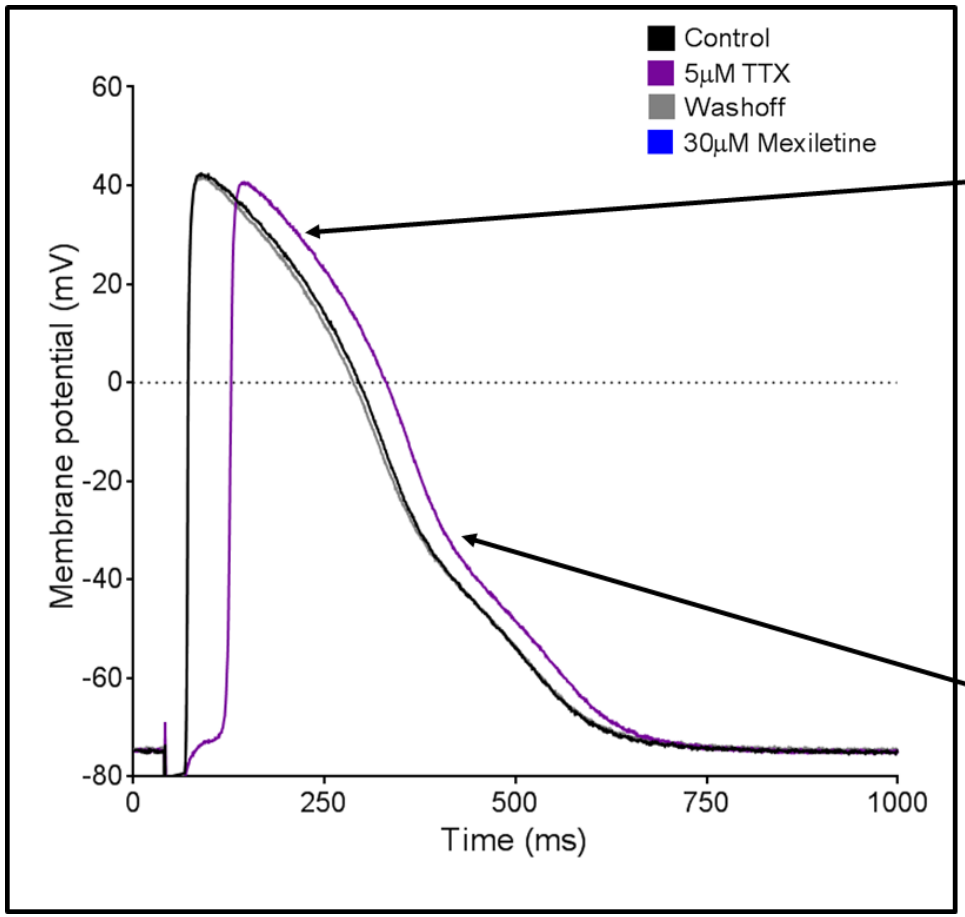


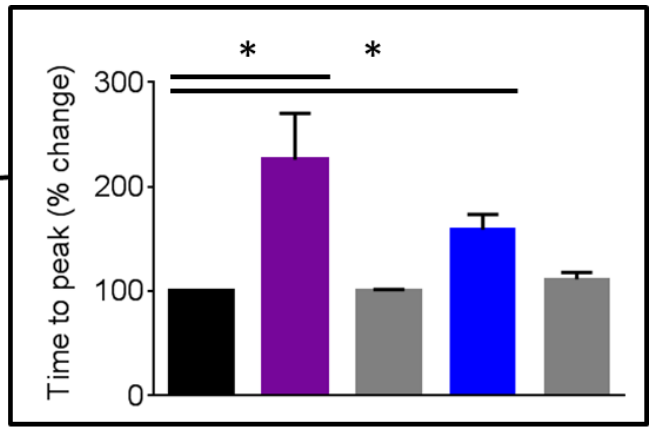
- Negligible effect on AP parameters (n=8)
  - Positive effect of carbachol observed with atrial-derived HL-1 cells
  - Suggests majority of cells do not display an atrial phenotype

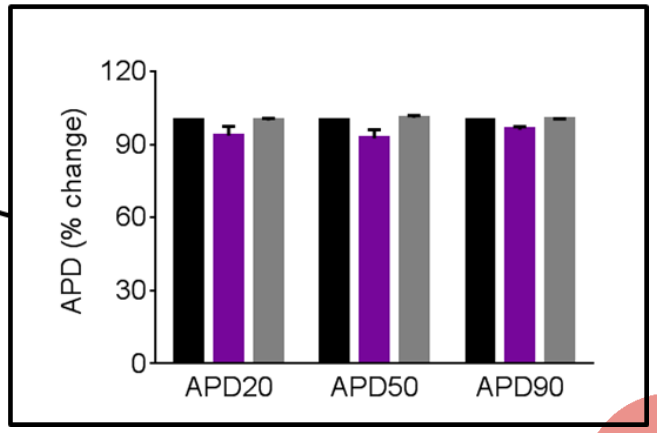
Ventricular myosin light chain (87%) and atrial myosin light chain (13%)

(Does not include nodal population)

TTX & Mexiletine (I_{Nav})

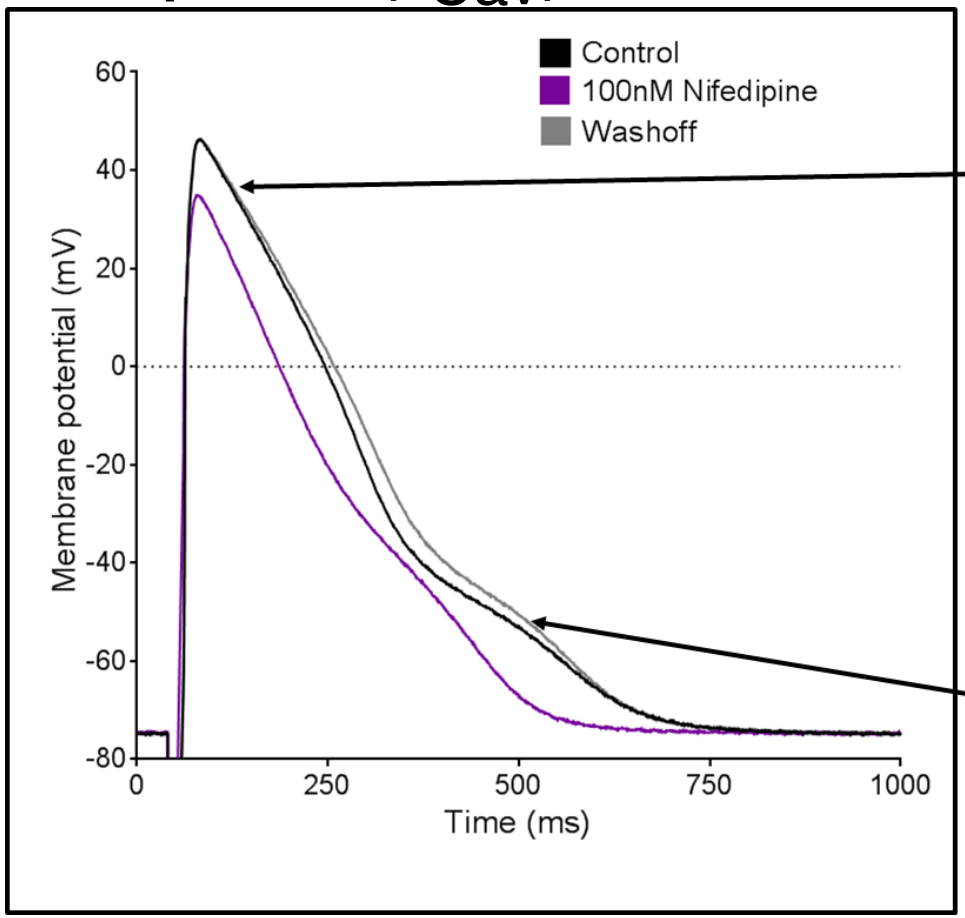


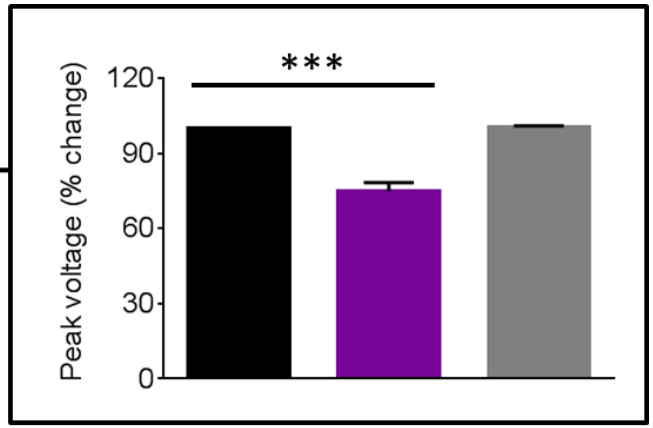


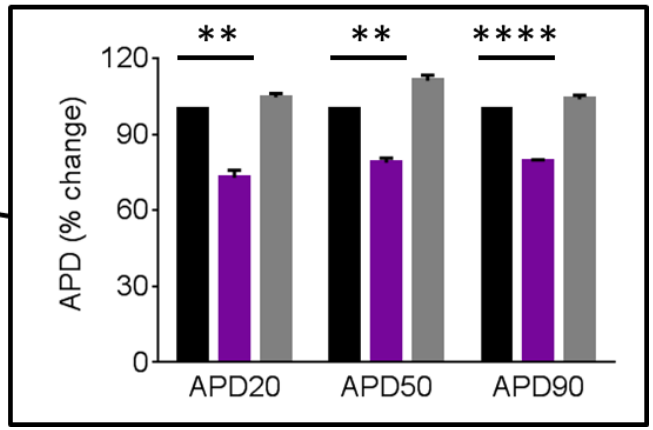


- Significantly prolonged the TTP
- Negligible effect on other AP parameters
- Similar effect observed with Mexiletine

Nifedipine (I_{Cav})

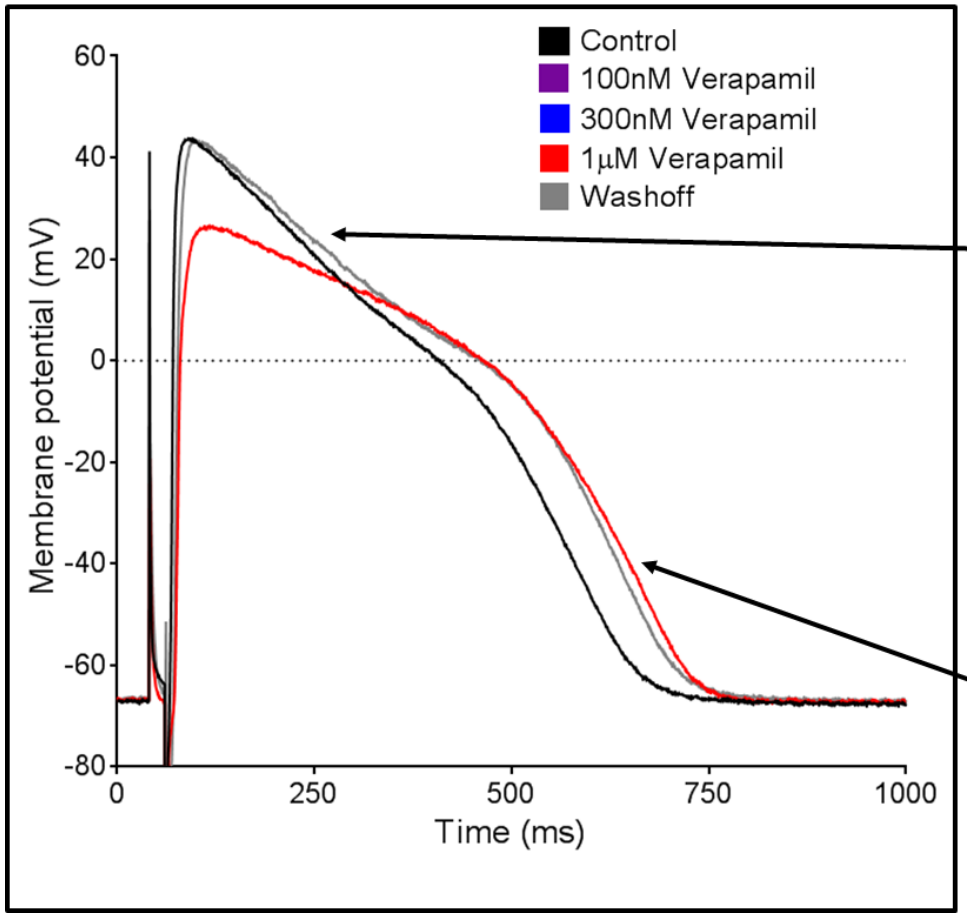


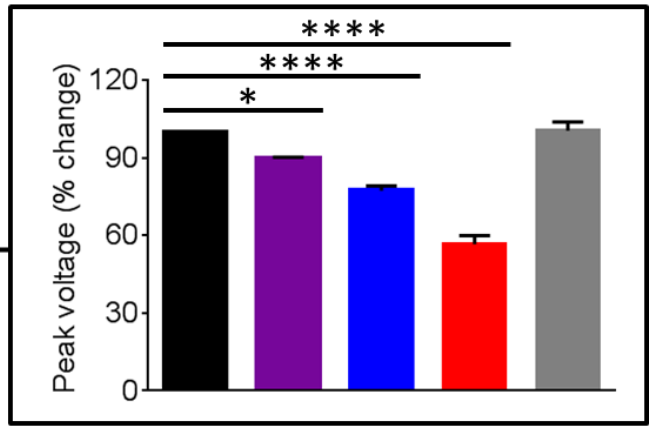


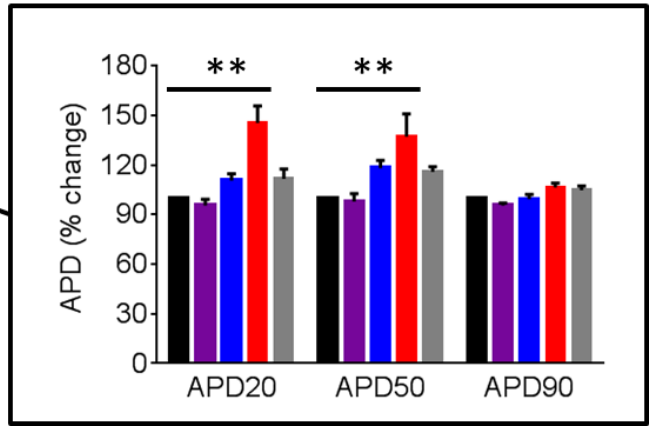


- Significantly reduced the peak voltage
- Significant shortening of APD20, APD50 & APD90

Verapamil (I_{Cav} & I_{Kr})

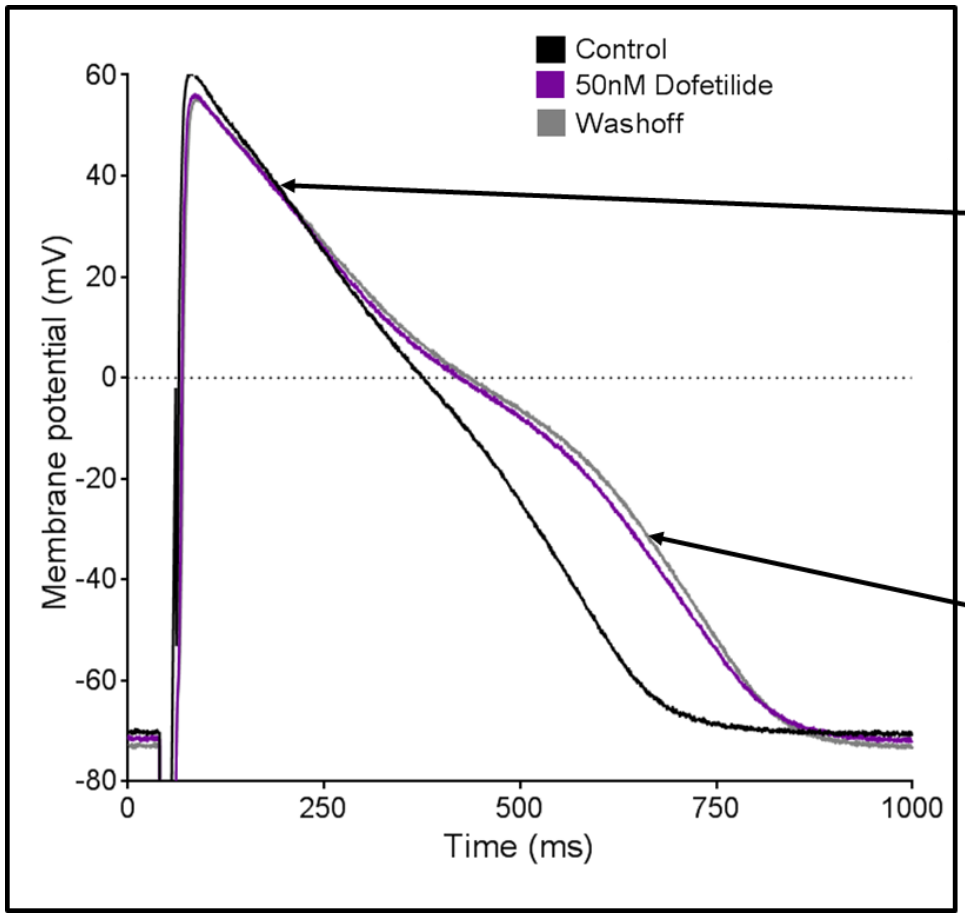


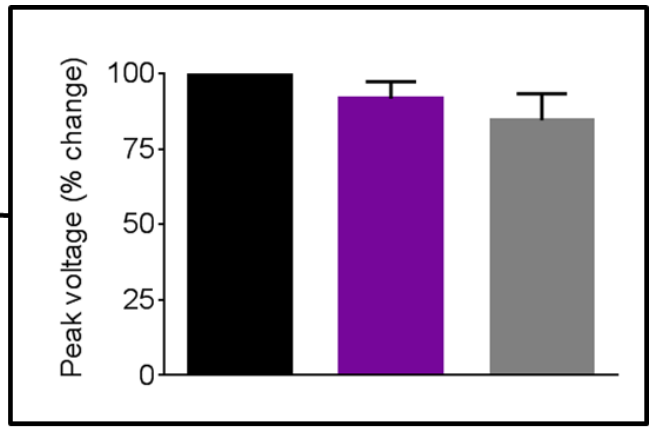


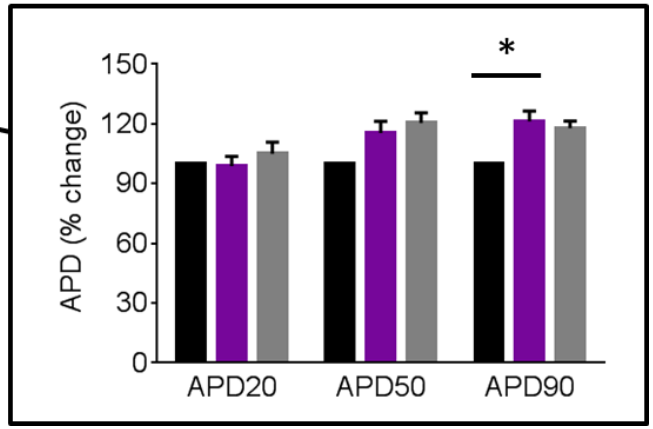


- Significant reduction to the peak voltage (all concentrations)
- Significant reduction in TTP(1µM)
- Significant prolongation of APD20 & 50 (1µM) but not APD90

Dofetilide (I_{Kr})





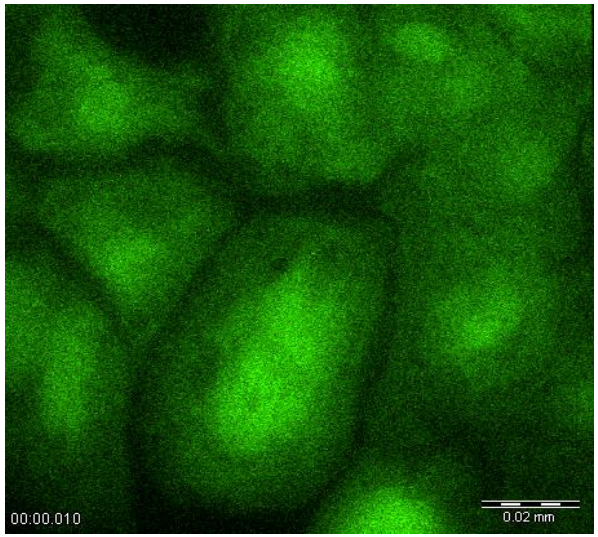


- Significant prolongation to APD90
- Negligible effect on other AP parameters

Effect of Dofetilide on Calcium Imaging

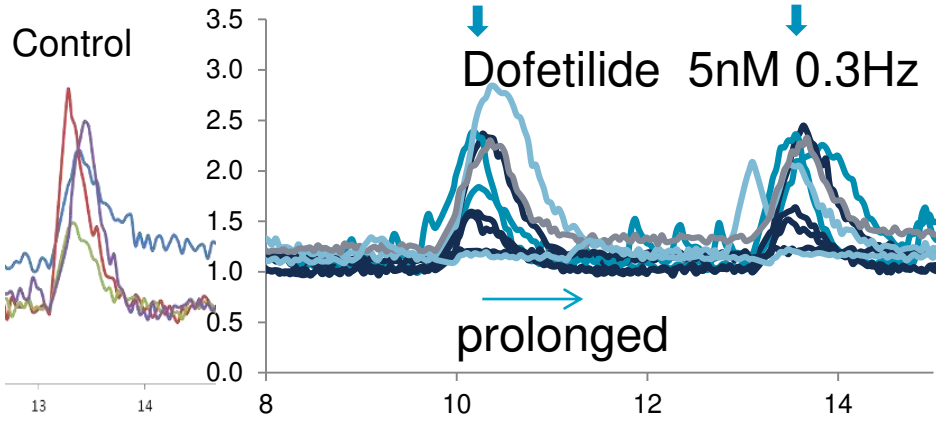
Without treatment

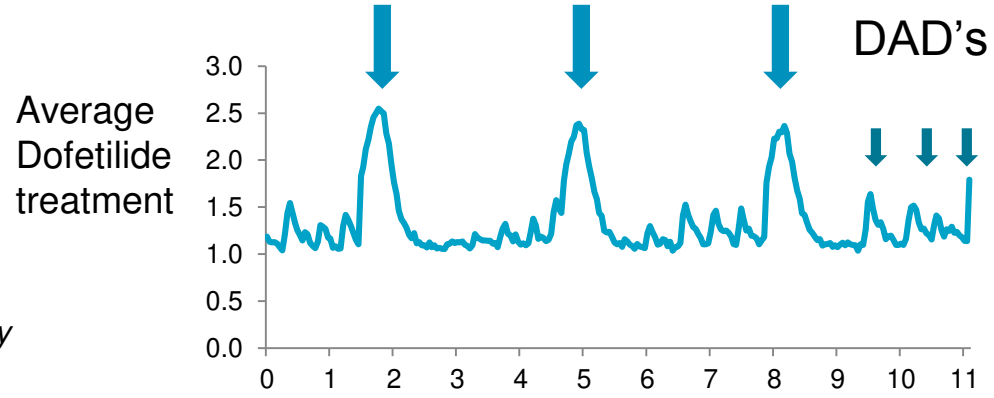
Using Fluo-4 calcium dye to measure calcium transients



Data provided by Dr Frances Brook at Oxford University

Dofetilide treatment prolongs the calcium transient

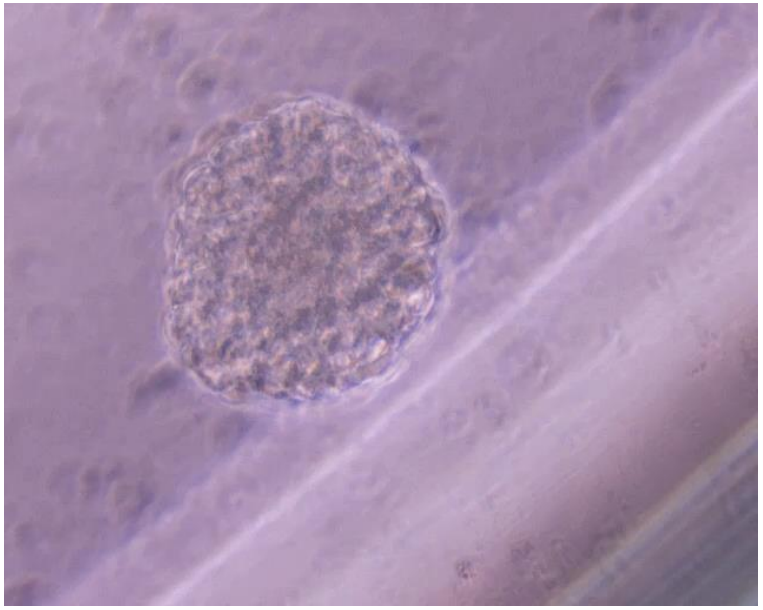




Delayed after depolarization (DAD) apparent in some cells

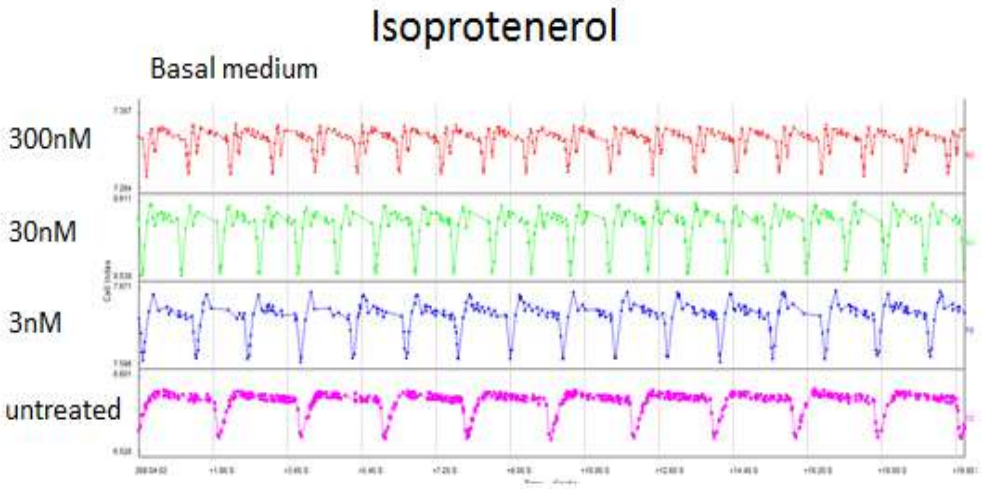
iPSC-Derived Cardiomyocytes in 3D culture

Cardiosperoids are essential for successful co-culturing of iPSC-derived cardiomyocytes & endothelial cells



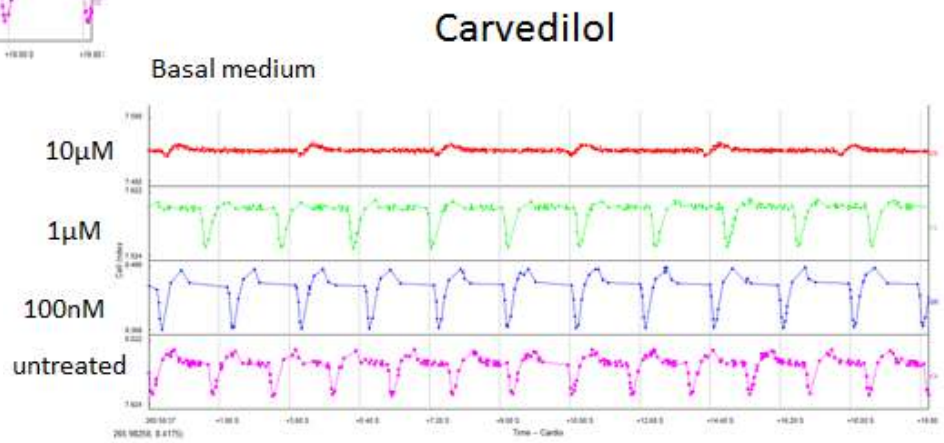
Dr Christian Zuppinger, University of Bern

In-Vitro Models for Cardiotoxicity Studies



Isoproterenol (β-adrenoceptor antagonist) & carvedilol (β-adrenoceptor agonist) were added at 265h

Data shared by Dr Jason Gill, Durham University



iPSC-derived cardiomyocytes responded to both compounds in a dose-dependent fashion & strongly indicates the clinical relevance of these cells & their utility for drug screening applications

Cardiotoxicity Summary

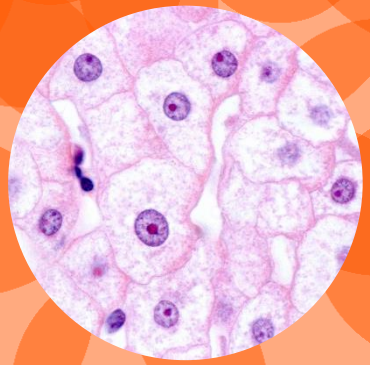
iPSC-derived cardiomyocytes (CMs) could be used in cardiotoxicity & cardiomyocyte pharmacology studies

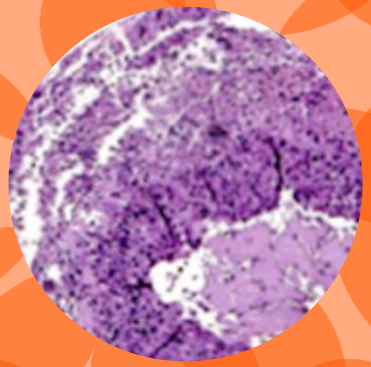
- iPSC-derived CMs express definitive cardiac markers & form organized sarcomeres
- iPSC-derived CMs show synchronized beating as a monolayer culture at high confluency
- Electrophysiological measurement of APs, pharmacology consistent with expression of INav, ICav & IKr
- Functional on xCelligence & for calcium imaging

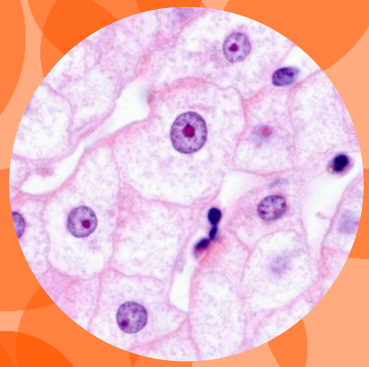
axol

your discovery stems from here

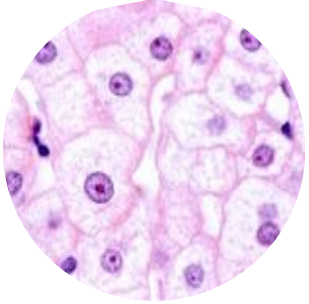
Hepatocytes







A way forward for more predictive toxicity testing

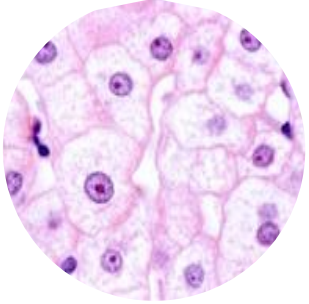


Hepatotoxicity in Drug Safety Testing

We need:

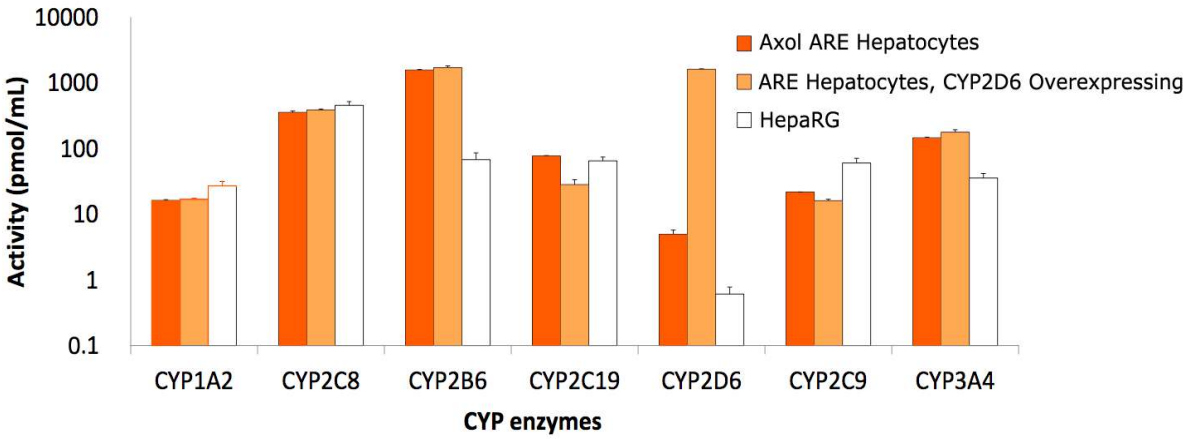
- Reliable genotoxicity testing, predictive hepatotoxicity screens
- Cells expressing adult hepatocyte markers & no fetal phenotype
- Large batch sizes from the same donor for consistency for toxicity and high-throughput screening

Human primary hepatocytes have much greater functionality than iPSC-derived hepatocytes

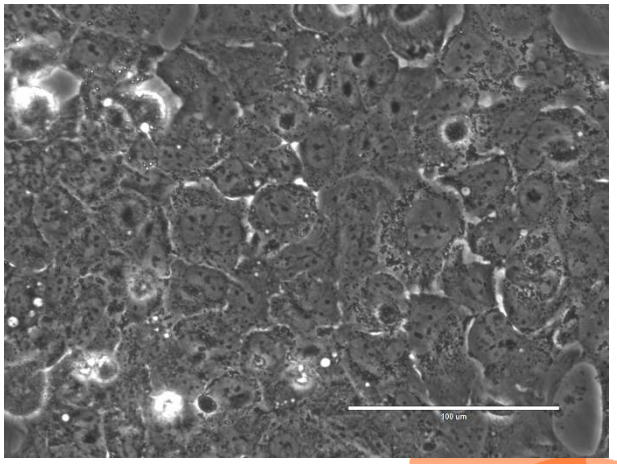


Assay-Ready Expanded (ARE) Hepatocytes

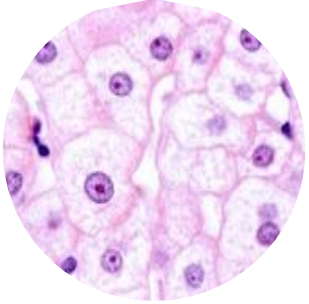
- Expanded hepatocytes that retain many characteristics of primary human hepatocytes
- Metabolically functional & express cytochrome P450 (CYP) enzymes



Comparison of the Phase I CYP enzyme activity between ARE Hepatocytes, ARE Hepatocytes (CYP2D6 Overexpressing) & HepaRG cells

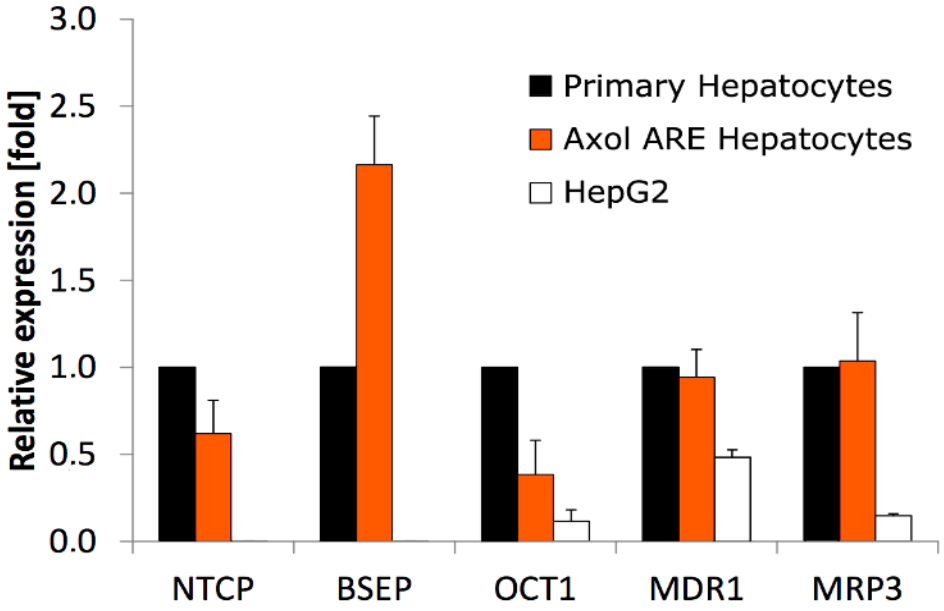


Cobblestone morphology



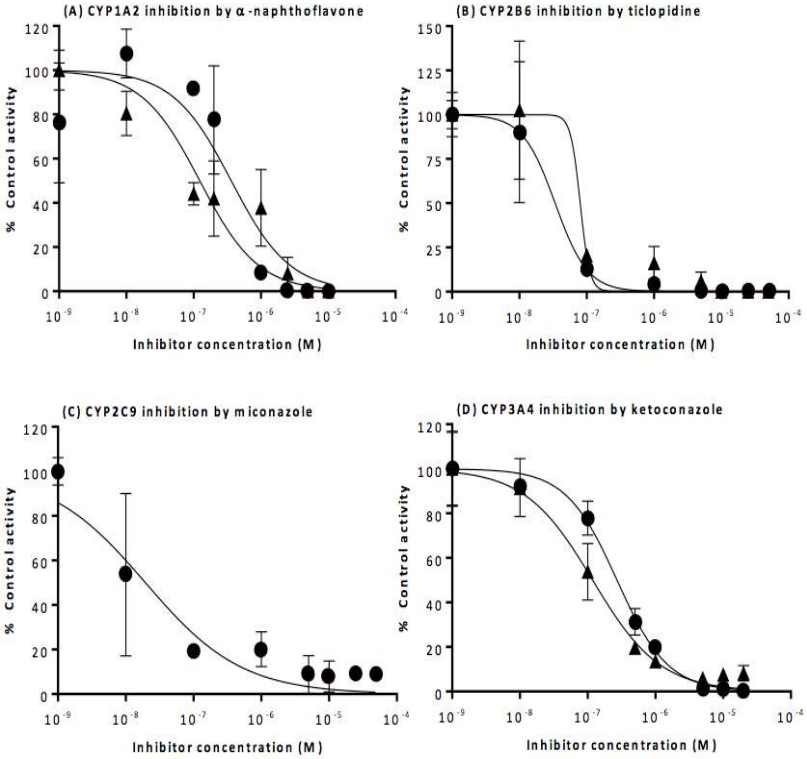
Assay-Ready Expanded (ARE) Hepatocytes

Compound uptake studies

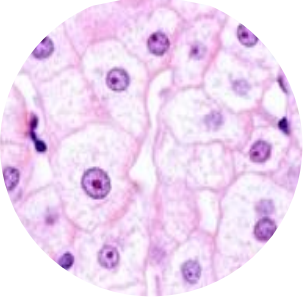


Expression of hepatic transporter genes in primary hepatocytes, ARE hepatocytes & HepG2 cells

Inhibition Studies

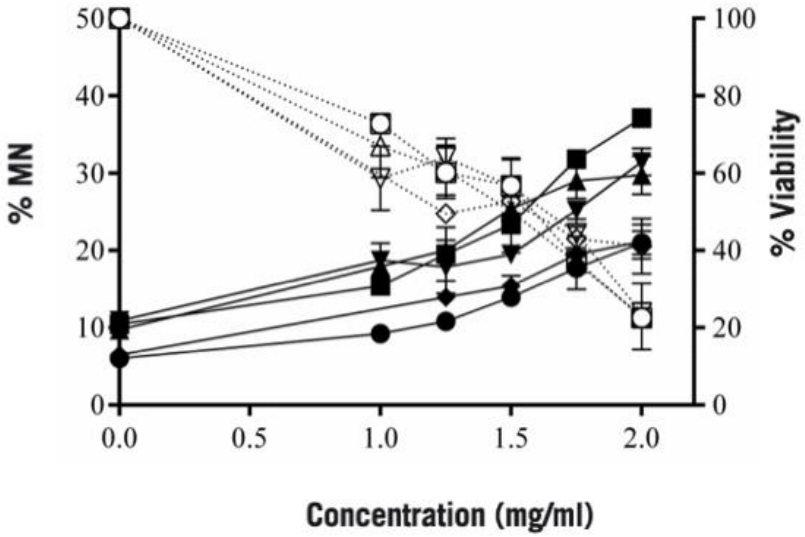


Reproducible CYP induction & inhibition in a donor-specific manner by prototypical inducers/inhibitors



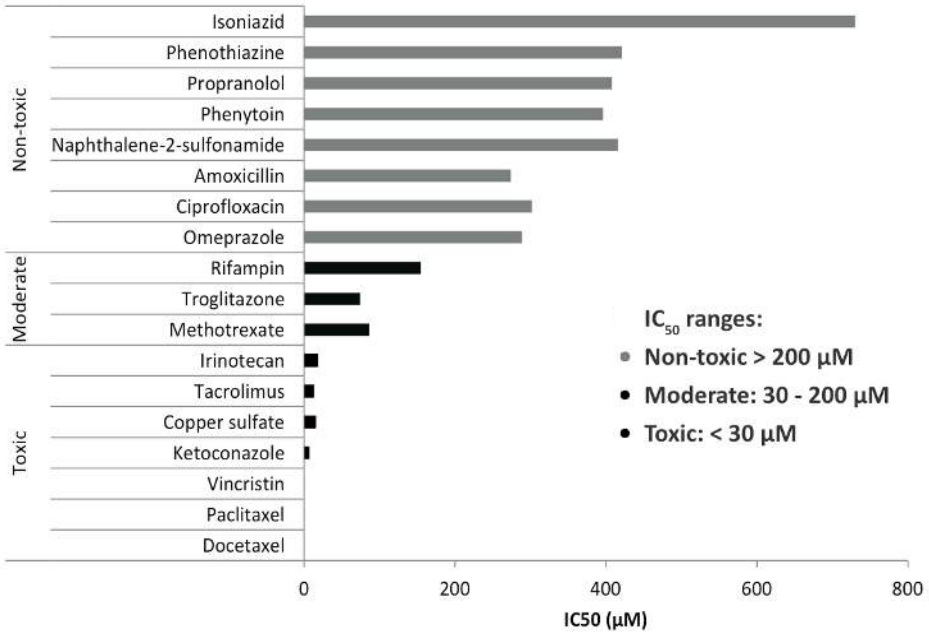
Assay-Ready Expanded (ARE) Hepatocytes

Genotoxicity studies

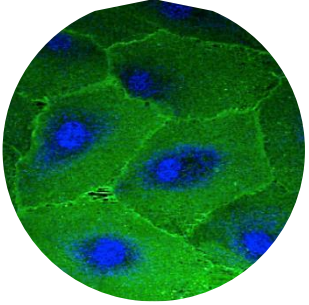


Increasing cyclophosphamide concentration affects the percentage of cells with micronuclei (% MN) & cell viability

Hepatotoxicity studies



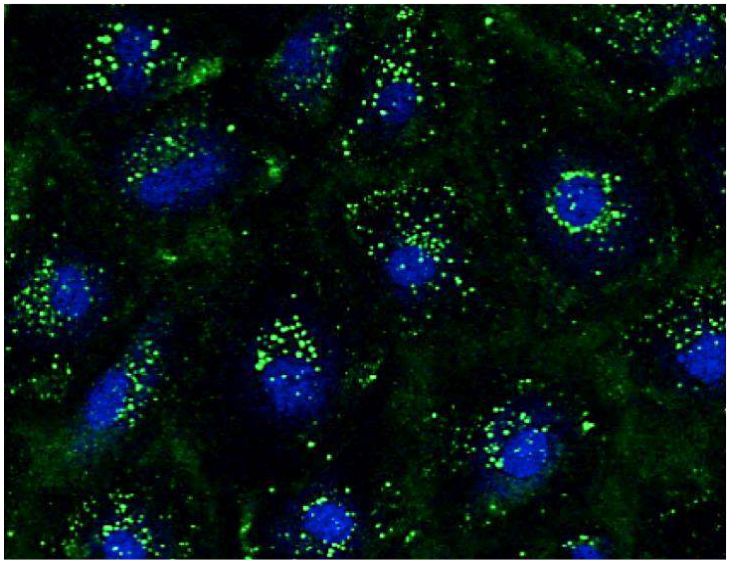
Sensitivity to hepatotoxic compounds



Assay-Ready Expanded (ARE) Liver Sinusoidal Endothelial Cells

ARE Liver Sinusoidal Endothelial Cells are primary liver endothelial cells that have been expanded *in-vitro*

3D cultures can be generated by co-culturing with ARE Hepatocytes



Low Density Lipoprotein (LDL) uptake in ARE Liver Sinusoidal Endothelial cells. LDL (green), DAPI (blue)

Hepatotoxicity Summary

- ARE Hepatocytes display a primary liver cell phenotype
- ARE Hepatocytes are metabolic competent cells expressing liver specific transporters and metabolizing enzymes
- Large batch sizes from the same donor for consistency for toxicity & high-throughput screening
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing
- ARE Hepatocytes can be co-cultured with liver sinusoidal endothelial cells

Conclusion

Our aim is to provide physiologically relevant *in-vitro* disease models for drug discovery & toxicity studies

Axol iPSC-derived NSC

Express neural markers at gene and protein level
Excellent neurite outgrowth
Electrophysiologically functional
Capable of synaptic plasticity

Axol iPSC-derived Cardiomyocytes

Expressing definitive cardiac markers and form organized sarcomeres
Synchronous beating monolayers, electrophysiologically functional
Functional on xCelligence & for calcium imaging

ARE Hepatocytes

Display a primary liver cell phenotype
Metabolic competent cells expressing liver specific transporters and metabolizing enzymes
Sensitivity to hepatotoxic compounds & reliable genotoxicity testing

axol
your discovery stems from here

Thank you!

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your discovery stems from here

For more information please contact us at:
support@axolbio.com

Or visit:
www.axolbio.com