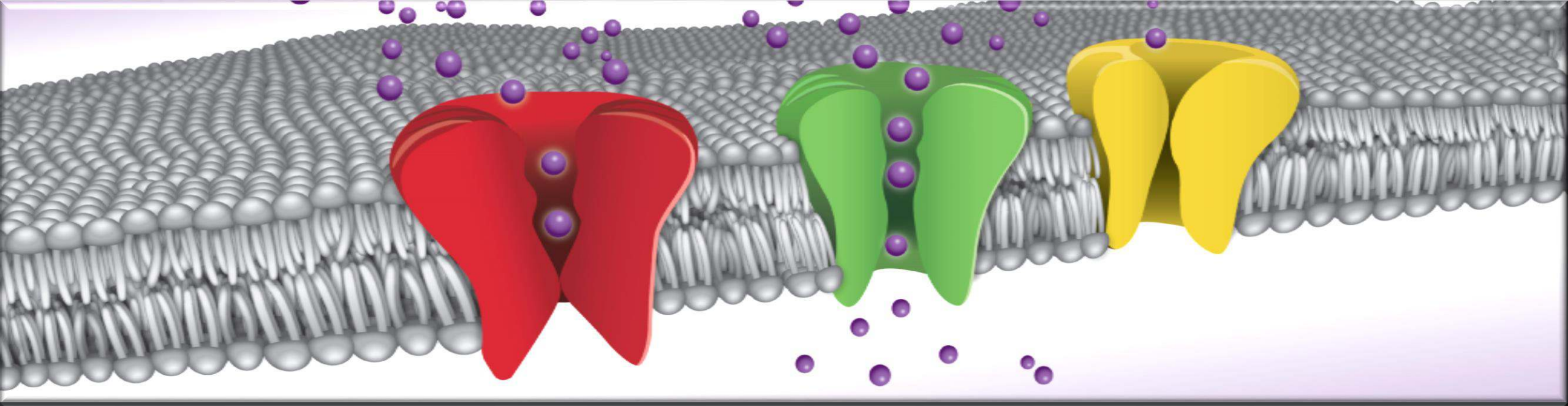

Metrion Biosciences: the ion channel specialists

In depth profiling of human iPSC-CM – from electrophysiology to phenotypic assays

Saïd El-Haou, PhD

08 March 2017

www.metrionbiosciences.com

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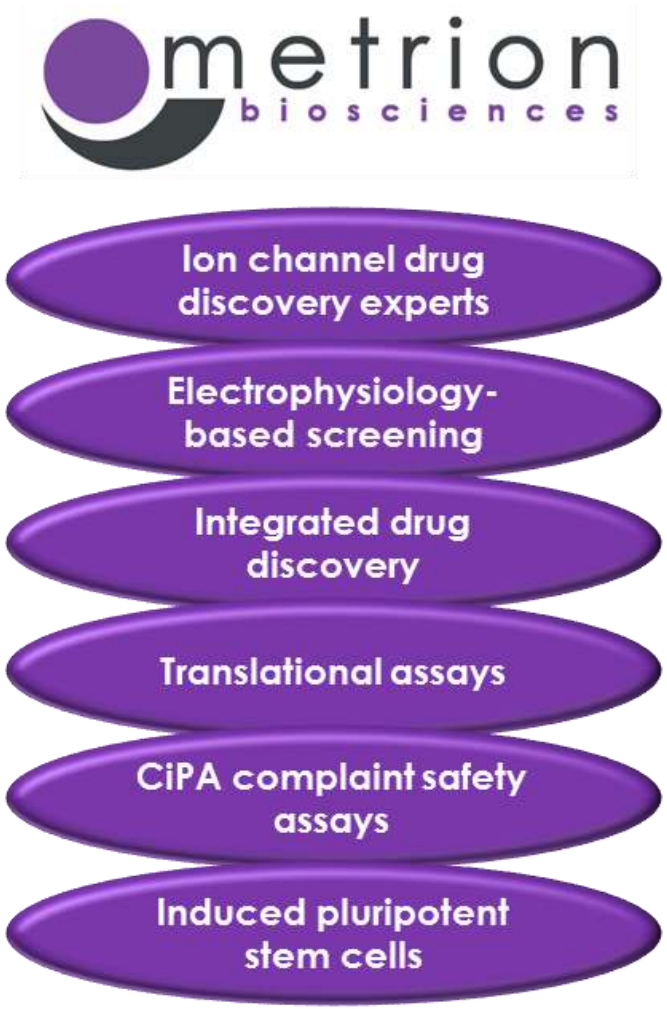
Metrion Biosciences: the ion channel specialists

- Established team of expert electrophysiologists
- Specialist in ion channel screening
- Expertise in both manual and automated patch clamp
- Part of CiPA consortium for HTS platform
- Expanding range of phenotypic and translational assays in the cardiac and neuroscience fields
- Integrated drug discovery capability
  - HTS to candidate nomination



Metrion

Eurostars CardioPredict Grant

- Eurostars CardioPredict is a European consortium composed of three partners:

Metrion Biosciences **Nanion Technologies** **Leiden University**

- Started in 2015 - 2 M€ over two years

- **Aim**: Optimised human stem cell cardiomyocytes & phenotypic assays using the latest generation of screening platforms to meet future market & regulatory requirements. Generate *in vitro* cardiac safety assay to reliably predict clinical cardiac risk

Presentation topics

1. **Manual Patch Clamp - Current Clamp**
2. Axion Maestro - MEA
3. Nanion CardioExcyte96 - Impedance

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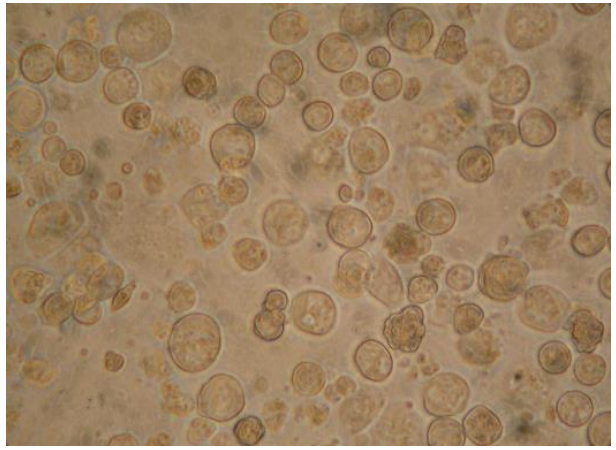
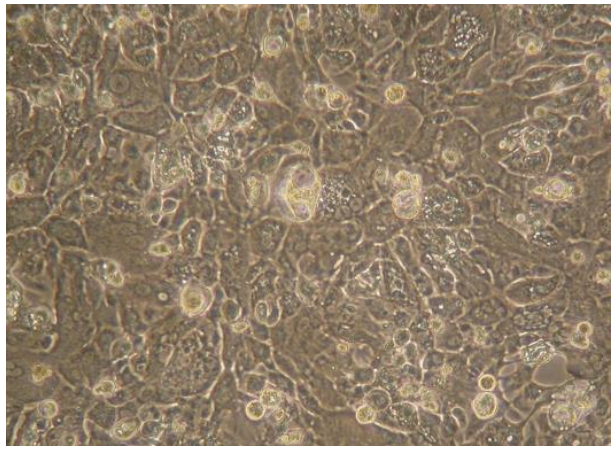
hiPSC-CM: Axiogenesis vCor.4U



Day 0



Day 7

- New generation of Axiogenesis ventricular induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM)
- Isolated from 26 year old Caucasian female
- 90 % ventricular
- Fibroblast free
- 7 days in culture - fibronectin (10 µg/ml)
- Cells form syncytial monolayer suitable for electrophysiological & phenotypical assessment
- Perforated patch clamp, current clamp configuration @ RT

Methodology

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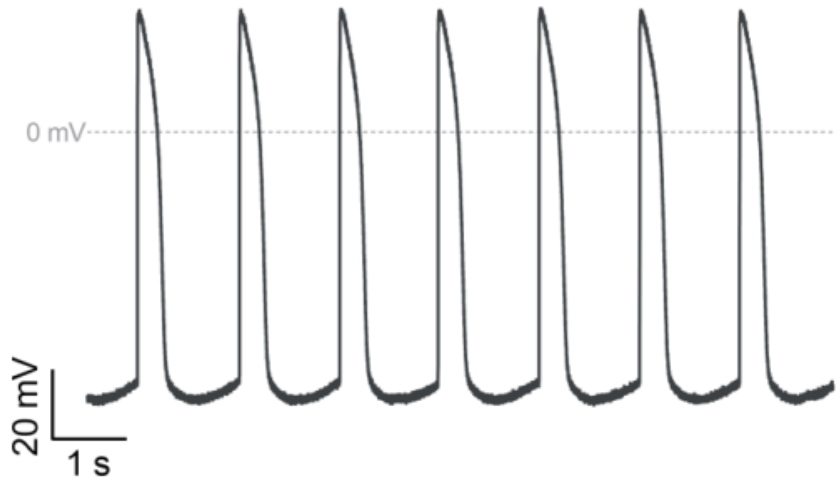
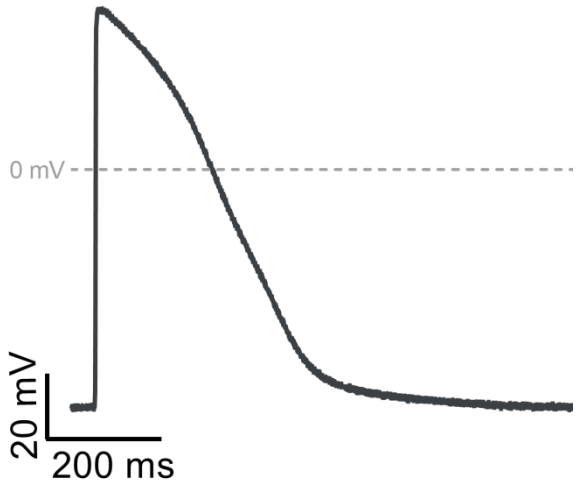
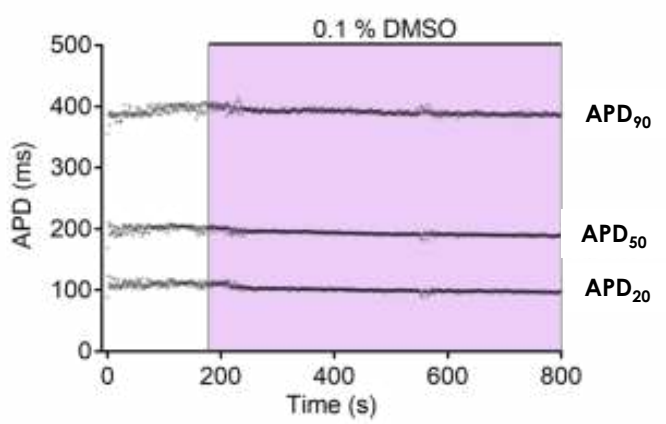
Current clamp recording on Manual Patch Clamp







AP parameters	Spontaneous (n=89)	Evoked 1 Hz (n=75)
RMP (mV)	-72.0 ± 0.3	-73.3 ± 0.6
Vmax ($V.s^{-1}$)	56.8 ± 3.7	50.1 ± 4.8
APA (mV)	106.5 ± 0.8	119.9 ± 1.7
APD_{20} (ms)	133.1 ± 3.8	99.3 ± 3.0
APD_{50} (ms)	249.4 ± 4.9	211.6 ± 3.8
APD_{90} (ms)	502 ± 16.1	426.8 ± 10.9
Frequency (Hz)	0.43 ± 0.01	1

- Suitable for recording of spontaneous & evoked activity (0.5 – 2 Hz)
- Very stable recordings > 30 min
- Good resting membrane potential ~ -73 mV
- Phase 0 upstroke of 50 $V.s^{-1}$ denoting good expression of $Na_V1.5$ channel
- Consistent APD_{90} ~ 500 ms

hiPSC-CM validation

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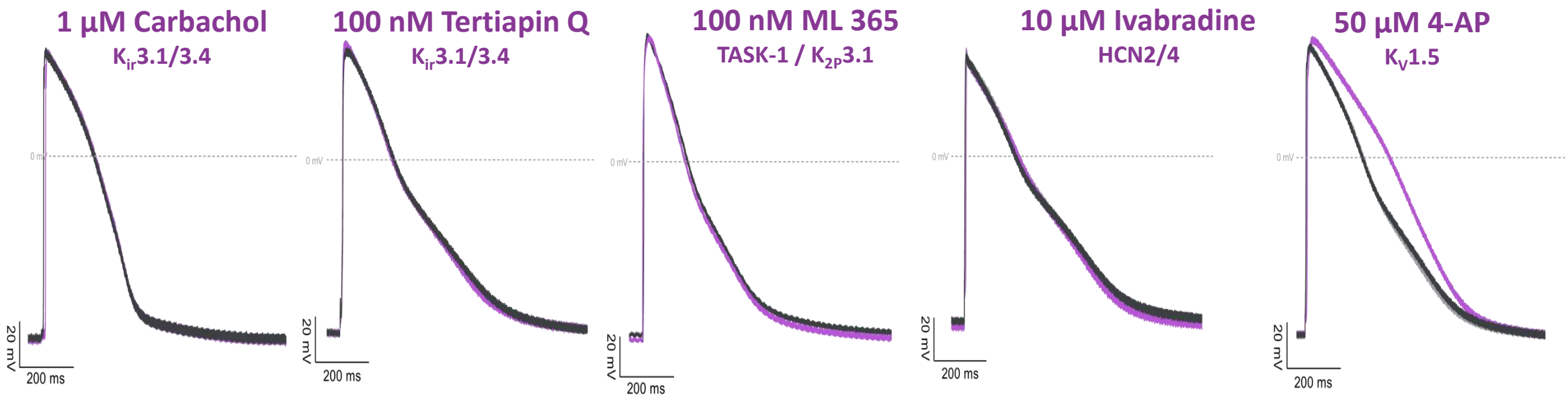
Action Potential phenotype: atrial vs ventricular (1 Hz)



- Insensitive to most atrial-selective ion channel blockers
- Small effect of 4-AP suggesting functional expression of K_V1.5 channels
  - K_V1.5 protein is expressed in the human ventricle, although no functional activity has been detected so far (Mays *et al*, 1995)
  - Common feature of all hiPSC-CM tested so far at Metrion

Atrial pharmacology

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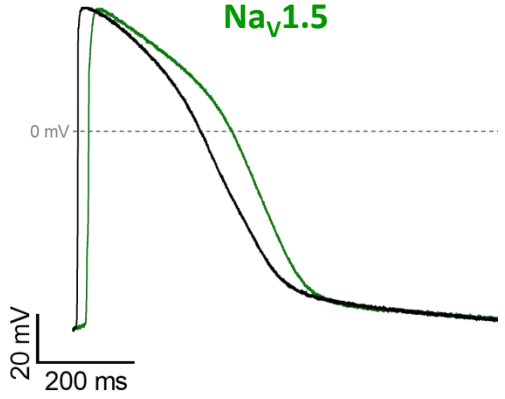
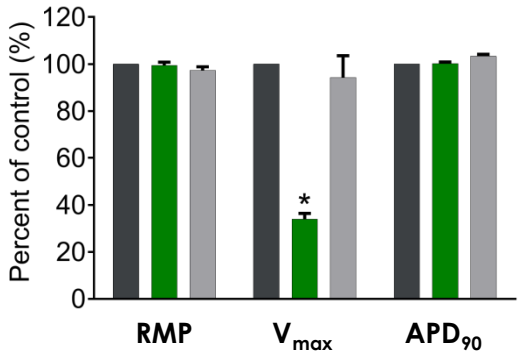
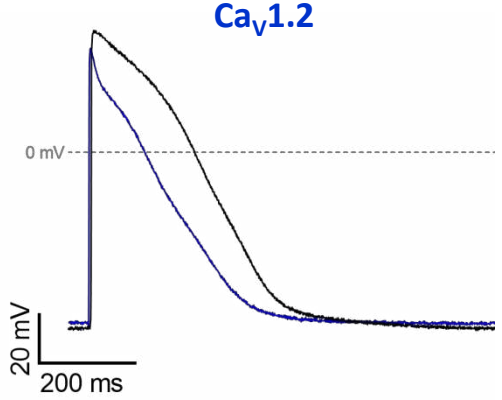
Pharmacology: evoked Action Potential (1 Hz)

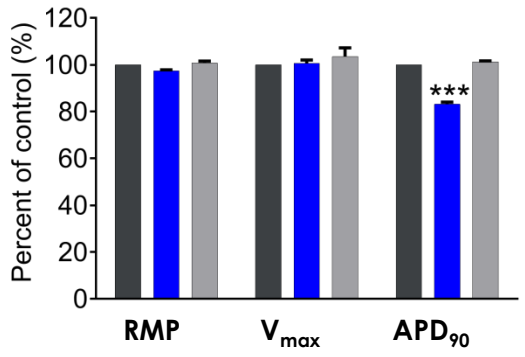
100 µM Lidocaine
$Na_V1.5$



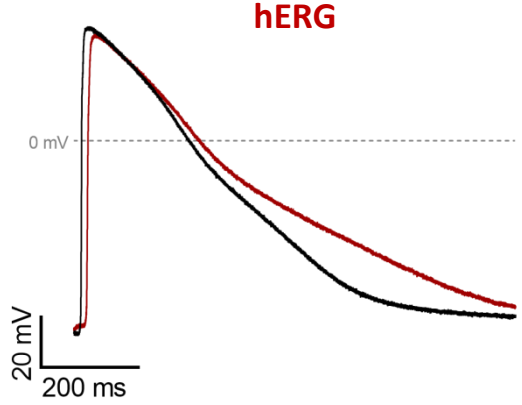


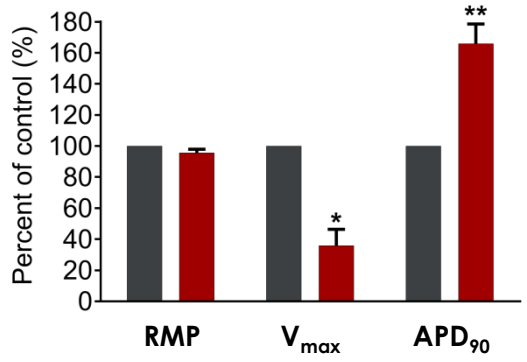
100 nM Nifedipine
$Ca_V1.2$





50 nM Dofetilide
hERG





- Expected effects of core CiPA reference compounds on AP:
  - Lidocaine slows the upstroke velocity by ~60%
  - Nifedipine decreases all APD values
  - Dofetilide prolongs APD_{90} by ~ 70% but does not trigger EAD at 1Hz

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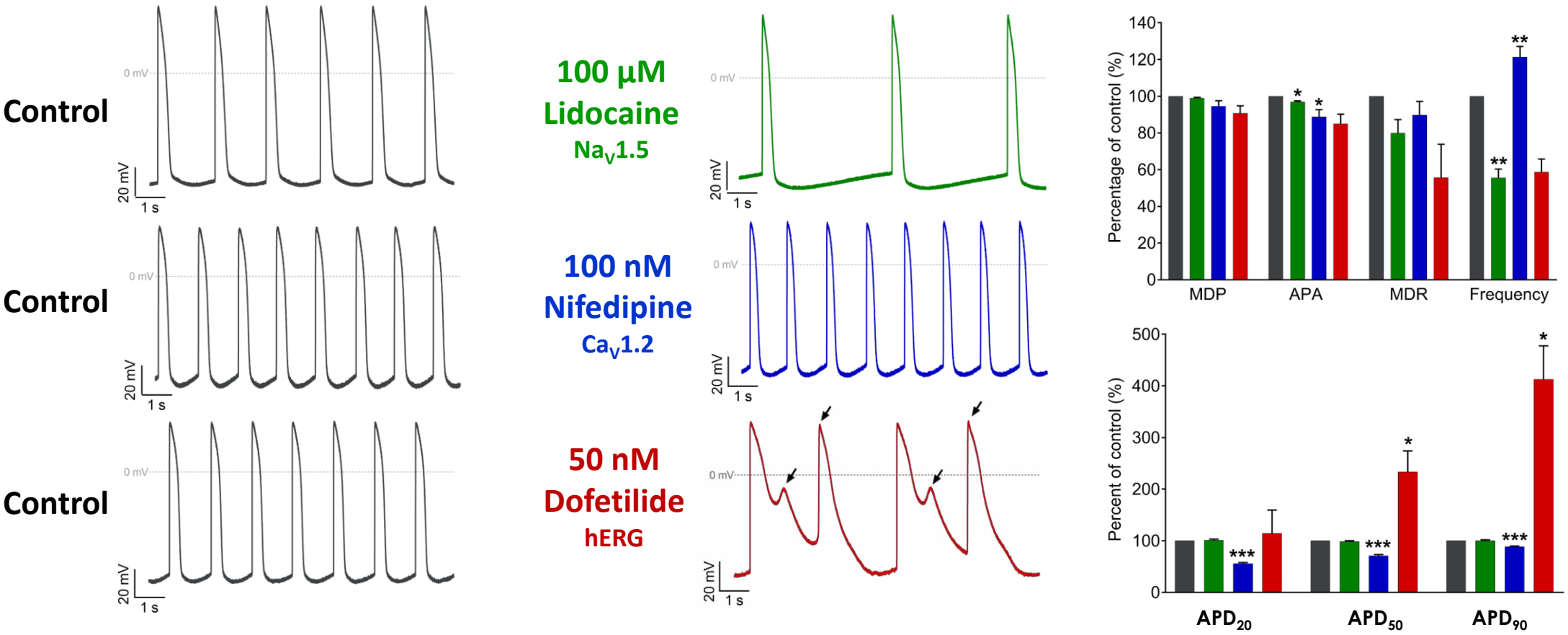
Pharmacology: spontaneous activity



- AP exhibit expected sensitivity to reference compounds
- EAD observed with dofetilide

Presentation topics

1. Current Clamp - Manual Patch Clamp

2. **Axion Maestro - MEA**

3. Nanion CardioExcyte96 - Impedance

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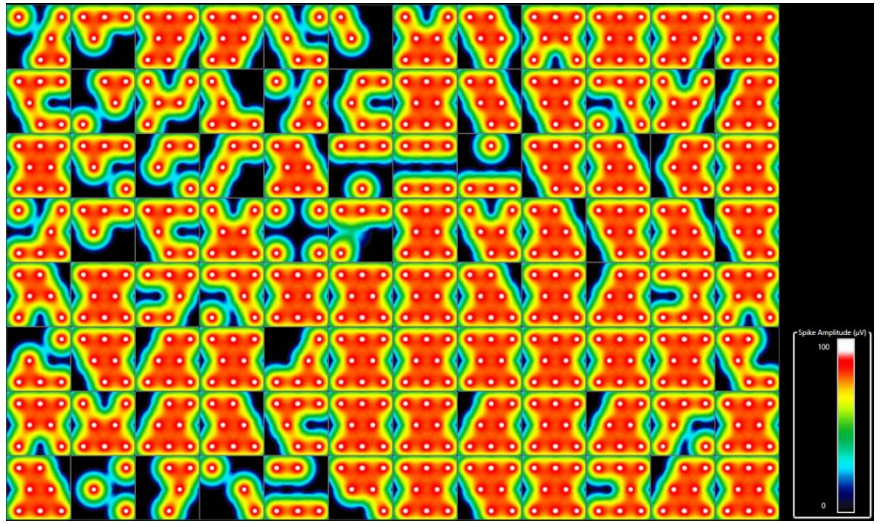
MEA platform: Axion Maestro



- Environment control plugin allows long term recording
  - 37°C / 5% CO_2
  - Acute / chronic pharmacology
- High success rate using 96 MEA well plate
  - 100% wells recorded MEA signal
  - >85% of the 784 electrodes

MEA parameters	vCor.4U
Spike Amplitude Mean (mV)	1.29 ± 0.4
Spike Slope Mean (V/s)	-2.4 ± 1
FPD Mean (ms)	363.1 ± 30.4
FPDcF Mean (ms)	405.7 ± 33.9
Beat Rate (bpm)	83.9 ± 2.4
Conduction Velocity Mean ($mm.ms^{-1}$)	0.22 ± 0.1
Beat Period Irregularity (%)	2.9 ± 5.8

Microelectrode array

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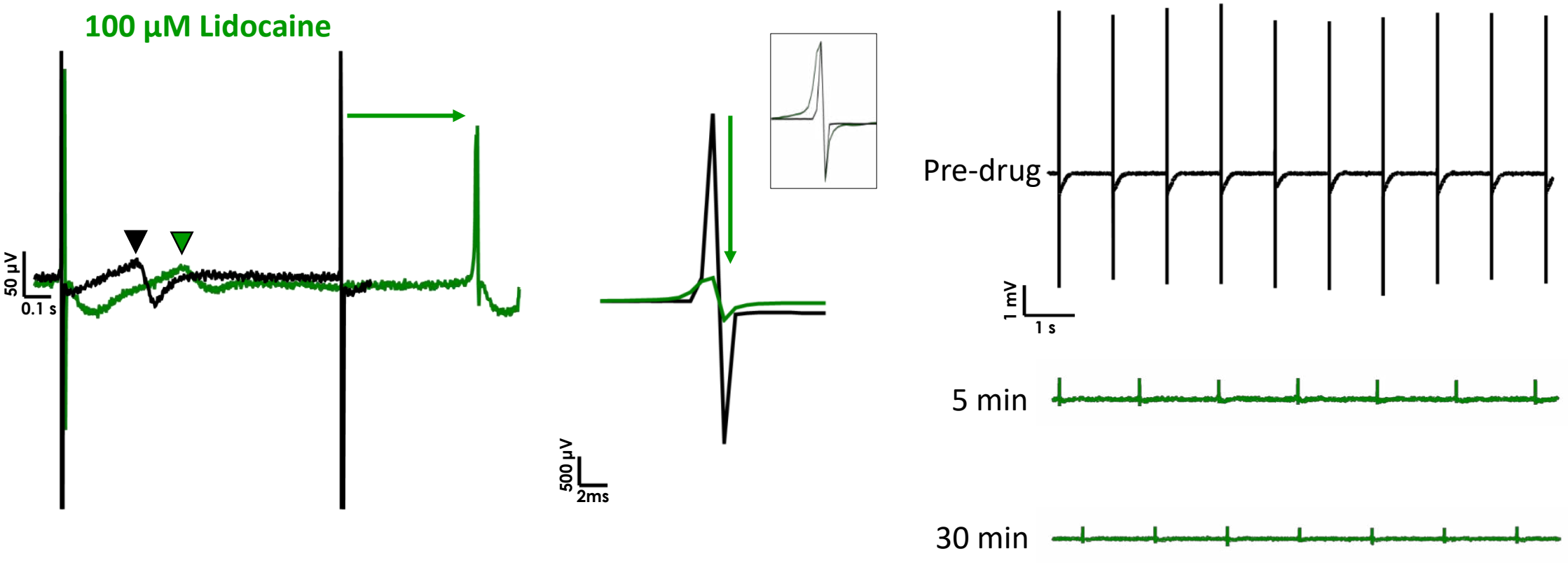
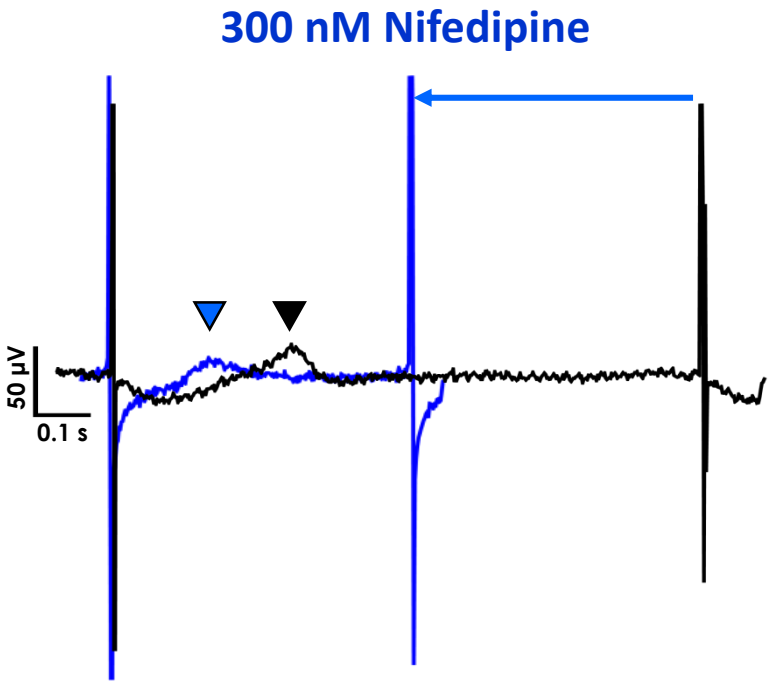
Maestro: Sodium channel blocker Lidocaine

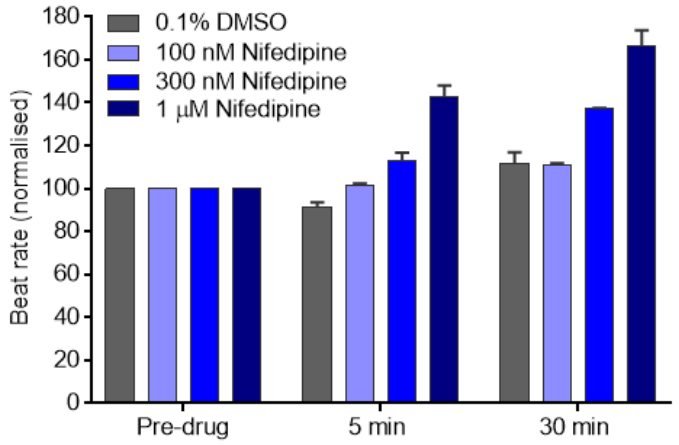


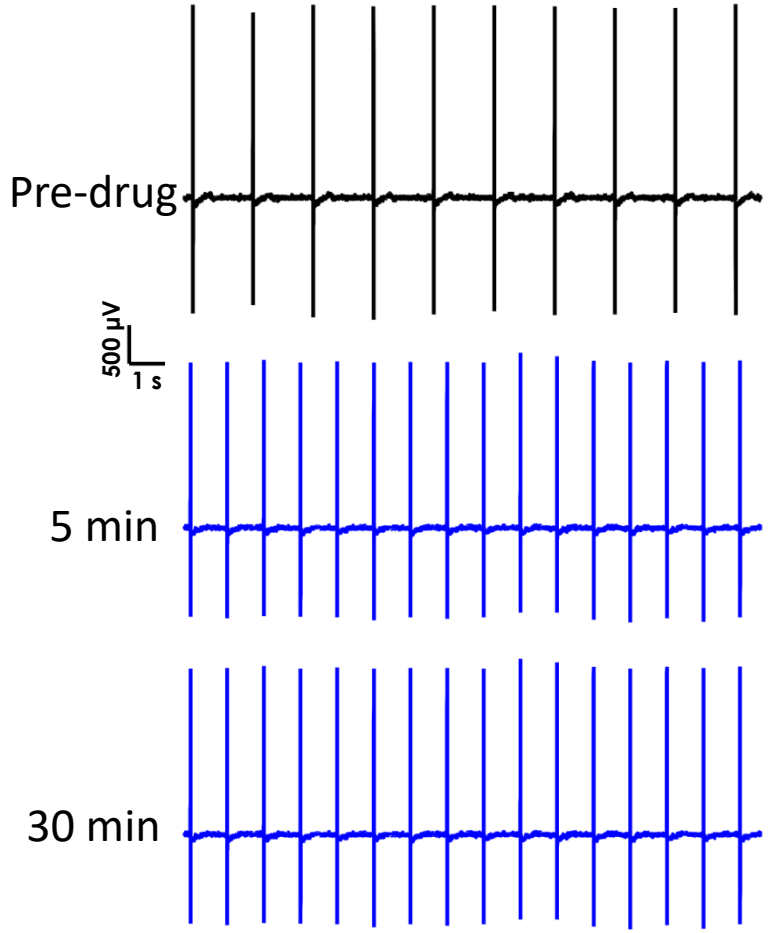
- Lidocaine increases inter-spike interval & QT interval but not the QTc
- Important decrease of peak amplitude & slope
- No arrhythmic event or EAD-like events

Microelectrode array

Maestro: Calcium blocker Nifedipine



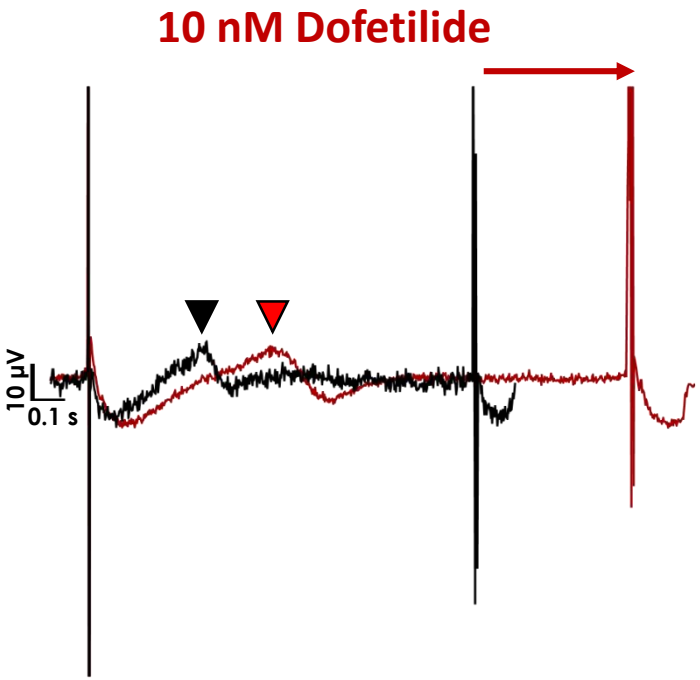


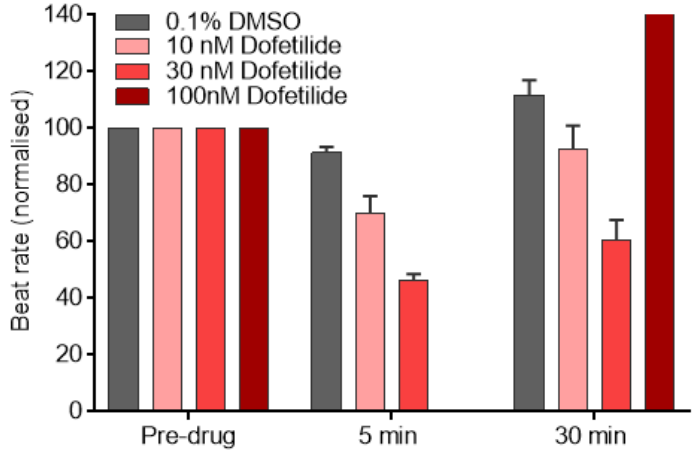


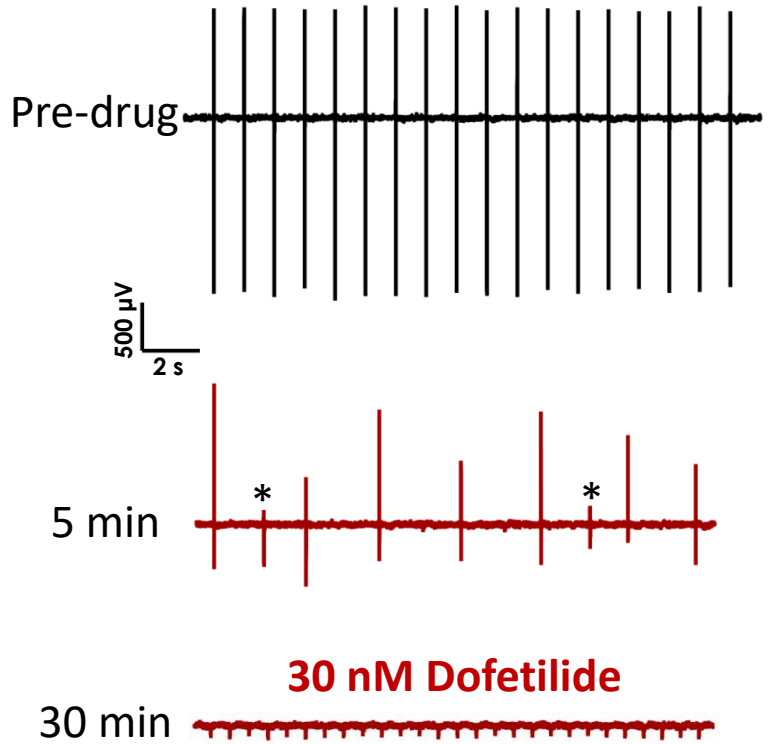
- Nifedipine decreases field potential duration (T wave) & inter-spike interval, with a small effect on the spike amplitude.
- Effects were observable after 5 min & stable over time

Microelectrode array

Maestro: hERG blocker Dofetilide







- Dofetilide increases QT & inter-spike interval . It also reduces the spike amplitude
- EAD were observable after 5 min & degenerate at higher concentration into fibrillation

Microelectrode array

Presentation topics

1. Current Clamp - Manual Patch Clamp
2. Axion Maestro - MEA
3. **Nanion CardioExcyte96 - Impedance**

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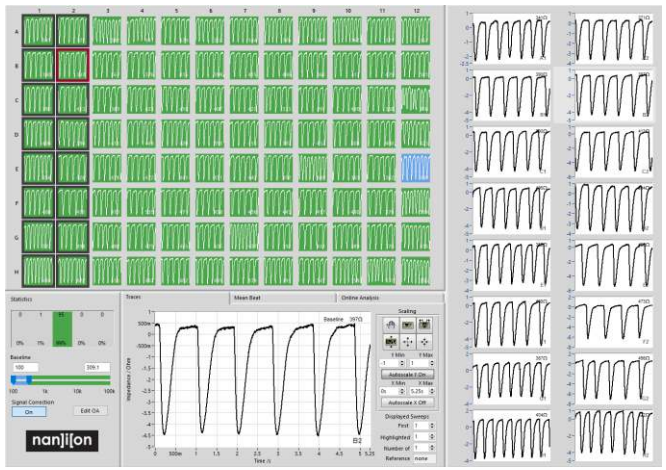
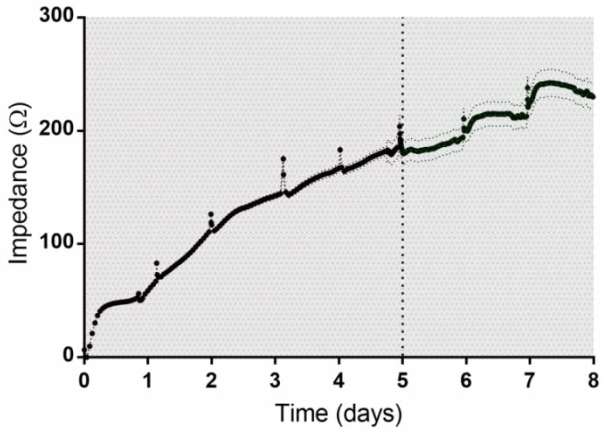
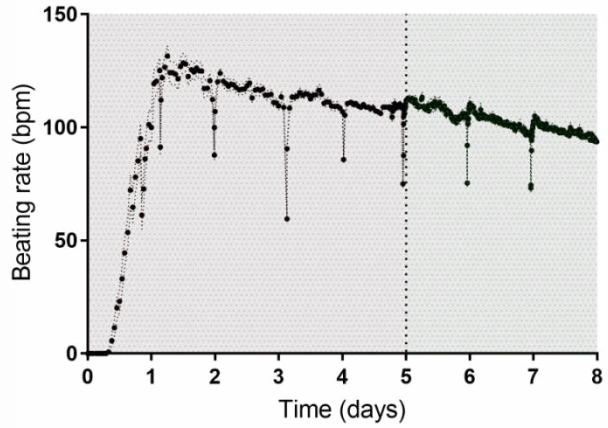
Impedance assay: Nanion CardioExcyte 96



- Dual mode platform: impedance & microelectrode array (MEA)
- High success rate ~ 100 % well display regular beating
- vCor.4U cells start to beat after 6h
- Base Impedance which represent the growth phase, is stable after 5 days
- BR stable after 72 hrs





CardioExcyte 96 parameters	vCor.4U
Base imp (Ω)	360.6 ± 4.1
Beat Rate (bpm)	108.5 ± 1.0
Amp (Ω)	3.6 ± 0.1
PW30 (ms)	195.1 ± 1.0
BR CoV	0.04 ± 0.0

CardioExcyte 96

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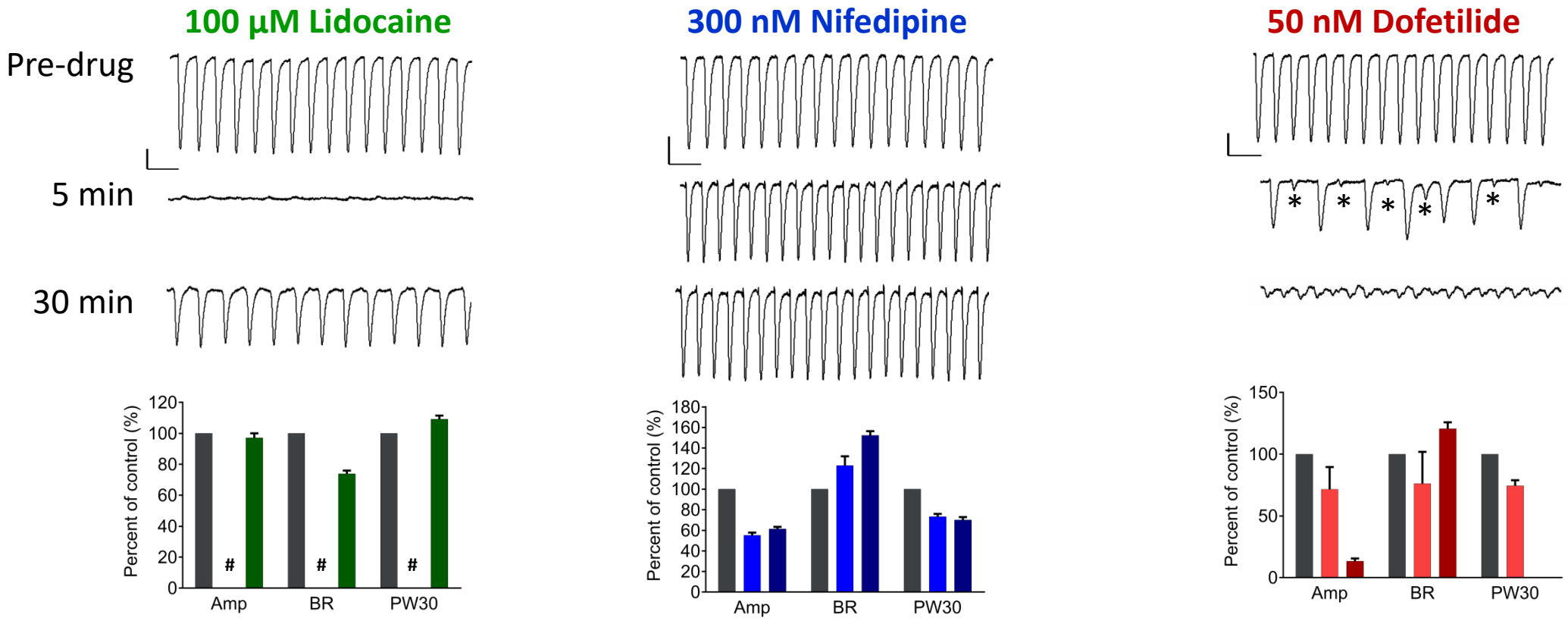
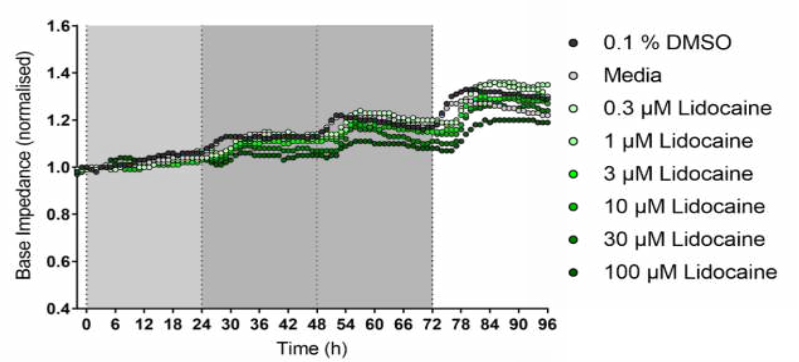
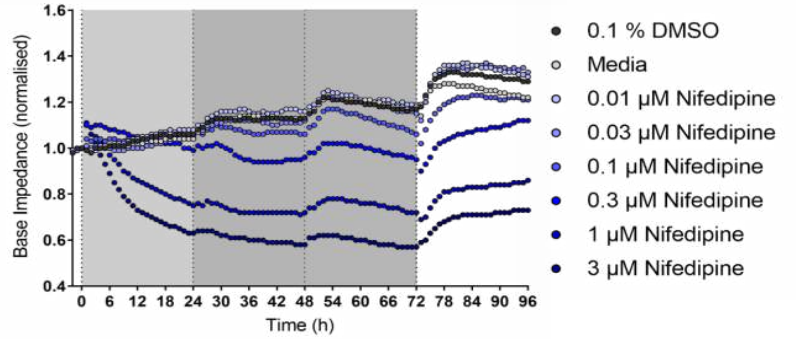
Impedance assay: Contractility & pro-arrhythmic effects

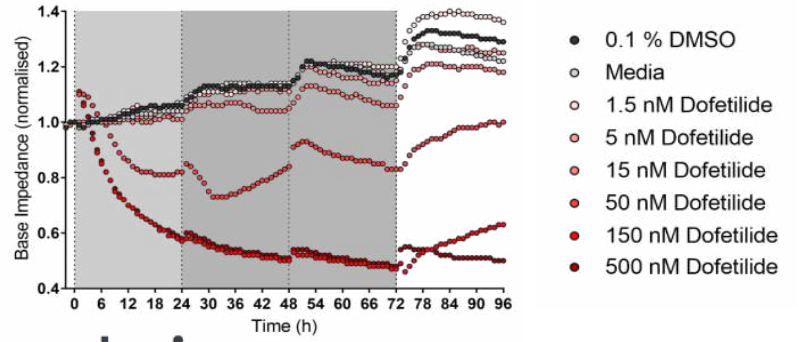


- Drug effect on contractility mirrored electrophysiological pharmacology
  - Lidocaine stops contraction but recovers with a decreased BR & amplitude
  - Nifedipine increases BR but decreases amplitude
  - Dofetilide decreases amplitude & BR. Secondary beats degenerate into fibrillation

Impedance assay: Chronic cardiotoxicity







- Base impedance is a good indicator of cell viability & possible cardiotoxicity effects can be evaluated
- vCor.4U cells are stable over 72 h with drugs
  - suitable for acute / chronic cardiotoxicity & safety screen experiments
- Lidocaine had no effect on cell viability
- Drugs with dose-dependant cardiotoxic effects
  - High concentrations of nifedipine or dofetilide decrease base impedance

Chronic impedance

Summary

- vCor.4U iPSC-CM are suitable for large range of assays:
  - Manual Patch Clamp: current clamp & voltage clamp
  - Impedance & cardiotoxicity assay using CardioExcyte 96 platform
  - Microelectrode array using the Maestro
- vCor.4U also displayed 'ventricular-like' pharmacology:
  - Mostly insensitive to atrial specific agents
  - Good responses to core pharmacological tools
  - Able to generate EAD and arrhythmic events (fibrillation)

Acknowledgment

- Eurostars Consortium:

  - Leiden University
    - Robert Passier
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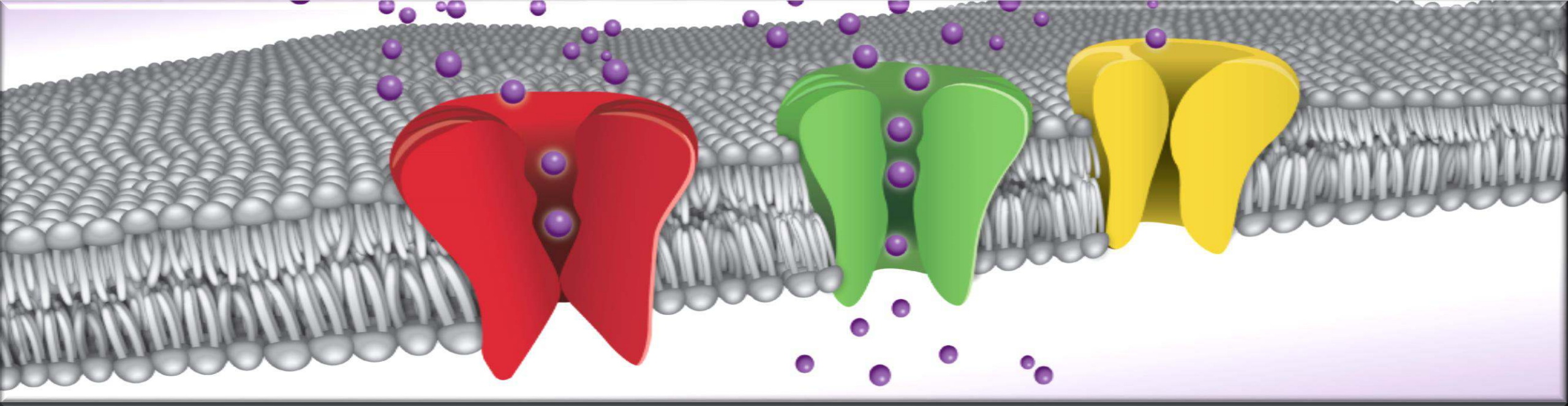
    - John Malcom Ridley
    - Louise Webdale
    - Kathy Sutton

  - Nanion technologies
    - Sonja Stölzle-Feix
    - Krisztina Juhasz

Thanks



Metrion Biosciences: the ion channel specialists

For enquiries please contact:
info@metrionbiosciences.com

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