axio GENESIS

# Cor.At®

## Ready-to-use Cardiomyocytes

Dr. Ralf Kettenhofen

ralf.kettenhofen@axiogenesis.com

Ion Channel Retreat June 24th 2008

Cor.At®

Ready-to-use Cardiomyocytes

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- **Introduction**
  - ***Embryonic stem cells***
  - ***Genetic Modification of ES Cells***
  - ***Production of Cor.At®***
- **Electrophysiology and Pharmacology of Cor.At® cells**
- **Other applications of Cor.At® cells (Tox/Hypertrophy)**

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# Introduction

**Embryonic Stem (ES) Cells**

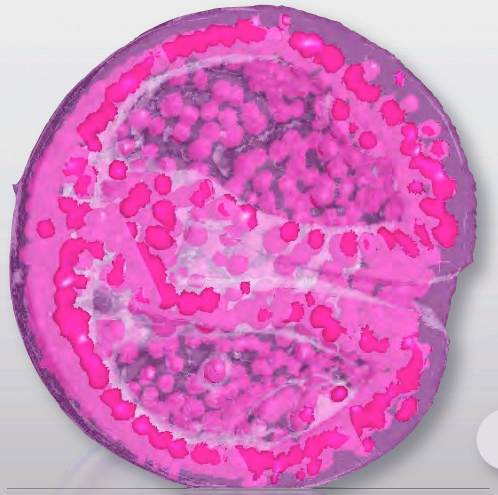
**Genetic Modification**

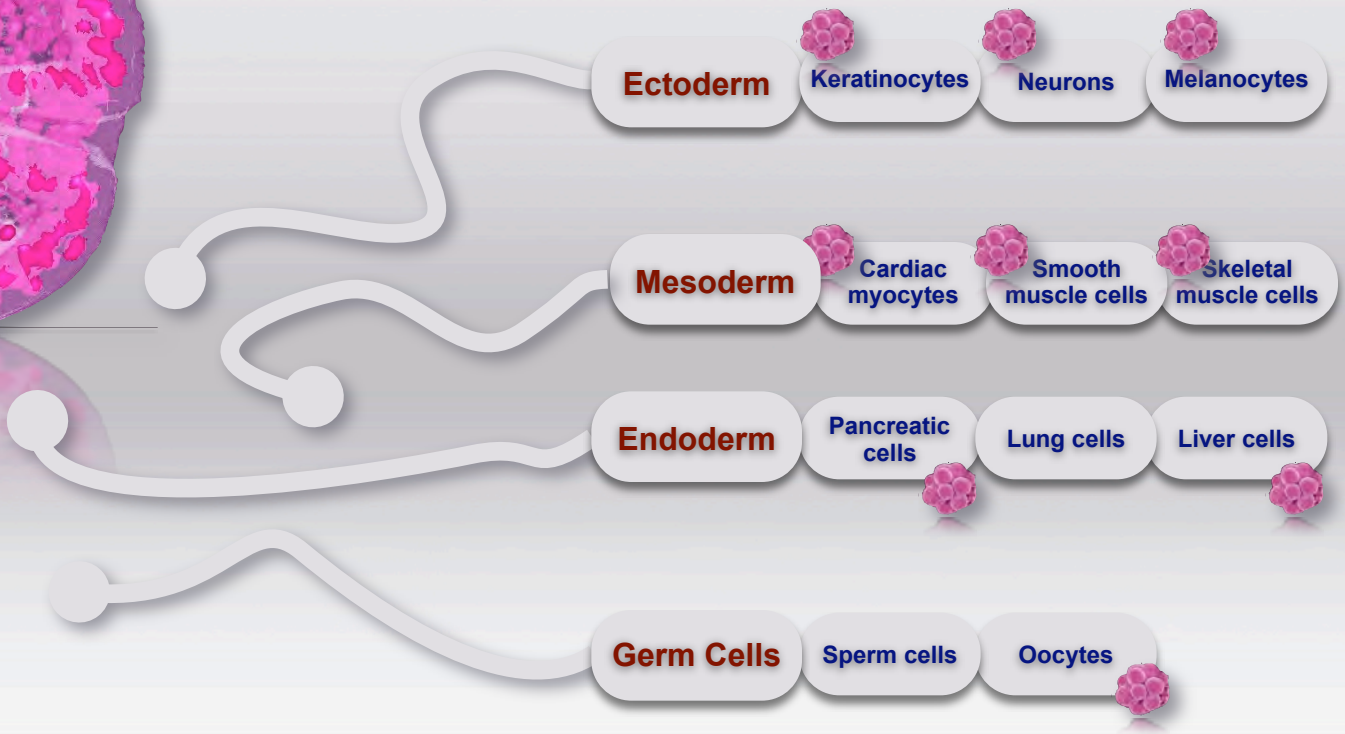
**Differentiation**

**Production of ES Cell-derived Cardiomyocytes (Cor.At®)**

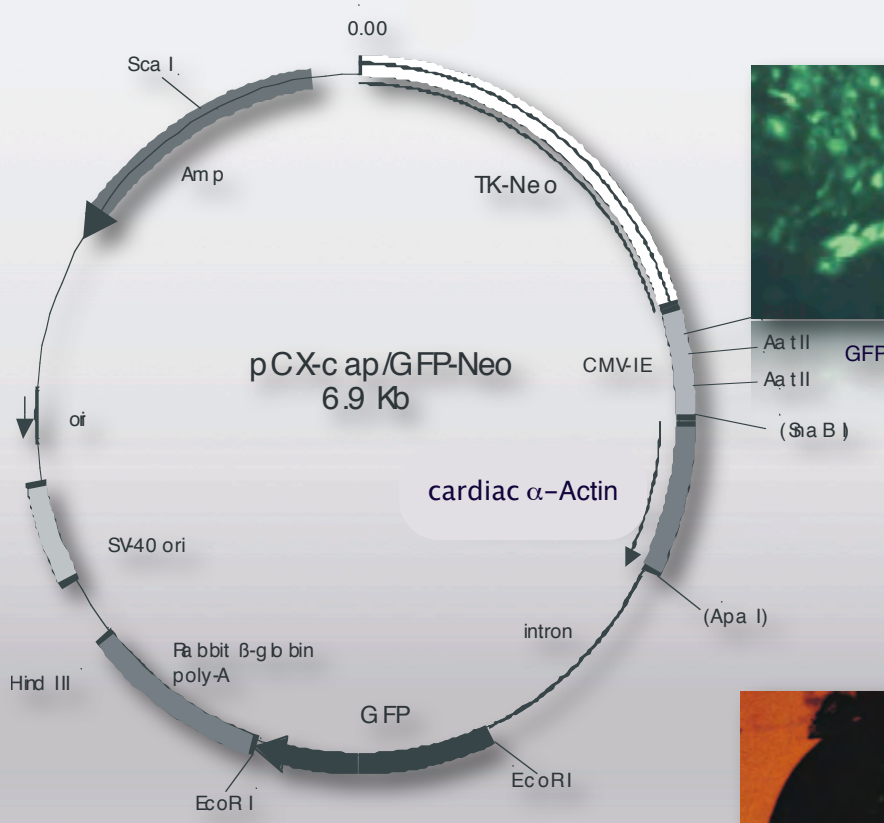
# Embryonic Stem Cells: Differentiation Capacity *in vivo*

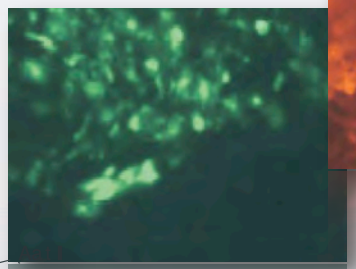
- ES cells are pluripotent and can differentiate into all cell types of the body.
- ES cells can be differentiated in vitro.
- Genetic modification of murine ES cells is achieved easily.





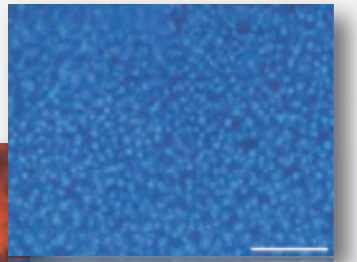
# Generation of ES Cell Clone Cardiac α-Actin



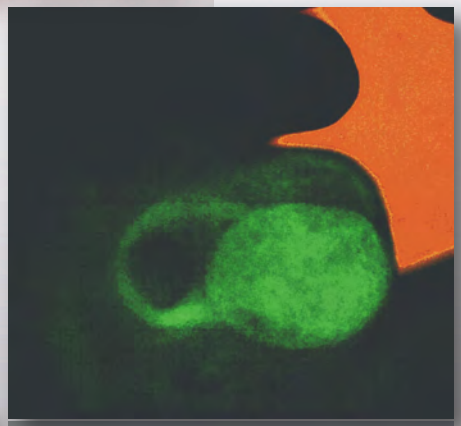



GFP-expression

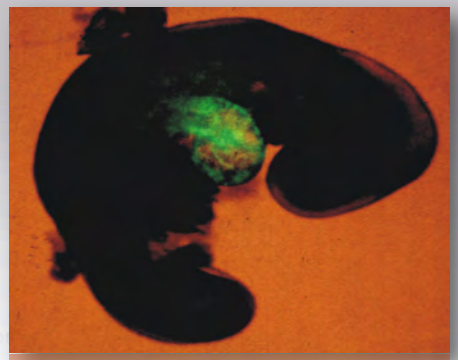
cardiac α-actinin staining



DAPI staining of nuclei



E 10.5



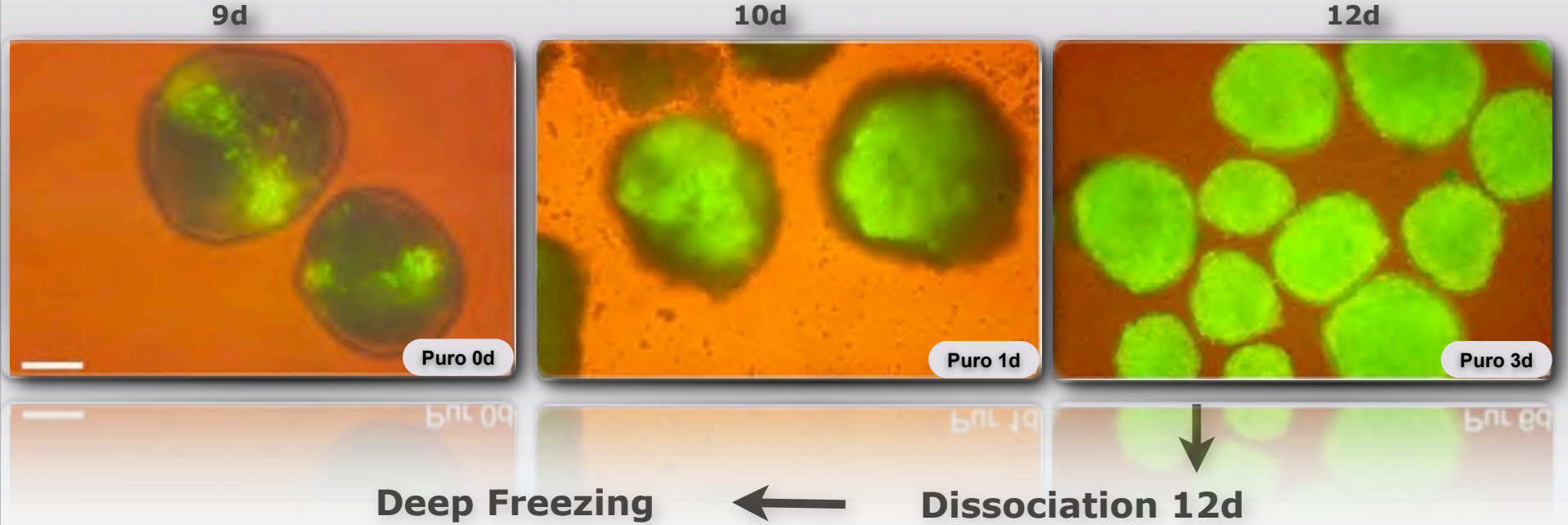
E 9.5

Transgenic mouse embryo

Fleischmann M et al. FEBS Lett. 1998 Dec 4;440(3):370-6

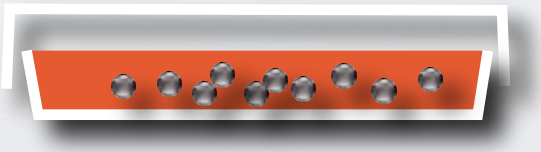
# Puromycin Selection of Cardiomyocytes from Genetically Engineered ES Cells


**Days after initiation of ES cell differentiation**



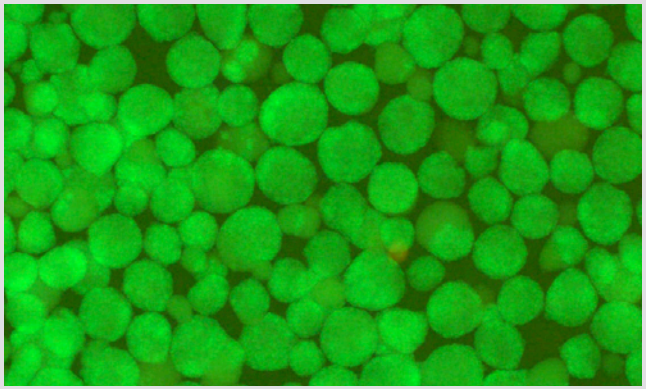
# High Yield Production

ES Cell Aggregation -> Embryoid Bodies 0 - 2d



Transfer of EBs in Cell Spin Bottles 2d

Initiation of Puromycin Selection 9d



Puromycin-selected Cardiac Bodies

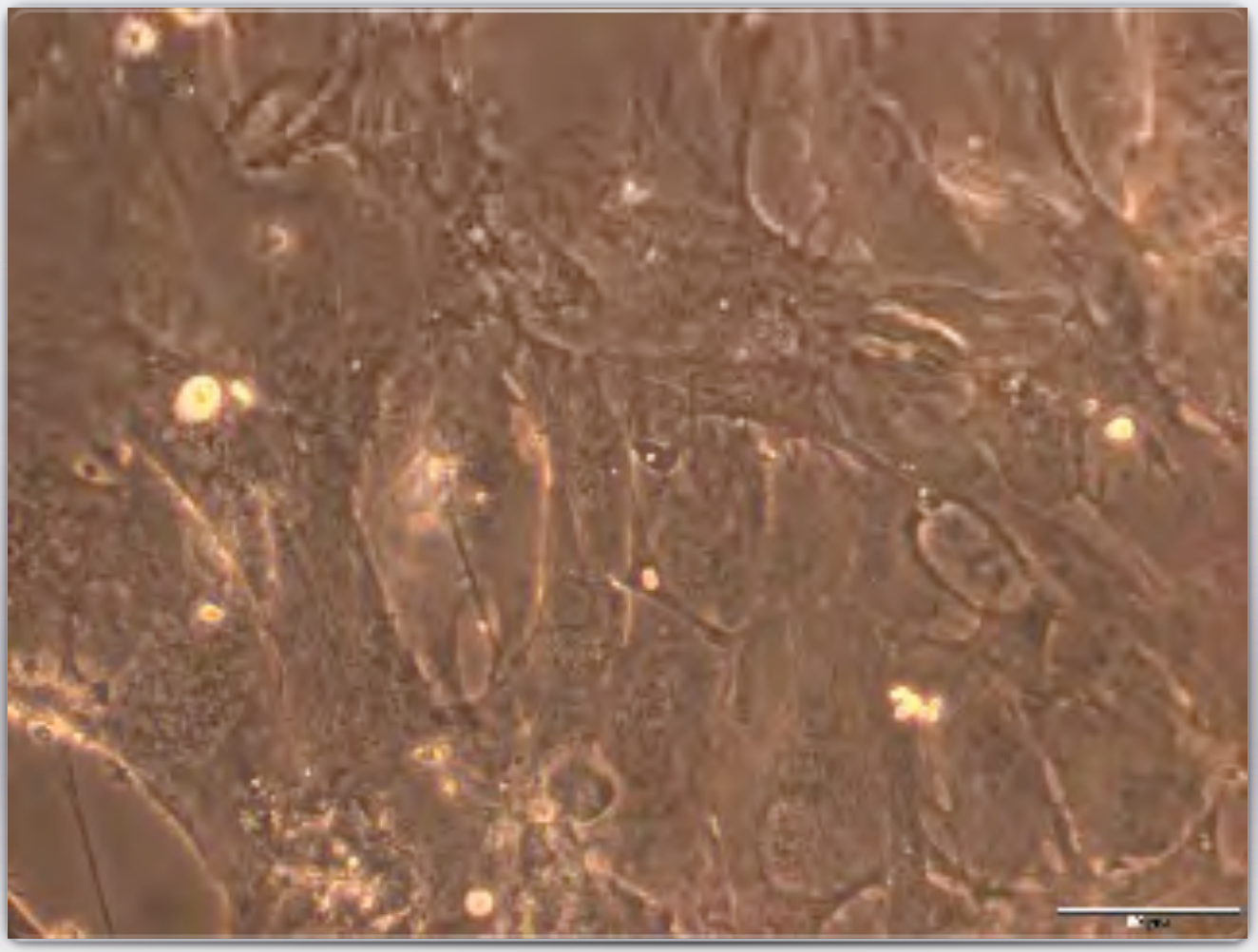
Dissociation of Cardiac Bodies (CBs) 12d
(up to 5x $10^7$ cardiomyocytes per liter)

Deep Freezing of Cardiomyocytes 12d

Thawing and Quality Control

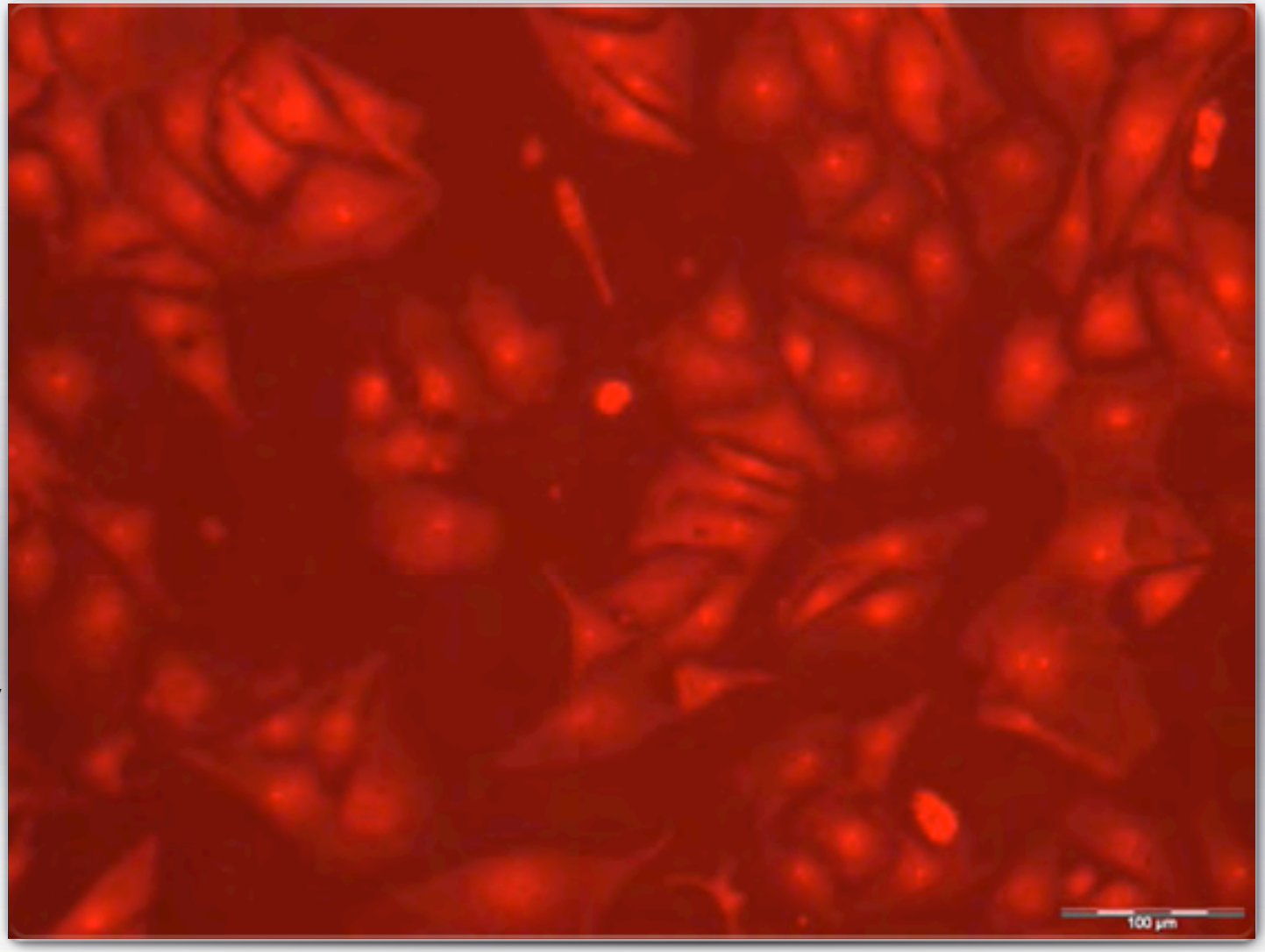
# ES Cell-derived, Genetically Selected and Purified Cardiomyocytes:

## 17d after thawing



**Frozen ES cell-derived and purfied cardiomyocytes are viable and retain their autonomous contractile phenotype for at least 3 weeks after thawing when cultured in monolayer.**

# ES Cell-derived, Purified, and Thawn Cardiomyocytes: ROD2 Visualization of Calcium Flux




**Cardiomyocytes first day after thawing. Clusters of already coupled cardiomyocytes show synchronous calcium flux.**

# Immunostaining

Week 3

Co Staining

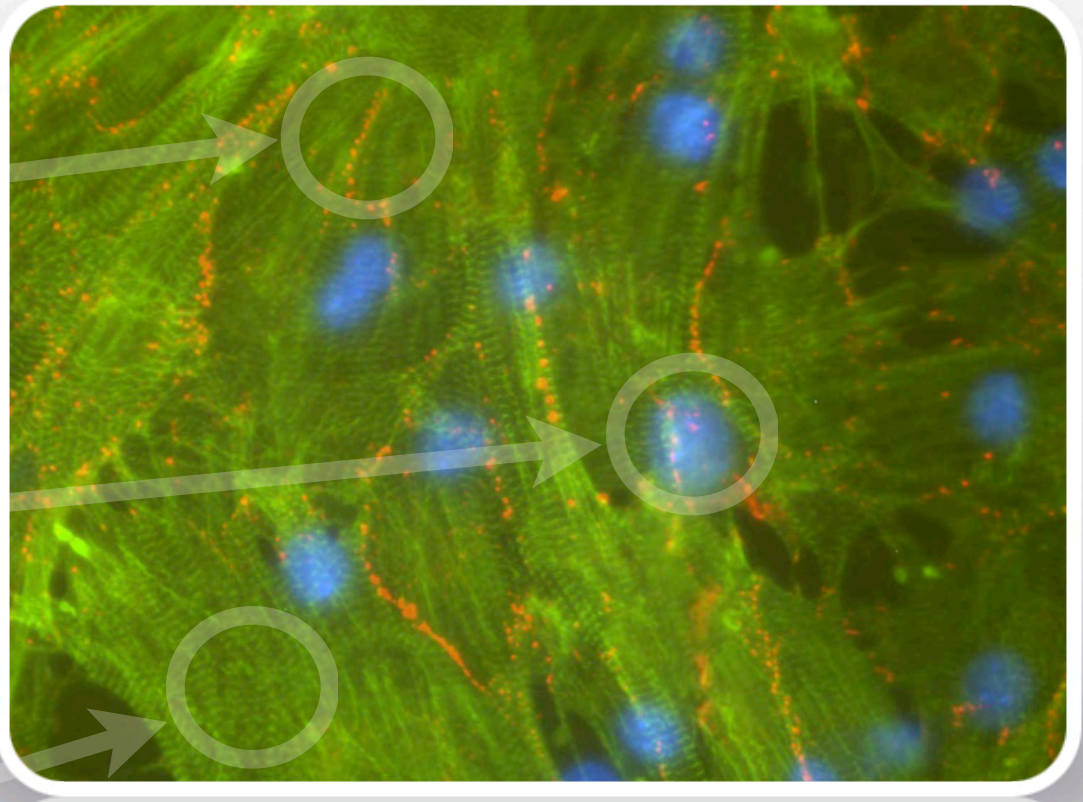
Cx43

+

DNA

+

Actinin



x64

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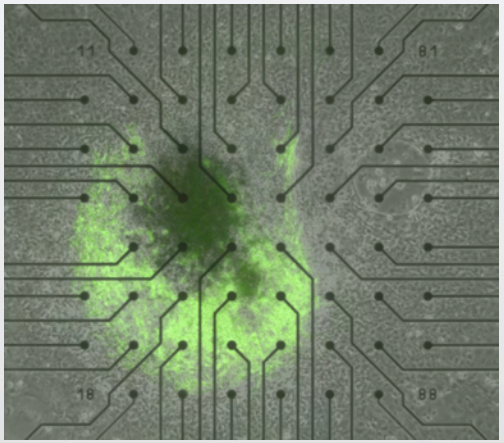
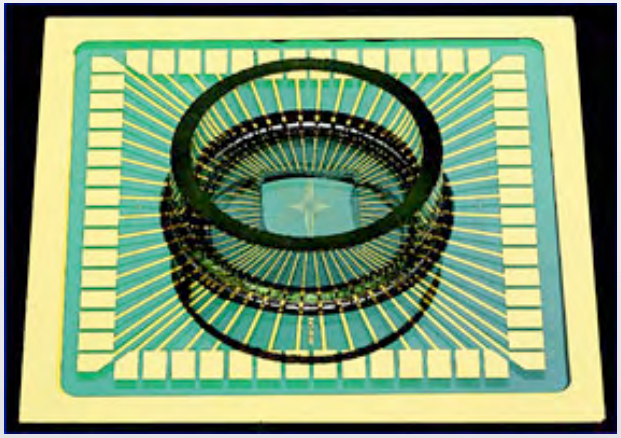
# Extracellular Recordings from ES cell-derived cardiomyocytes on Micro Electrode Arrays (MEAs)

- non-invasive
- long-term observations of cardiac cell
- Goal: identification of specific ion channel blocker activators and humoral agonists

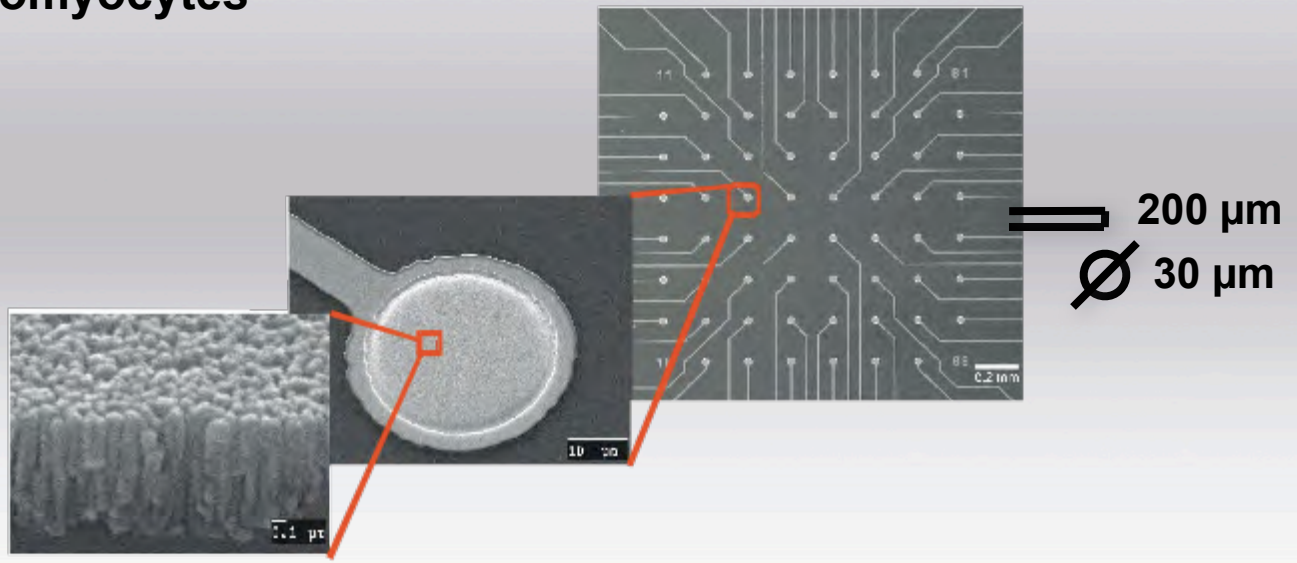
# Micro Electrode Array



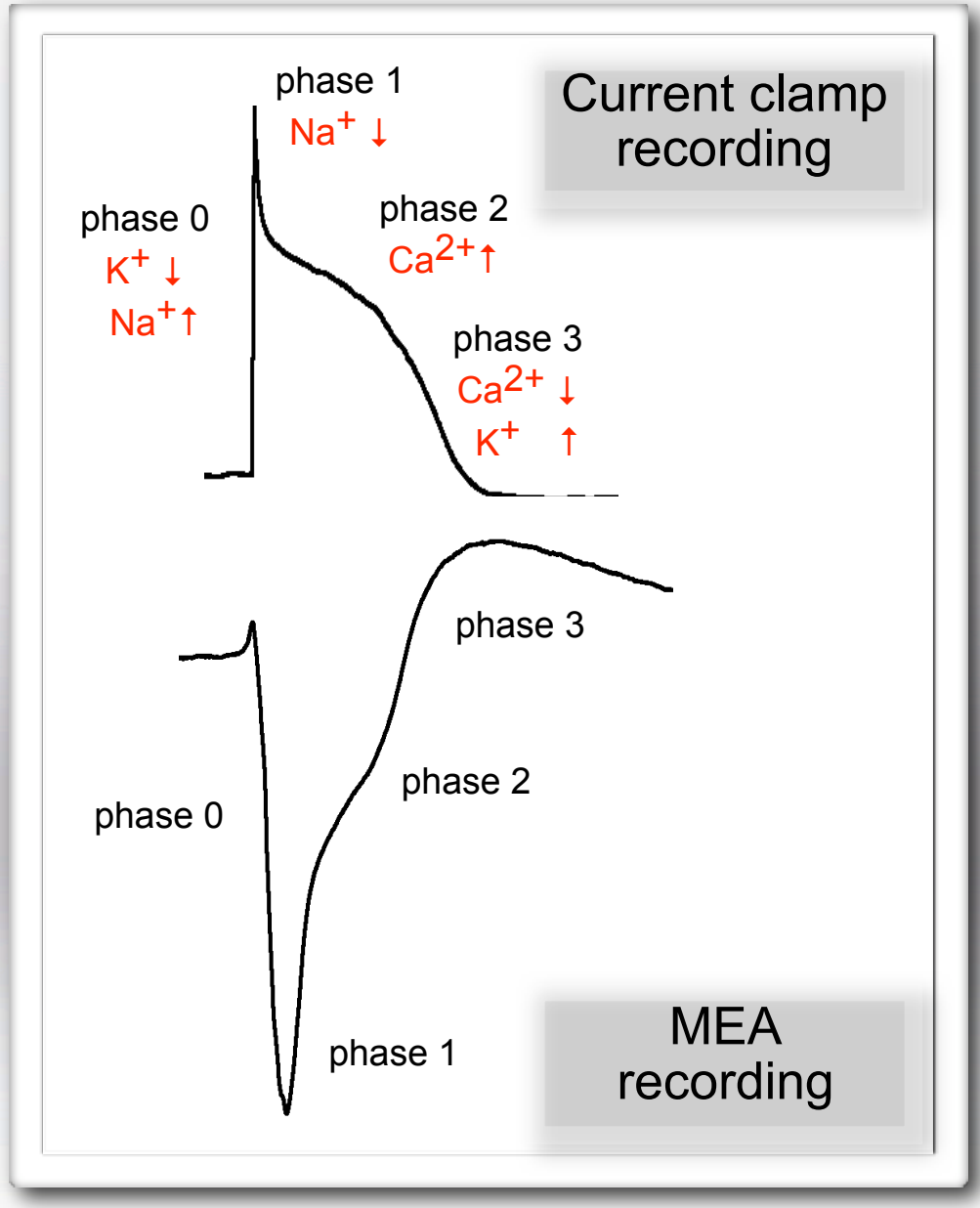
Clusters of EGFP-expressing cardiomyocytes



Microelectrode-Array (MEA)



# Correlation: Action Potential - Field Action Potential



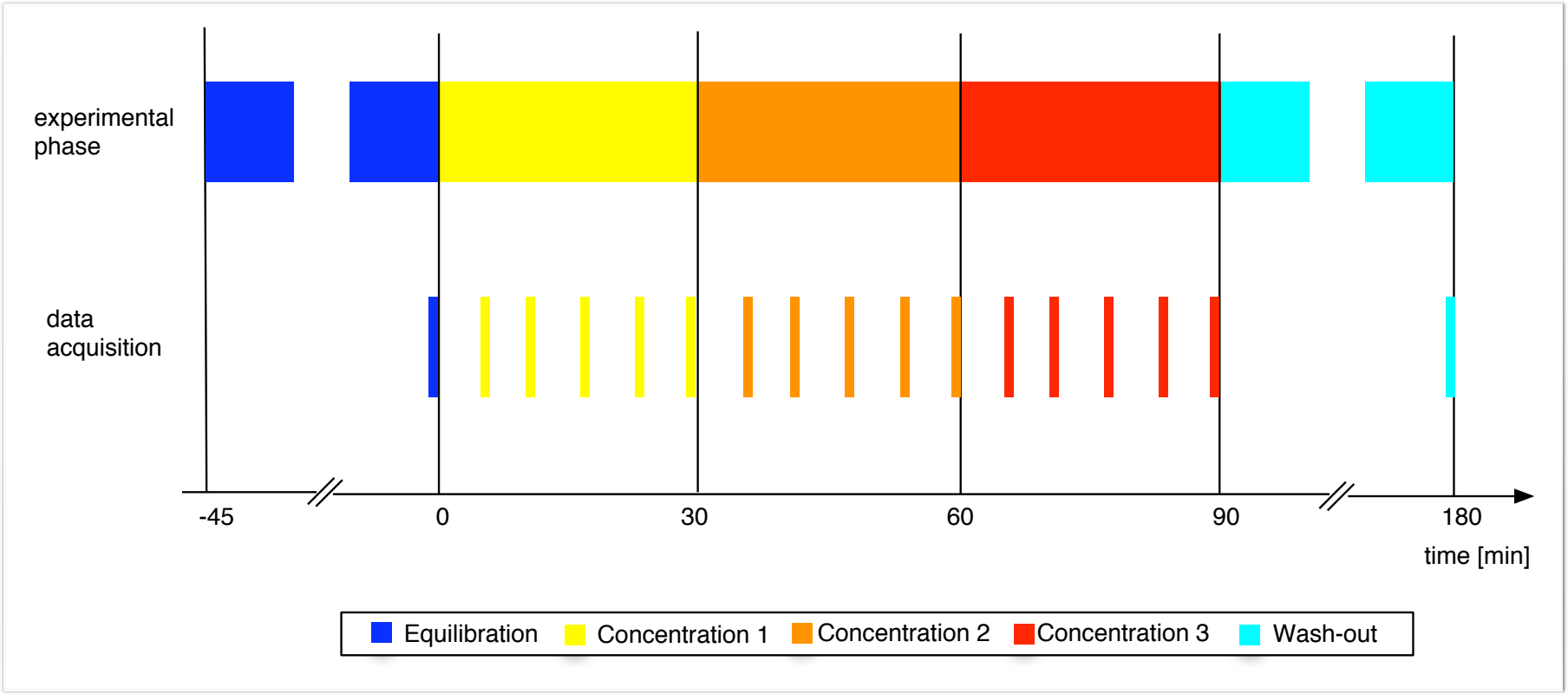
# Research Project:

# Blinded Validation Study

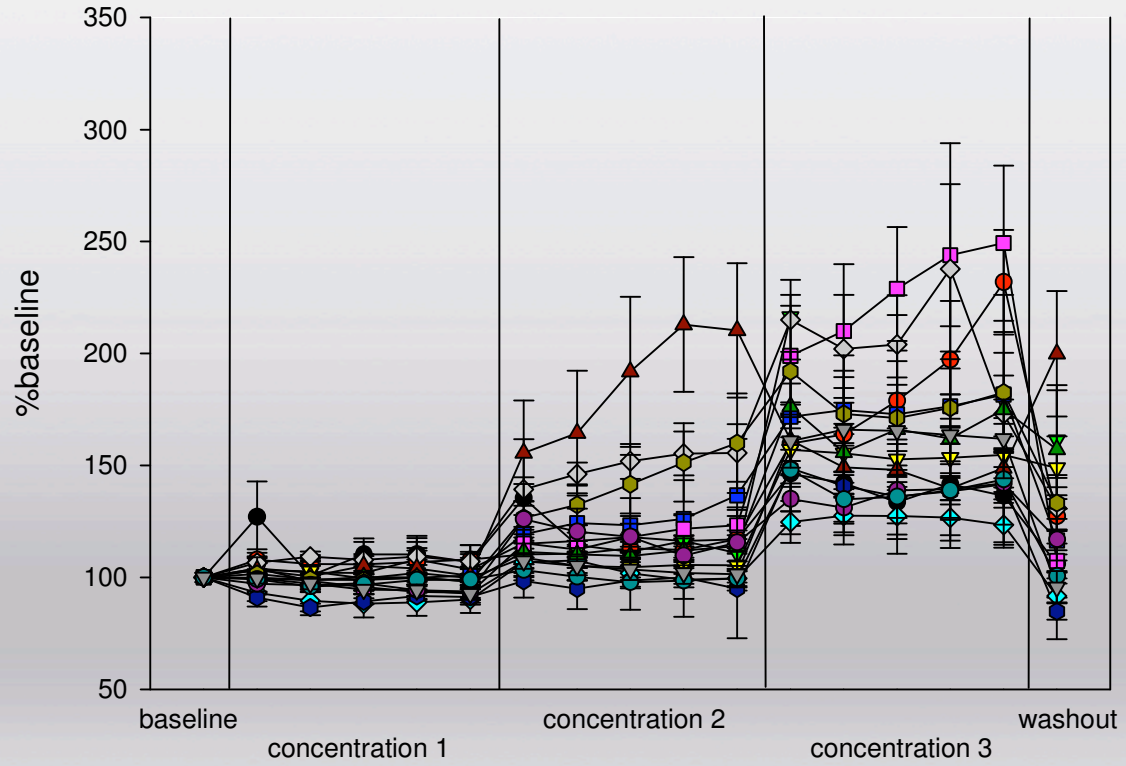
# Substances Tested in the Validation:

- 25 compounds overall
  - 15 mixed ion channel blockers (in concentrations tested)
  - 5 specific hERG blocker
  - 1 Sodium channel activator
  - 1 $I_{Ks}$ blocker
  - 1 Noradrenalin uptake inhibitor
  - 2 Negative controls

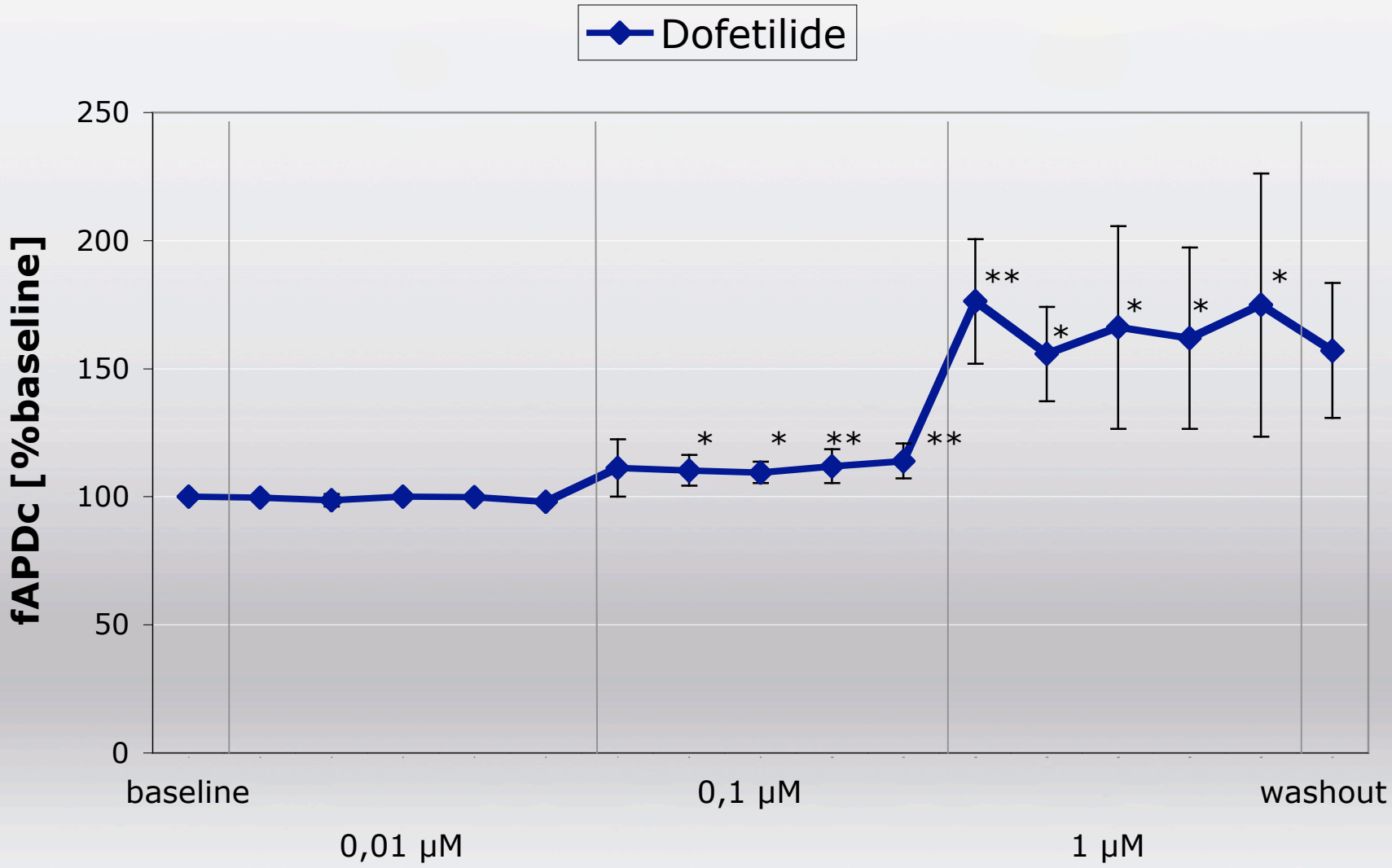
# Study Protocol



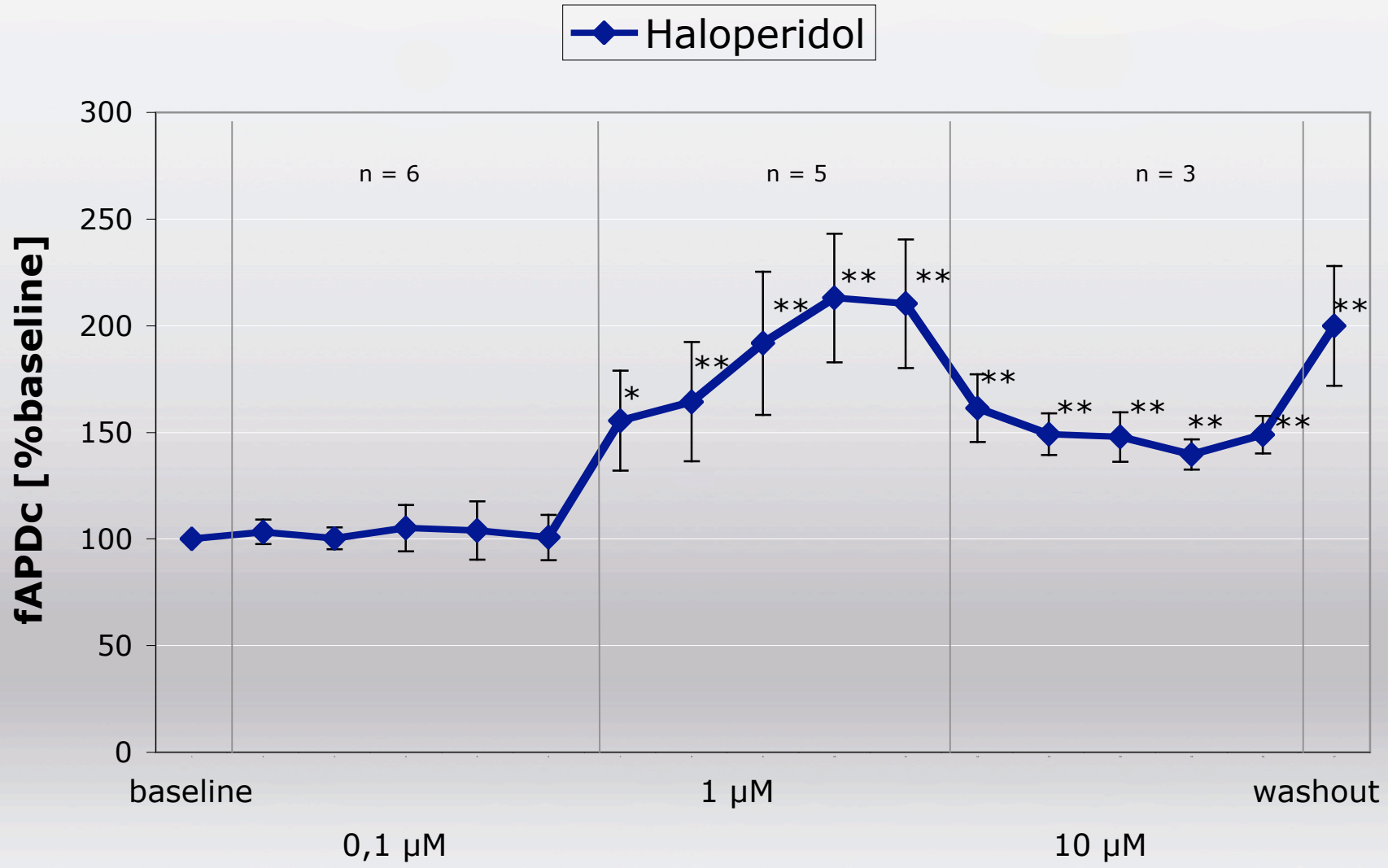
# 15 Compounds Induced fAPDc Prolongation



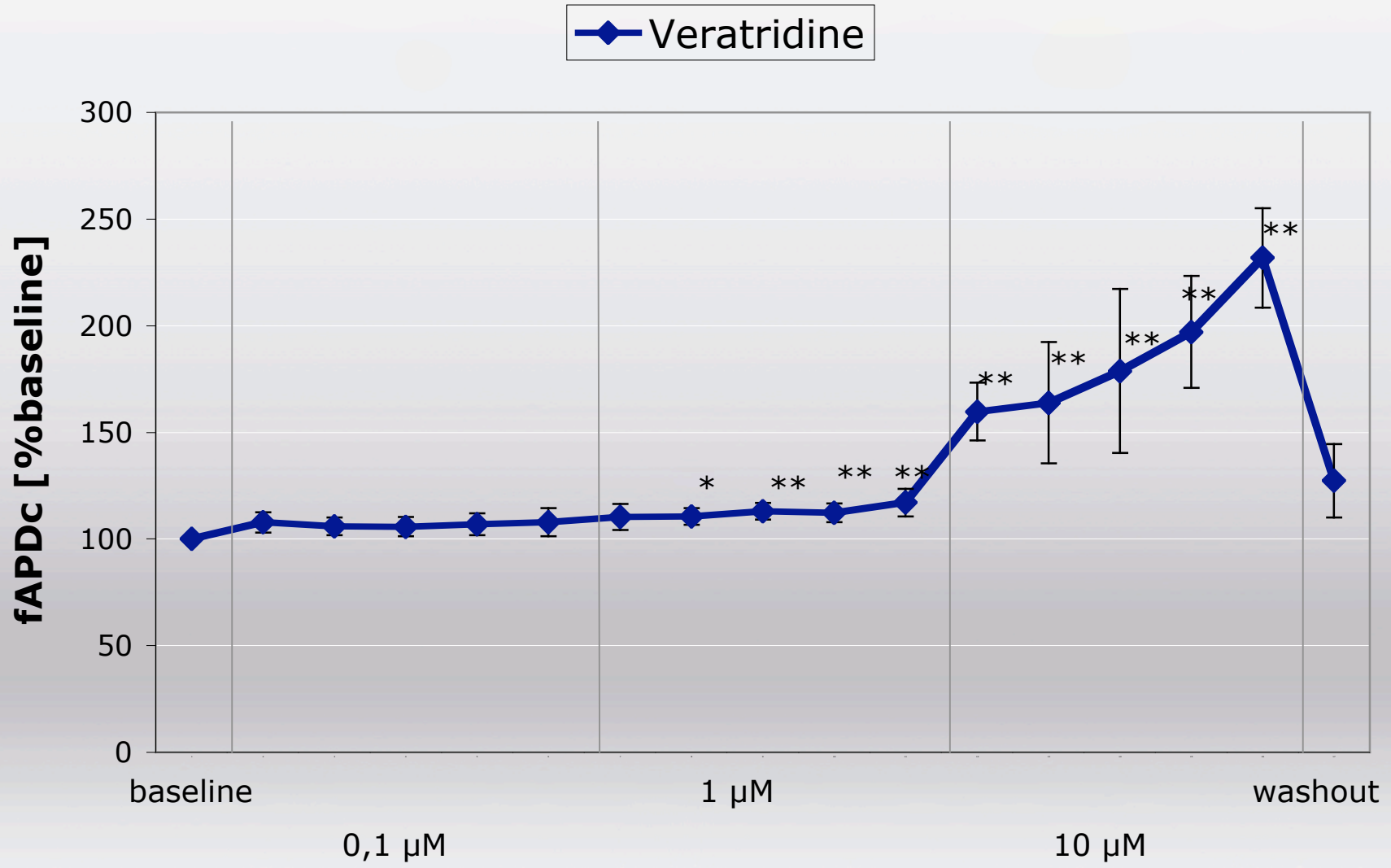

125 - 1 µM, 10 µM, 100 µM
127 - 0.1 µM, 1 µM, 10 µM - Veratridine
129 - 0.3 µM, 3 µM, 30 µM - Fluoxetine
130 - 0.1 µM, 1 µM, 10 µM
131 - 0.1 µM, 1 µM, 10 µM
133 - 1 µM, 10 µM, 100 µM
135 - 0.1 µM, 1 µM, 10 µM
136 - 0,3 µM, 3 µM, 30 µM - Nisoxetine
137 - 0.1 µM, 1 µM, 10 µM - Haloperidol
138 - 0.01 µM, 0.1 µM, 1µM - Dofetilide
140 - 0.1 µM, 1 µM, 10 µM
143 - 0.1 µM, 1 µM, 10 µM
145 - 3 µM, 30 µM, 300 µM
148 - 1 µM, 10 µM, 100 µM
149 - 0.1 µM, 1 µM, 10 µM



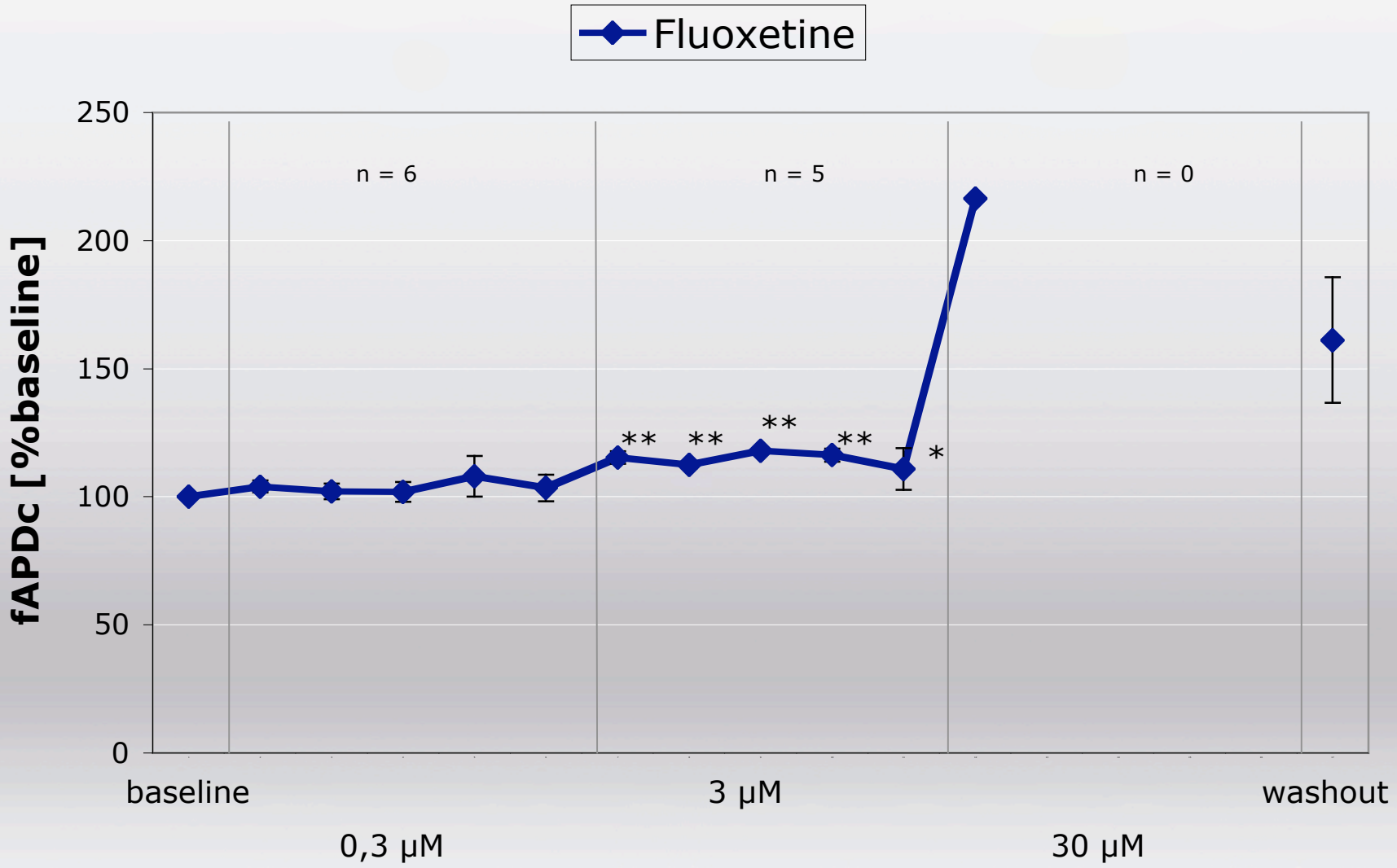

specific hERG blocker




**anti psychotic: hERG blocker, $I_{Ca,L}$ blocker**

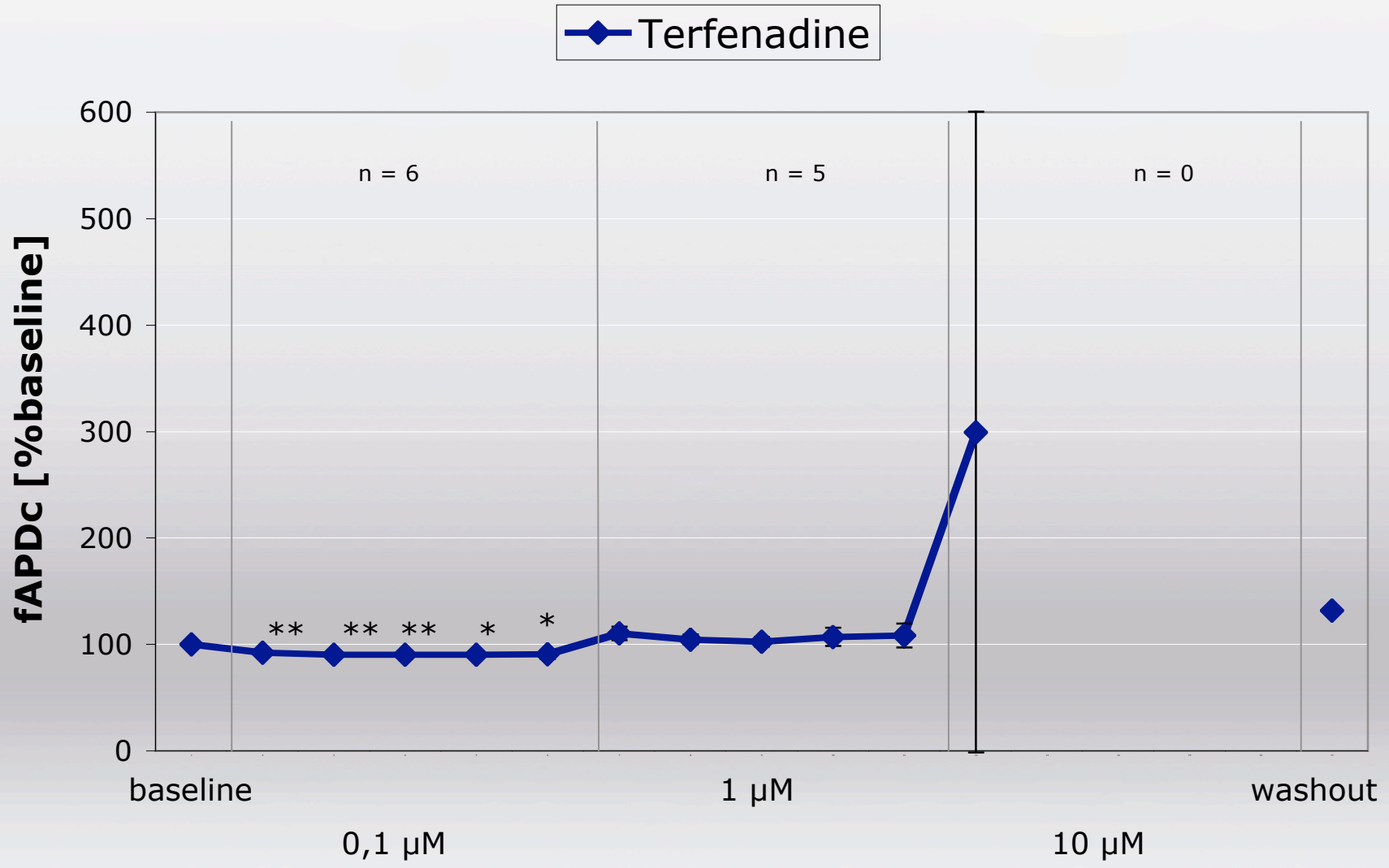



**neurotoxic steroid alkaloid: $I_{Na}$ aktivator**

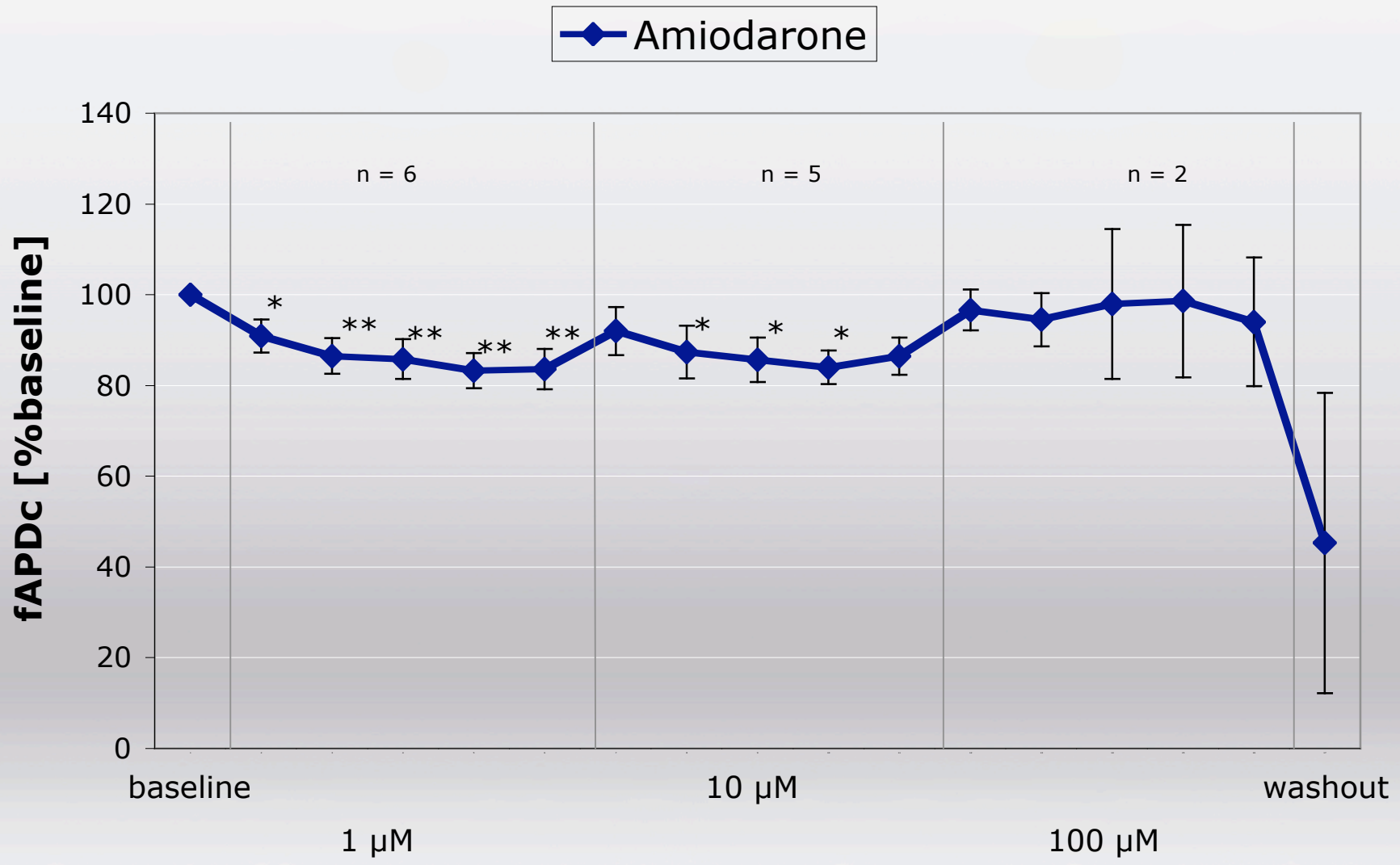



**anti psychotic, serotonine uptake inhibitor: hERG blocker, $I_{Ca,L}$ blocker**

Witchel 2002 FEBS Lett.



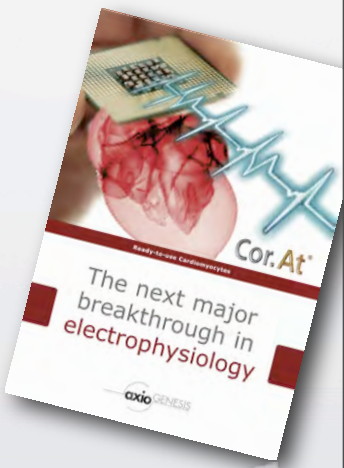

**Anti-histaminic compound: hERG blocker, $I_{Ca,L}$ blocker**




**antiarrhthmic compound: hERG blocker, $I_{Ca,L}$ blocker**

# Results:

- 15 compounds prolonged fAPDc
  - all 5 pure hERG blockers were identified
  - 9 mixed channel blockers with hERG blocker properties prolonged fAPDc including Haloperidol
- 2 negative control were negative
- No effect of $I_{Ks}$ blocker chromanol 293B


# Cor.At®

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# Electrophysiology

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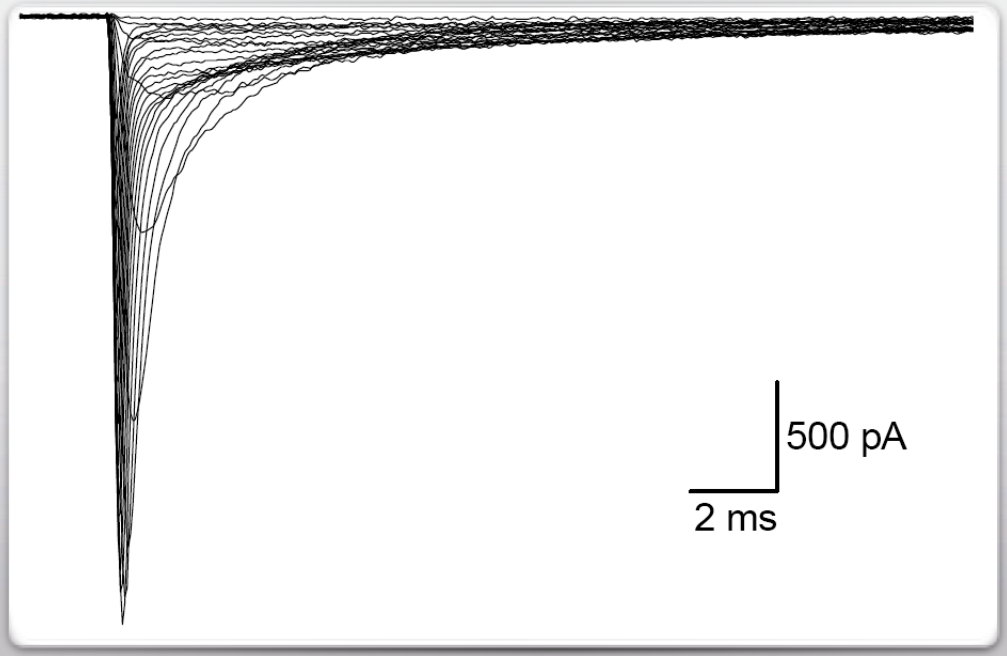
# Electrophysiological Characterization of Cor.At® Cardiomyocytes

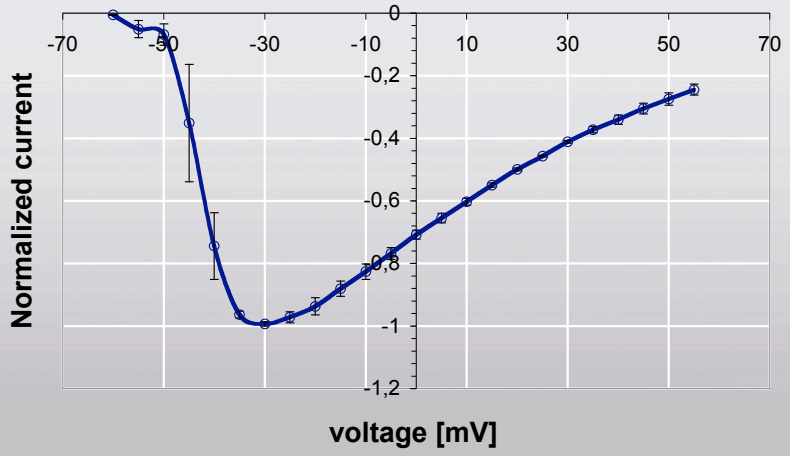
- **Detection of Cardiac Currents:**
  - Voltage Clamp
- **Pharmacology of Cor.At® cells**
  - Patch Clamp
  - Microelectrode Array Recording
  - Impedance Spectroscopy

# Voltage Clamp Recording of $I_{Na}$




## I/V Diagram

**(Normalized to Maximum Amplitude)**

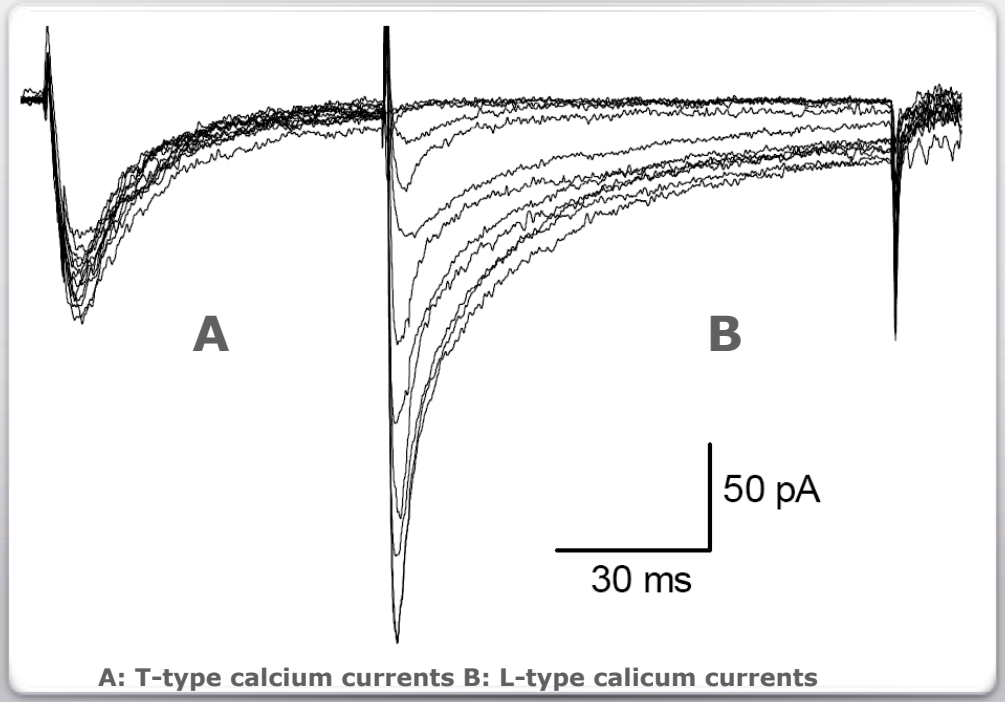



**Current Density$_{Max}$ = 435 ± 47 pA/pF**

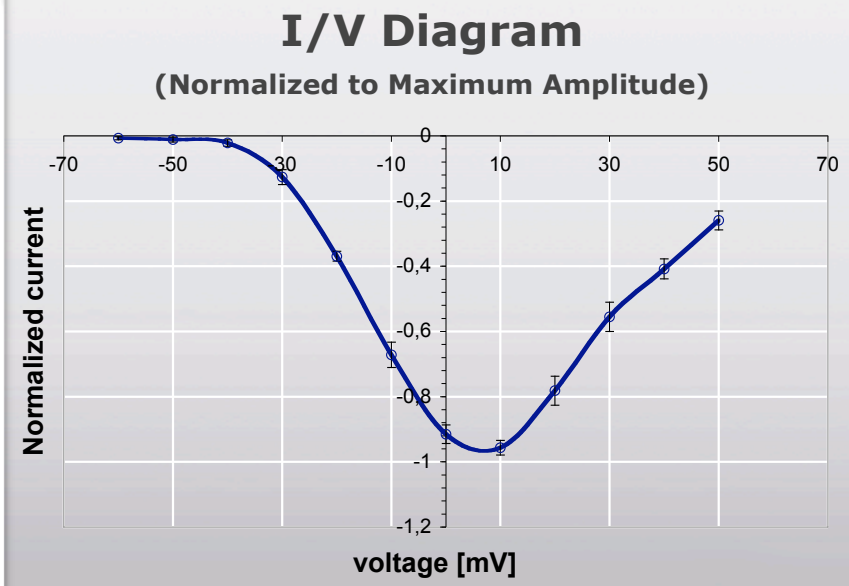
**Current amplidute = 4,5 ± 0,5 nA**

**(n = 4)**

# Voltage Clamp Recording of $I_{Ca}$




A: T-type calcium currents B: L-type calicum currents

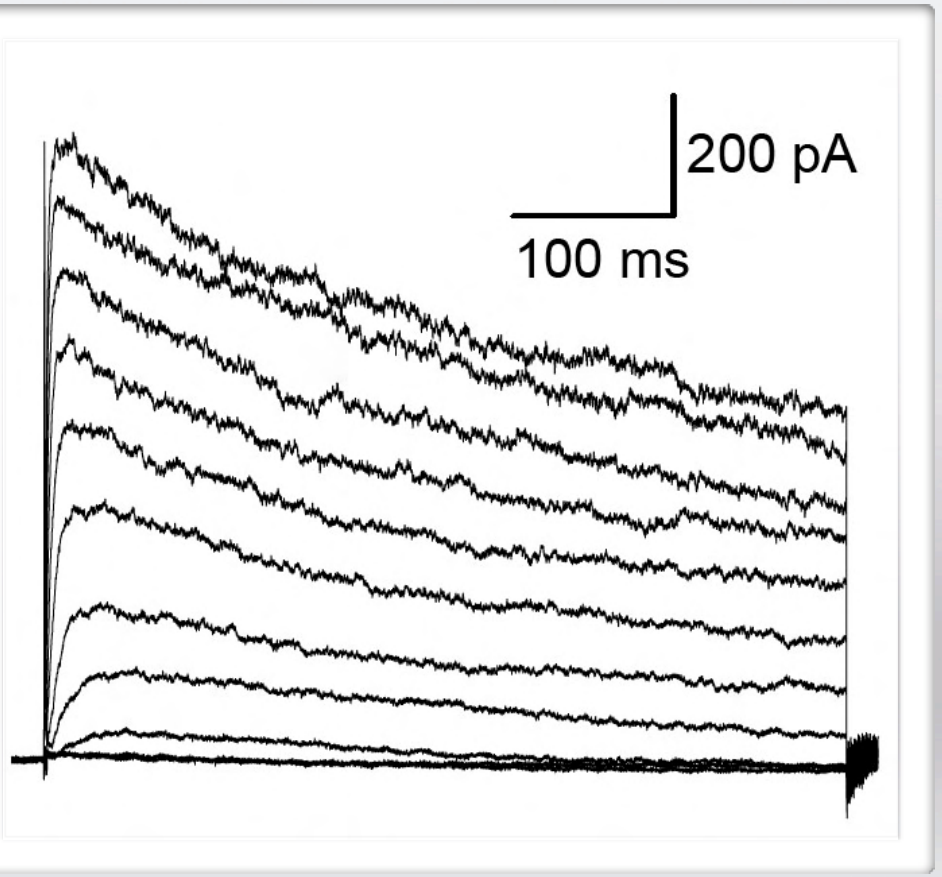



**Current Density$_{Max}$ = 18,3 ± 4 pA/pF**

**Current amplidute = 182 ± 24 pA**

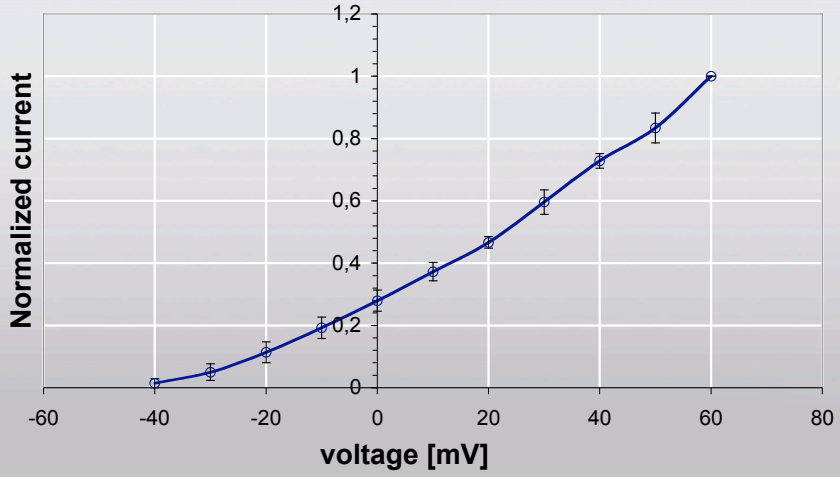
**(n = 4)**

# Voltage Clamp Recording of $I_K$




## I/V Diagram

**(Normalized to Maximum Amplitude)**




**Current Density$_{Max}$ = 24,8 ± 5 pA/pF**

**Current amplidute = 348 ± 70 pA**

**(n = 5)**

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# Pharmacology with Cor.At Cells®

## Assays Systems:

- Patch Clamp
- MEA Recordings
- Impedance Spectroscopy

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# Application of Potassium Ion Channel Blockers

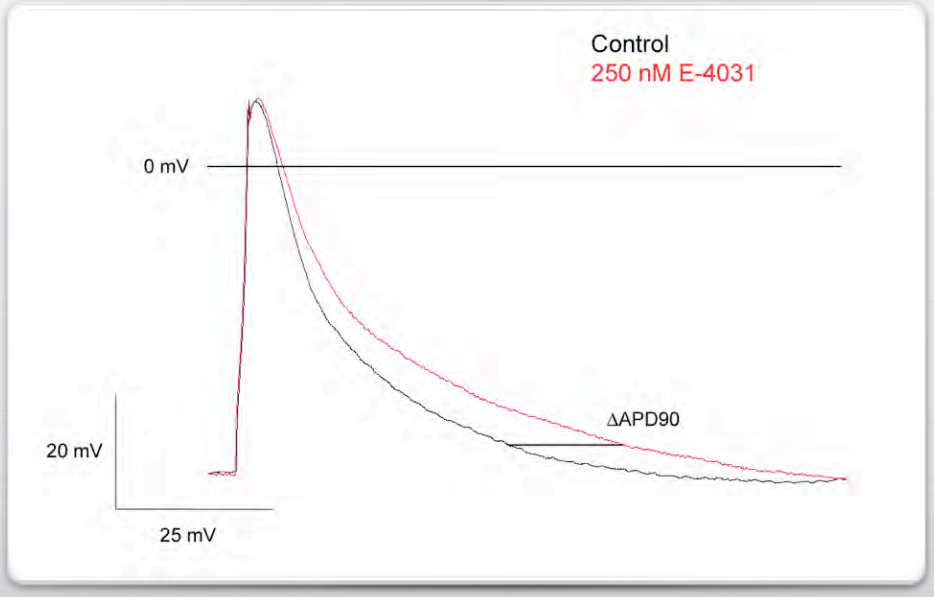
- hERG blocker: E-4031
- Kv4.3/hERG blocker: Flecainide

# Current Clamp/MEA Recording

## E-4031

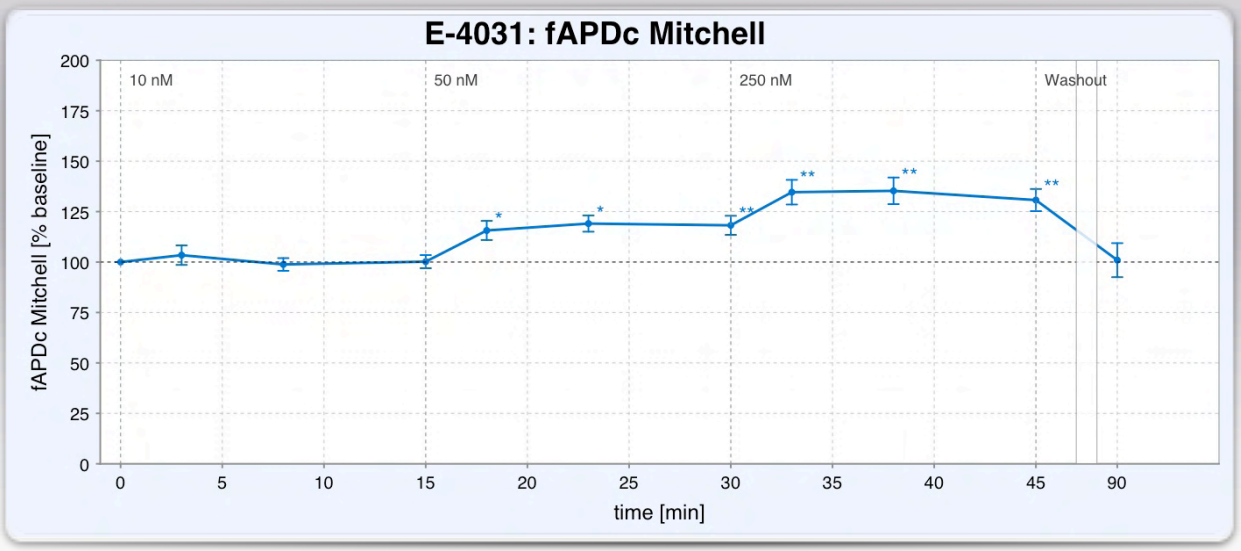
**Effect:**

**$APD_{90}$ prolongation**



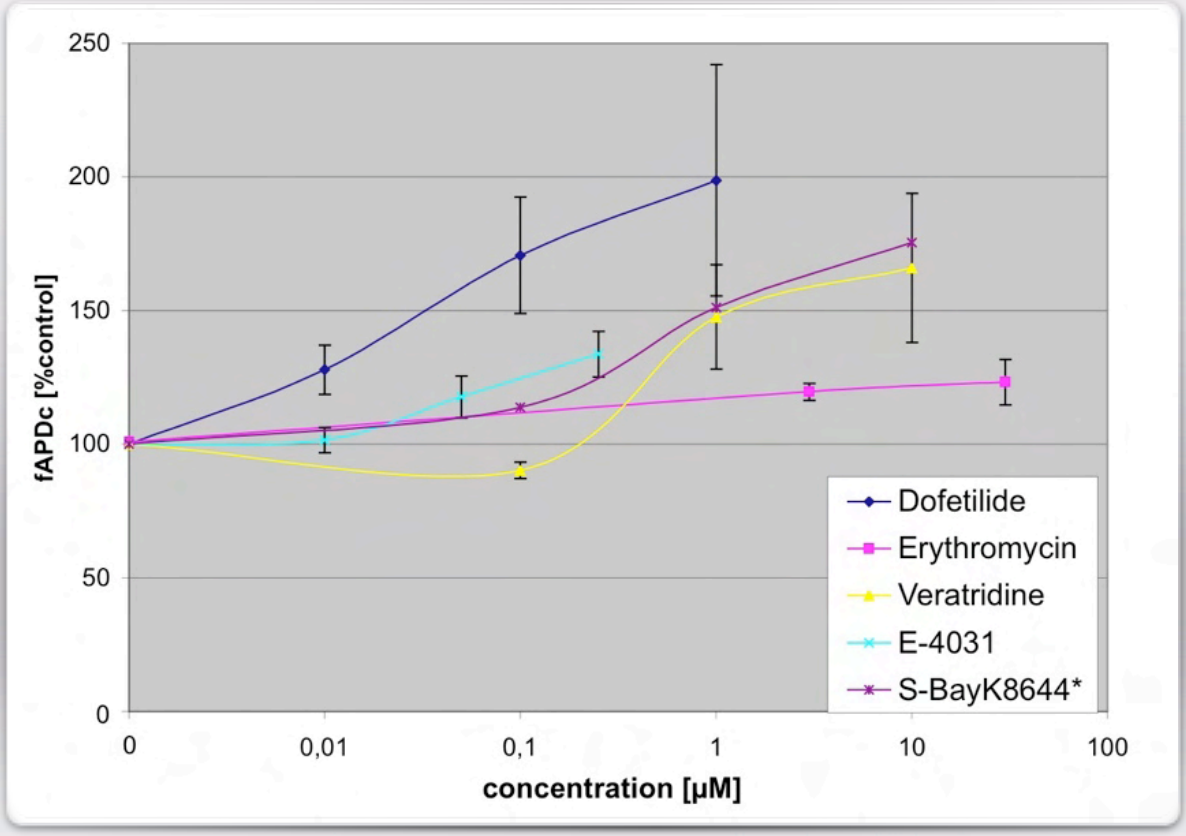

**Effect:**

**fAPD prolongation**



# MEA Recording

## Drugs inducing fAPDc Prolongation in Cor.At® cells




**hERG blocker:**

E-4031, Dofetilide, Erythromycin

**Sodium channel activator:**

Veratridine

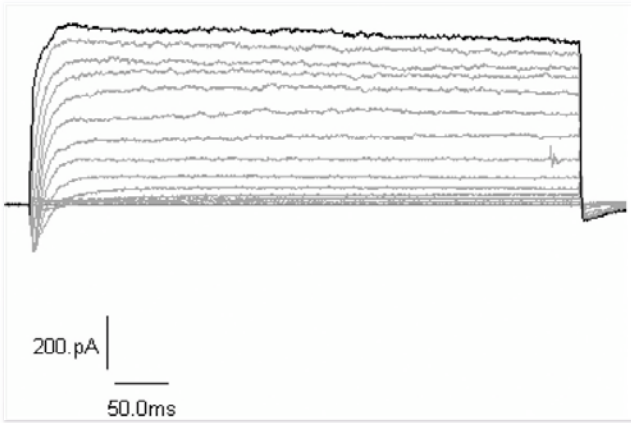
**Calcium channel activator:**

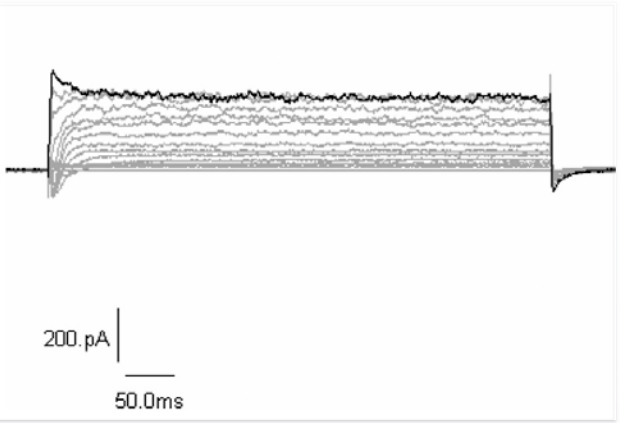
S-BayK 8644

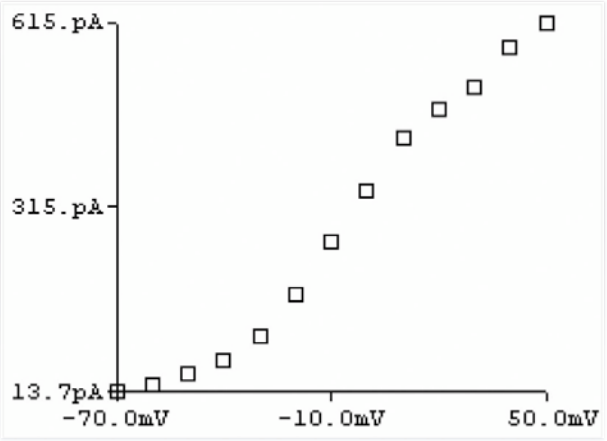
# Voltage Clamp

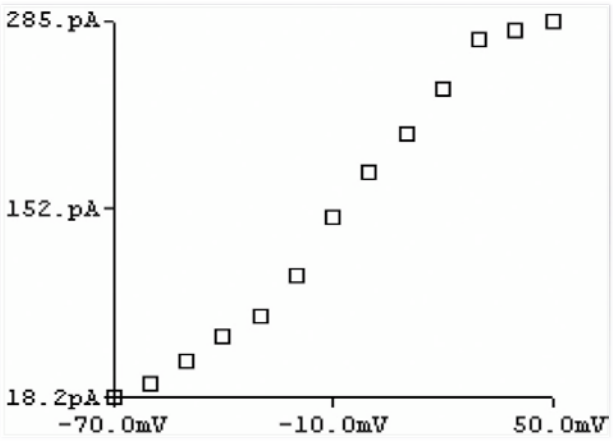
## Flecainide: Ic antiarrhythmic agent ($I_{to}$ and hERG blocker)

**Initial Recording** | **10 µM Flecainide**



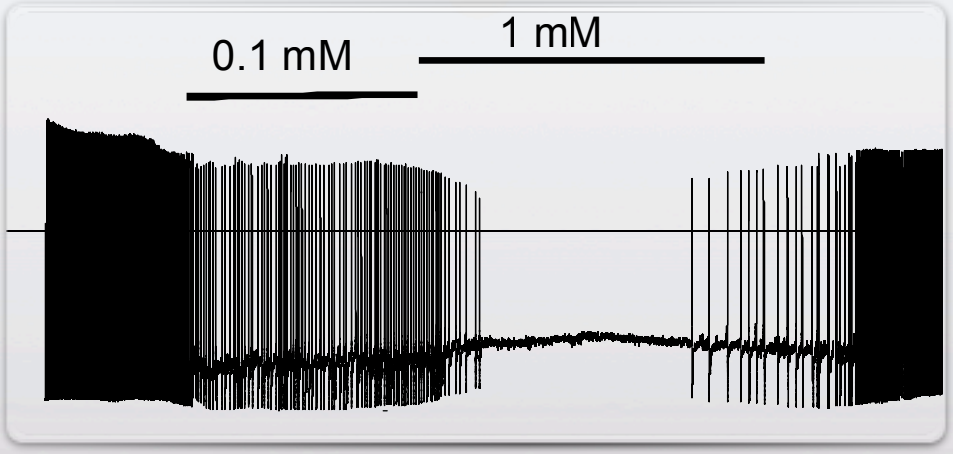




Data was kindly provided by Dr. Clemens Möller from EVOTEC AG, Germany.

# Current Clamp Recording

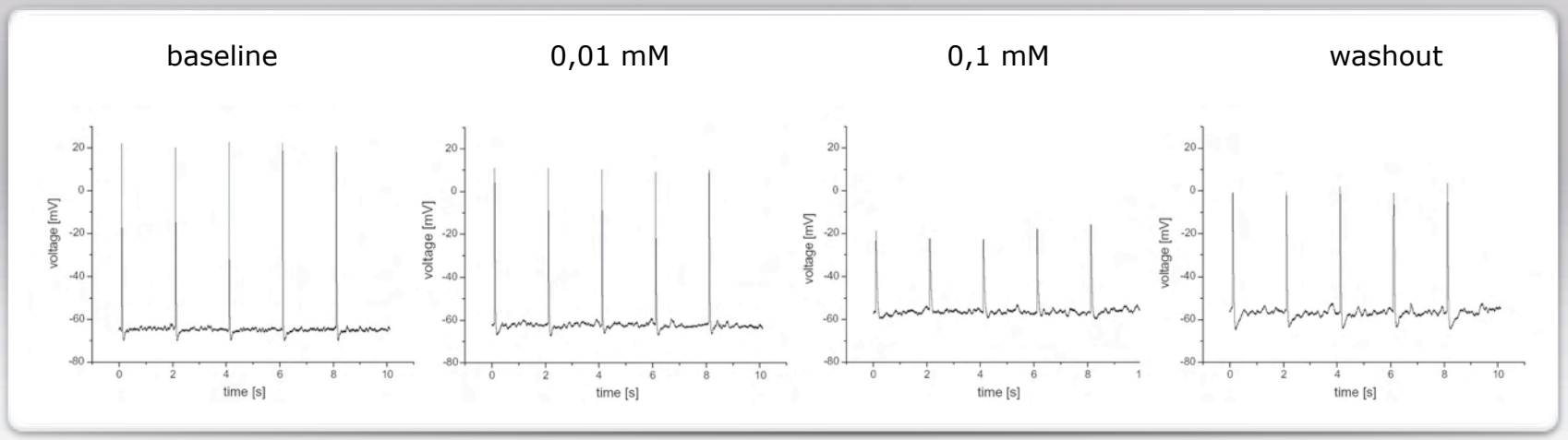
## Lidocaine: $I_{Na}$ and Mixed Channel Blocker




**Continuous recording:**

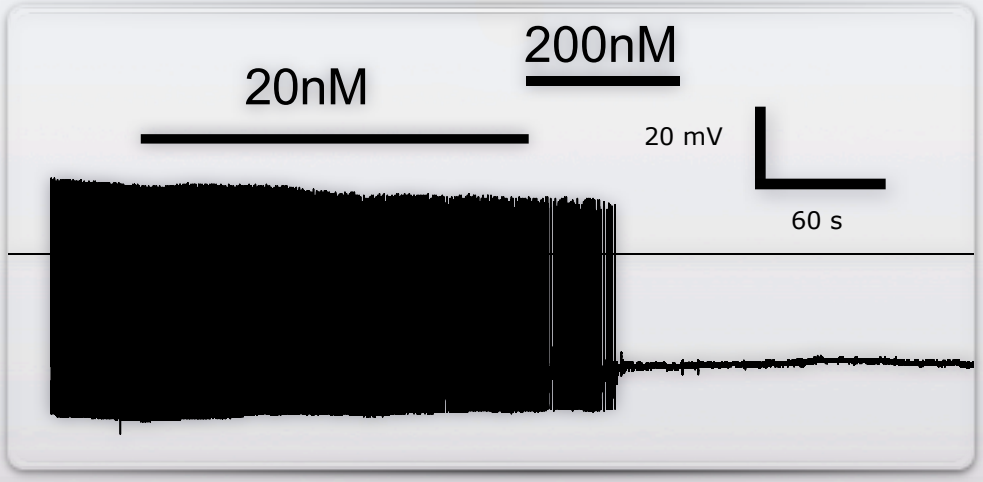
- **0.1 µM decrease of:**
  - **frequence and**
  - **AP amplitude at**
- **Arrest at 1 mM**

**Evoked Action Potential (0.5 Hz):**



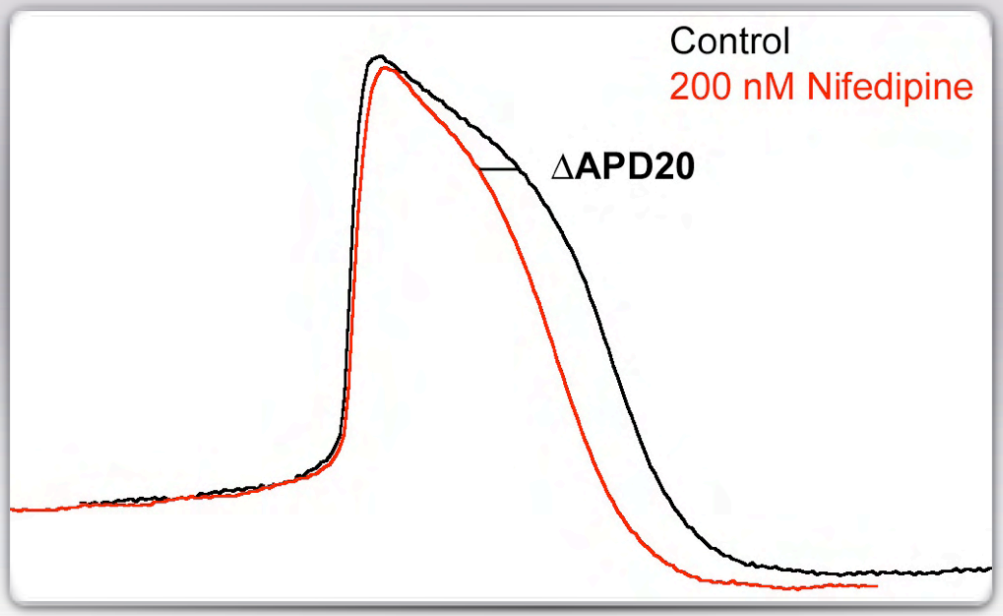
# Current Clamp Recording

## Nifedipine: $I_{Ca,L}$ blocker




**Continuous recording:**

**Nifedine induced beating arrest at 200 nM.**




**Nifedipine-induced $APD_{20}$ shortening**

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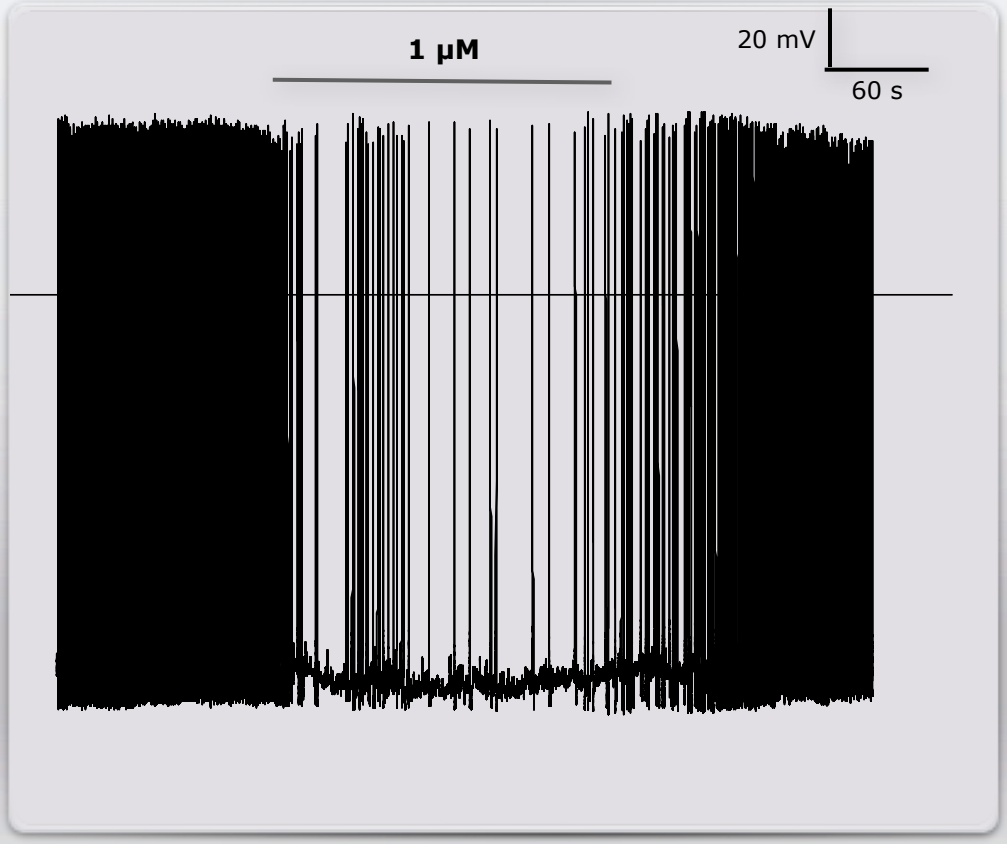
# Humoral Regulation

## G-protein Coupled Receptor (GPCR) Agonist

# Continuous Current Clamp Recording

## Carbachol: Muscarinic Agonist



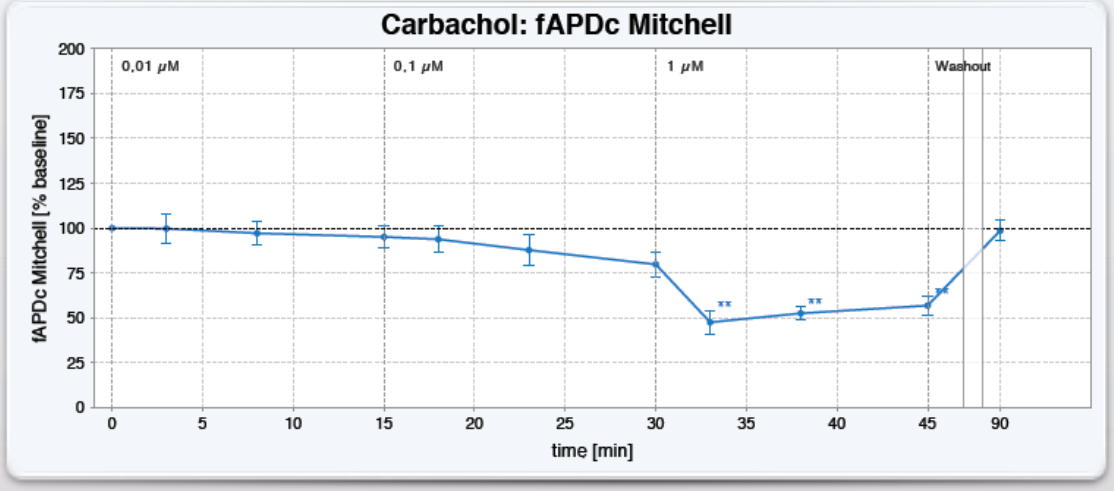

**Effect:**

**- negative chronotrope**

# MEA Recording

## Carbachol

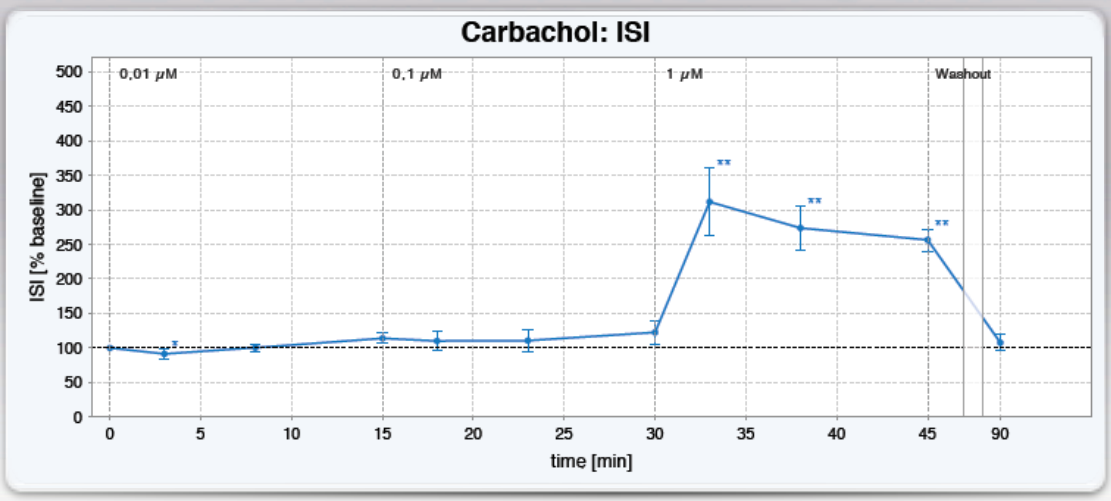
fAPDc




**Effect:**

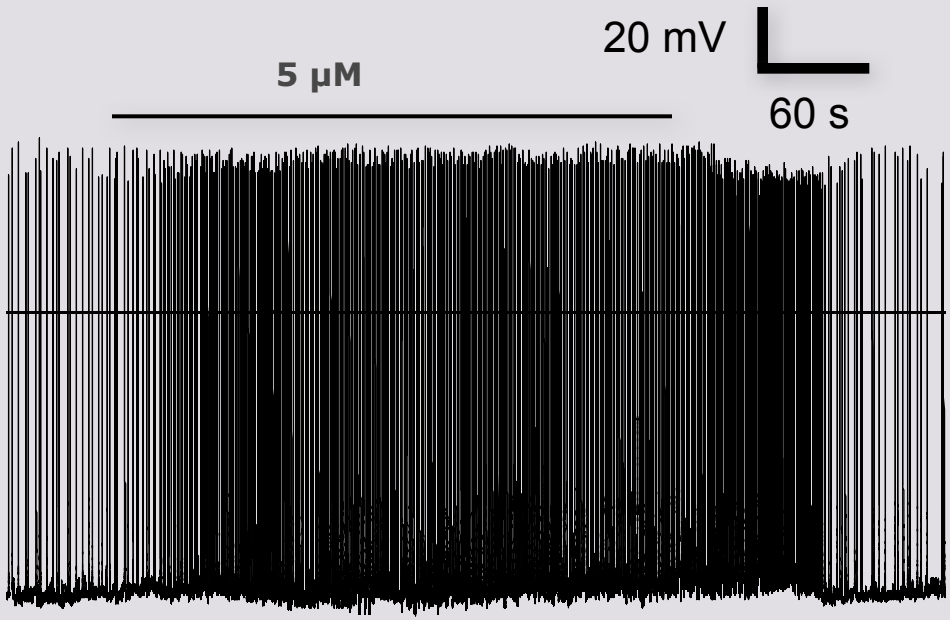
- fAPDc shortening
- negative chronotrope

ISI: interspike interval



# Continuous Current Clamp Recording

## Suprarenine: ß-adrenergic Agonist



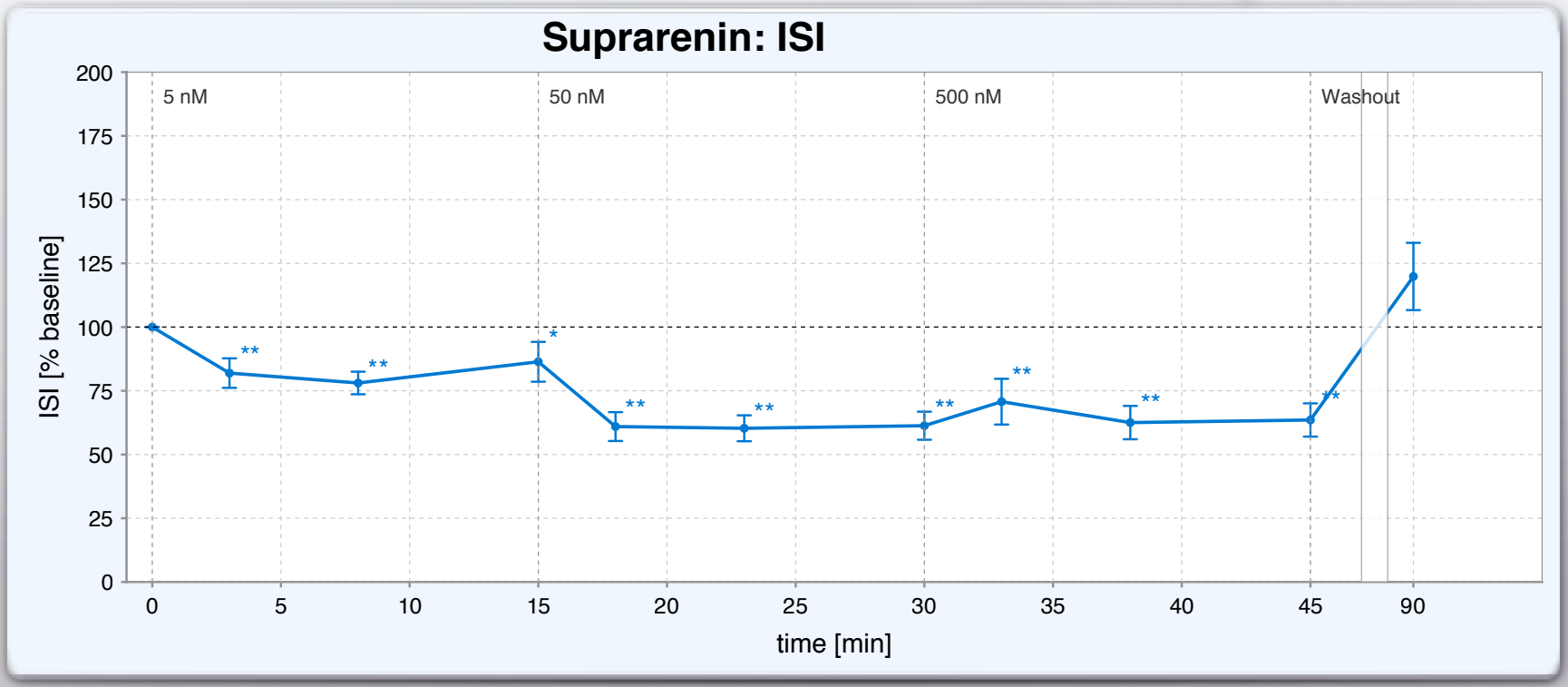

**Effect:**

**- positive chronotrope**

# MEA Recording

## Suprarenine

ISI: Interspike Interval




## Effect:

- positive chronotrope

Cor.At®

Ready-to-use Cardiomyocytes

# RT-CA/xCELLigence System
## (ACEA/Roche)

## Dynamic Monitoring of GPCR Agonists on Cor.At® Cells

# Principle of Impedance Technology Applied to Cell Biology

## Impedance Sensor:

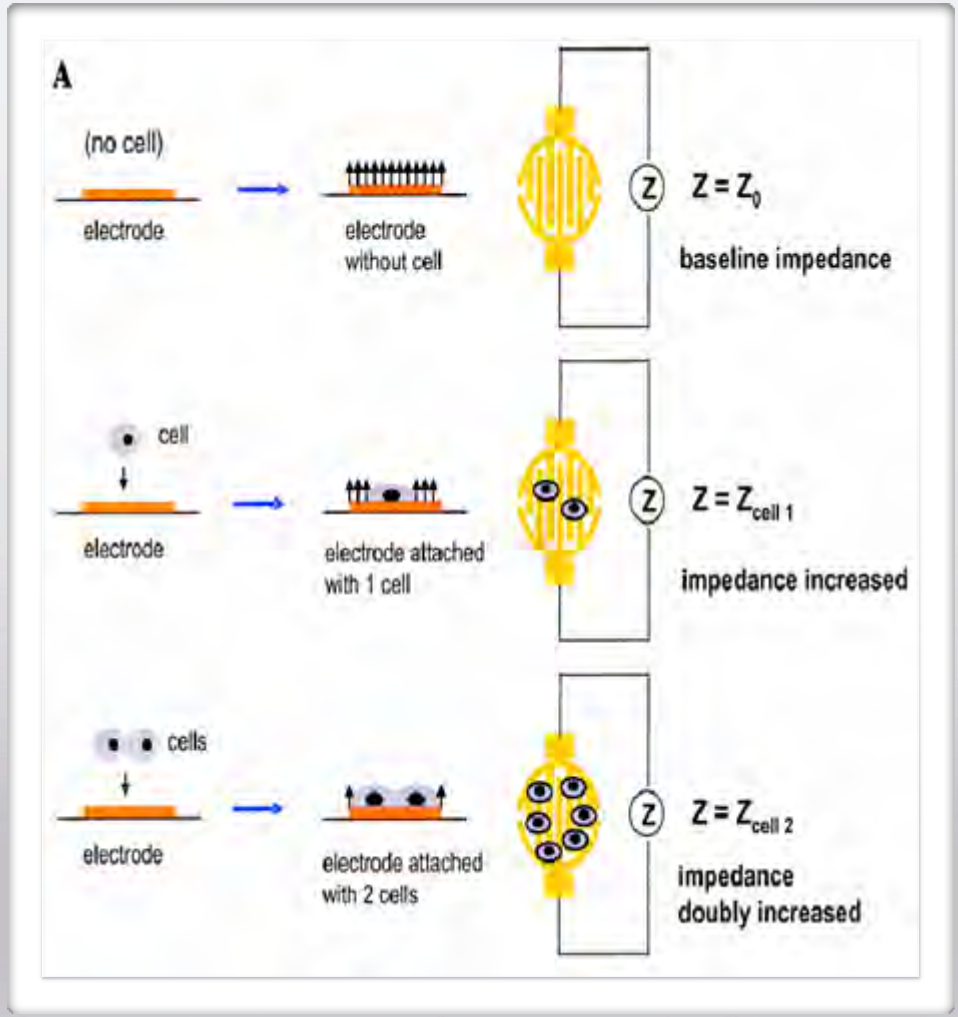
- Application of 20 mV

=> Background impedance in presence of media

=> Substraction by software

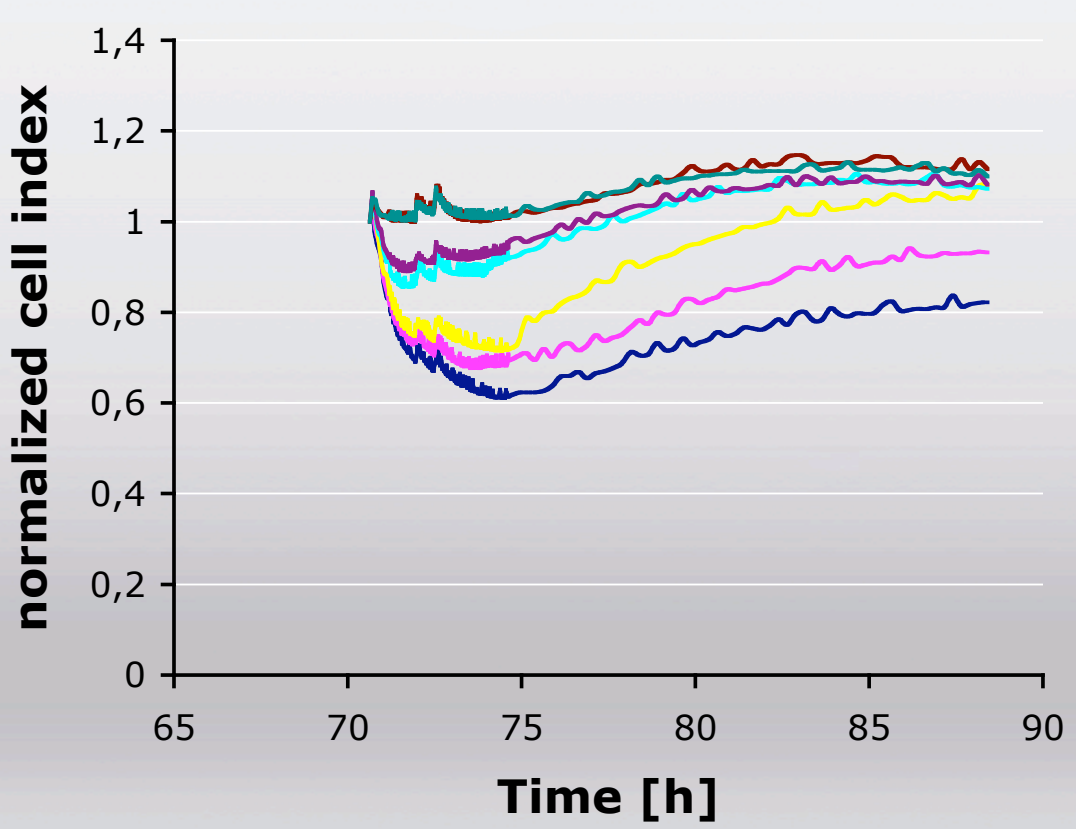
## Changes of Impedance:

- Proportional to cell density
- Sensitive to cellular events

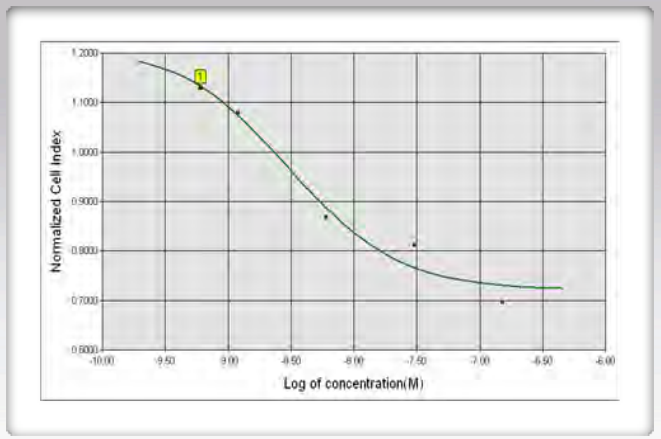


# Impedance spectroscopy

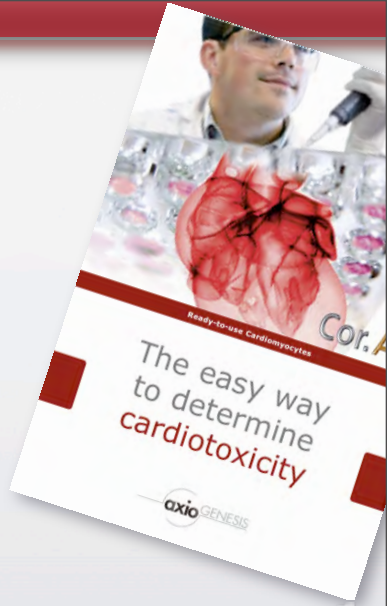
## Isoproterenol: ß-adrenergic Agonist




$EC_{50}$ = 3.1 nM




Data was kindly provided by Dr. Yama Abassi from ACEA Biosciences

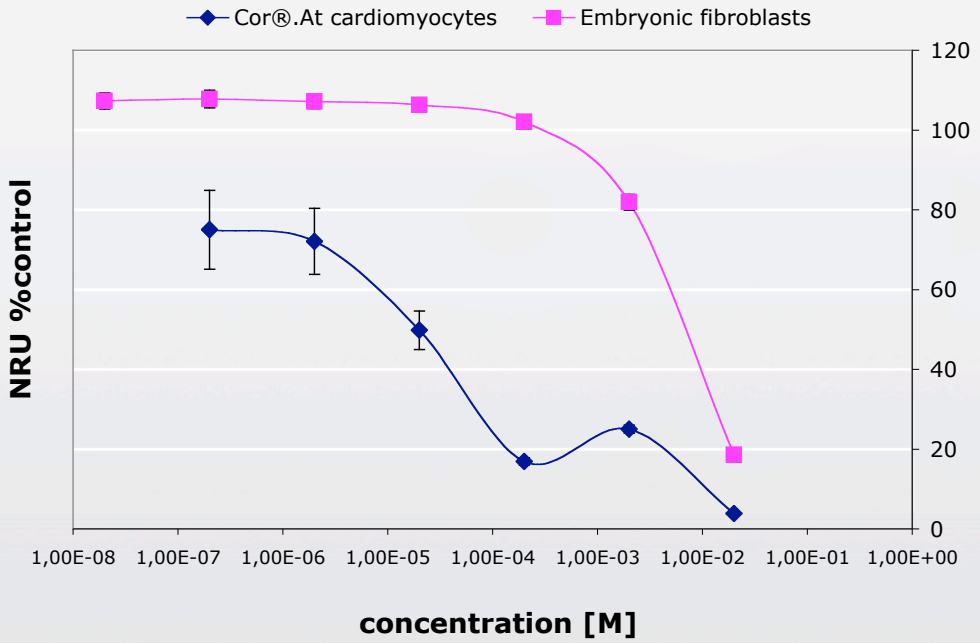




# Cor.At® Tox

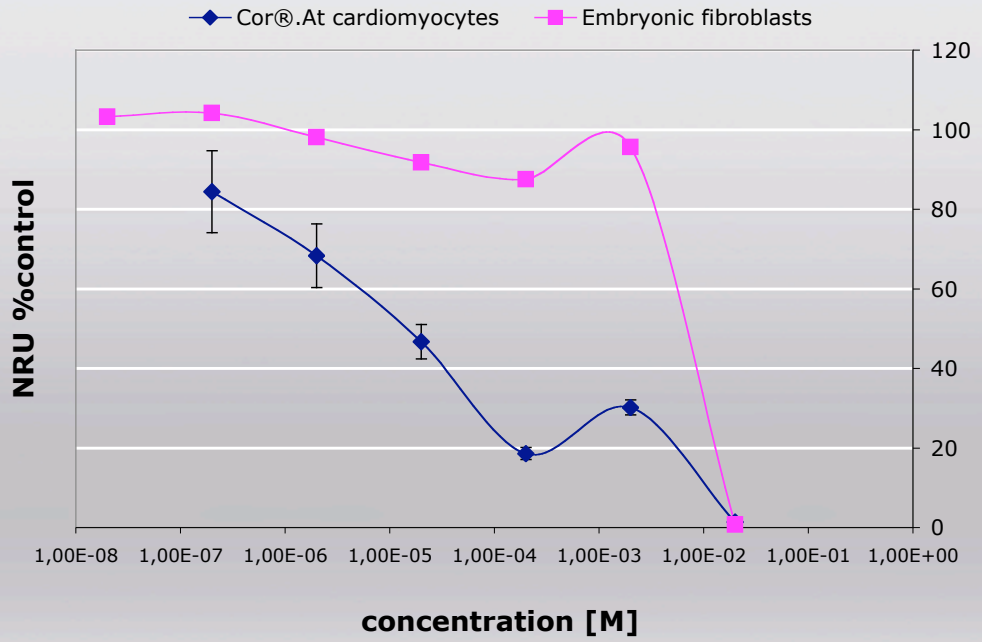
## Ready-to-use Cardiomyocytes

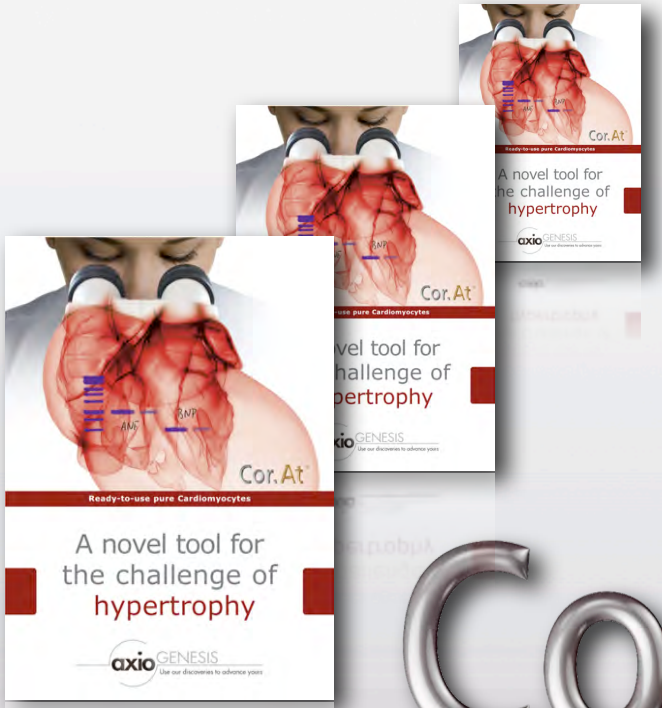
# Specific Cardiac Cytotoxicity

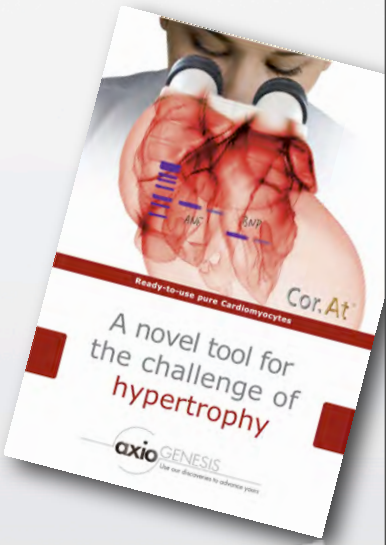
## Doxorubicin Hydrochlorate




## Epirubicin Hydrochlorate






# Cor.At® Mod

## Ready-to-use Cardiomyocytes

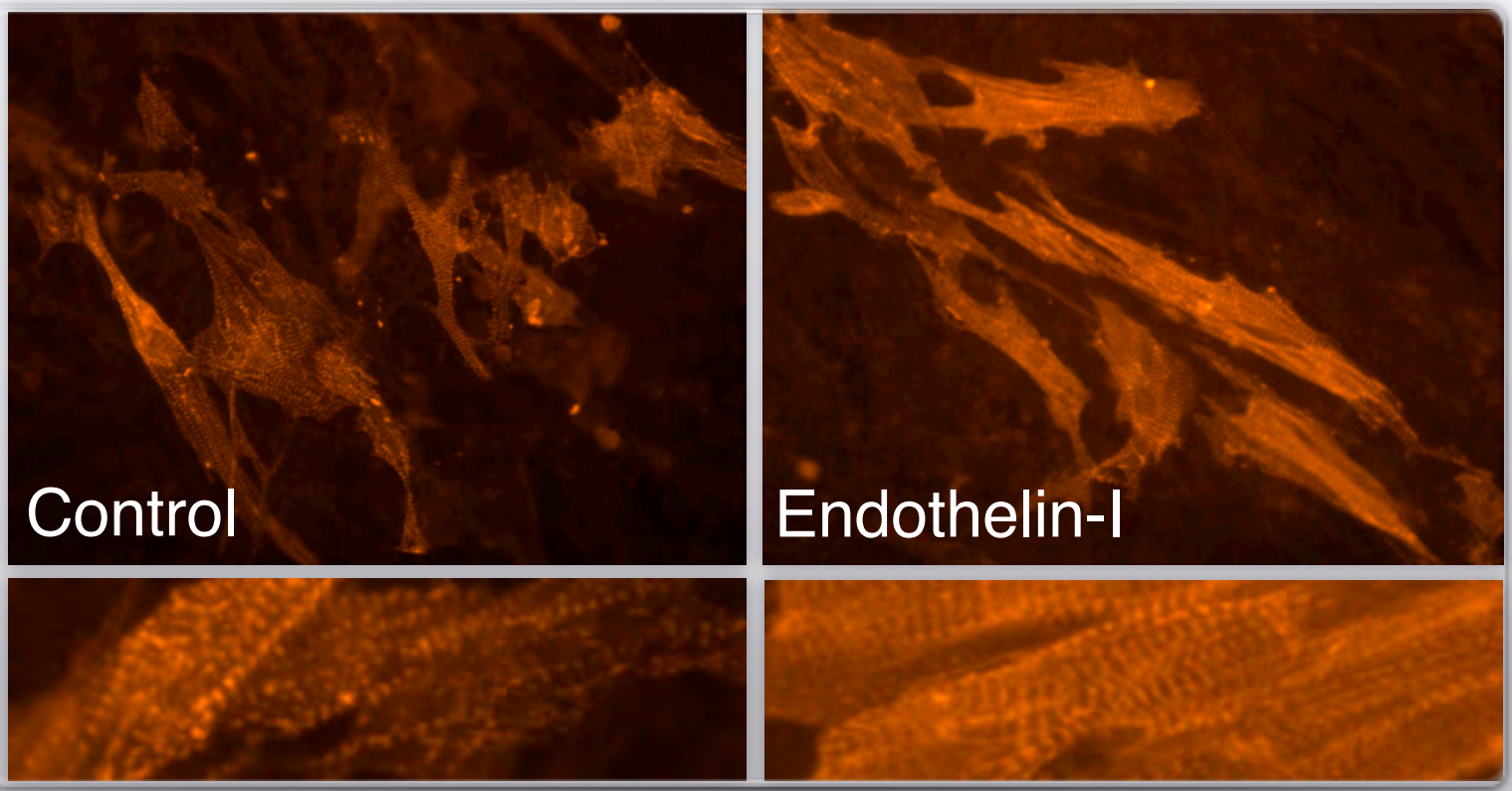
# Displaying the disease

Cor.At®

Ready-to-use Cardiomyocytes

# Induction of Hypertrophy

## Immunostaining: cardiac α-actinin




**Enhanced enhanced sarcomeric organization upon endothelin-1 treatment .**

# Summary

## Cor.At® ready-to-use cardiomyocytes:

- Highly standardized production (in vitro)
- Availability of large, frozen stocks
- Inter lot uniformity
- primary-like cardiomyocytes
- 100% purity, no fibroblast contamination
- Highly predictive

# Partners:

**Axiogenesis AG:**

- Dr. Eugen Kolossov
- Dr. Silke Schwengberg
- Josef Tenelsen
- Peter Metzger

**Institute for Neurophysiology University of Colone:**

- Alexey Kuzmenkin
- Huamin Liang

**ACEA Biosciences:**

- Dr. Yama Abassi

**EVOTEC AG:**

- Dr. Clemens Möller

Scottish Biomedical

amaxa biosystems

vivoMEDICA SCIENCES FOR LIFE

Bionas®

# Special thanks to

**Eric Atkinson and Lynn MacIntyre from our North Amerikan Distributor ReachBio for the warm welcome in Vancouver, their assistance and guidiance, and the great job they are doing.**

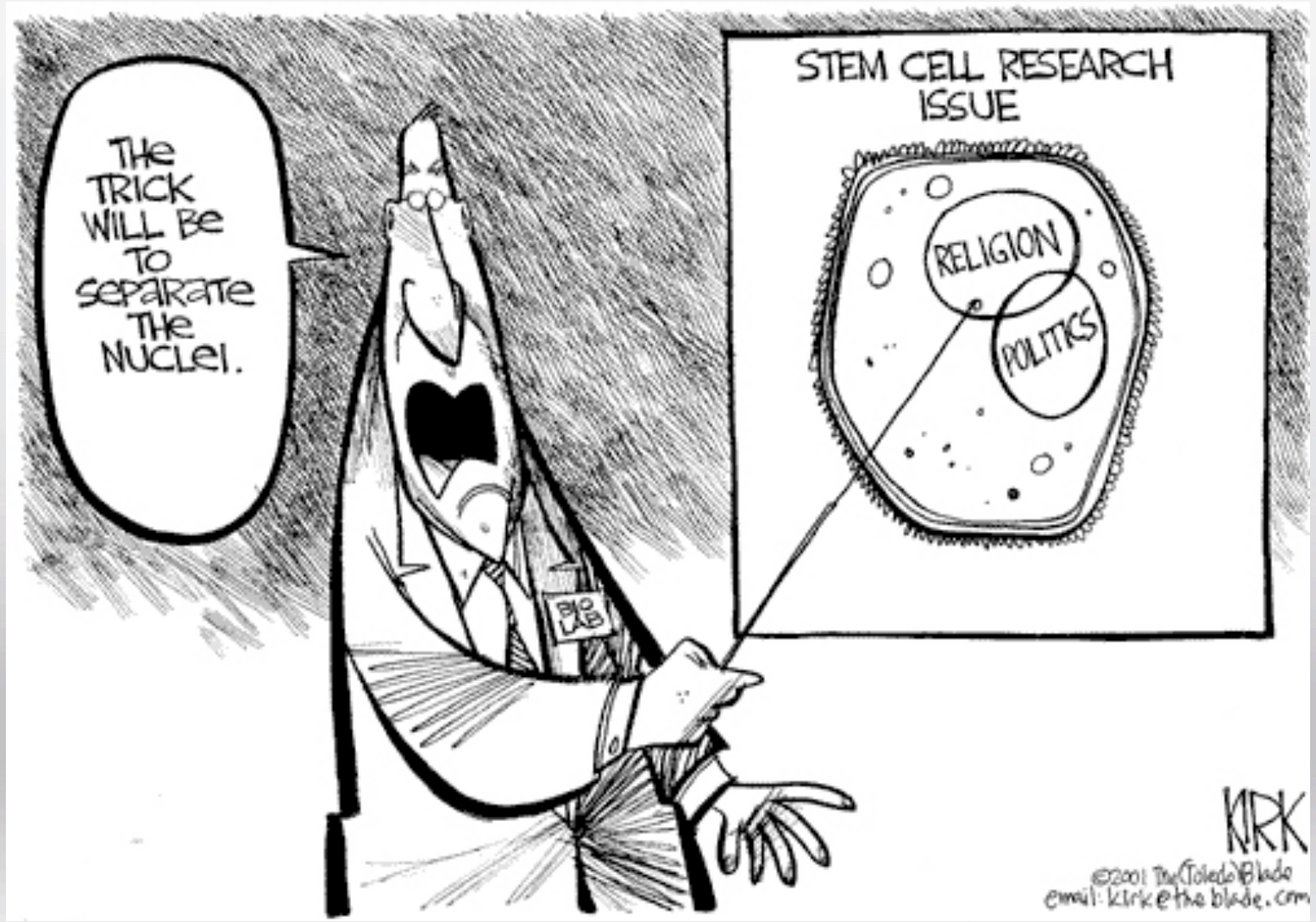
www.reachbio.com

**Our Japanese distributor Veritas**

www.veritastk.com

**Our European distributor CellSystems**

www.cellsystems.de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RELIGION
POLITICS
BIO LAB
KIRK
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