

BioPacemakers: Therapy for Cardiac Arrhythmias

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Disclosure

Hee Cheol Cho holds stock interest and serves as program director for Excigen which licensed BioPacemaker-related technologies from Johns Hopkins Univerisity.

Modern therapy for cardiac arrhythmias

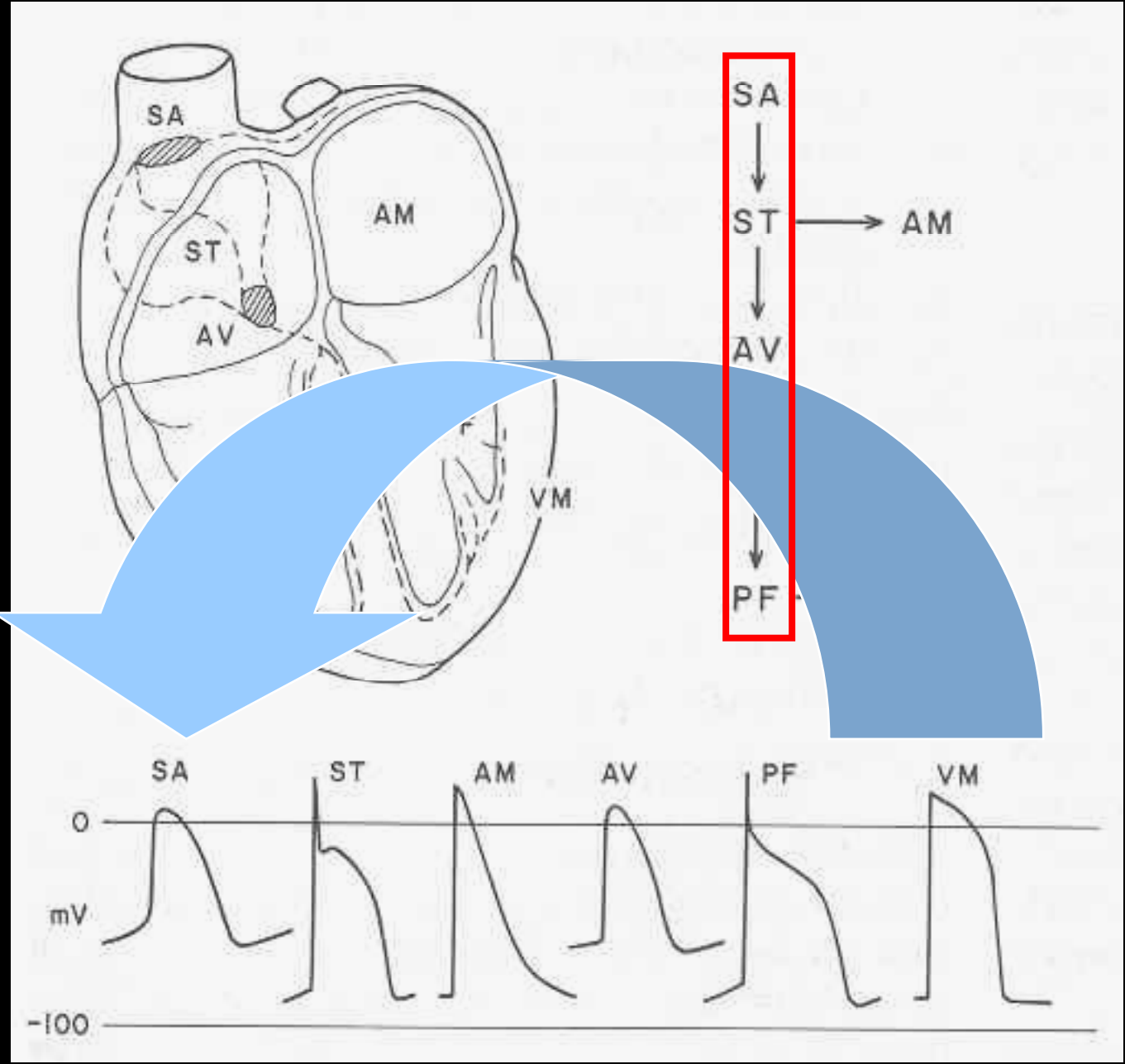
- Pharmacotherapy
 - Variably effective, bedeviled by proarrhythmia
- Radiofrequency Catheter Ablation
 - Potentially curative, but less reliable for most common arrhythmias
- Implantable Devices
 - Palliative for bradyarrhythmias, life-saving for tachyarrhythmias
 - Lifelong commitment to repeated procedures, significant expense, potentially catastrophic complications
- **Gene therapy? Cell therapy?**

Potential Advantages of Biological Therapy

- Highly Localizable
- Therapeutic action responsive to endogenous signal transduction
- Avoidance of implantable hardware
- Reversible by conventional therapy

Cardiac conduction system

Action potentials



Goal

To create a biological pacemaker

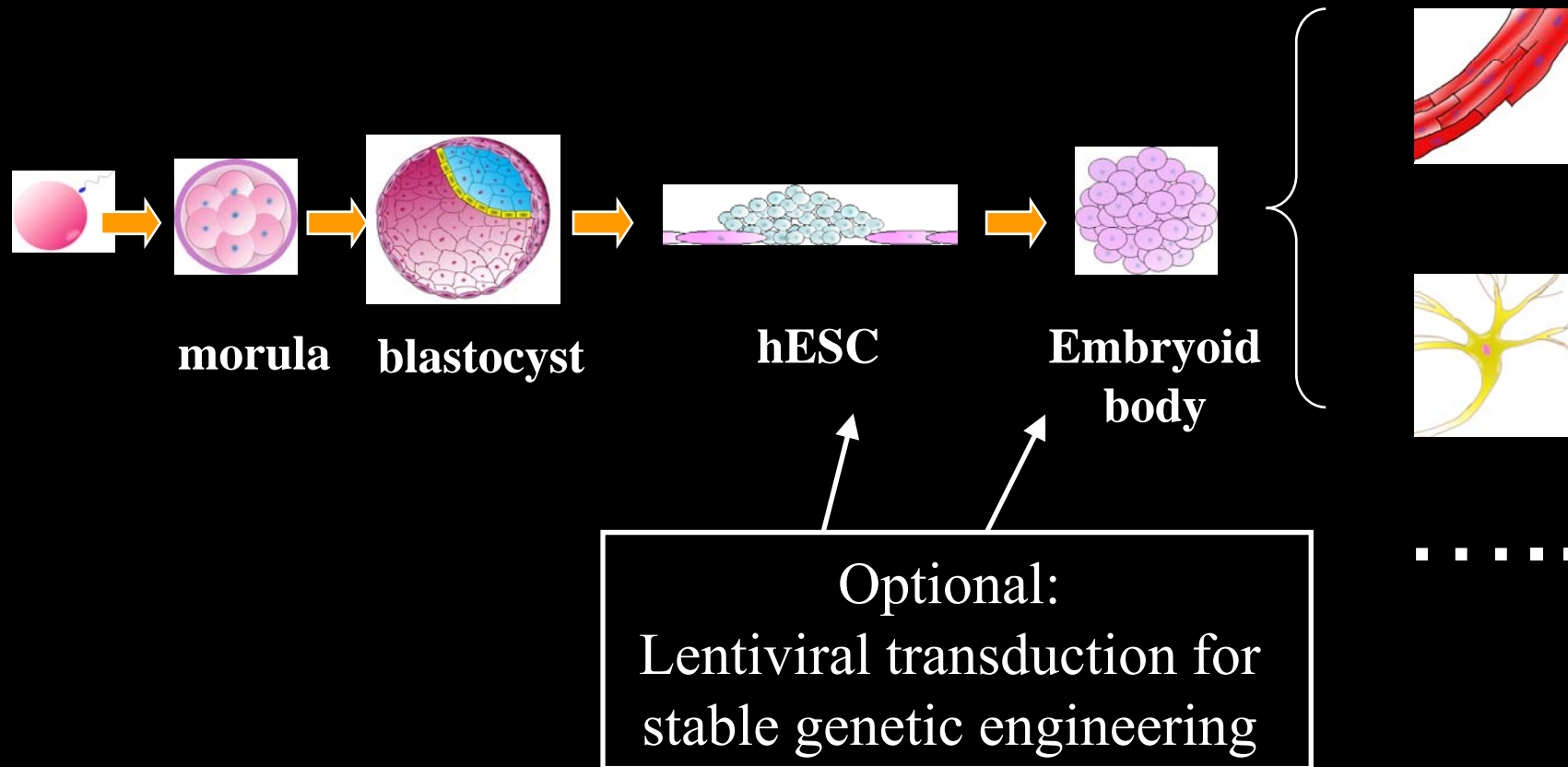
I. Stem cell therapy

- human embryonic stem cells
- human adult cardiac stem cells

II. Gene therapy

- Kir2.1AAA
- HCN-channel/cell fusion
- Kv1.4-based synthetic pacemaker channel

Human embryonic stem cells



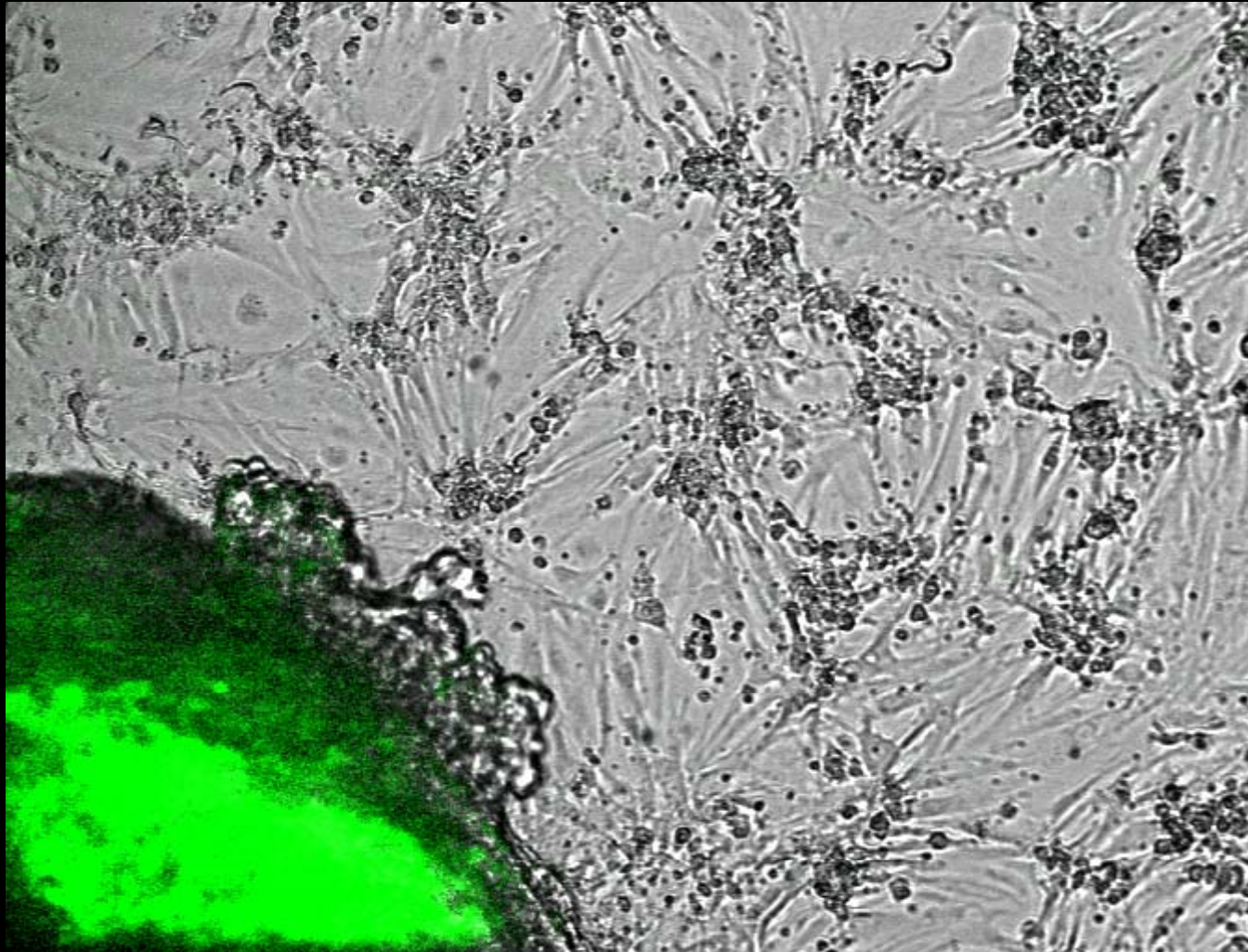
Objective

- Do spontaneously beating hEBs (BioPacemakers) integrate with recipient tissue?

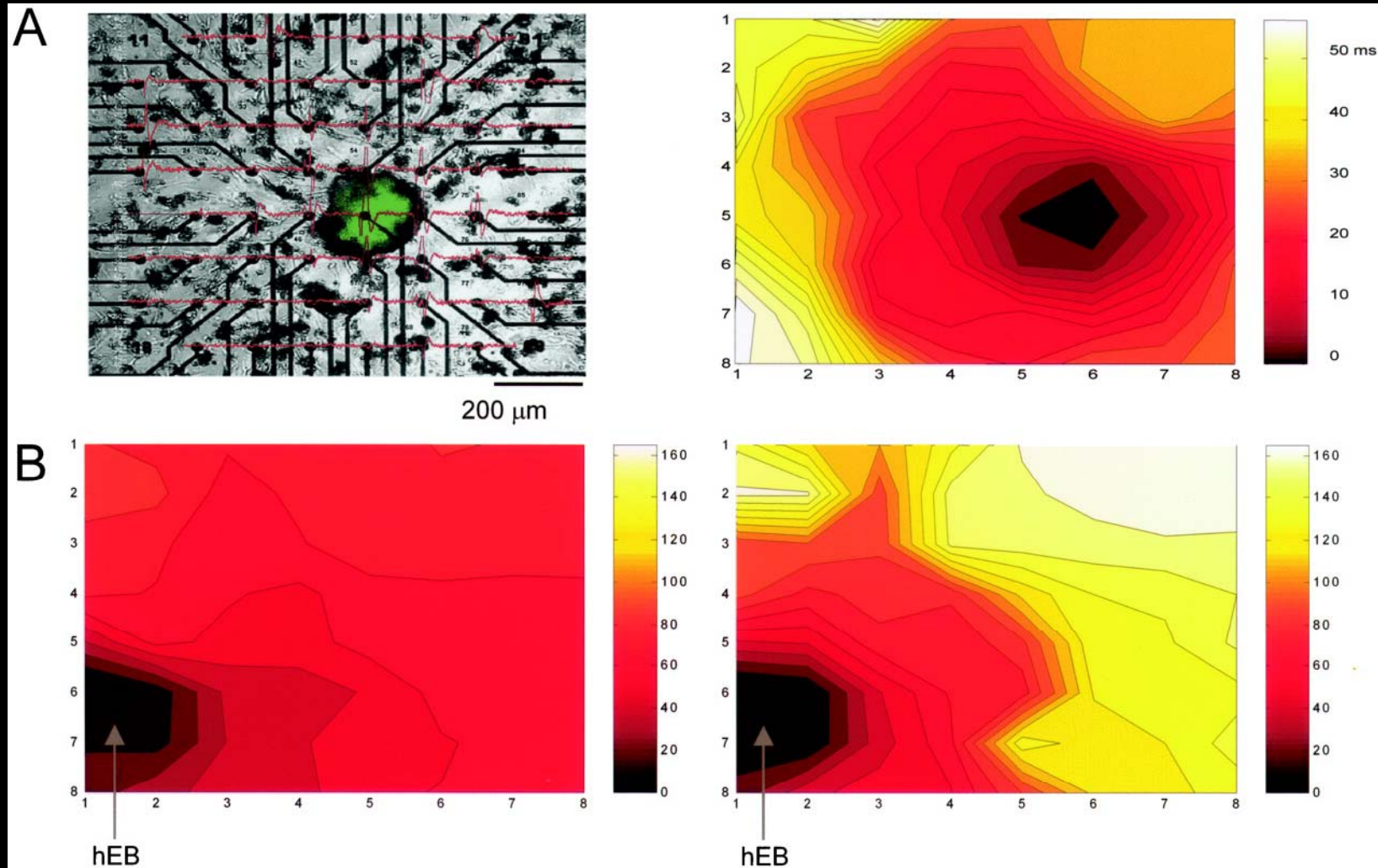
Strategy

- GFP-transduced hESCs differentiated *in vitro* into EBs
- Beating cardiac mass microdissected and transplanted onto quiescent rat heart cell monolayers

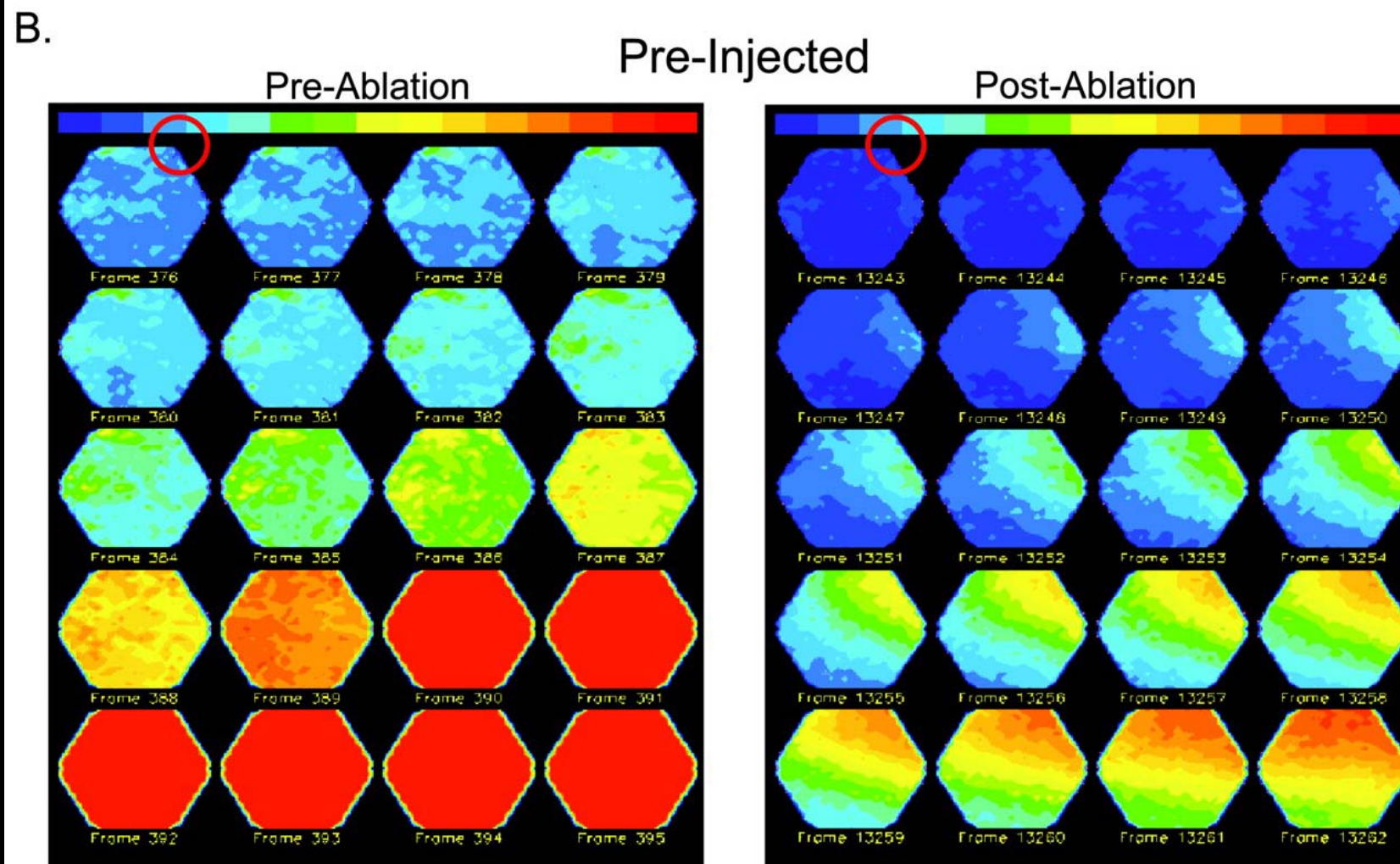
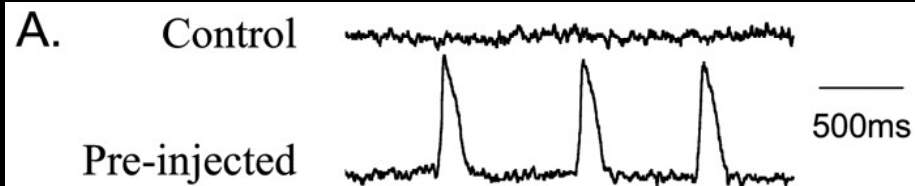
GFP-hEB coupled with a monolayer of NRVMs



Synchronous electrical activity in the human EB and rat cardiomyocytes: MEA study



Optical-mapping: isolated heart



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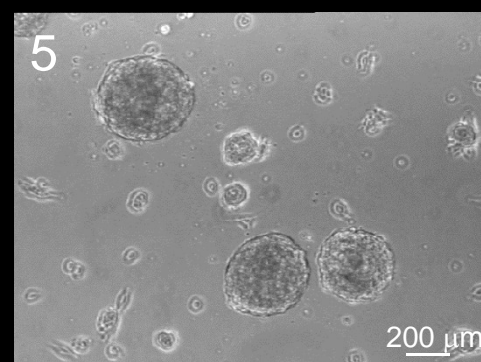
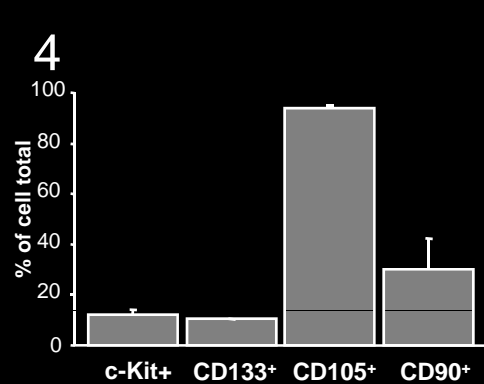
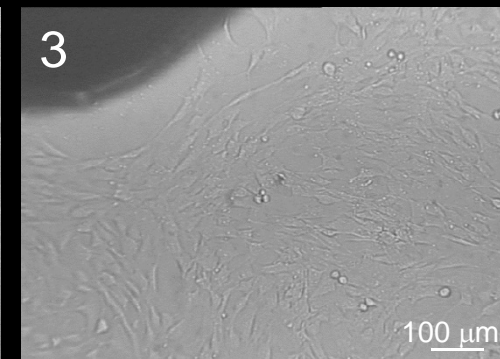
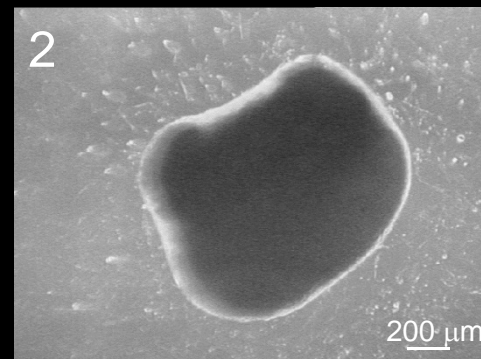
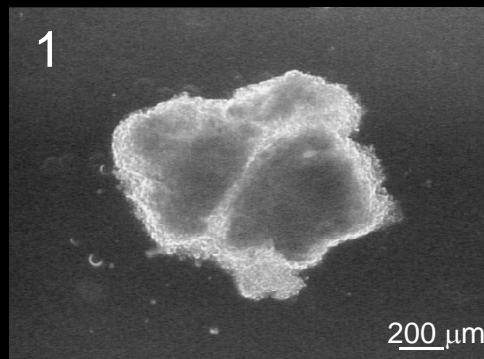
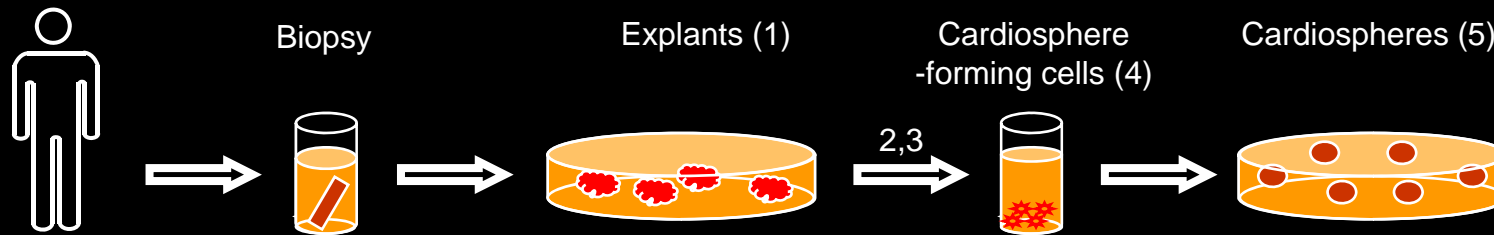
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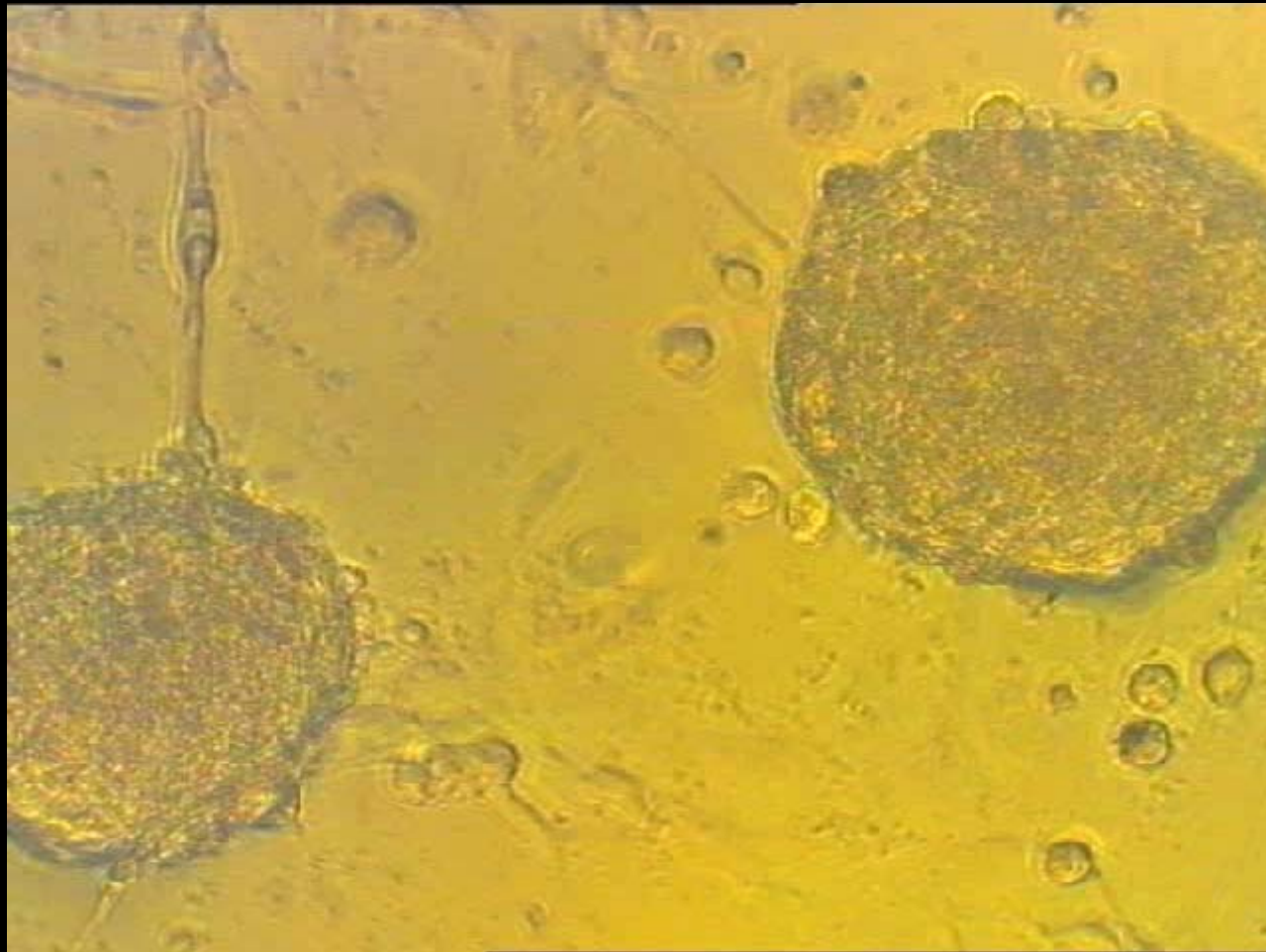
Alternative cell source: Cardiac stem cells and Cardiospheres

- Cardiac stem cells (CSCs)
 - Spontaneously shed from explants
 - Can regenerate heart muscle
- Cardiospheres (CSps)
 - Multicellular spherical clusters derived from CSCs
 - Partially-differentiated in vitro
- Unique advantages for cell transplantation
 - Autologous
 - Cardiogenic
 - Can be induced to beat spontaneously

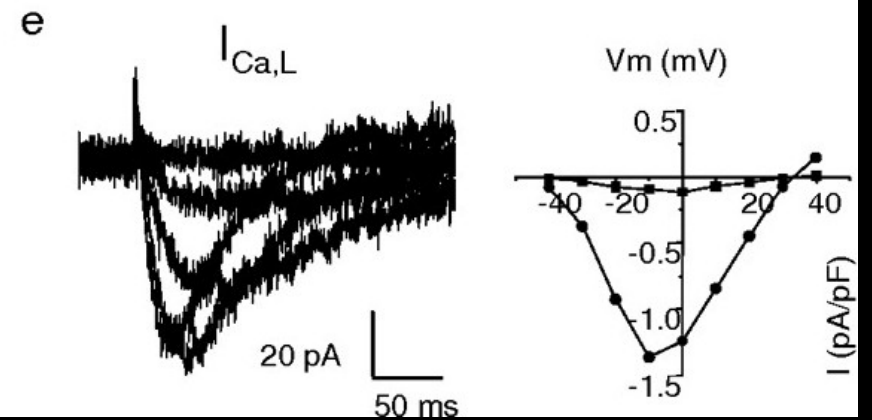
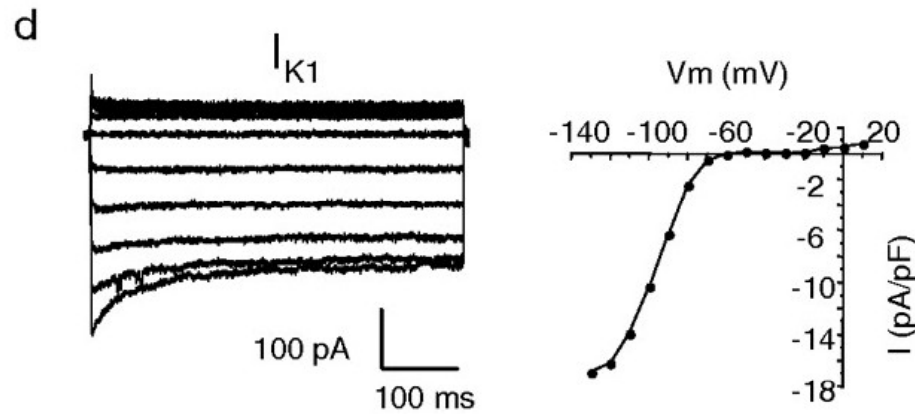
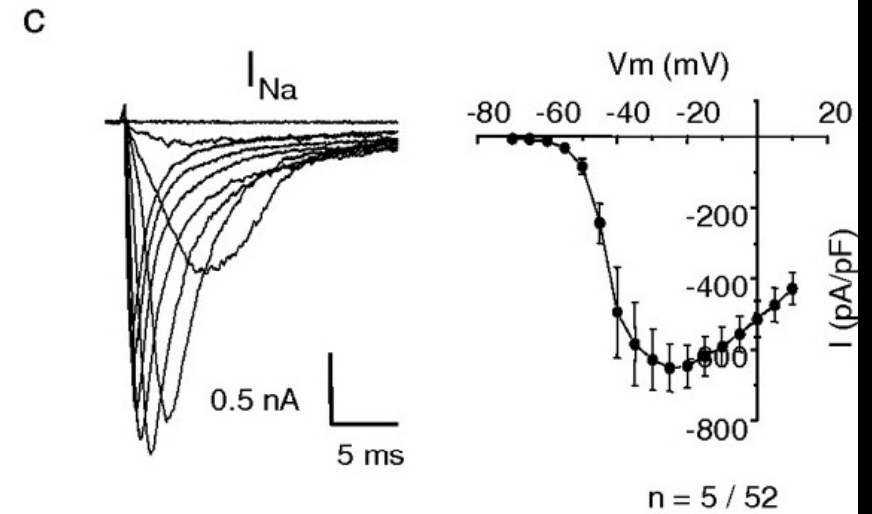
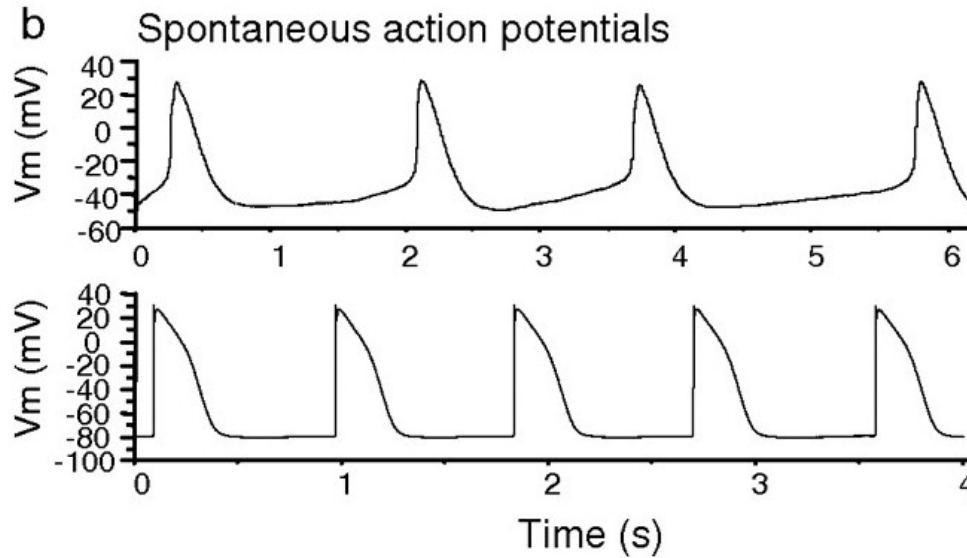
Harvest and grow cardiospheres



Spontaneous rhythmic electrical activity from a cardiosphere



Electrophysiology of cardiosphere



Limitations with stem cell therapy

- Stem cells present risk of teratomas, immune rejection
- Gap-junctional coupling may or may not be stable long-term
- Frequency tuning desirable
- Most injected cells may not reside at the site of cell injection: <30%, Rosen AB et al. Stem Cells 2007.

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Current

Probable gene

I_{Na}



SCN5A

$I_{Ca,L}$

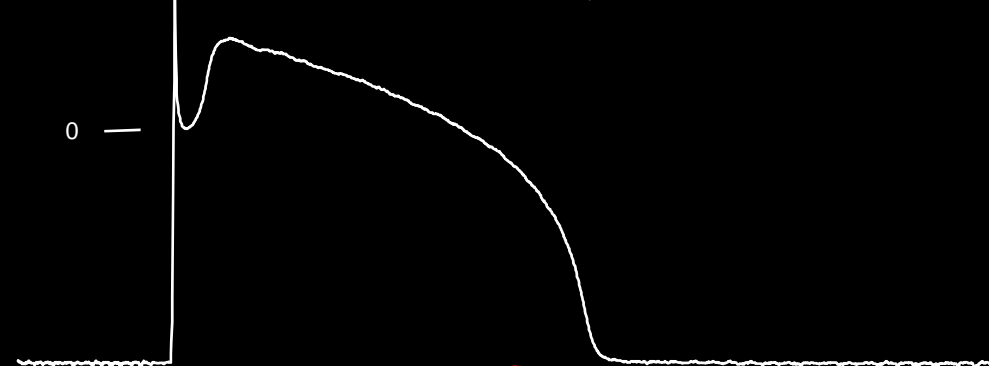


DHP receptor

$I_{Na/Ca}$



NCX



I_{K1}



Dominant-negative
Kir2.x
Kir2.1AAA

$I_{to,1}$



Kv4.x

$I_{to,2}$



I_{Kr}



HERG/mirp

I_{Ks}

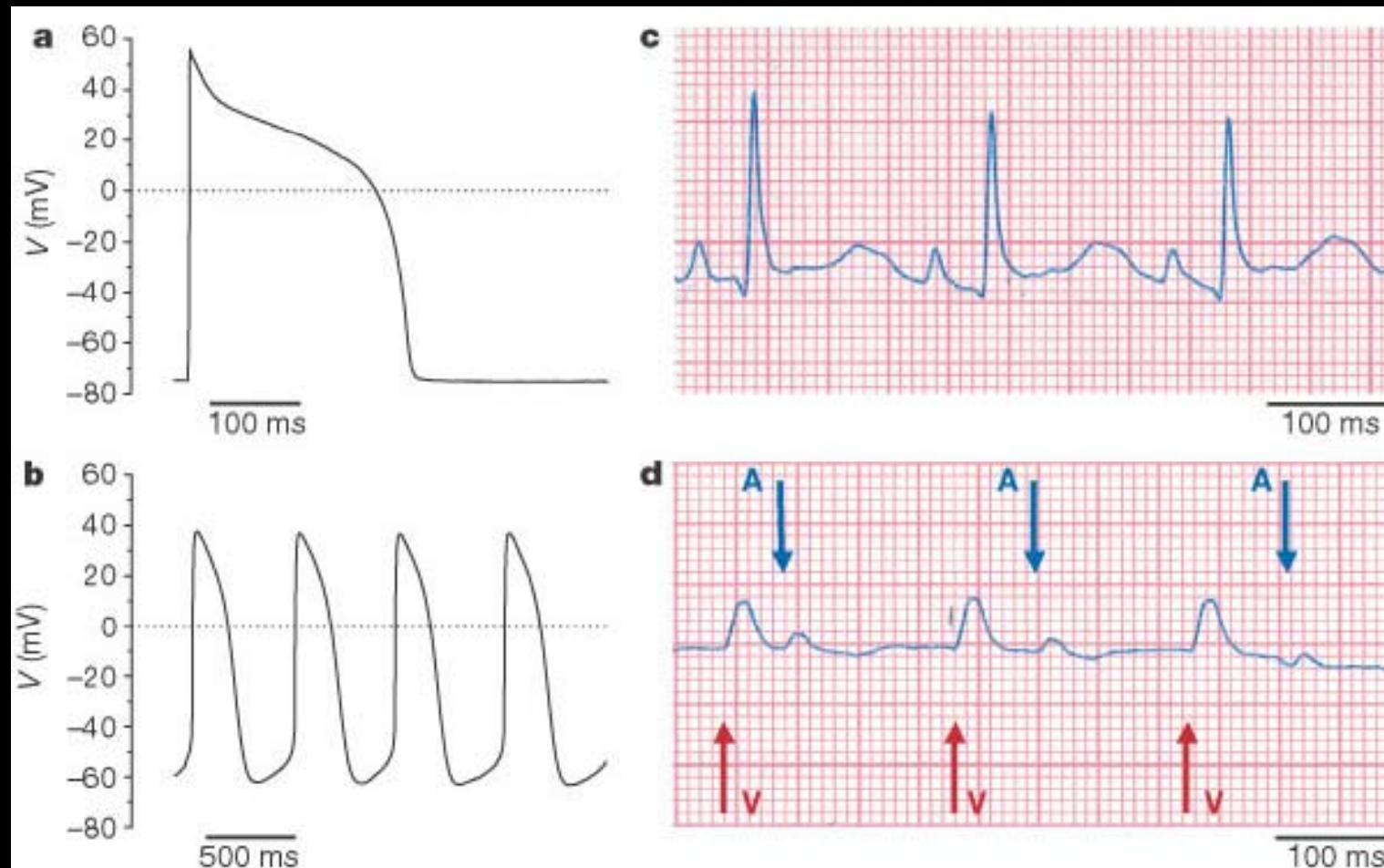


KvLQT1/minK

I_{Kp}



Conversion of ventricular myocytes into pacemaker cells: Kir2.1 dominant negative strategy



Miake, Marbán & Nuss, *Nature* 419:132-133, 2002

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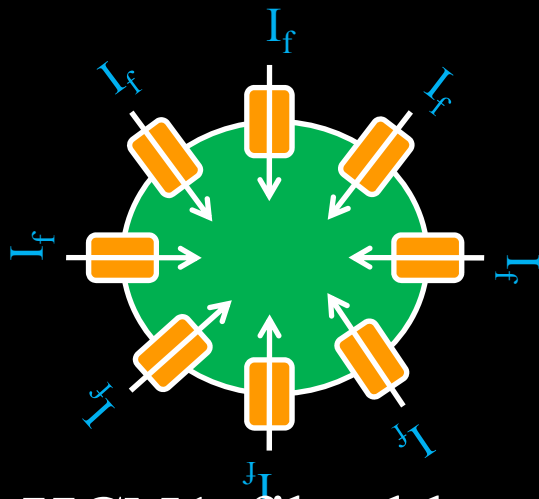
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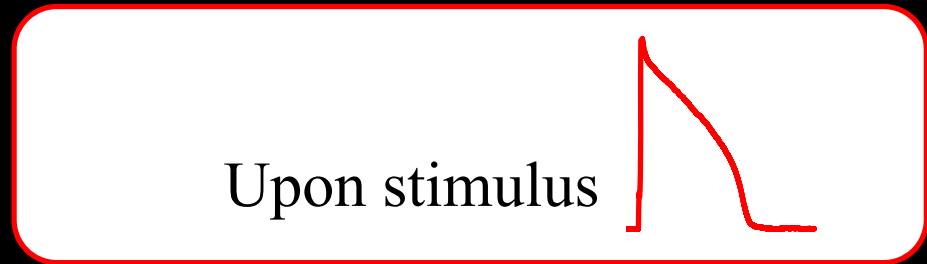
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BioPacemaker by somatic cell fusion

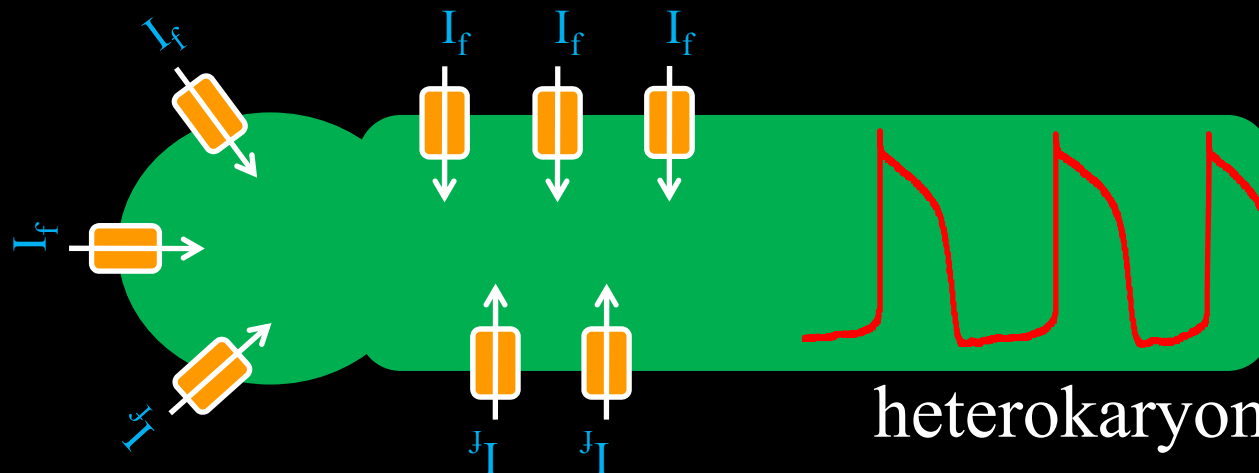


HCN1-fibroblast



cardiomyocyte

PEG-assisted cell fusion

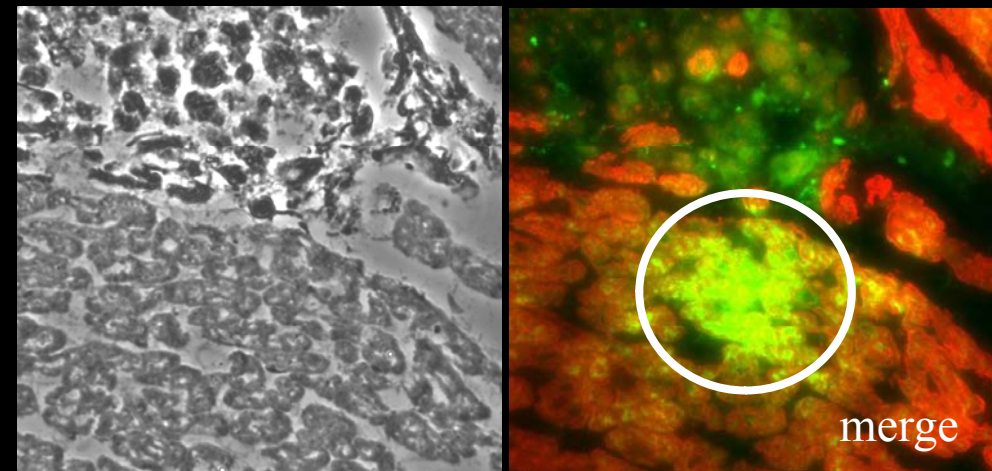
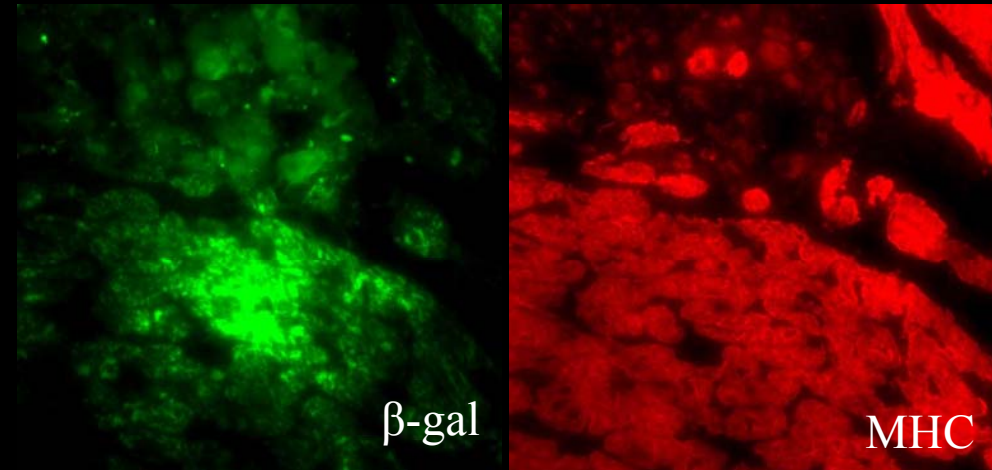
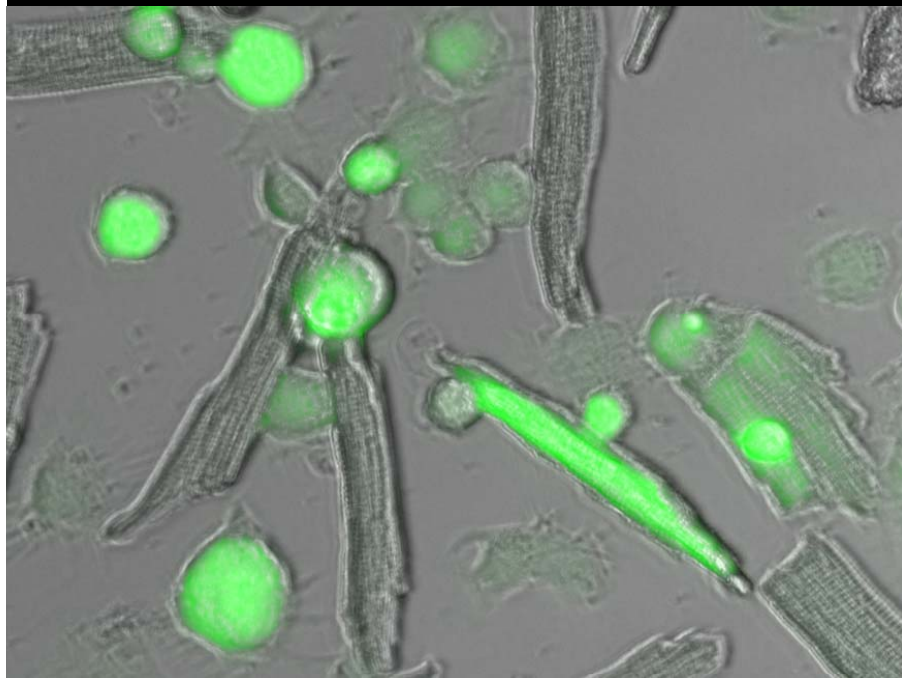


heterokaryon

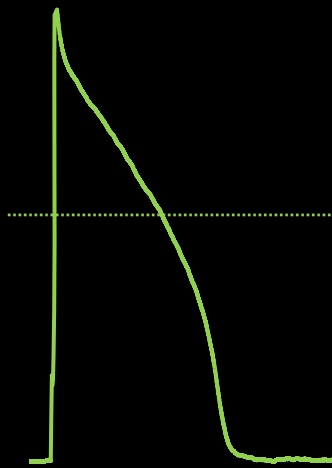
BioPacemaker by somatic cell fusion

in vivo

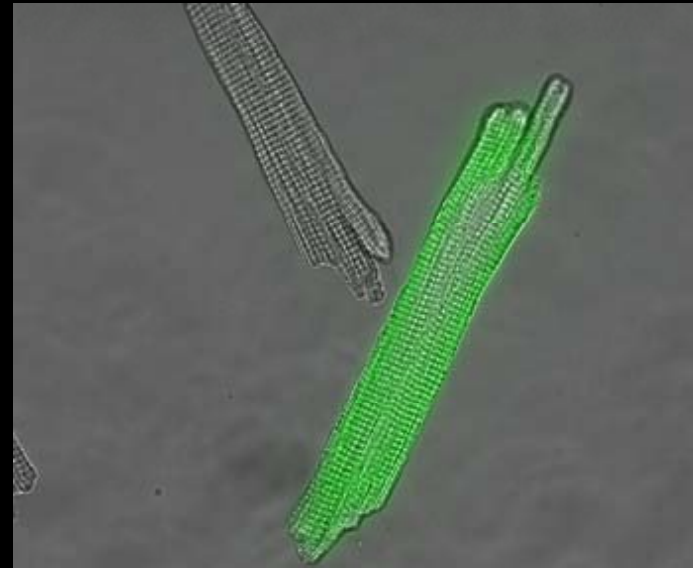
in vitro



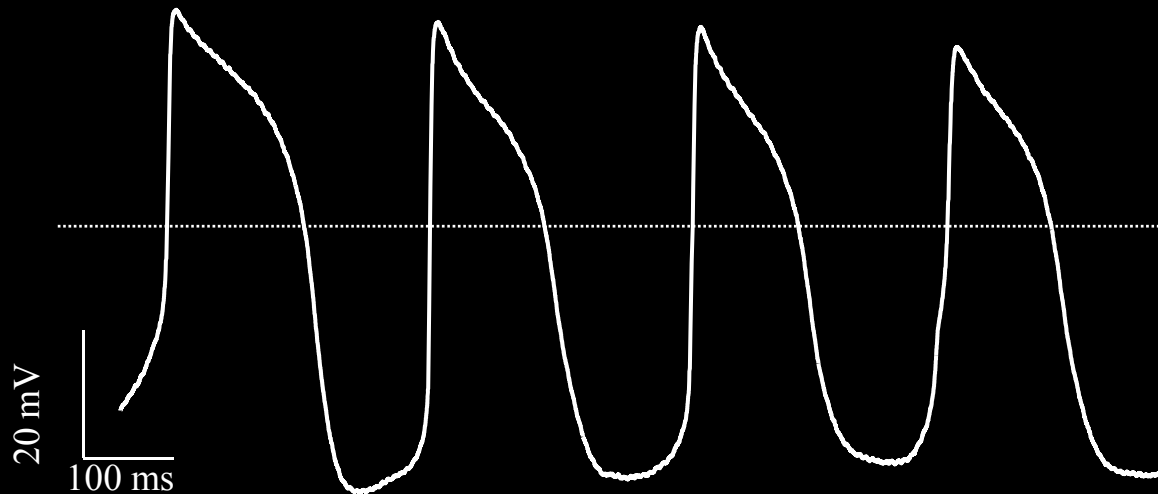
Cell fusion converts cardiomyocytes to pacemaker cell



Myocyte fused with
control fibroblast



6-days post cell fusion:
myocyte + GFP-fibroblast

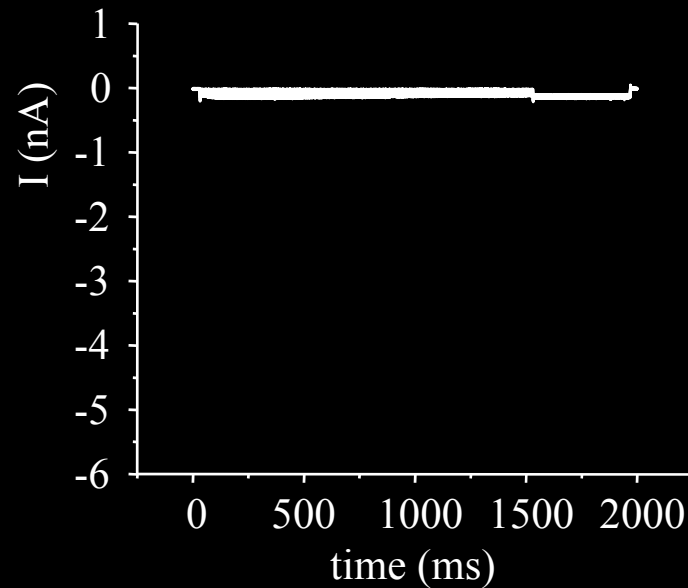


Myocyte fused with
HCN1-fibroblast

I_f from *in vivo* PEG-induced heterokaryons

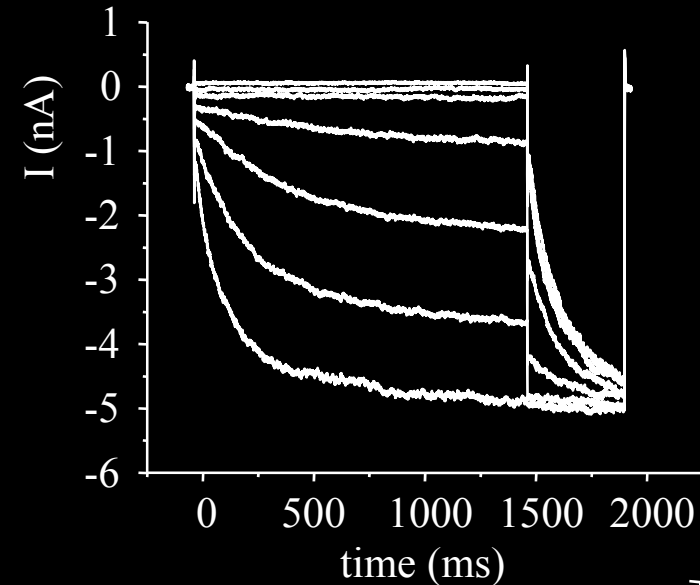
GFP-fibroblasts

-0.2 ± 0.5 pA/pF at -130 mV ($n=7$)



HCN1-GFP-fibroblasts

-12 ± 2 pA/pF at -130 mV ($n=9$)

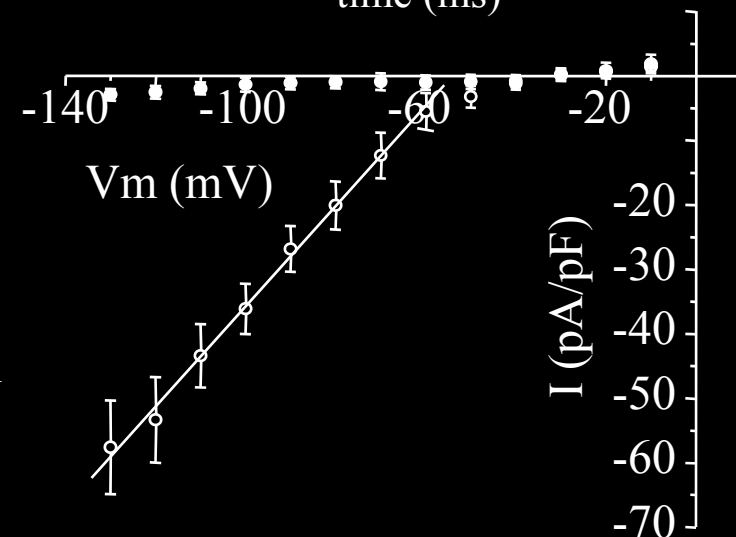


$\gamma = 770 \pm 10$ pS/pF, $n=9$

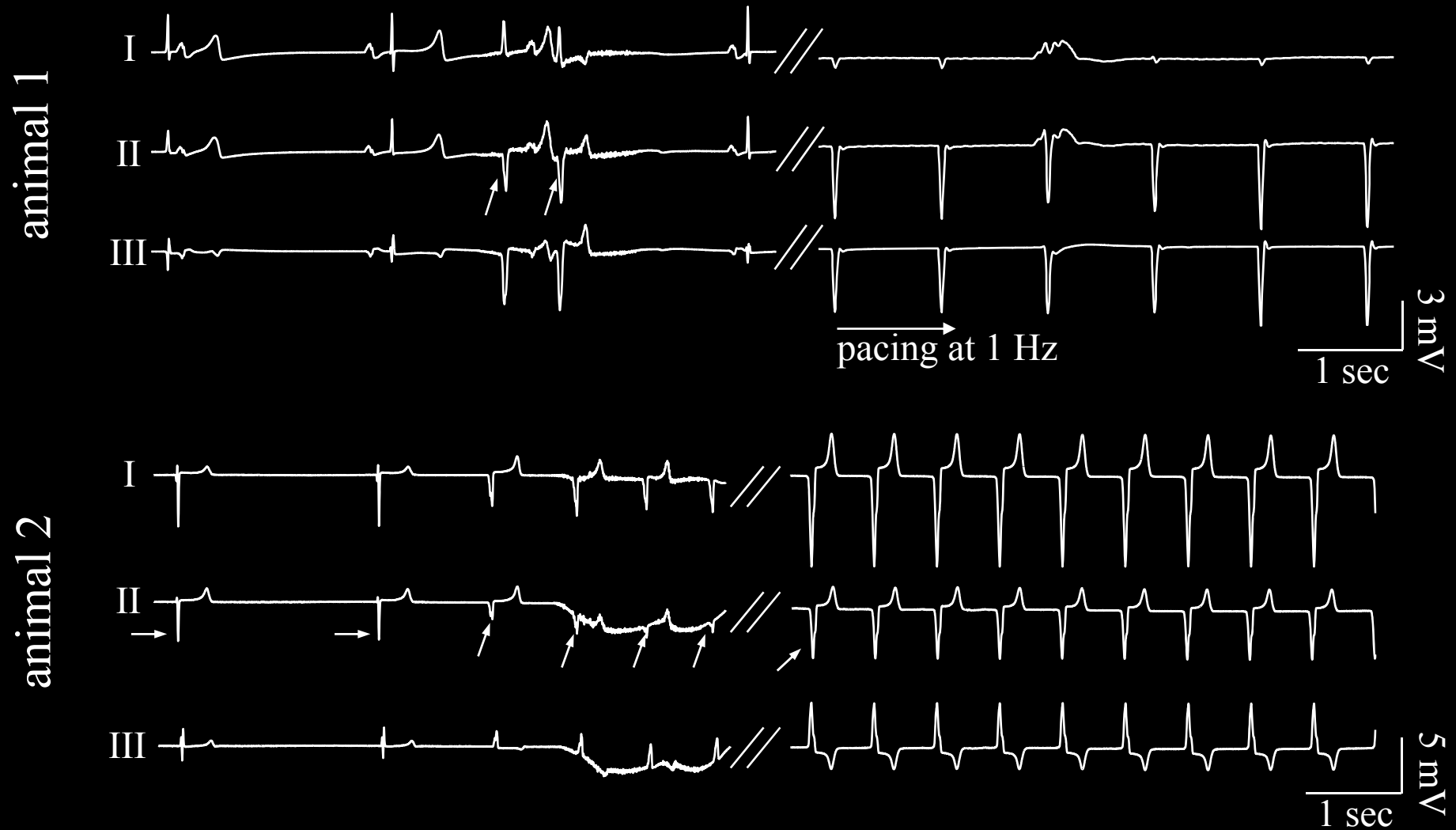
In rabbit SA nodal cells

- $I_f = 12$ nS (Honjo et al., 1996)
- cell capac. = 38 pF (van Ginneken et al., 1991)

→ 316 pS/pF



BioPacemaker activity by *in vivo* somatic cell fusion



BioPacemaker by somatic cell fusion

- Convenient cell source: stem cells not required
- No reliance on gap junctions
- Cell fusion with ventricular myocytes
 - Allows the fused cells to stay at the site of cell injection
 - Could achieve precise location and stable activity of BioPacemaker.

Goal

To create a biological pacemaker

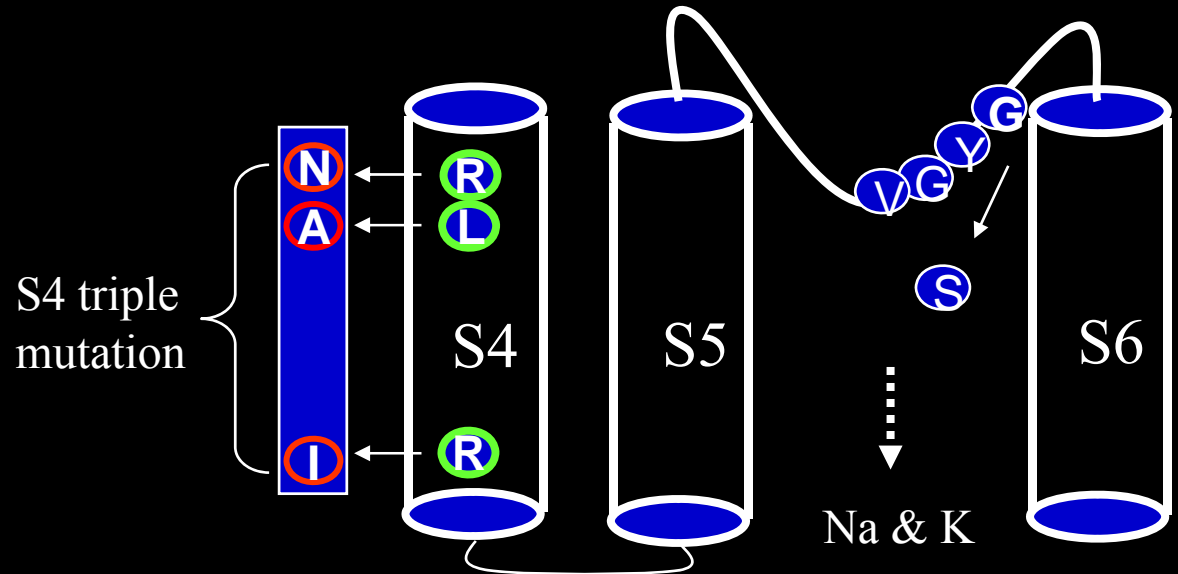
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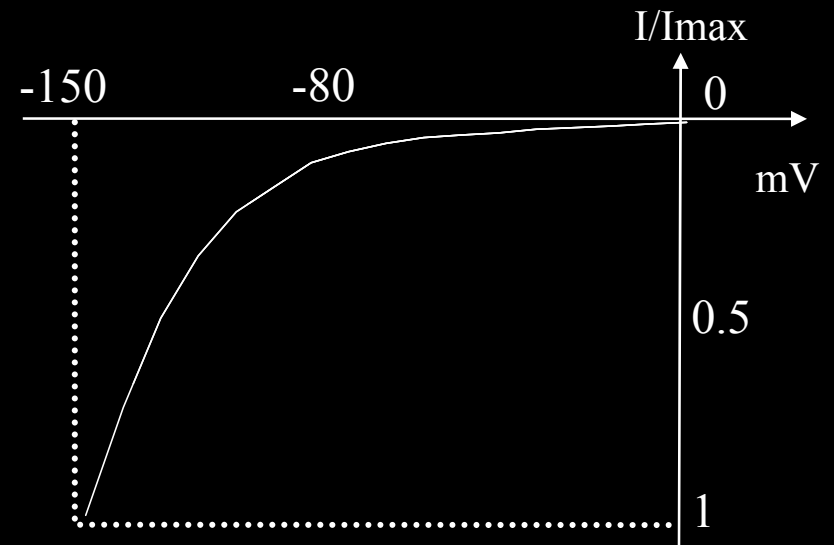
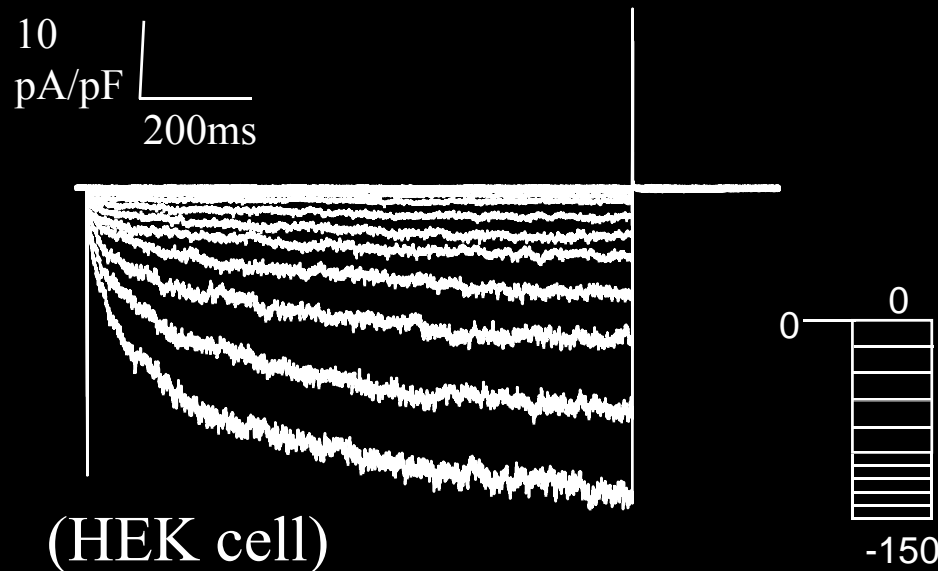
Creation of a synthetic pacemaker channel (SPC)



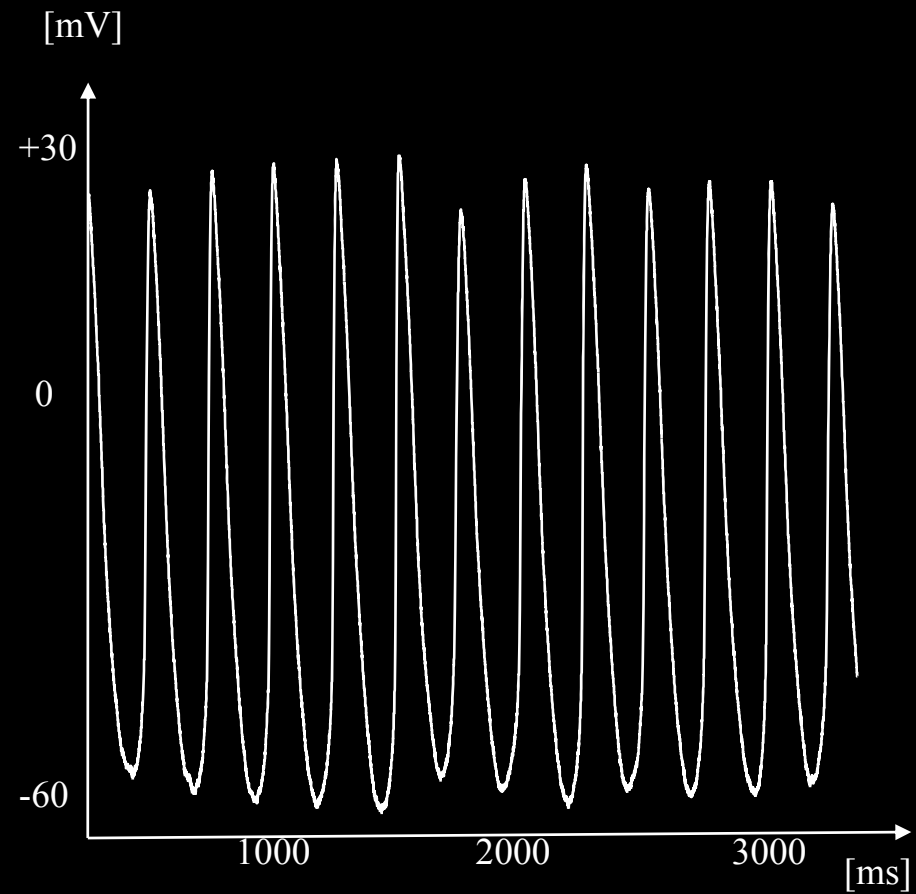
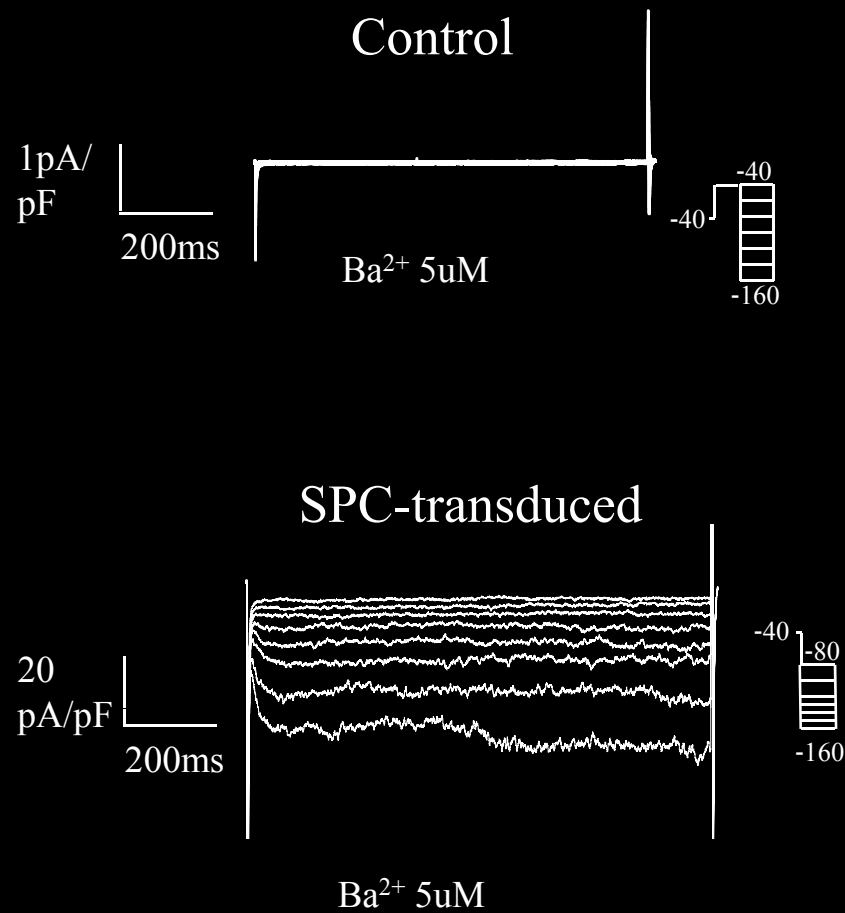
Kv1.4 selective mutagenesis:

S4: R447N, L448A, and R453I

Pore: G528S



Pacemaker activity in SPC-transduced adult ventricular myocytes



BioPacemaker: Summary

1. Novel, cell- and gene-based alternatives to electronic pacemakers
2. Stem cells or fibroblasts or HEK293 cells
3. Kir2 suppression, HCN, SPC, triple-gene vector

BioPacemaker: Next steps

- Autonomic regulation: HCN-channel constructs
- Optimization of permutations of genes, cells and engraftment strategies
- Stability of therapy
 - e.g. fusion approach stable for at least 3 months.
- Bio-safety: minimal risk for inflammation and neoplasia
 - Adv, AAV, lentivirus
 - helper-dependent adeno virus

Acknowledgements

Johns Hopkins University

Michelle K. Leppo

Yuji Kashiwakura

Rachel R. Smith

Eduardo Marbán

Excigen, Inc.

Hui Sun

Brian Taylor

Jun Huang

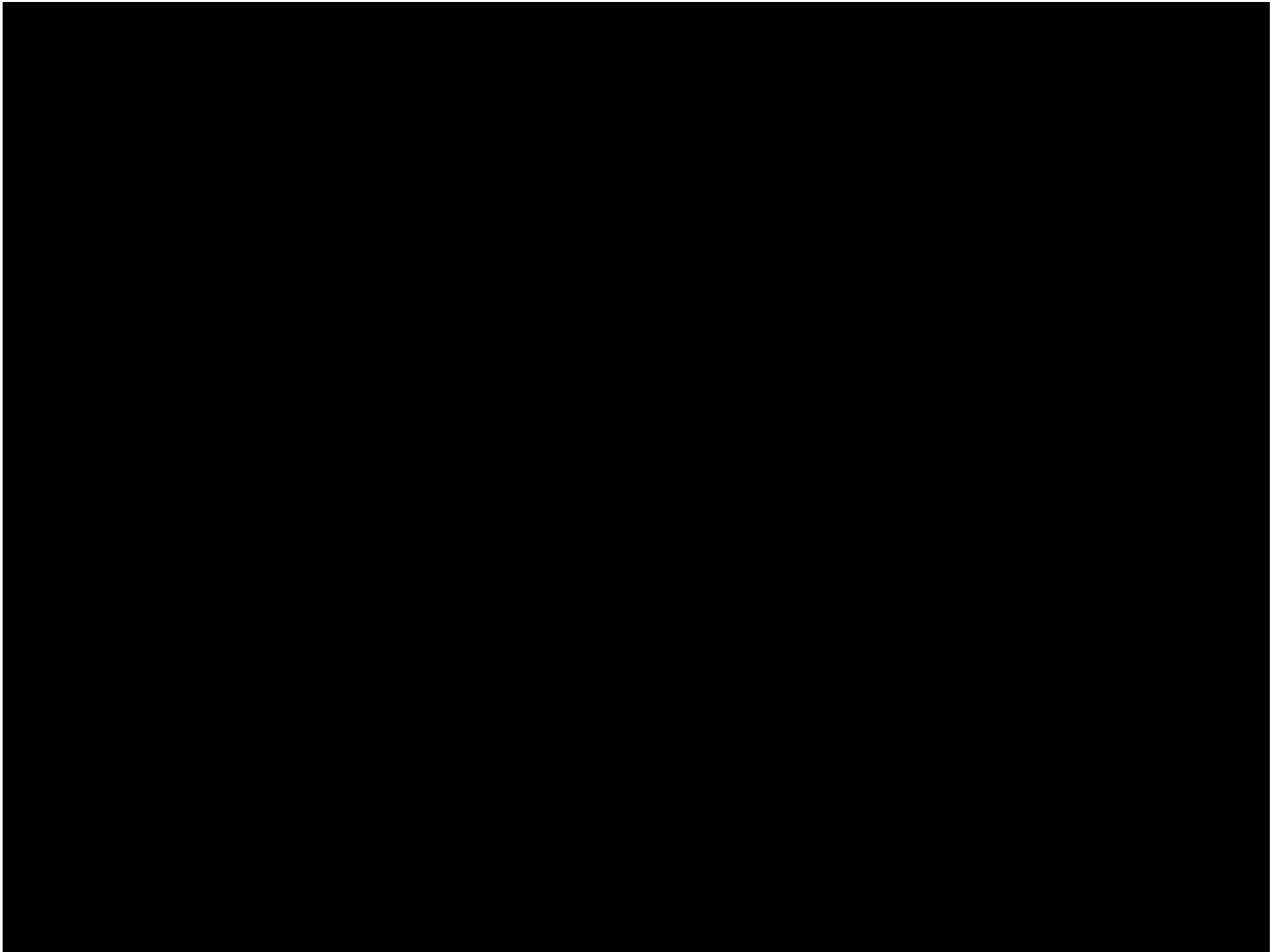
Lin P. Chen

Heart Rhythm Society

NHLBI

Donald W. Reynolds Foundation

Medtronic



Biopacemaker

- Rationale:
 - Demonstrable clinical need
 - Proximity to endocardium allows access by intracardiac injection
 - Phenotypic changes readily quantifiable
 - Adverse effects can be rescued by conventional electrophysiological methods

