Generation and Regulation of Cardiac Pacemaker Activity

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Master 1 D2HP
Dec 10th, 2024

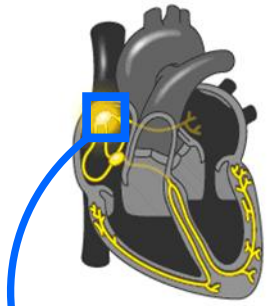
Outline

Cardiac Conduction System

Cardiac Action Potentials

Electrical Coupling of Myocytes: Gap Junctions

In vivo
Heart (Organ)

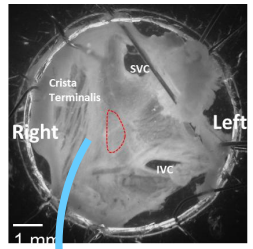


Sinoatrial Node (SA Node): Description

Cell types in the Rabbit SA node

Two conceptual models for the sinoatrial transition

Ex vivo
SA node (Tissue and Cells)

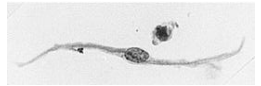


Genesis of cardiac automaticity

- Voltage Clock
- Ca^{2+} Clock

Autonomic Regulation of Cardiac Automaticity

- Sympathetic Regulation of Pacemaker Activity
- Parasympathetic Regulation of Pacemaker Activity



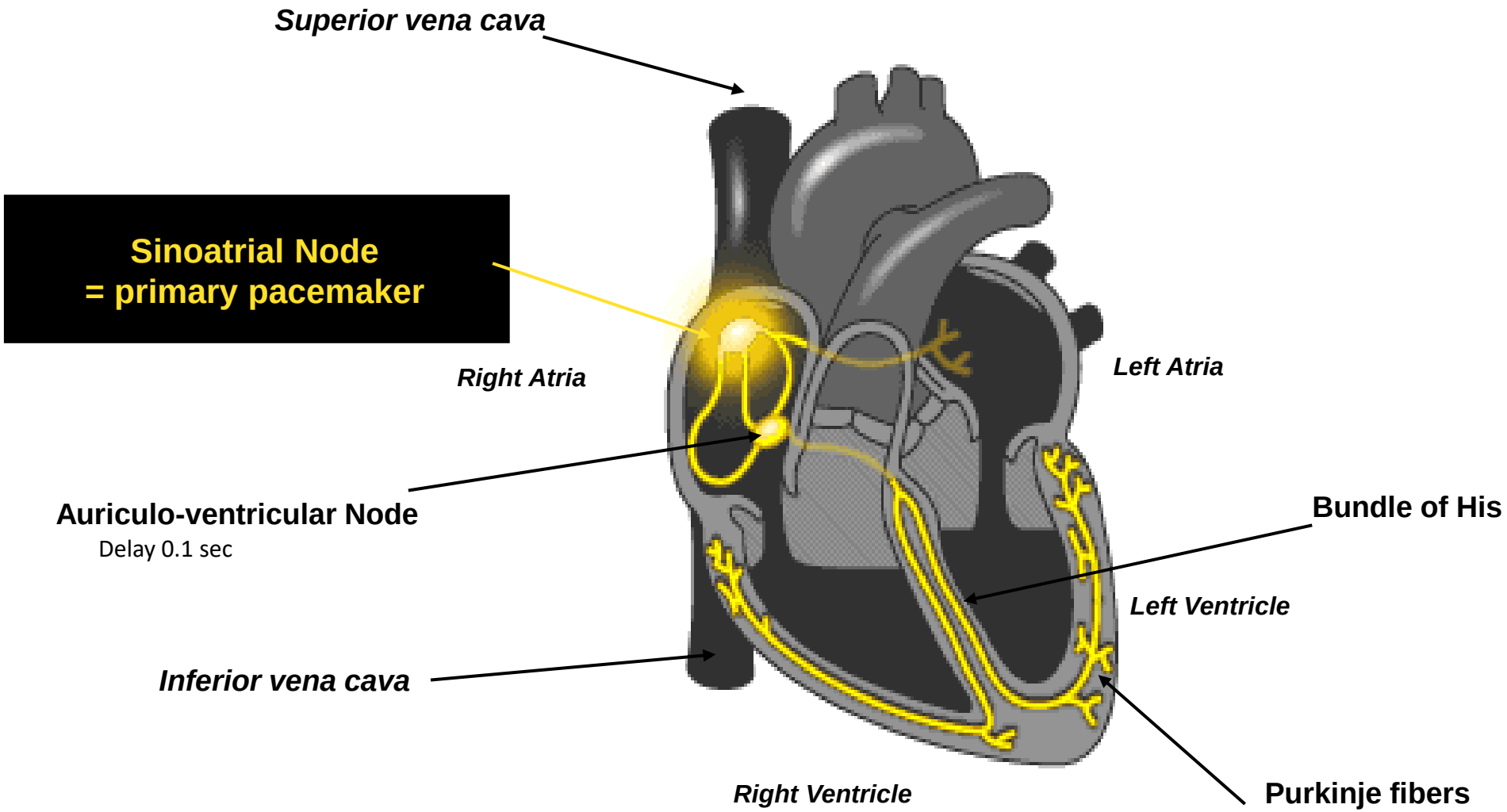
In cellulo
Generation and regulation of Action Potential (Cells)

Focus 1: I_f current – HCN channels

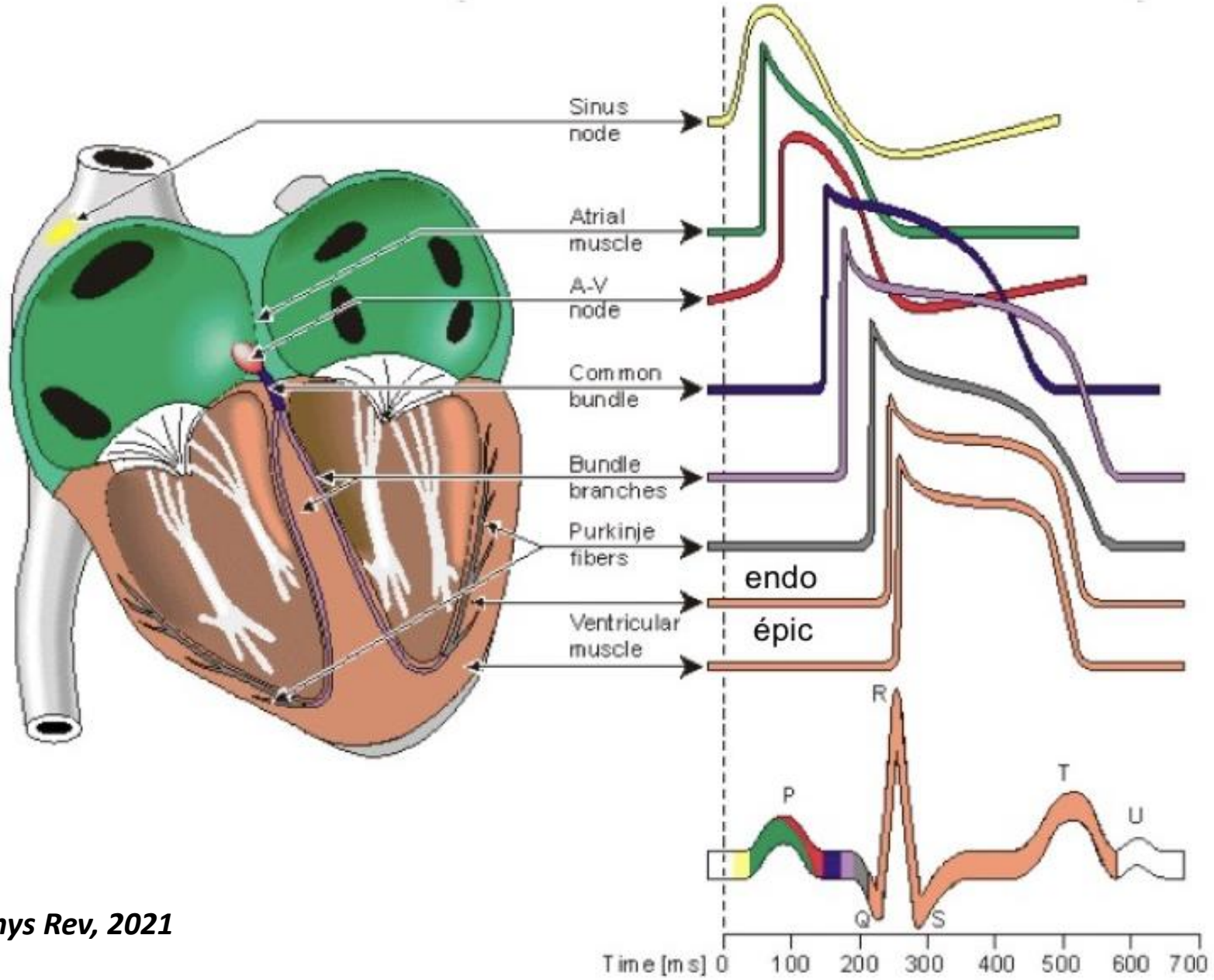
Focus 2: $I_{Ca,L}$ current – $Ca_V1.3$ channels

Focus 3: Ryanodine receptor

Cardiac Conduction System

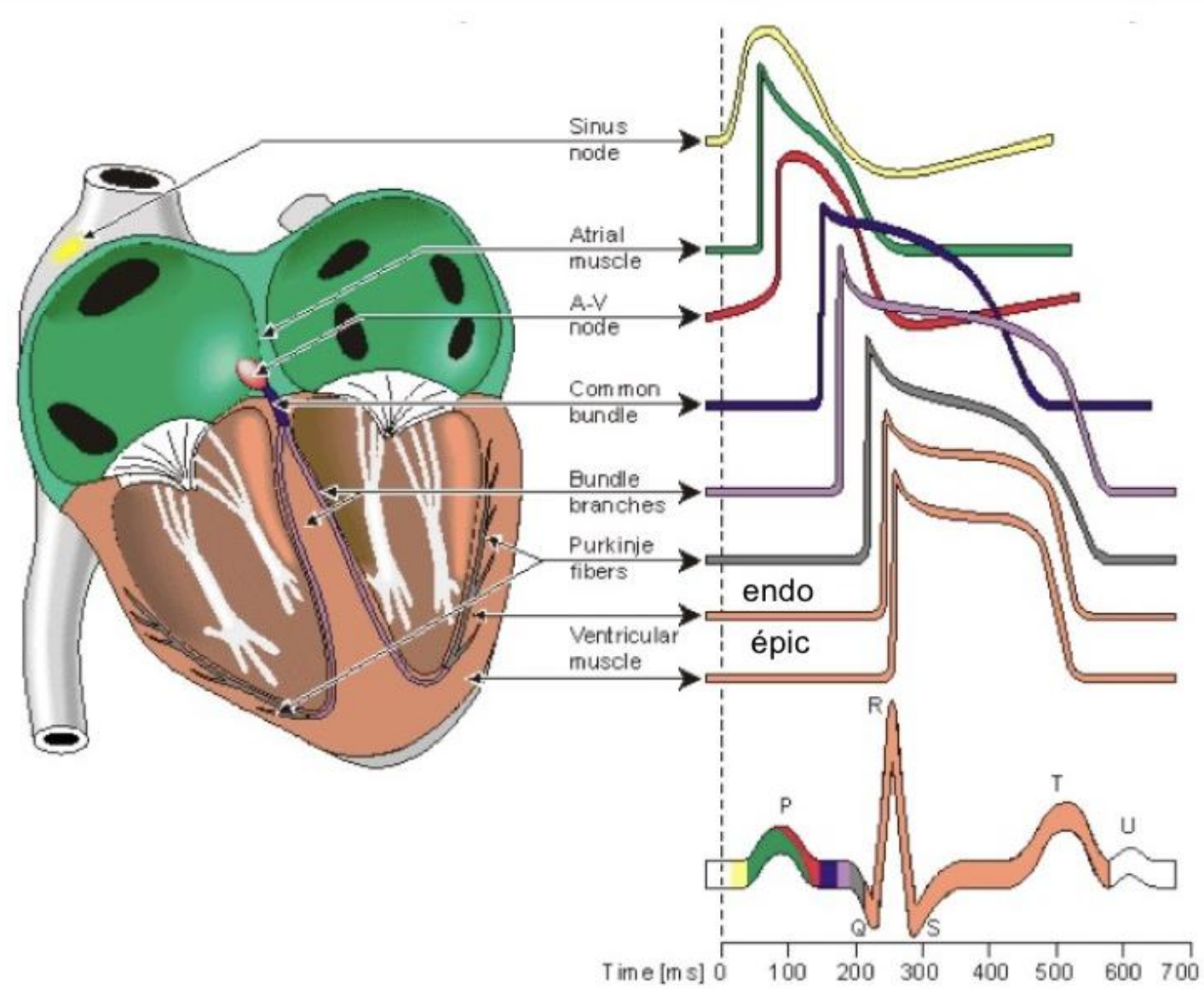


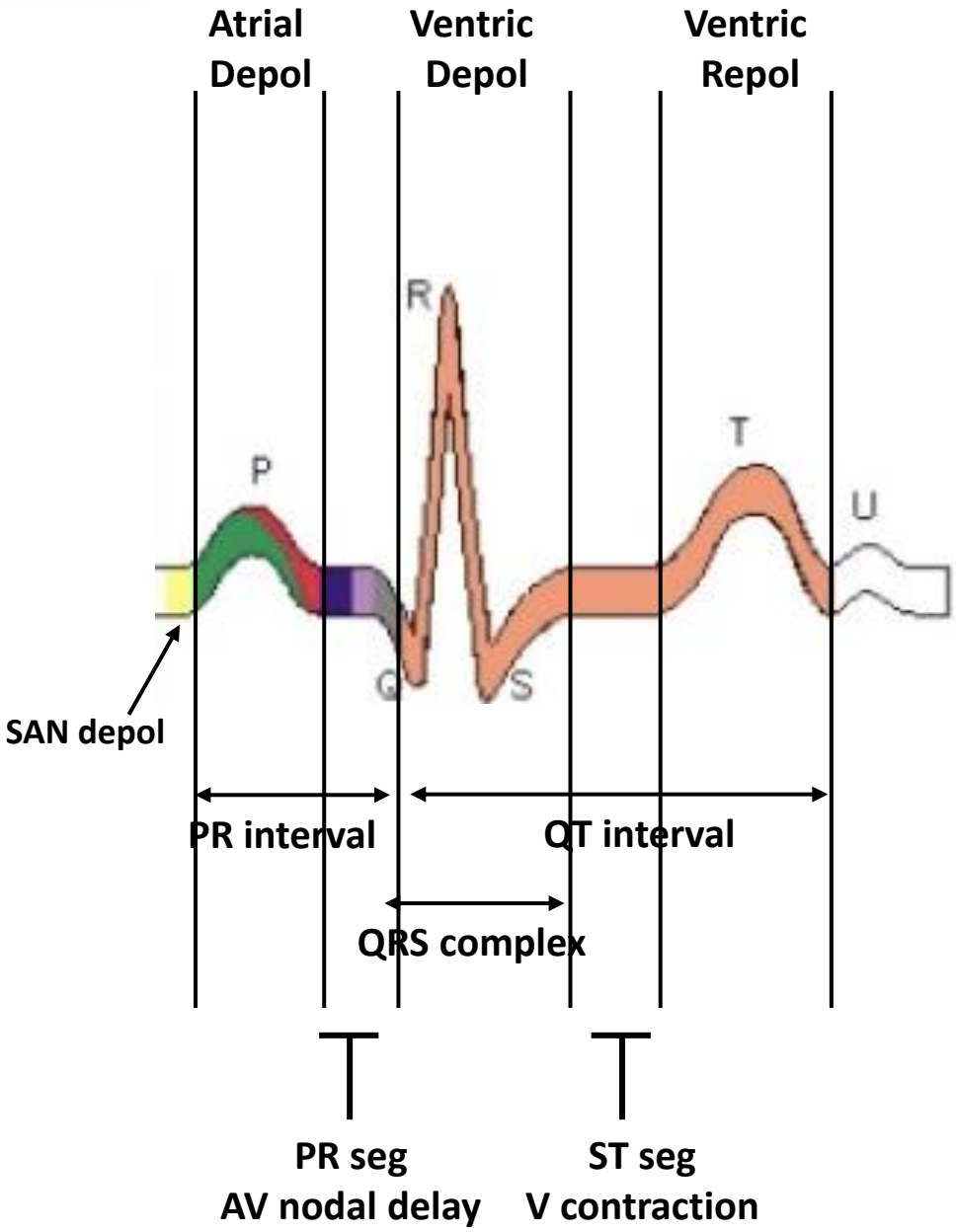
Cardiac Action Potentials



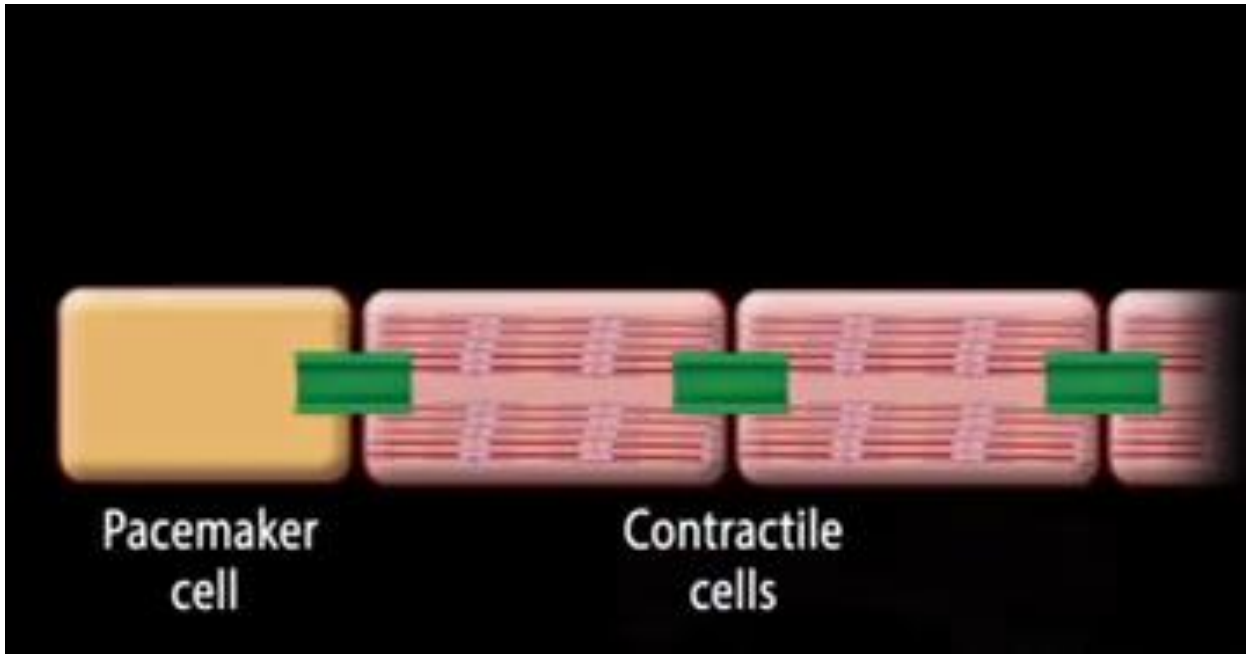
Varro et al. Phys Rev, 2021

Cardiac Electrocardiogram (ECG)





Electrical Coupling of Myocytes: Gap Junctions



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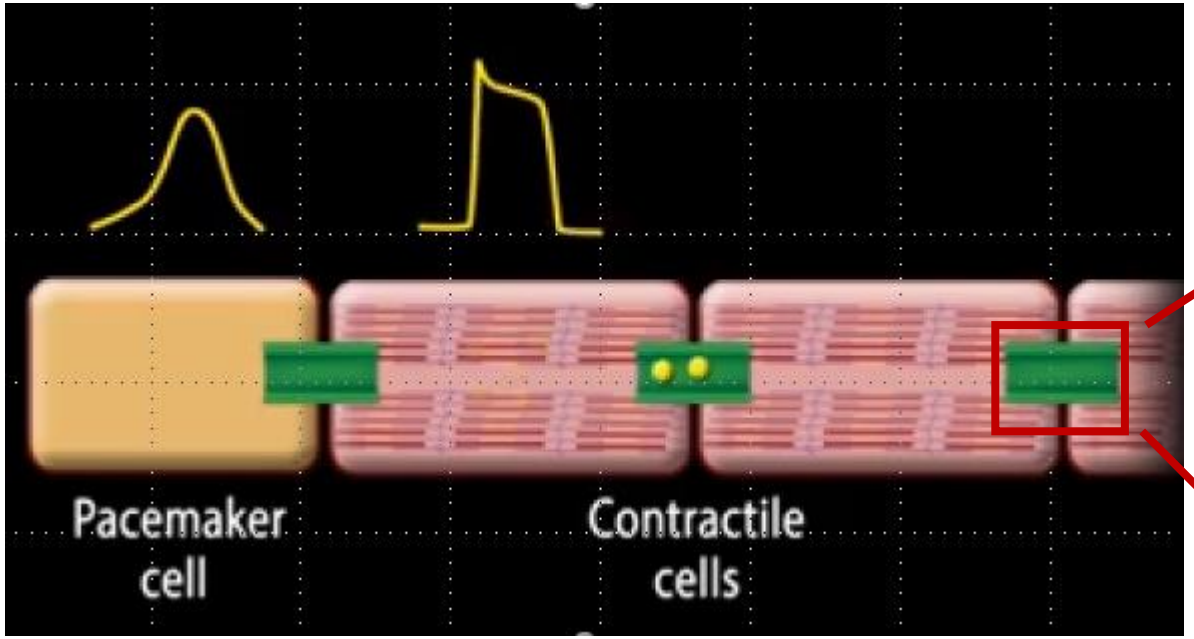
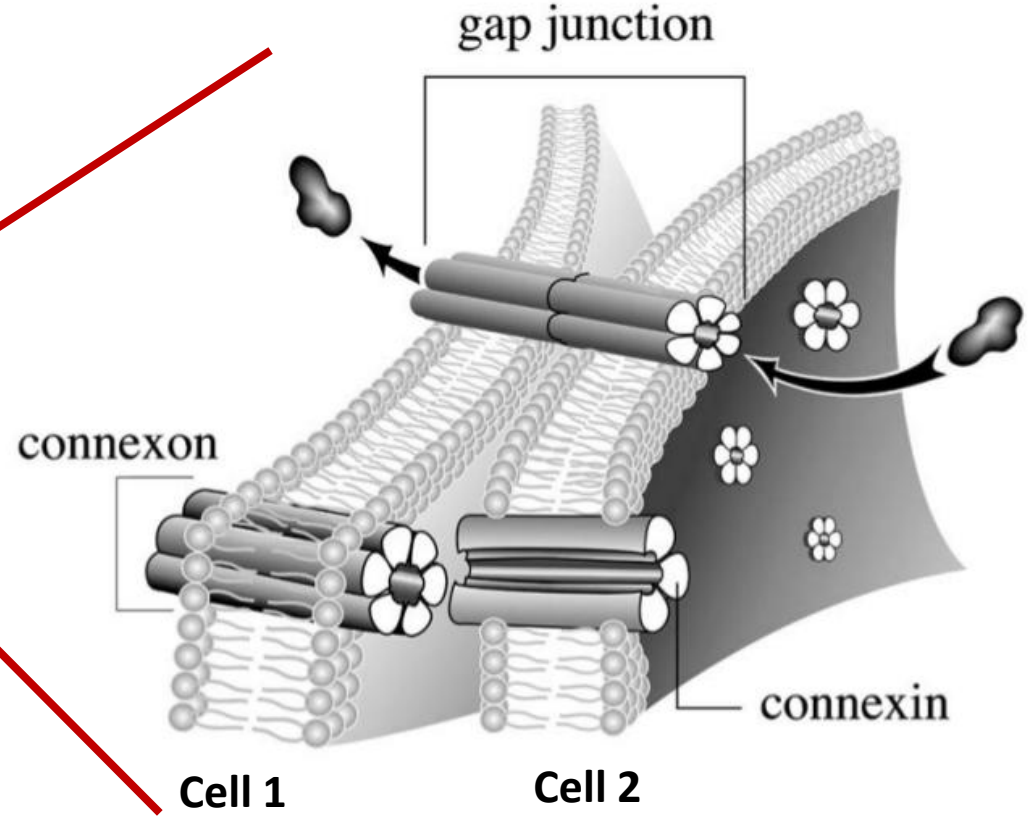
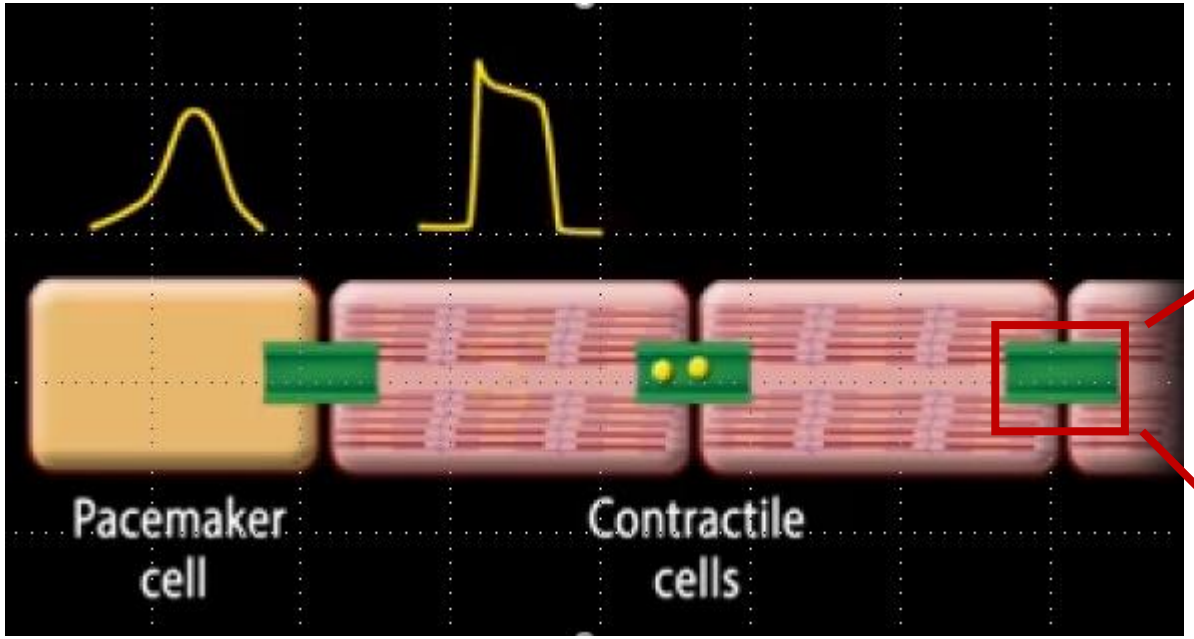


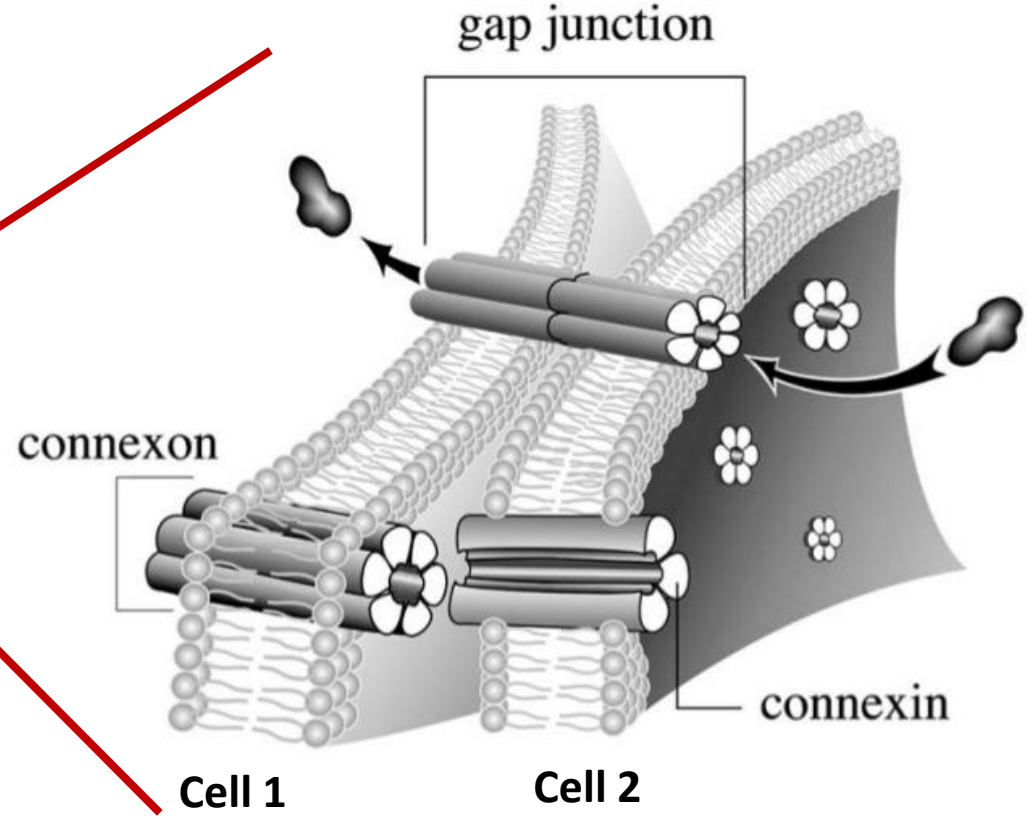


Gap Junctions

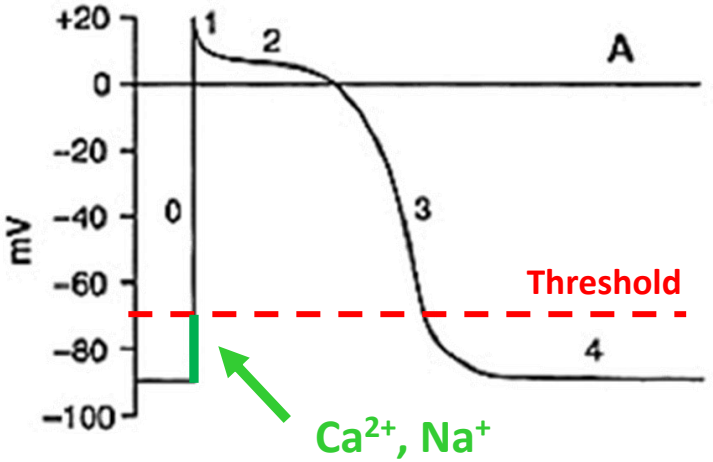
➔ Large-conductance pores permeable for ions and small molecules
➔ 2 sets of 6 subunits = connexons
➔ Electrical coupling of myocytes
➔ Connexin: several isoforms (cell type specificity)

Electrical Coupling of Myocytes: Gap Junctions





Ventricular Action Potential



Gap Junctions

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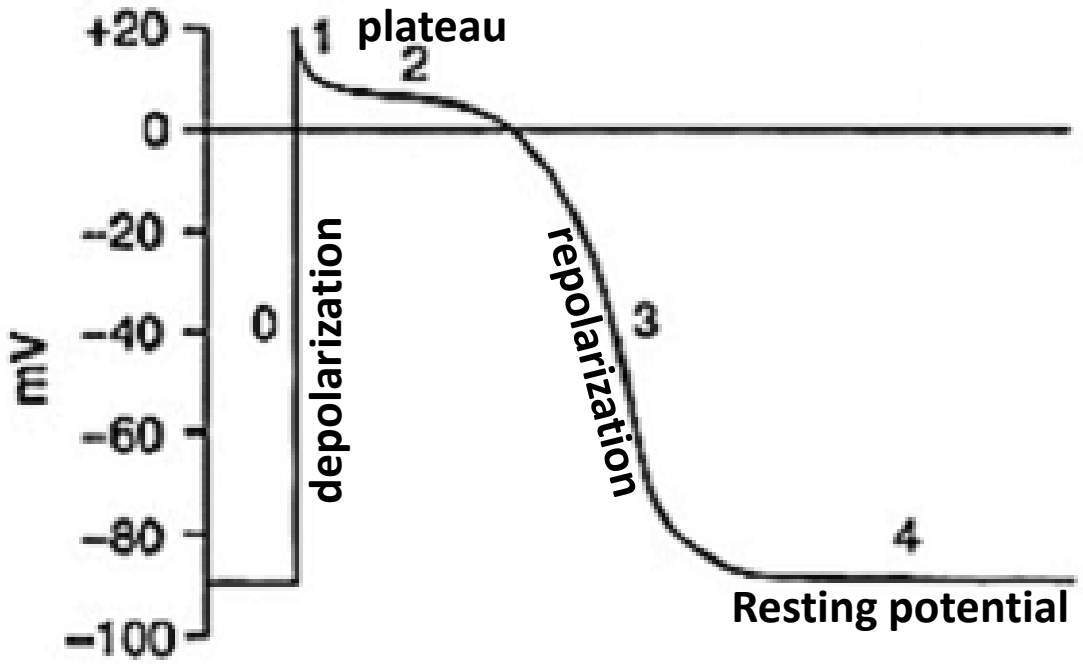
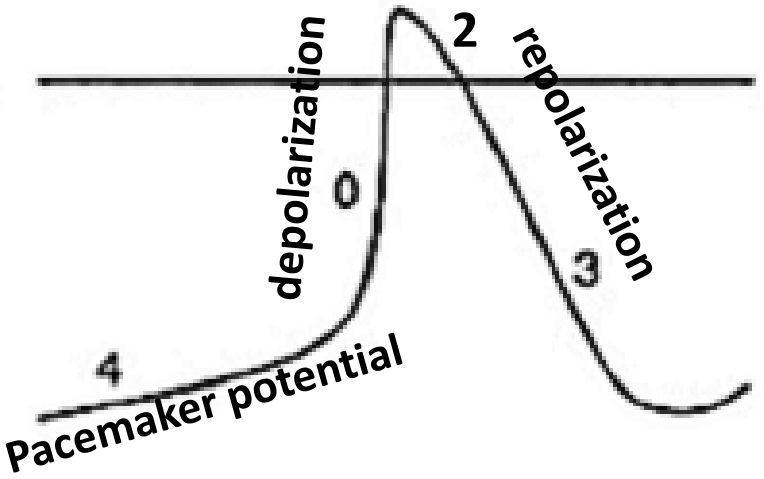
Ventricular AP *vs* Nodal AP

Ventricular Myocardium



« Sodium AP »

Sinus Node



« Calcium AP »

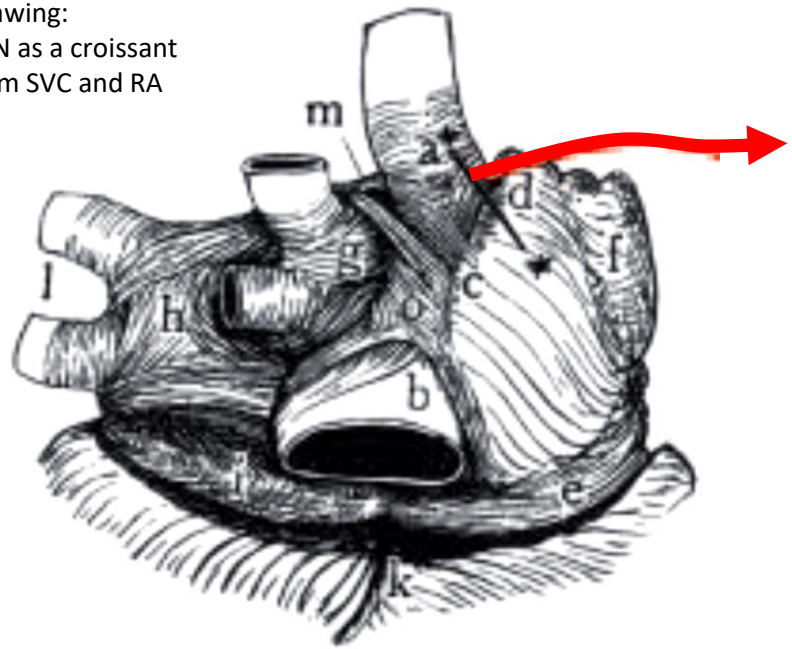
⇒ *R. Perrier course*
Nov 19th 2024

No resting potential
Pacemaker potential = phase 4
Absence of phase 1

Sinoatrial Node (SA Node): Description

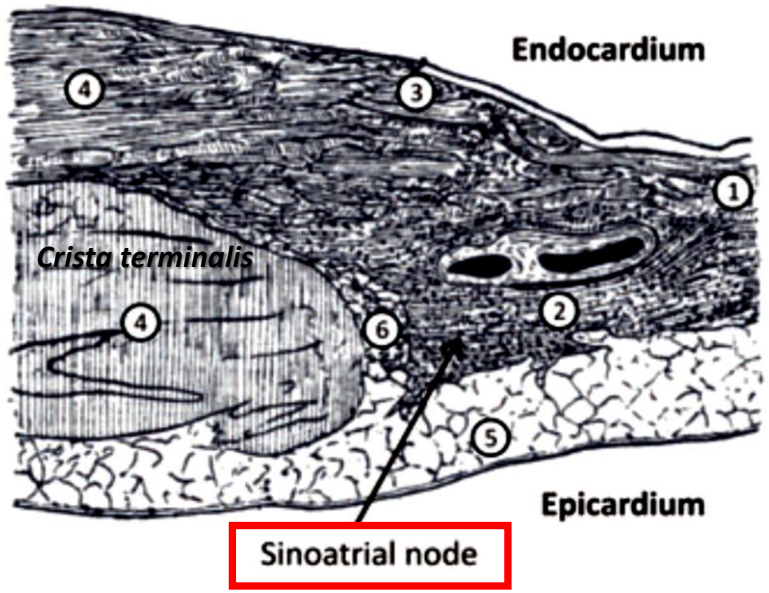
Discovered by Keith & Flack in 1907
Point of initial cardiac excitation by Wybau and Lewis 1910

Drawing:
SAN as a croissant
from SVC and RA



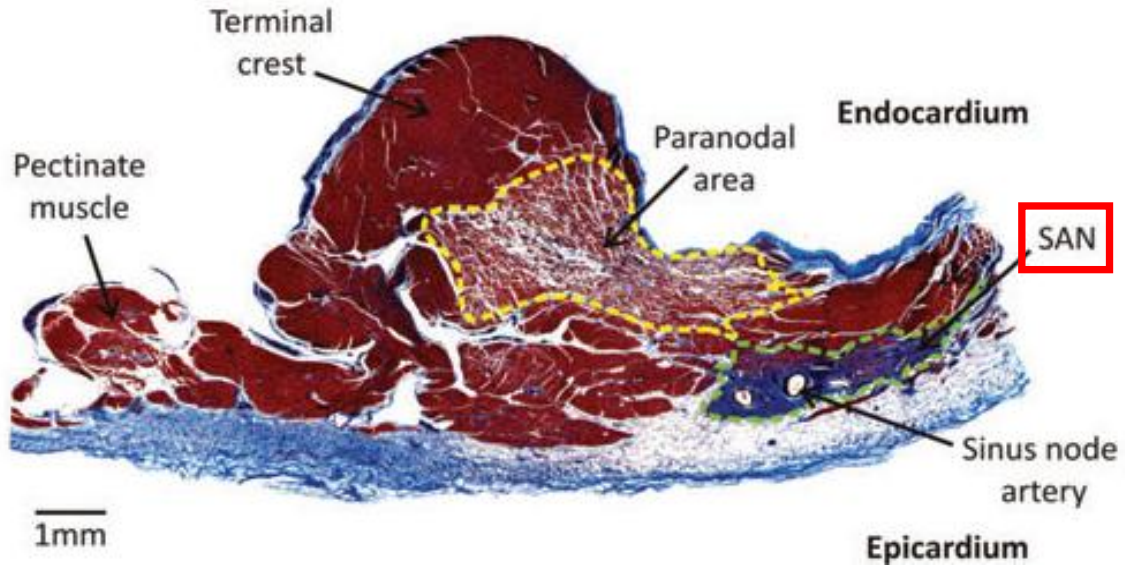
a, superior caval vein
b, inferior caval vein
c, terminal crest
i, coronary sinus
k, base of ventricles
l, left pulmonary veins

Keith & Flack J Anat Physiol 1907



1 - musculature of superior vena cava
2 - artery and surrounding musculature at sino-auricular junction
3 - position of venous valve
4 - auricular muscle (crista terminalis)
5 - subepicardial tissue/fat
6 - connective tissue between sinus and auricle

Keith & Flack J Anat Physiol 1907



Human Heart

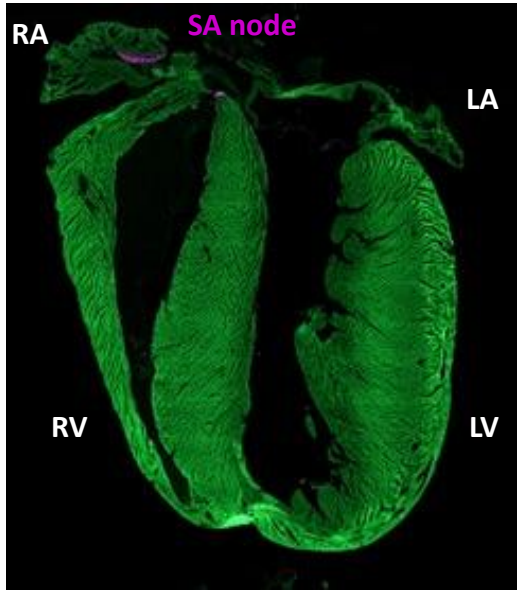
Myocytes
Dense Connective Tissue

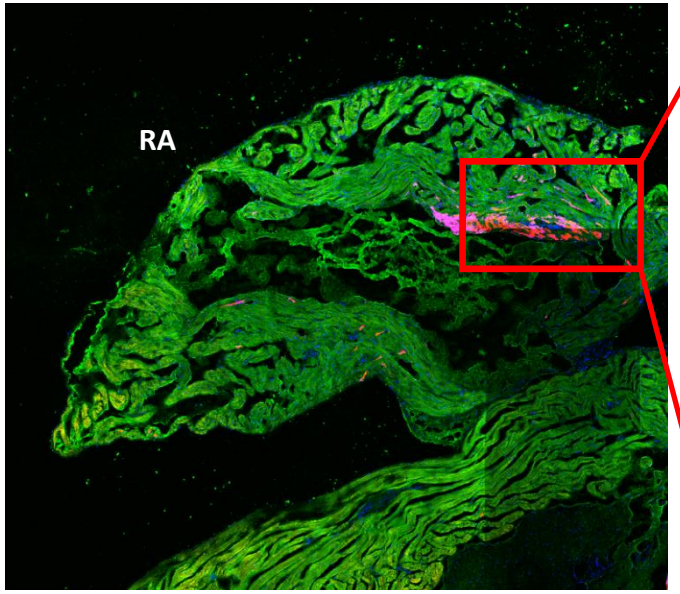
Chandler et al. Circulation 2009

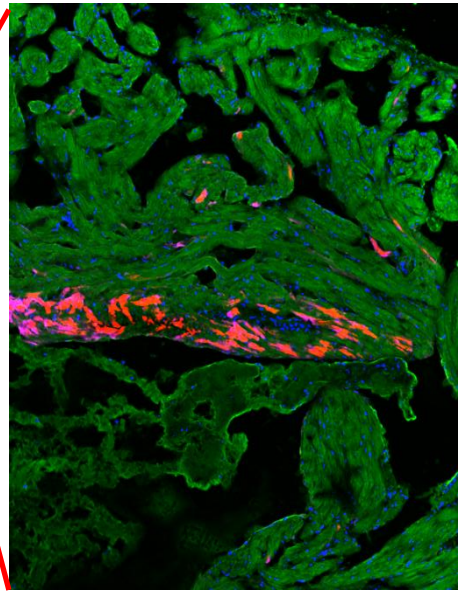
Sinoatrial Node (SA Node): Description

(Mezzano et al. Cardiovasc Res, 2016)

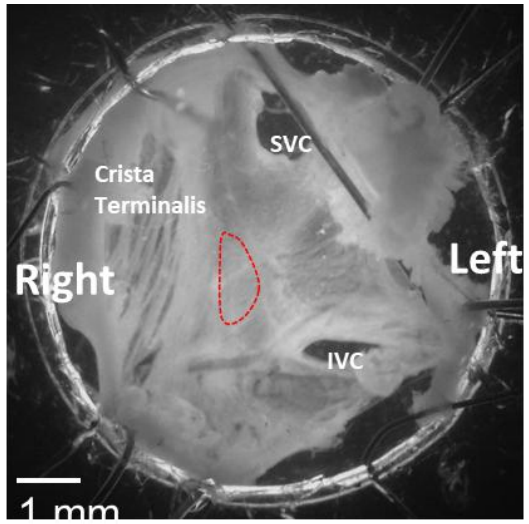
HCN4Cre-ERT2-tomato mice **HCN=marker of automatic centers**





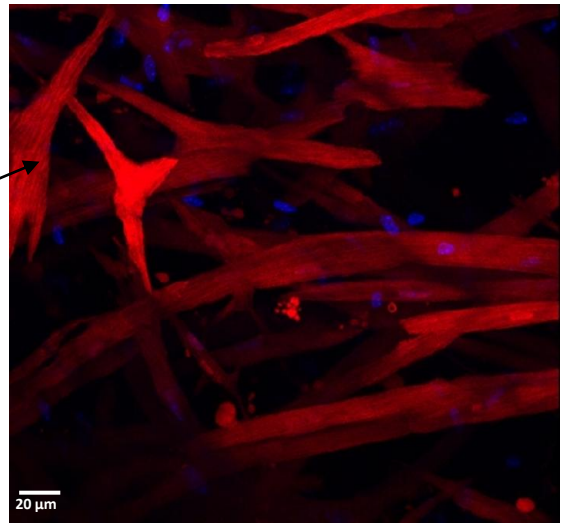


Staining
Blue: DAPI
GFP: MF20
Cy3: Endogenous Tomato
Cy5: Tomato



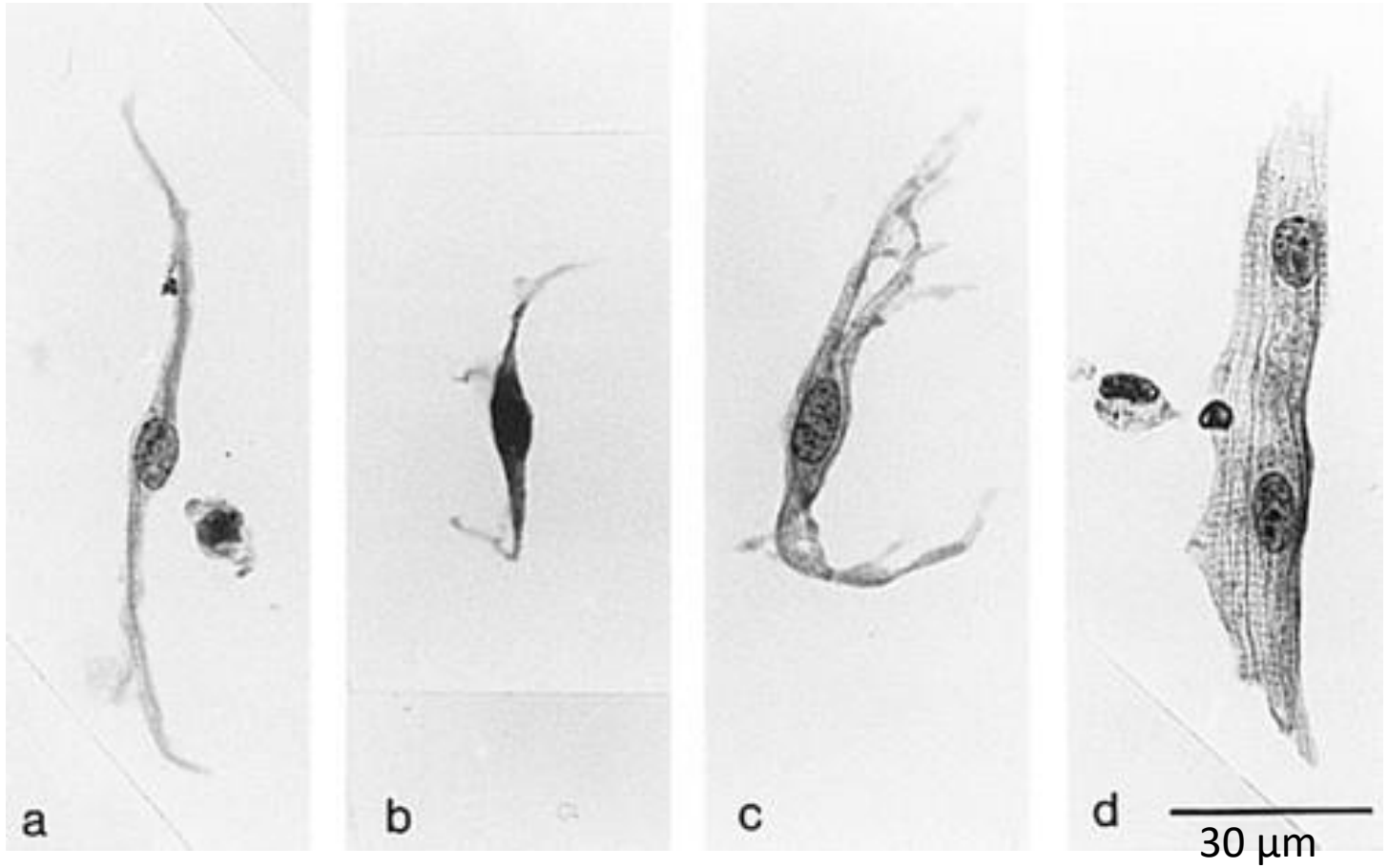
Pacemaker cells within the tissue

Confocal Imaging



Delphine Mika (Orsay)
Francesca Rochais (Marseille)

Cell types in the Rabbit SA node

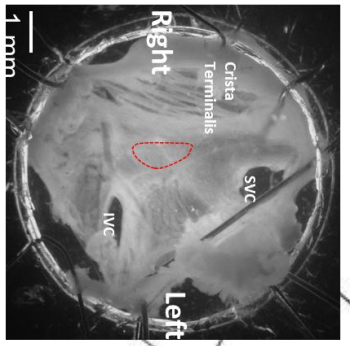


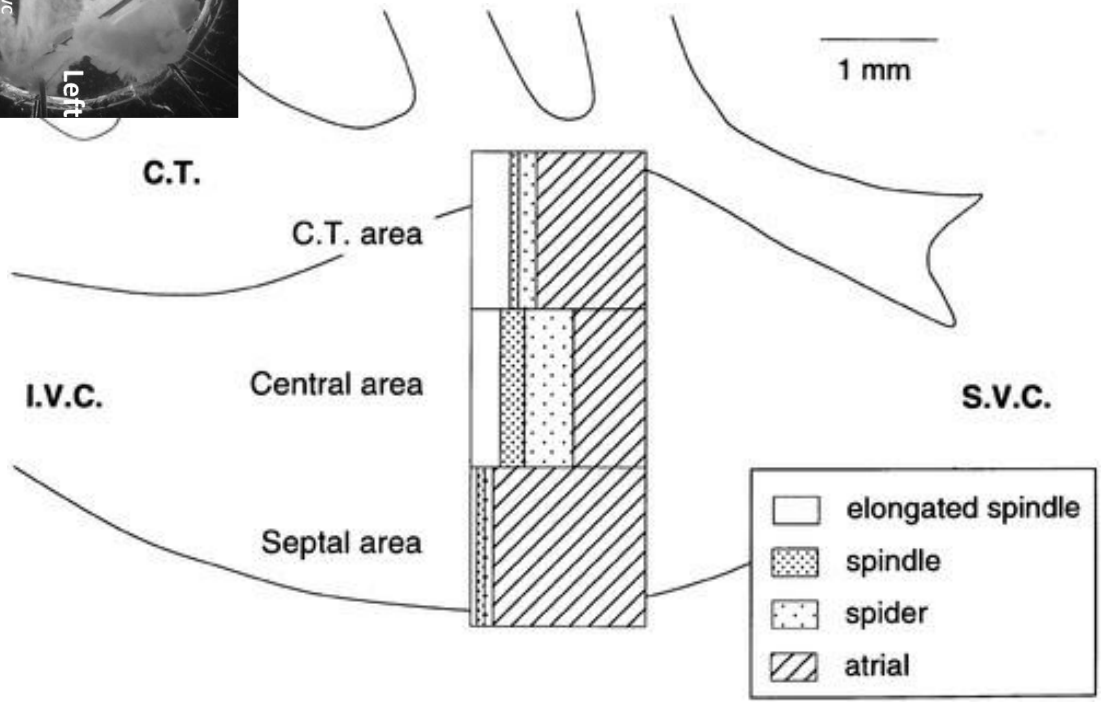
Elongated spindle cell | **Spindle cell** | **Spider cell** | **Non nodal Atrial cell**

Verheijck et al. Circulation 1998

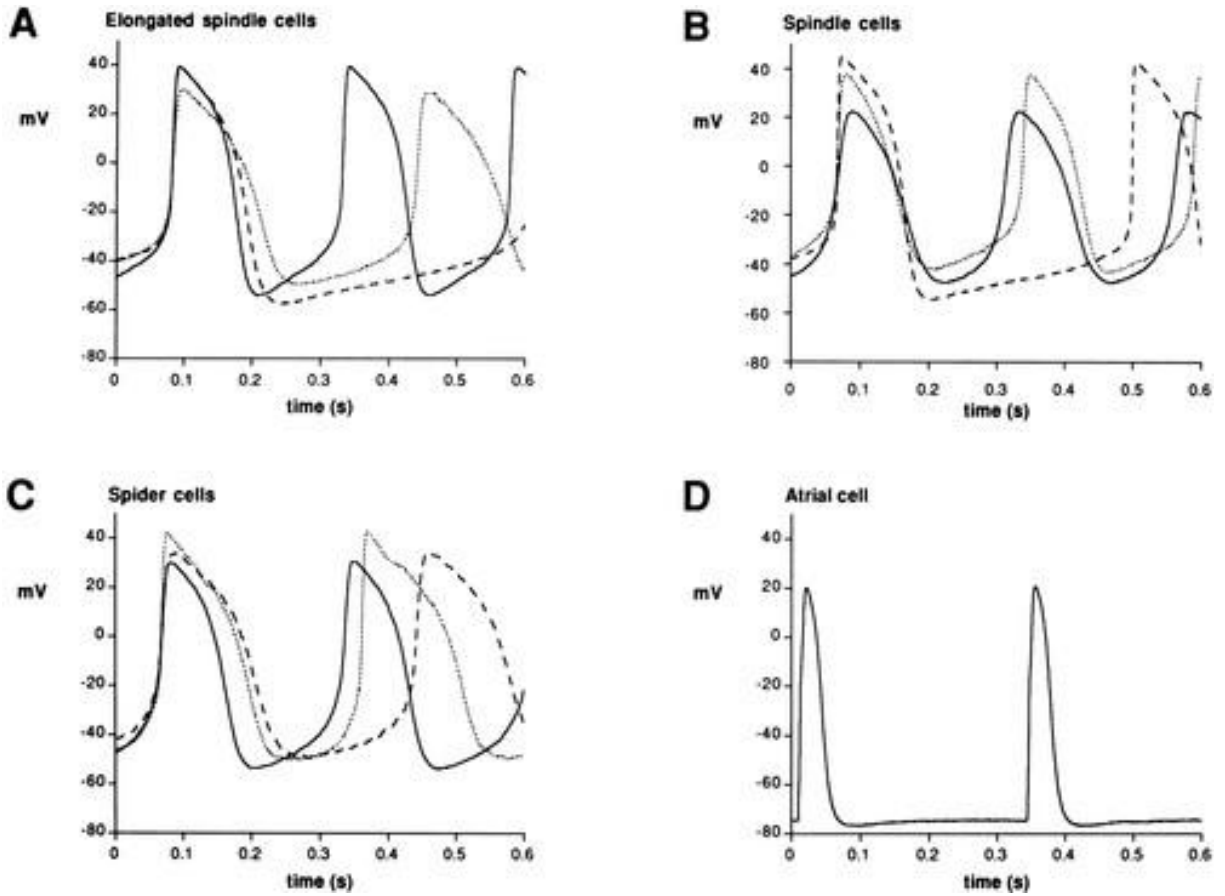
Cell types in the Rabbit SA node

Relative contribution of the four different cell types in the sinoatrial node



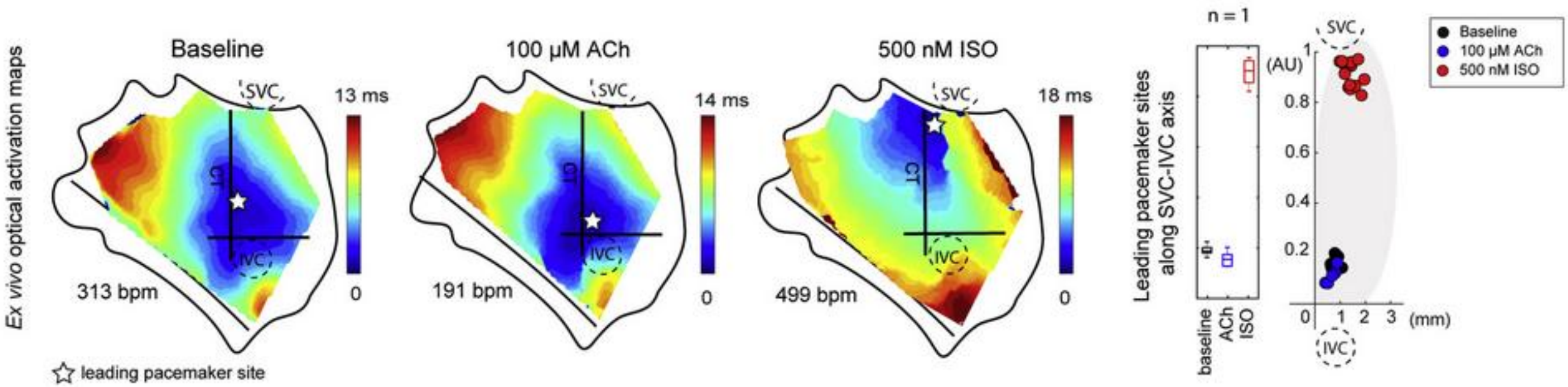


Action potentials of morphologically different nodal cells



Verheijck et al. Circulation 1998

Sinoatrial Node (SA Node): leading pacemaker site



Ach = Acetylcholine

ISO = Isoprenaline

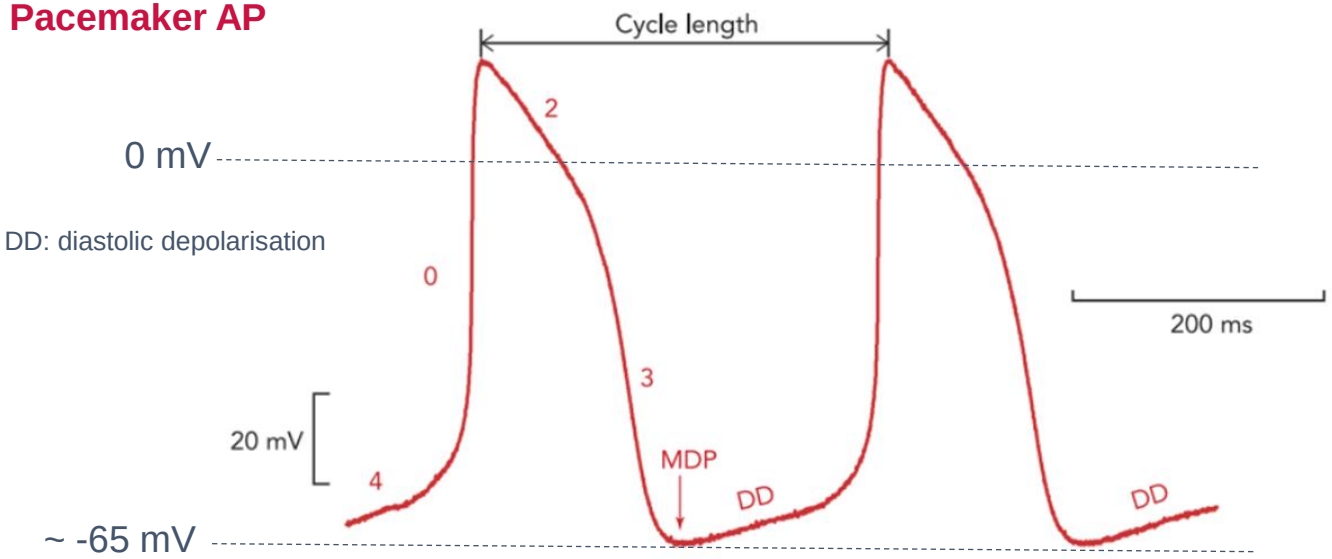
Pacemaker Shift in response to **ANS modulation** or in **pathological context**

Voltage Fluorescent probe : di-4-ANEPPS

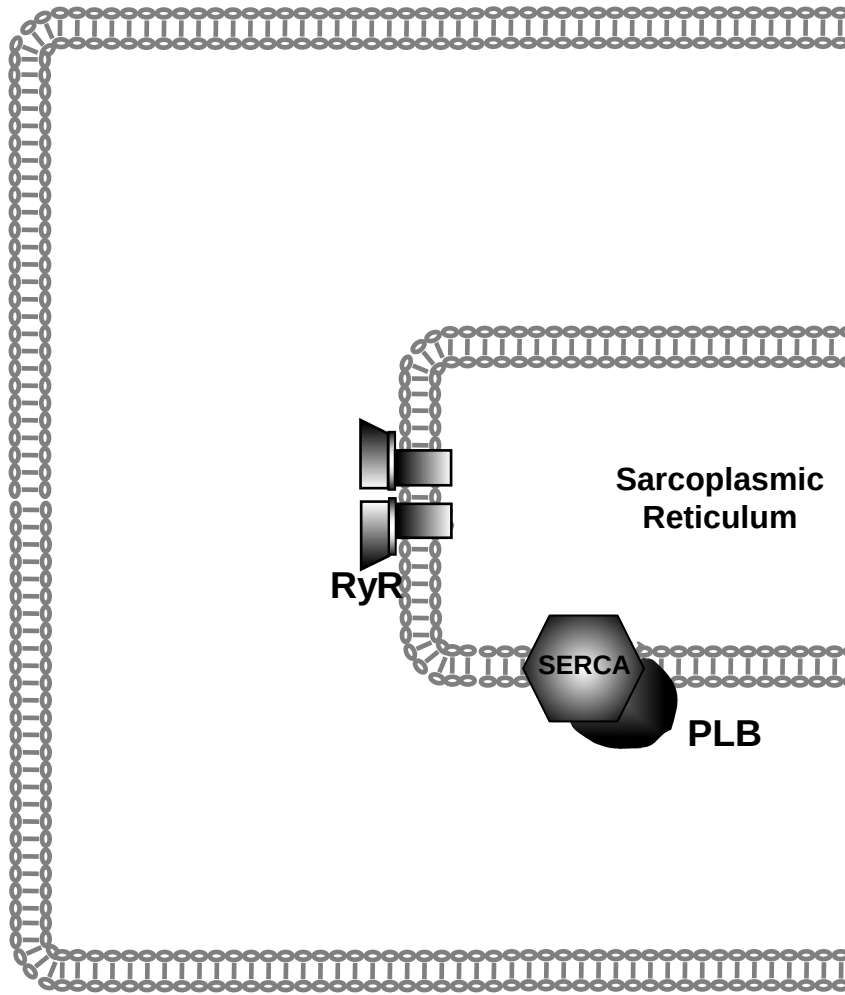
Brennan et al. JACC: clin electrophysiol 2020

Generation of pacemaker activity

Pacemaker AP



SA node cell



Modifié de Monfredi et al., Physiology, 2013

Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

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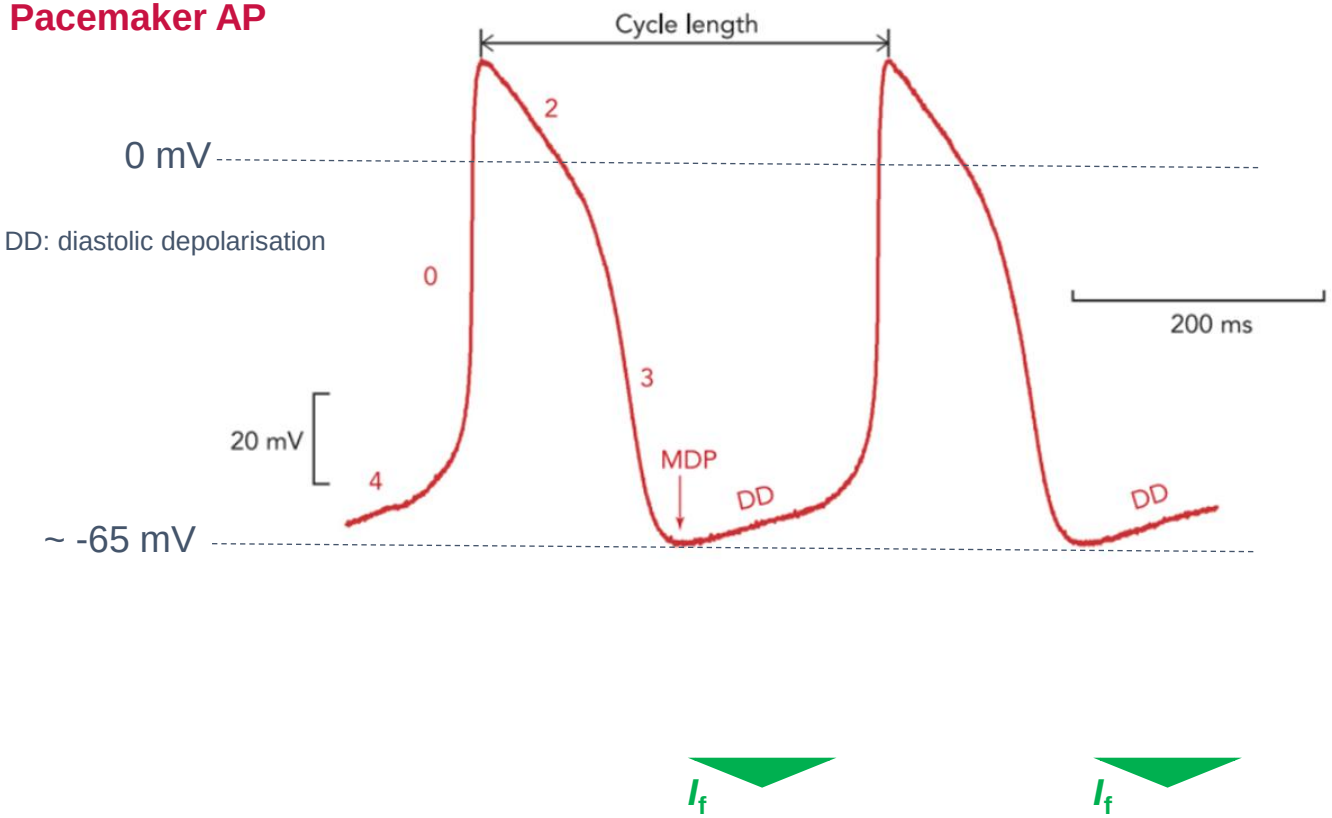
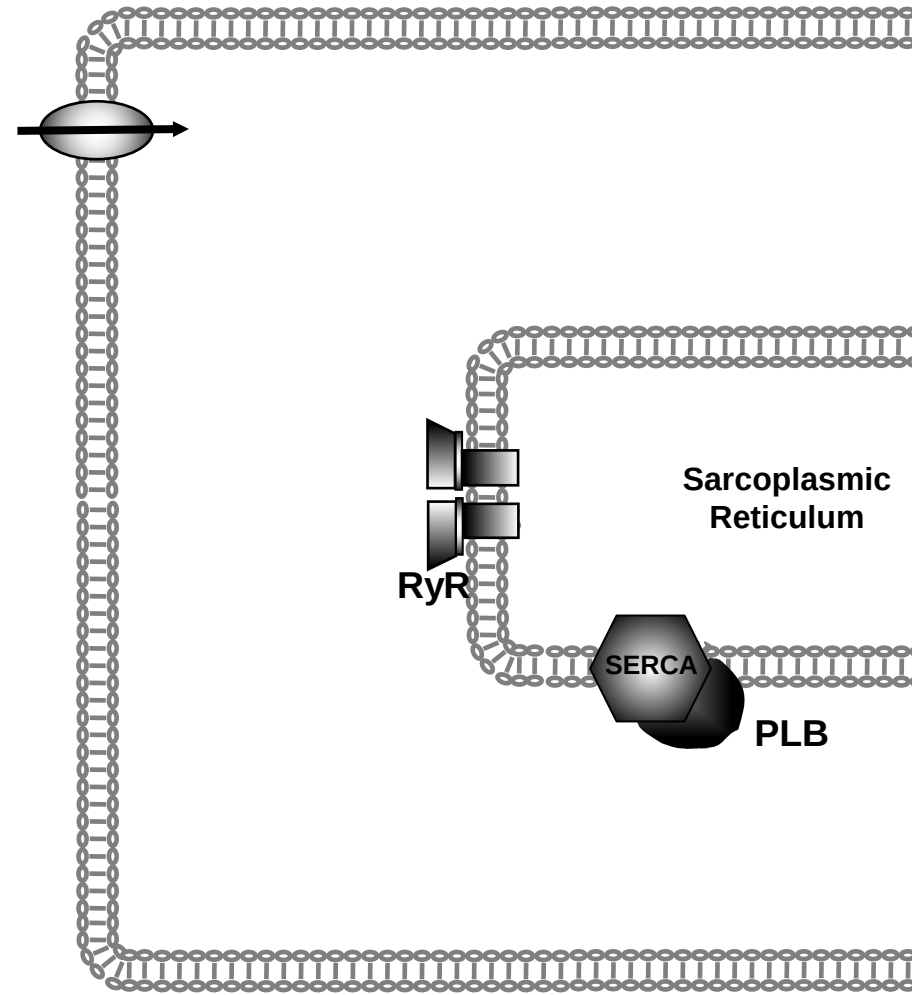


HCN2
HCN4 I_f

SA node cell



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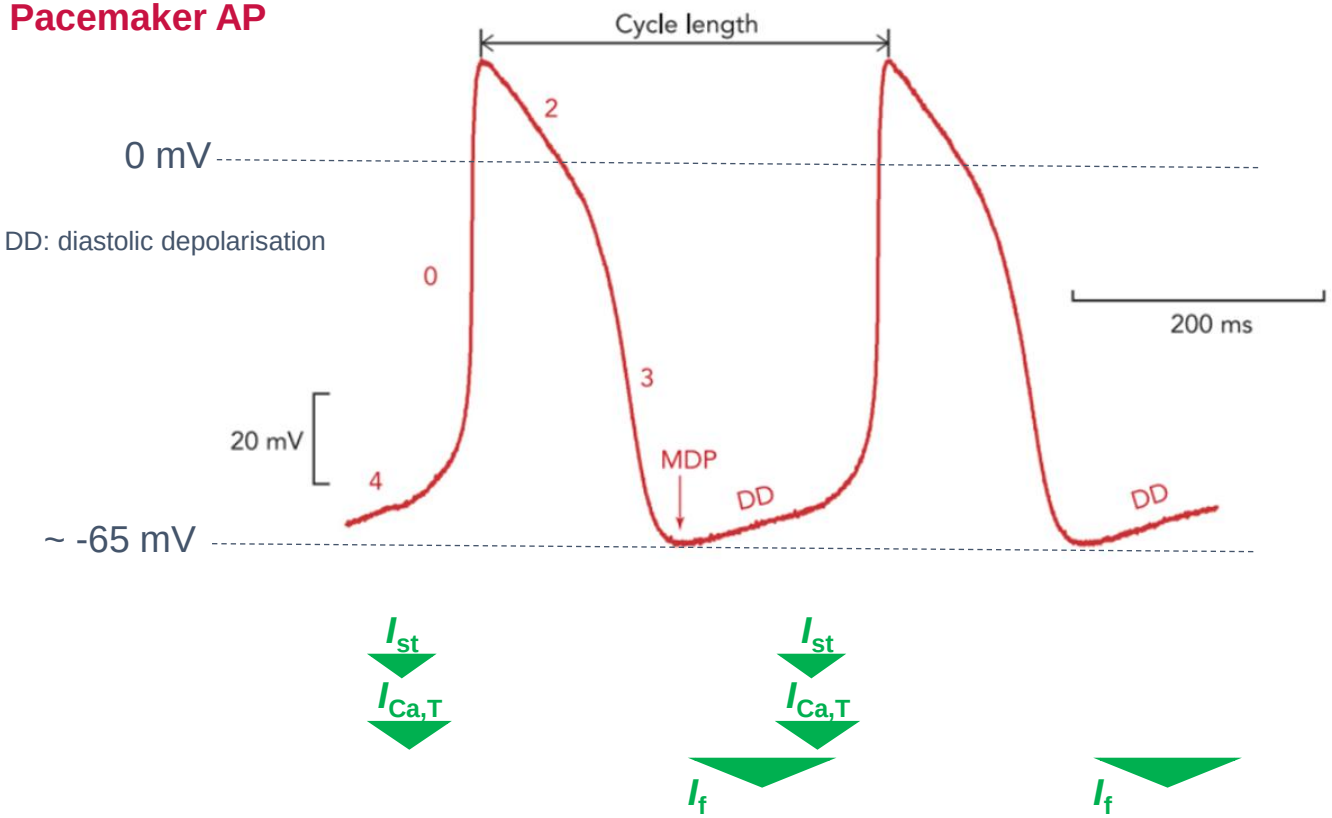

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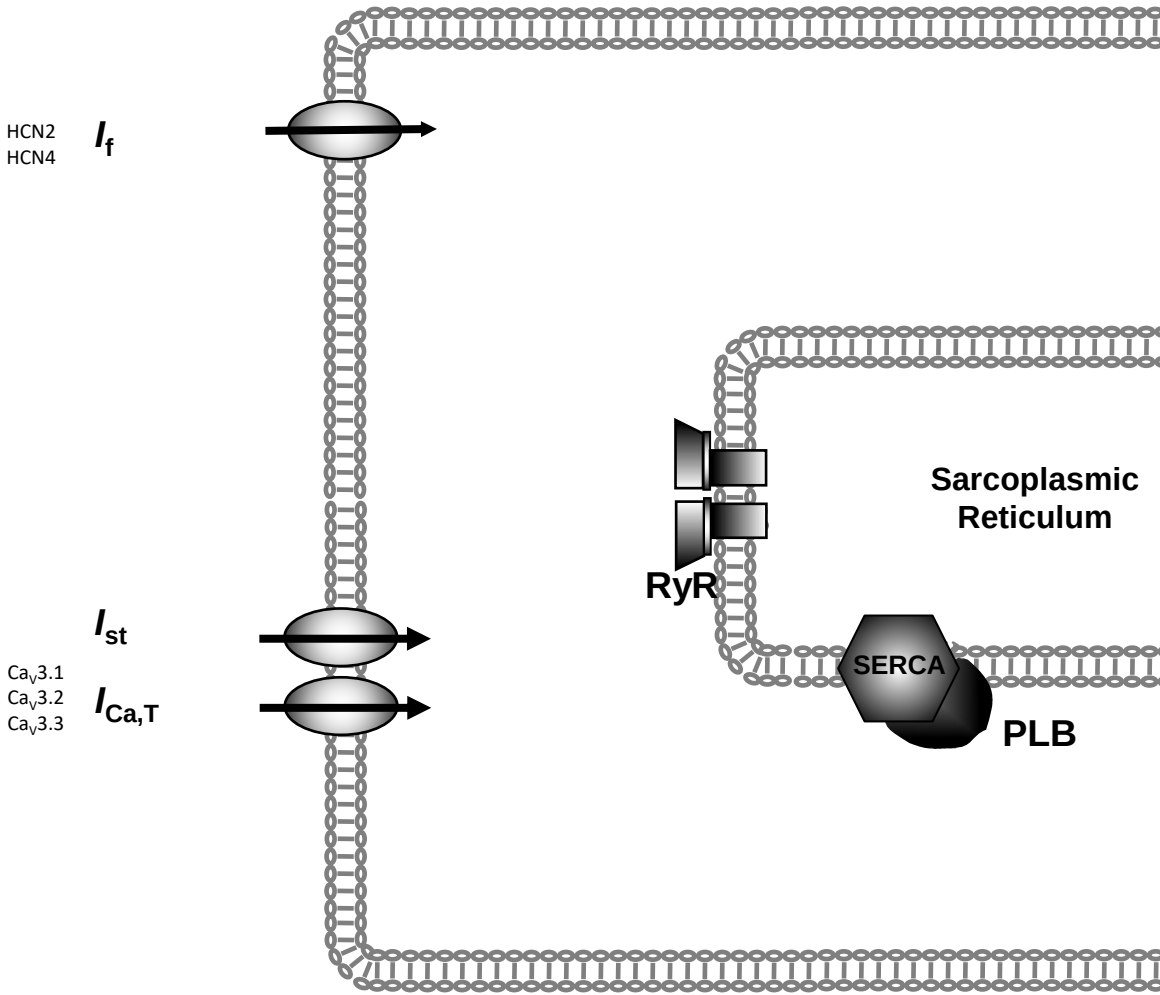

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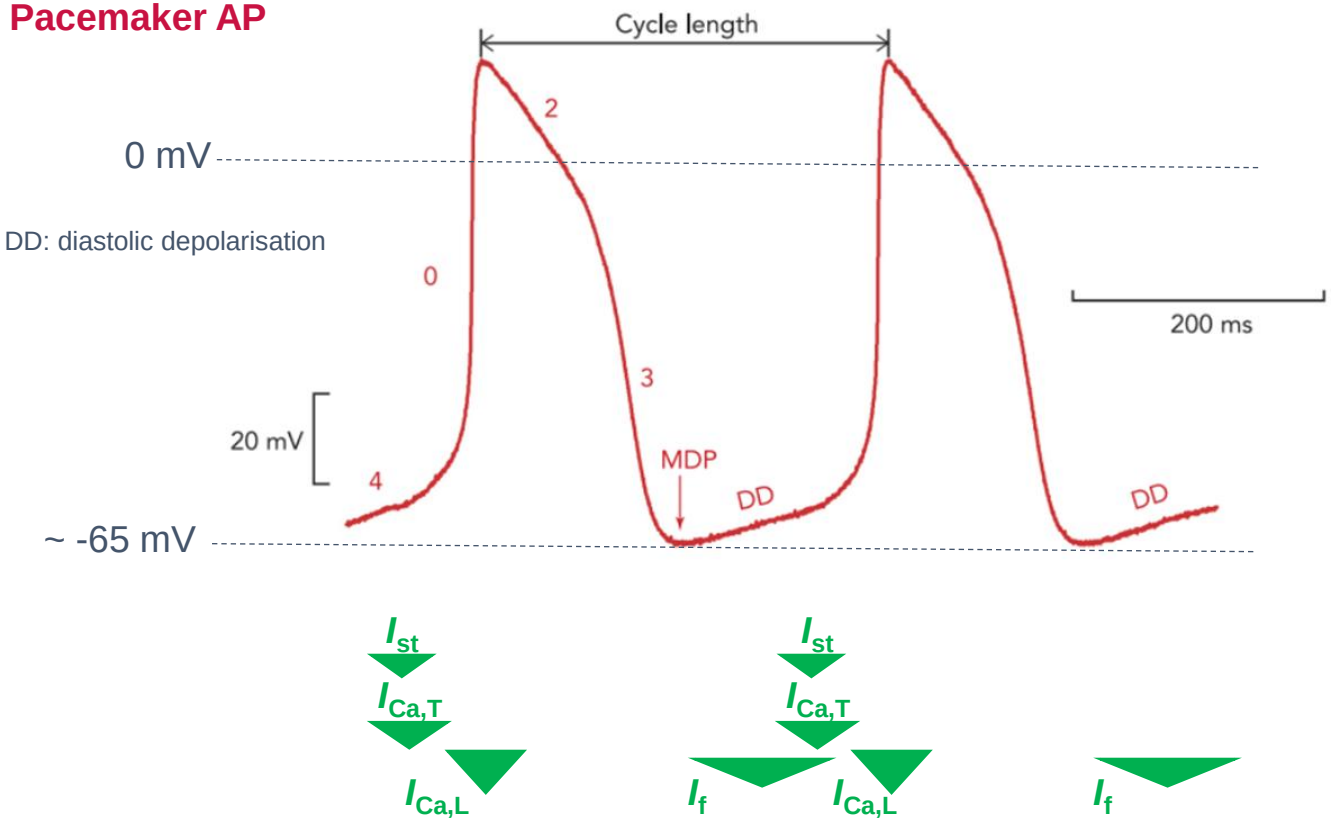

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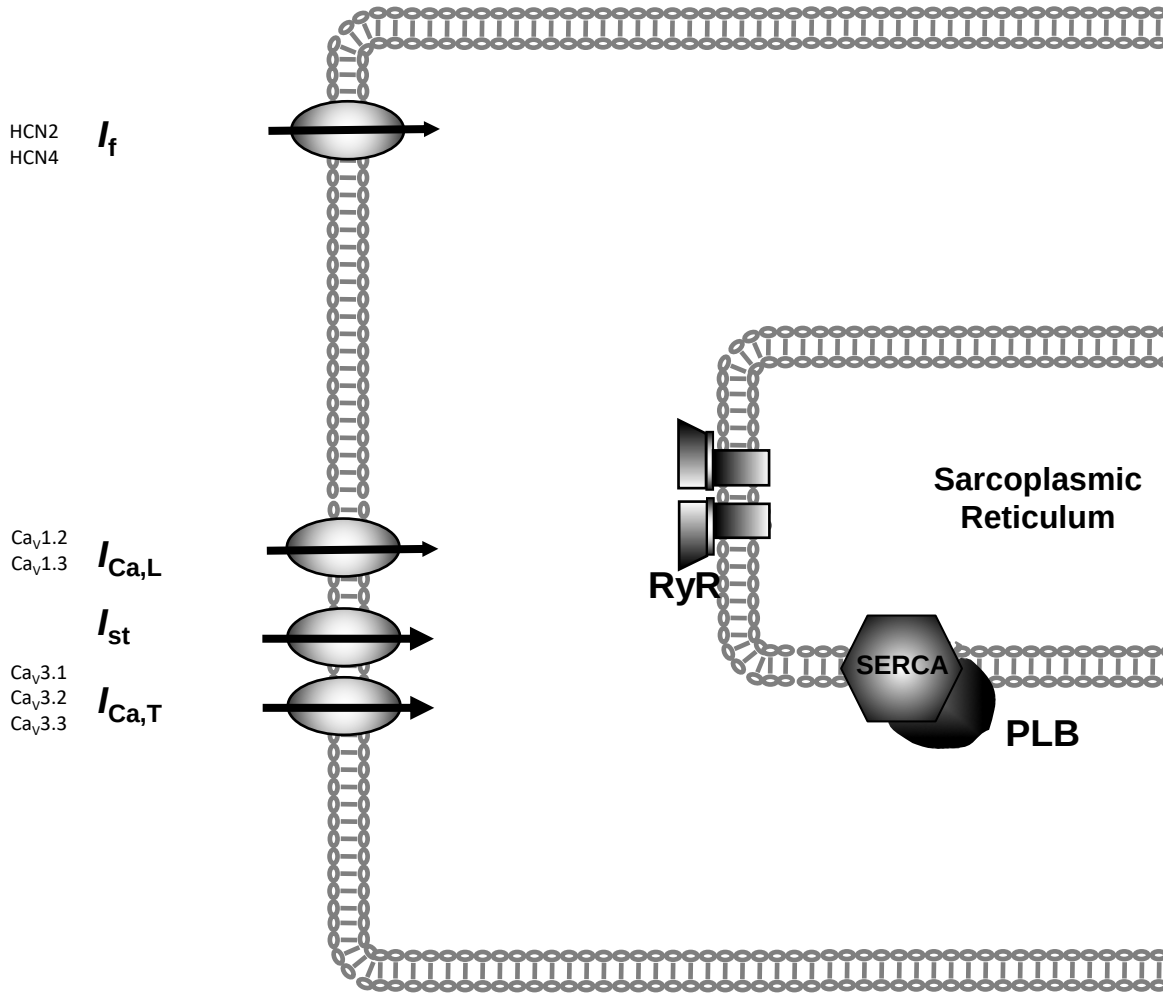

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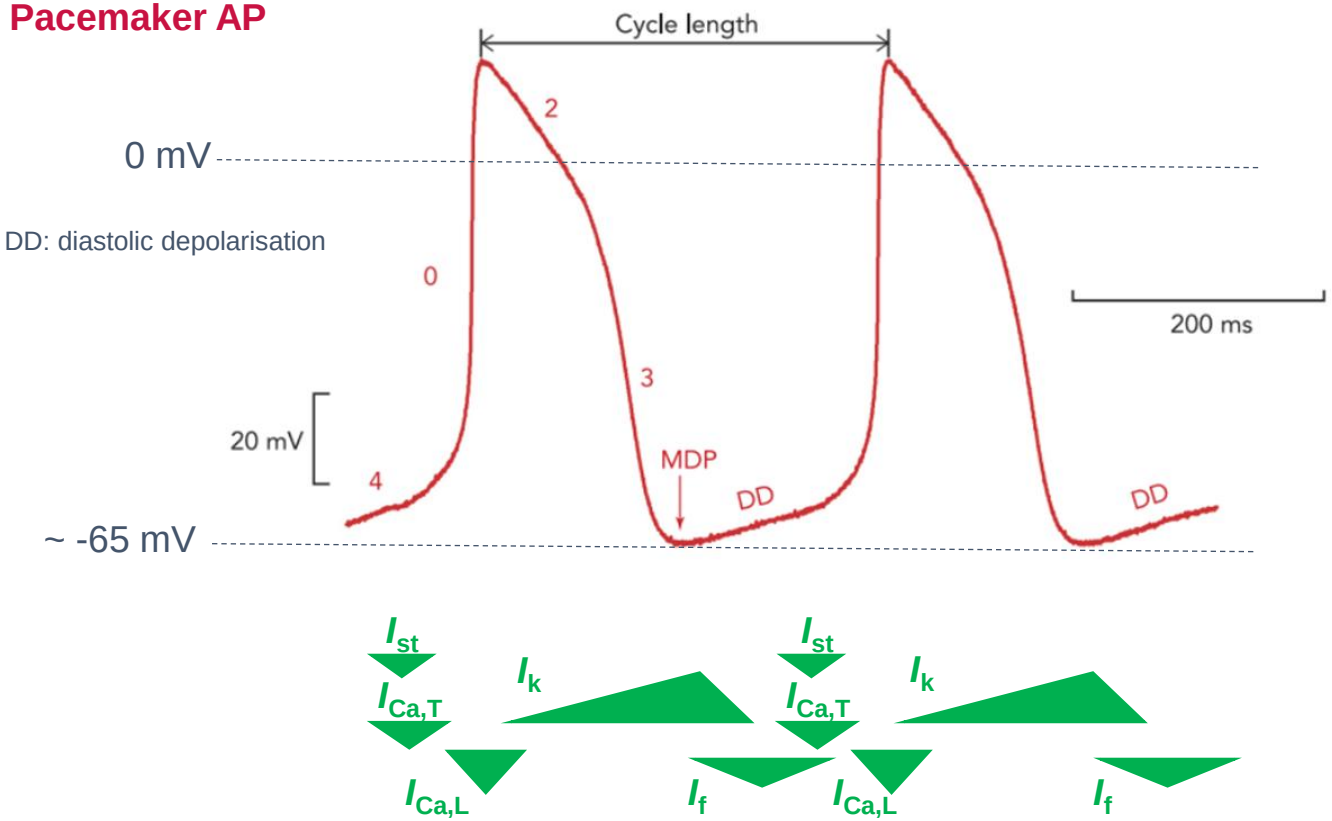

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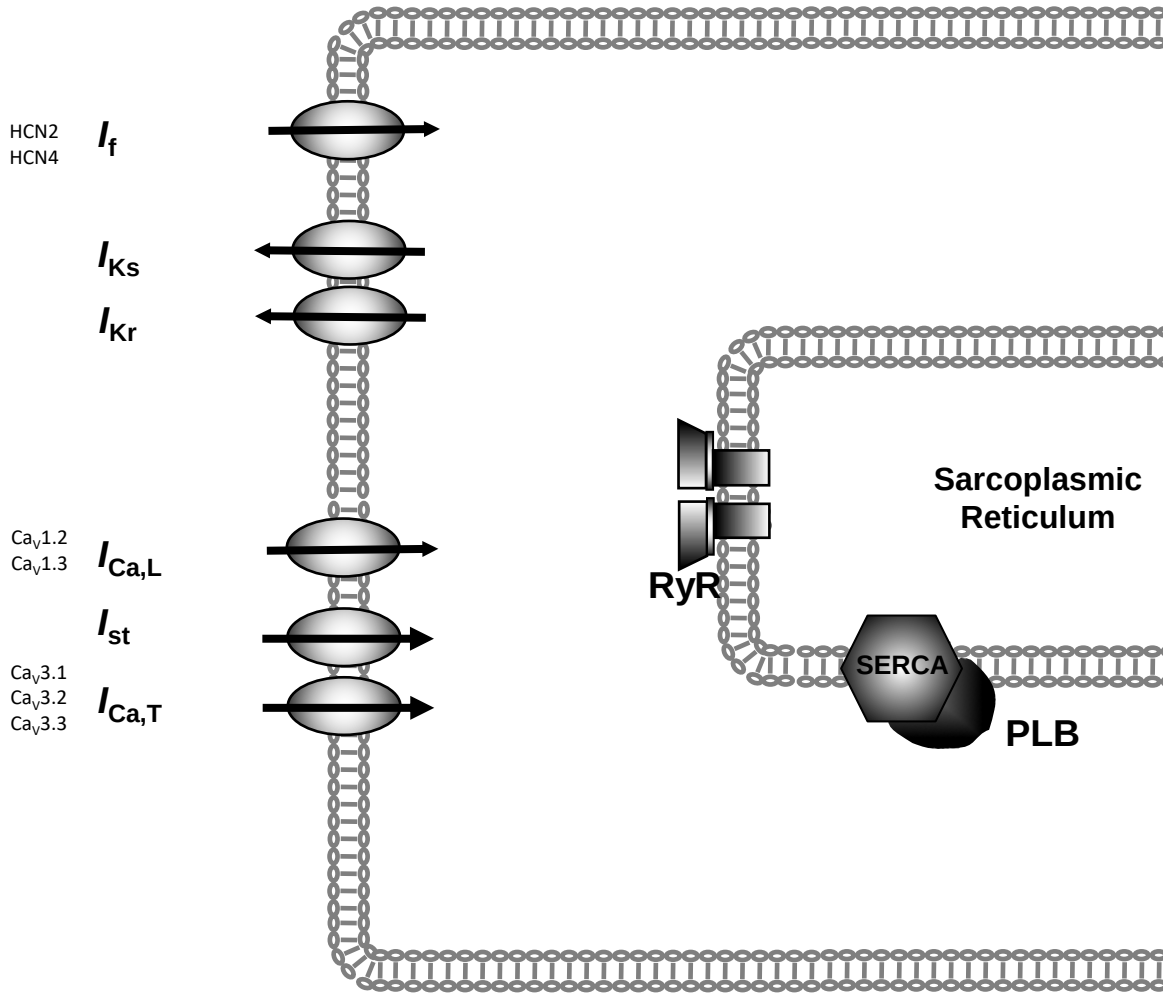

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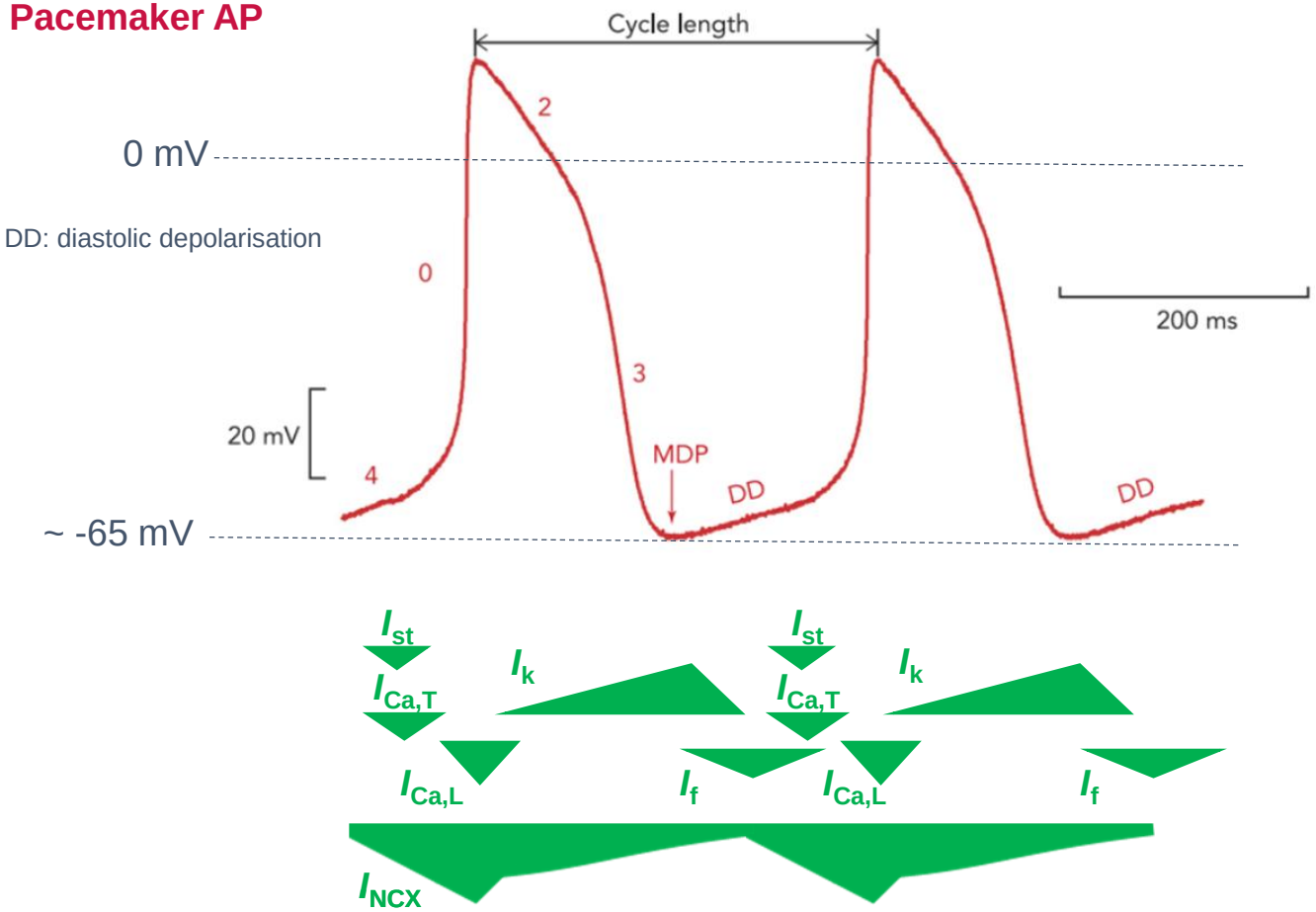

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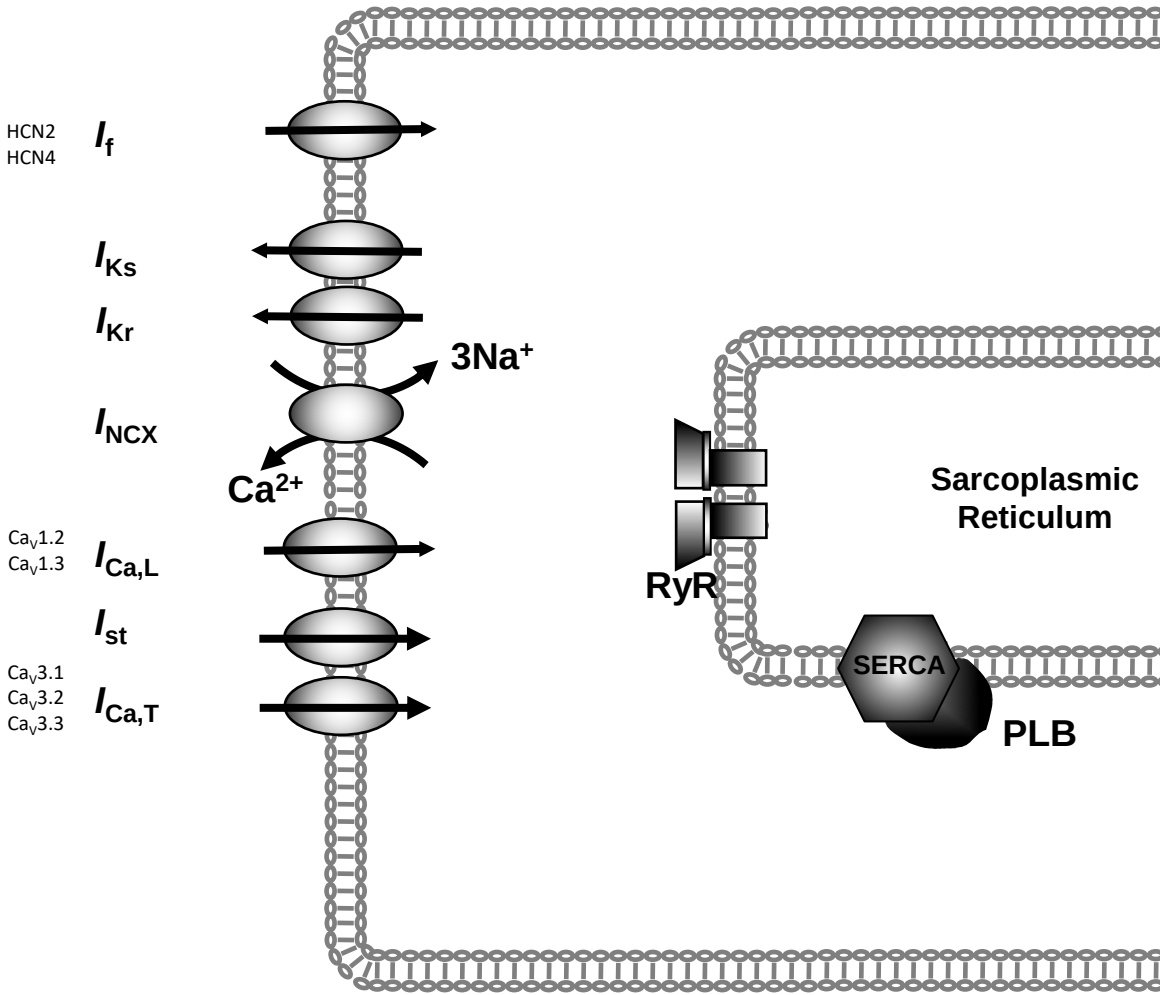

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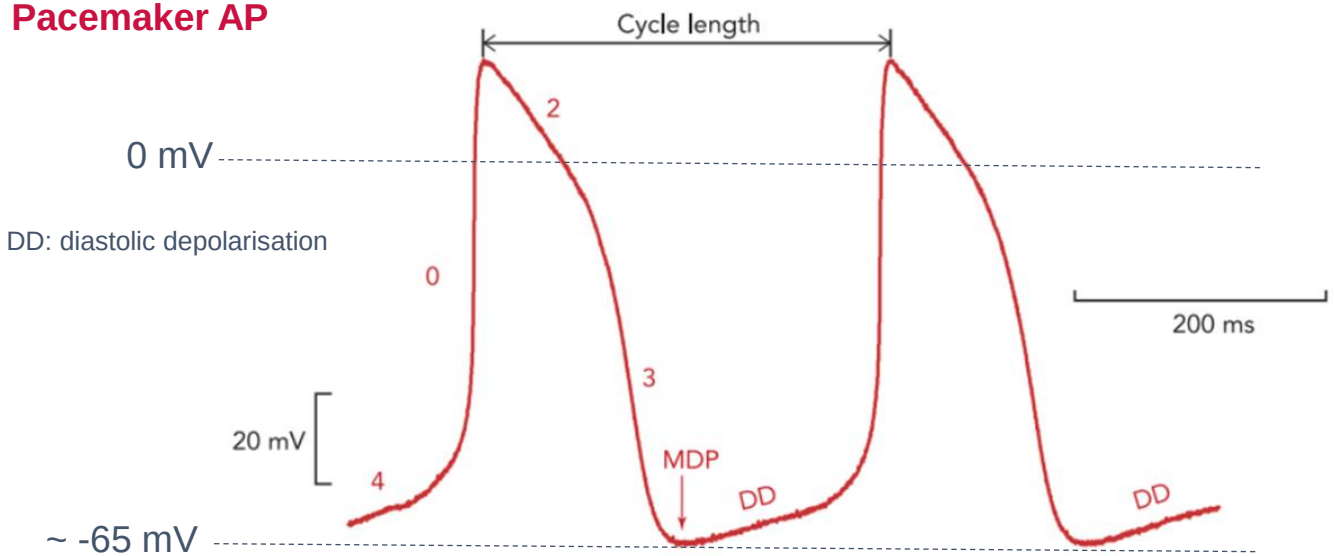

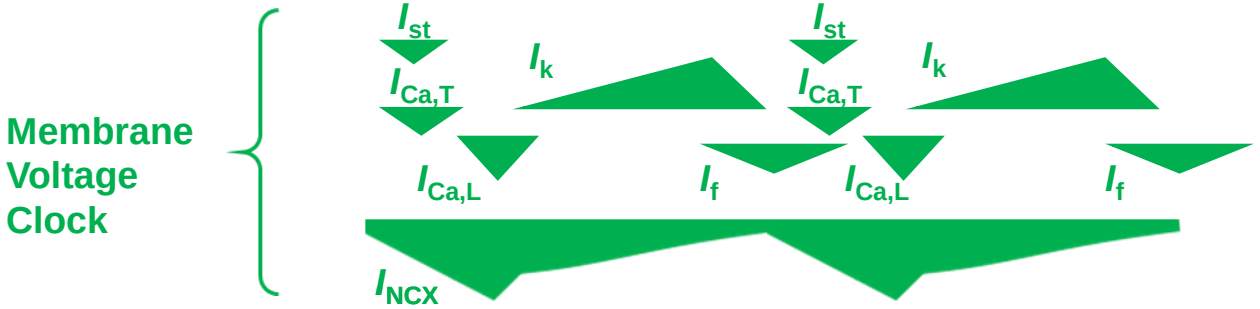

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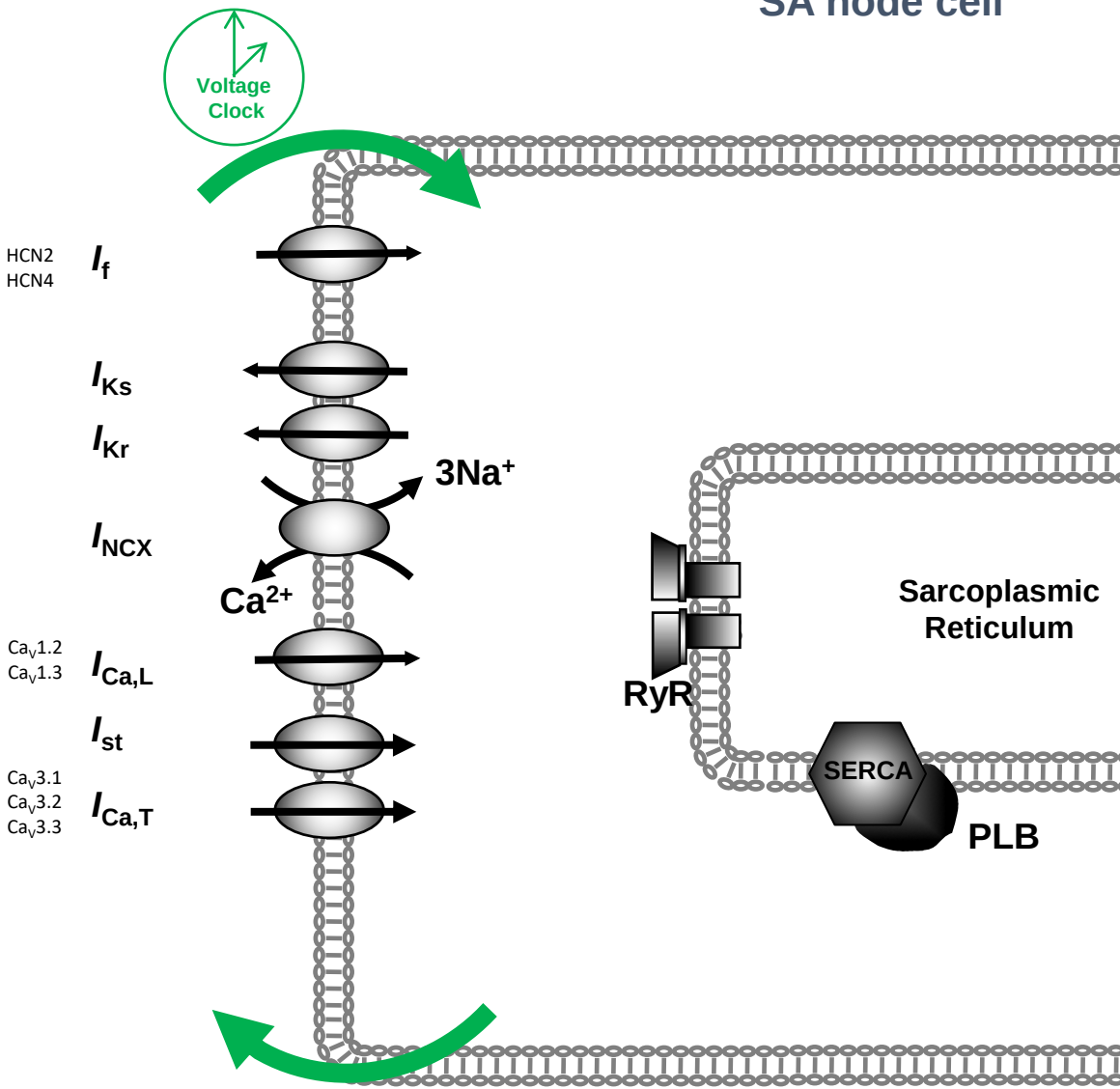

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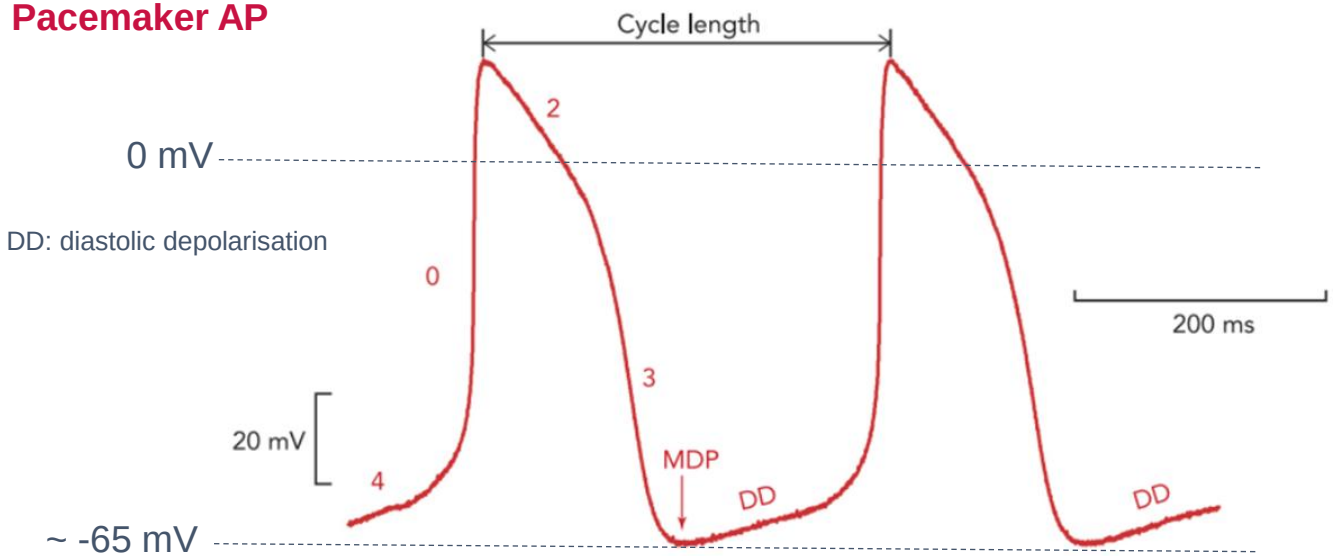

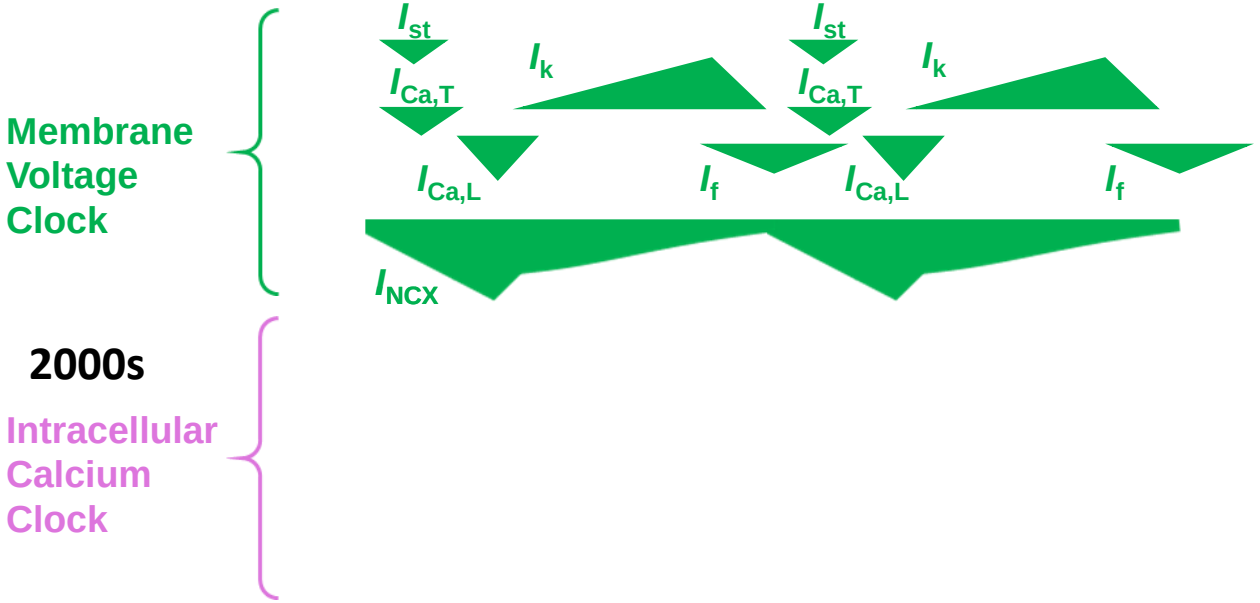

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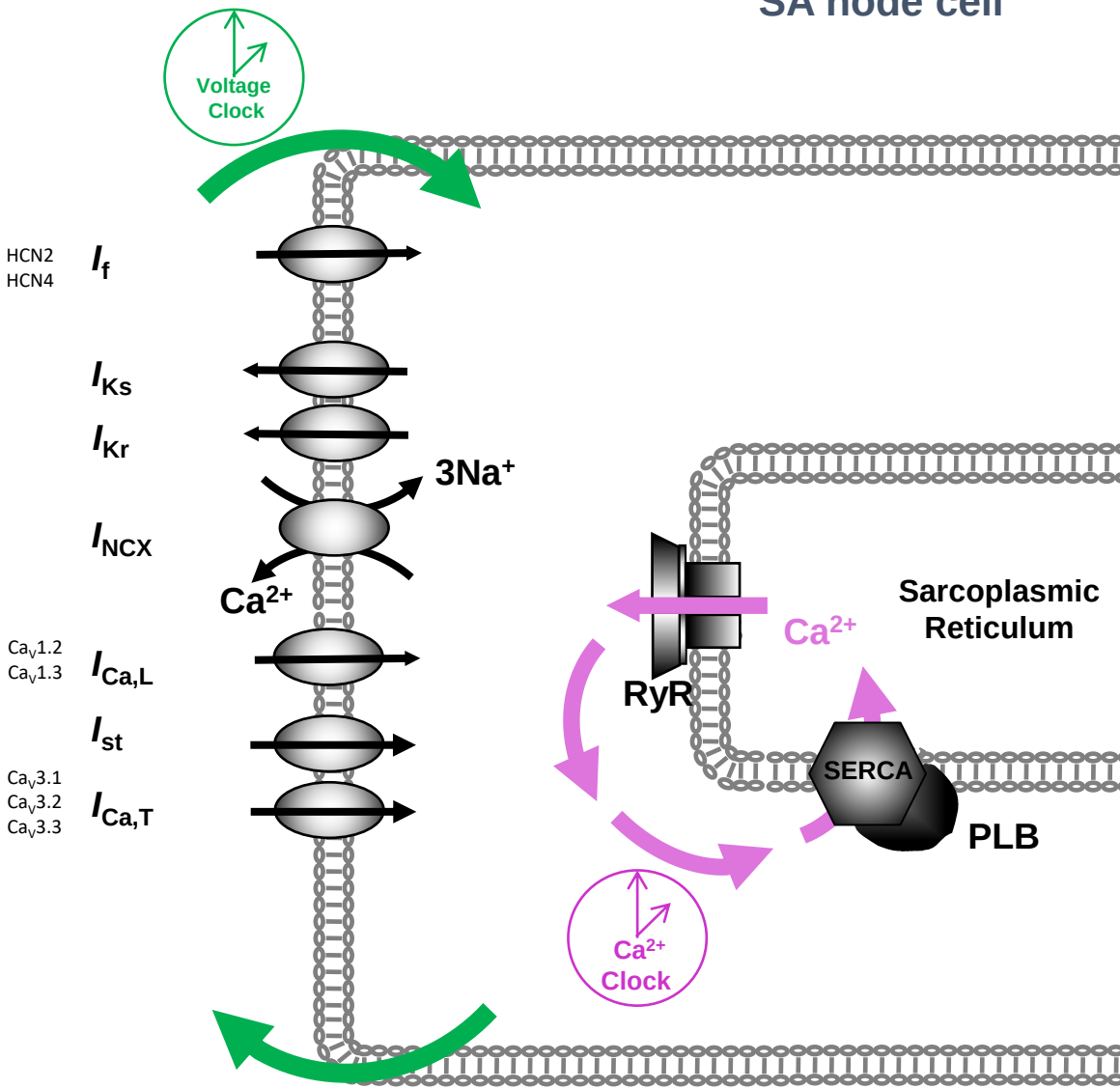

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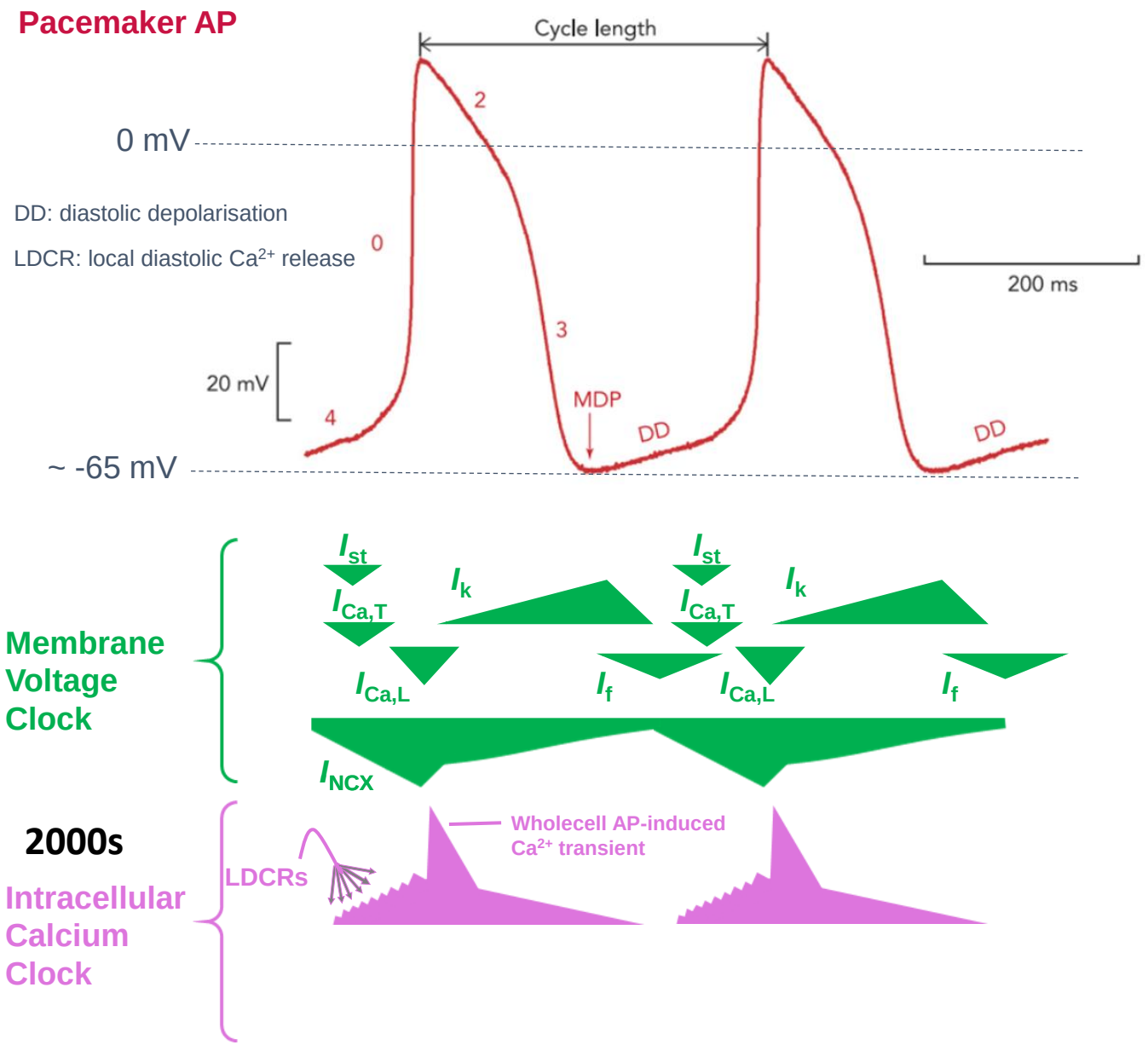

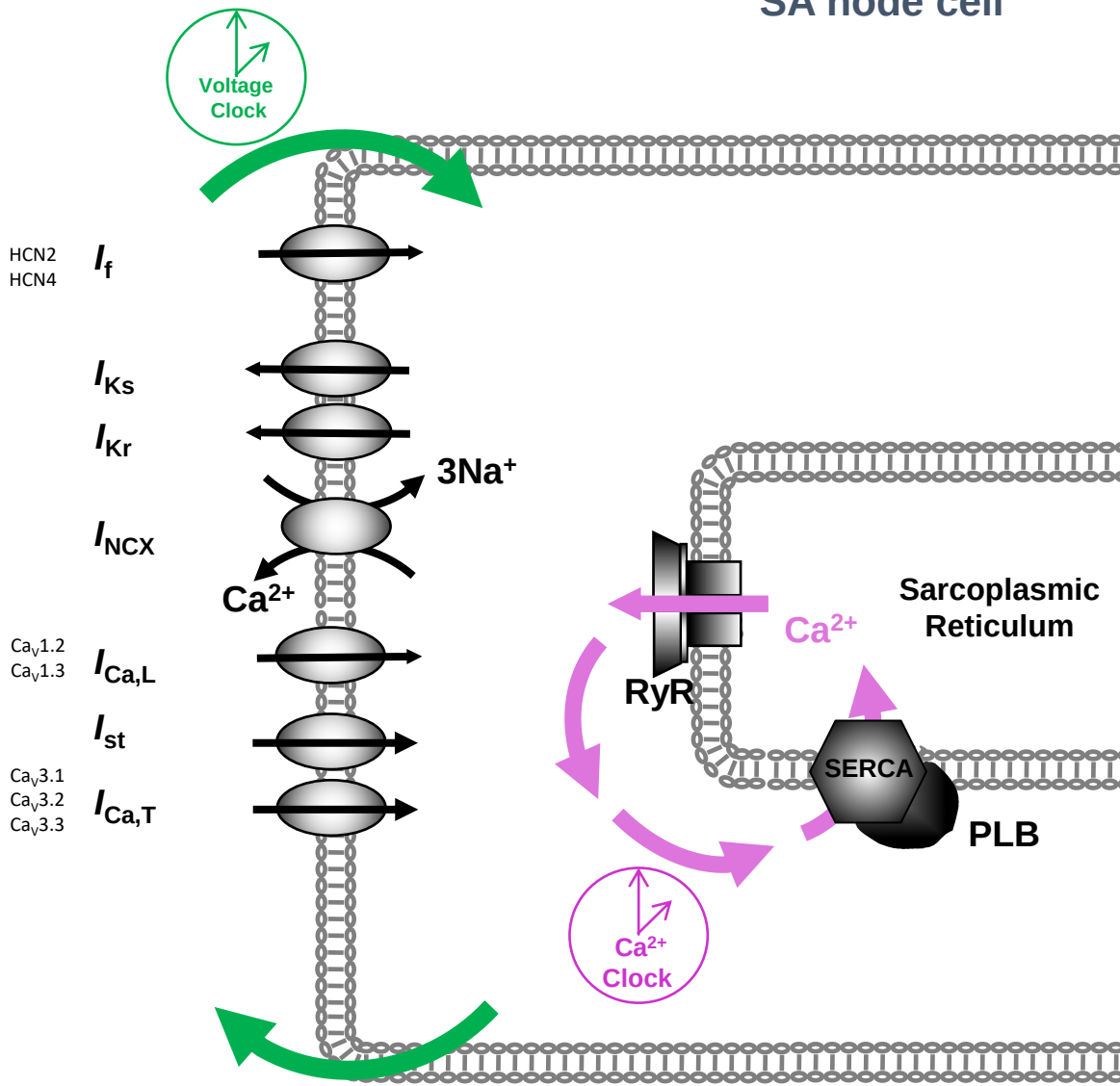

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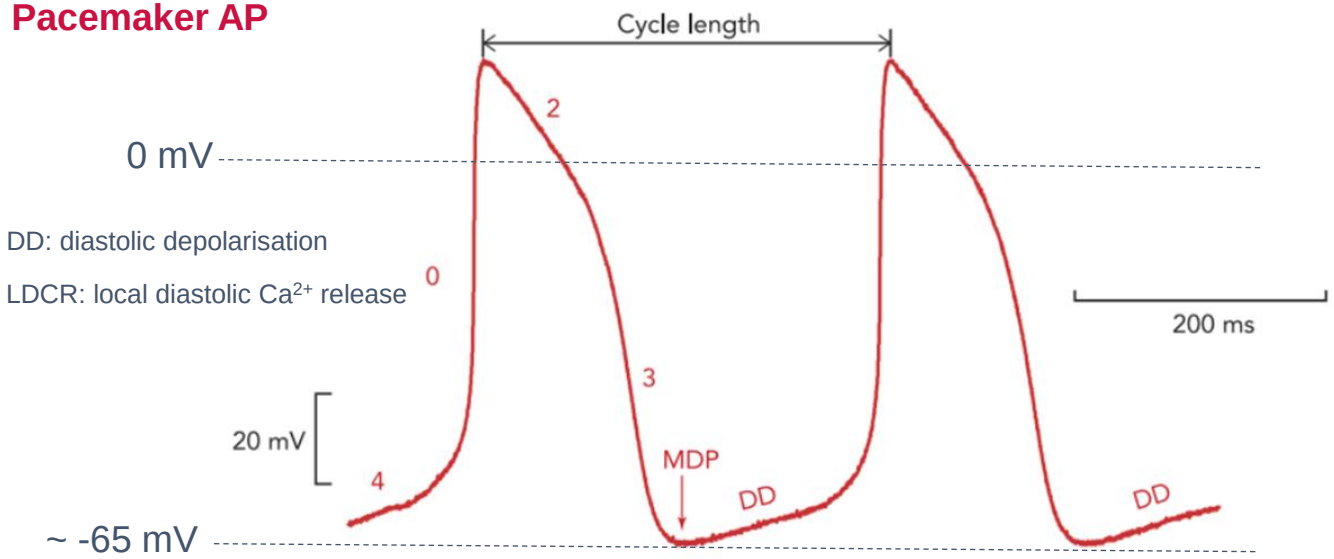
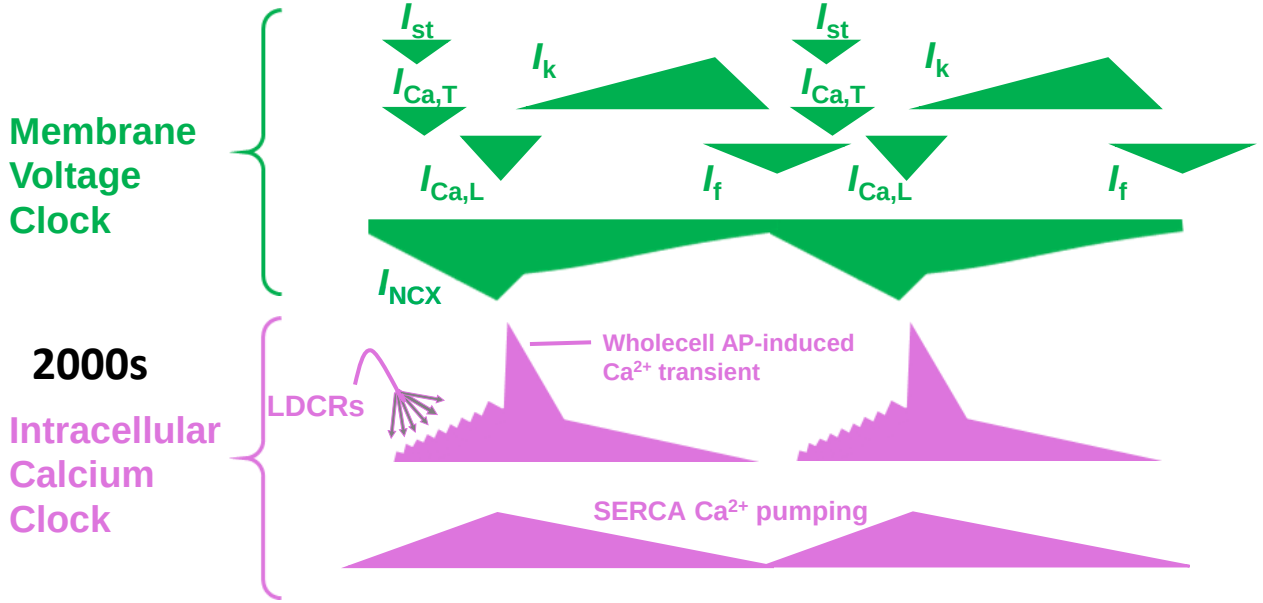


DD: diastolic depolarisation

LDCR: local diastolic Ca^{2+} release



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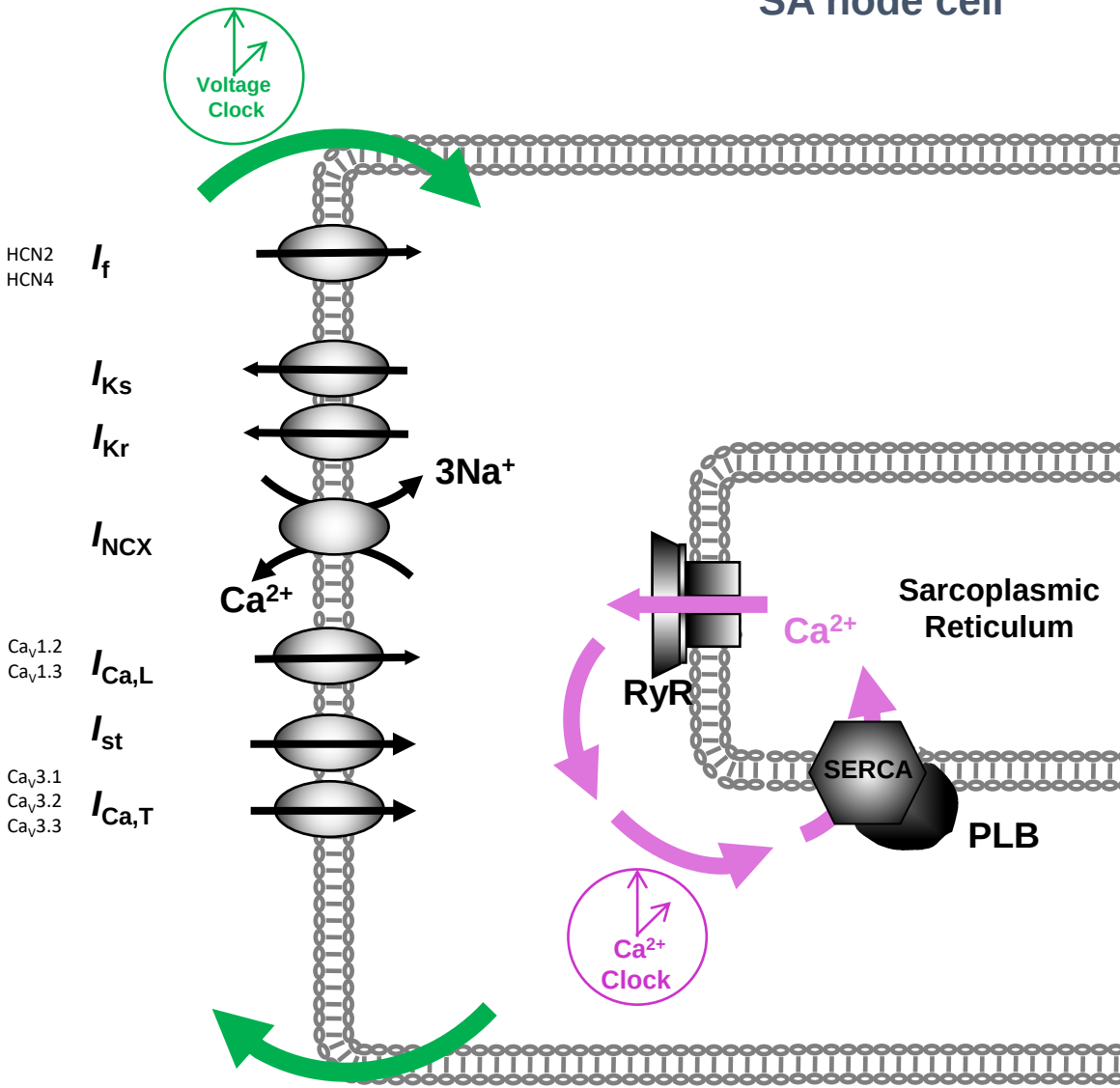


Modifié de Monfredi et al., Physiology, 2013

Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

Dario DiFrancesco

Voltage Clock

"A NEW CONTENDER: THE CA CLOCK"
« Whether the Calcium clock is a pacemaking mechanism and how this relates to the If –based "membrane clock" mechanism are debated questions »

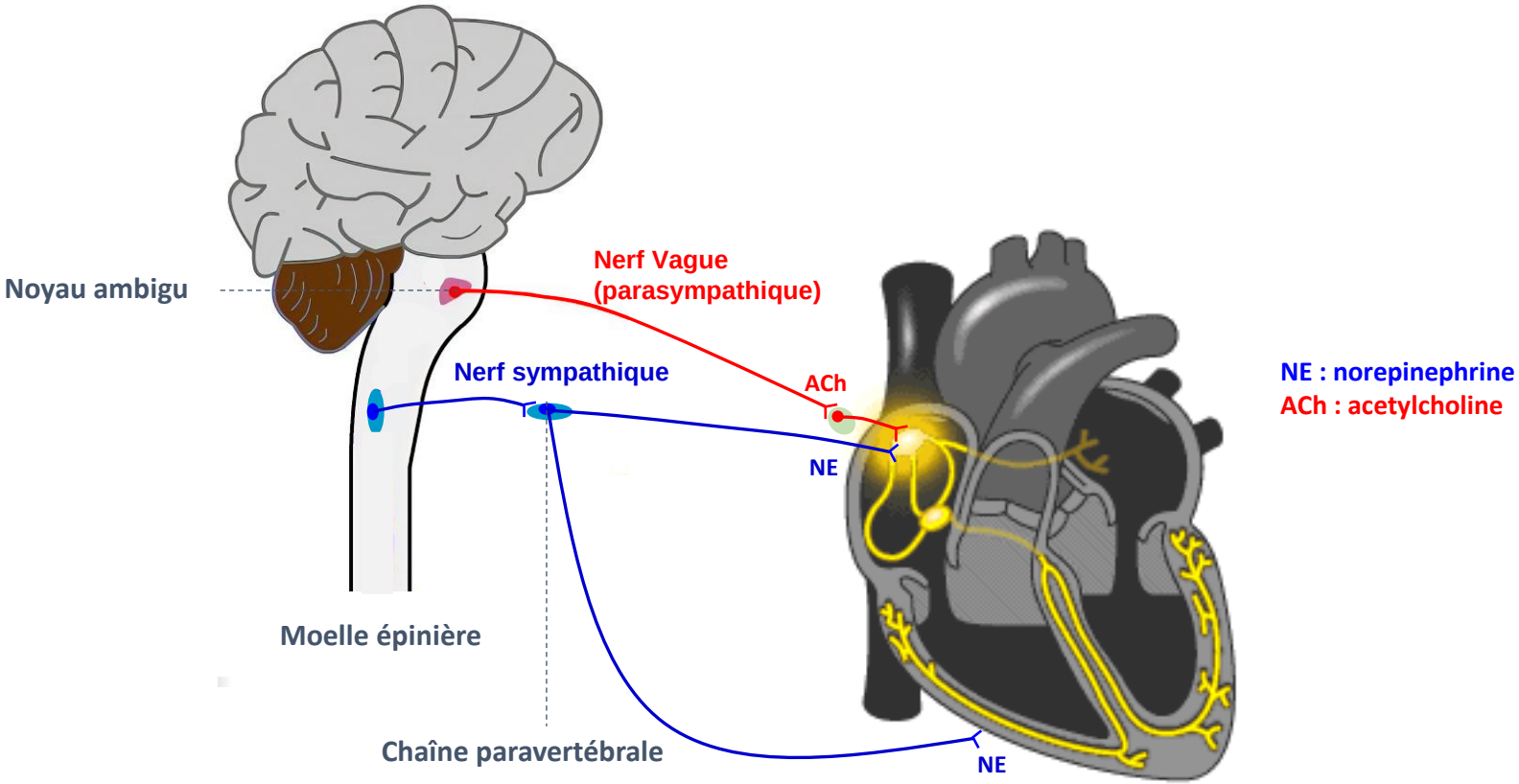
VS

Edward G. Lakatta

Calcium Clock

« intracellular Ca^{2+} cycling ("Ca^{2+} clock") ignites membrane clocks, effecting rhythmic APs. »

Autonomous Nervous System regulation of cardiac function

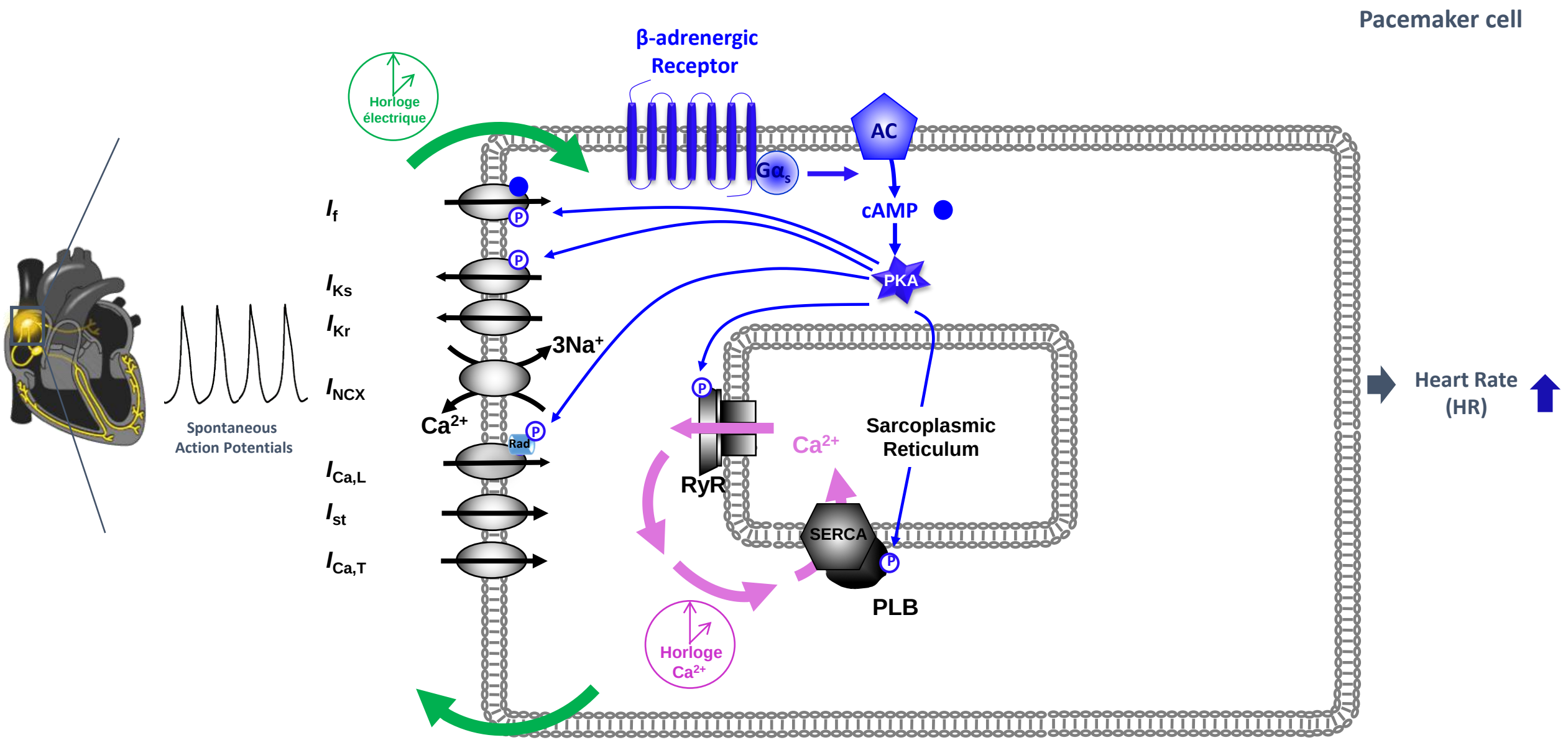


Système Nerveux Autonome

Sympathique : augmente la fréquence cardiaque (effet chronotrope positif)

Parasympathique : diminue la fréquence cardiaque (effet chronotrope negatif)

Regulation of pacemaker activity by the autonomous nervous system



Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

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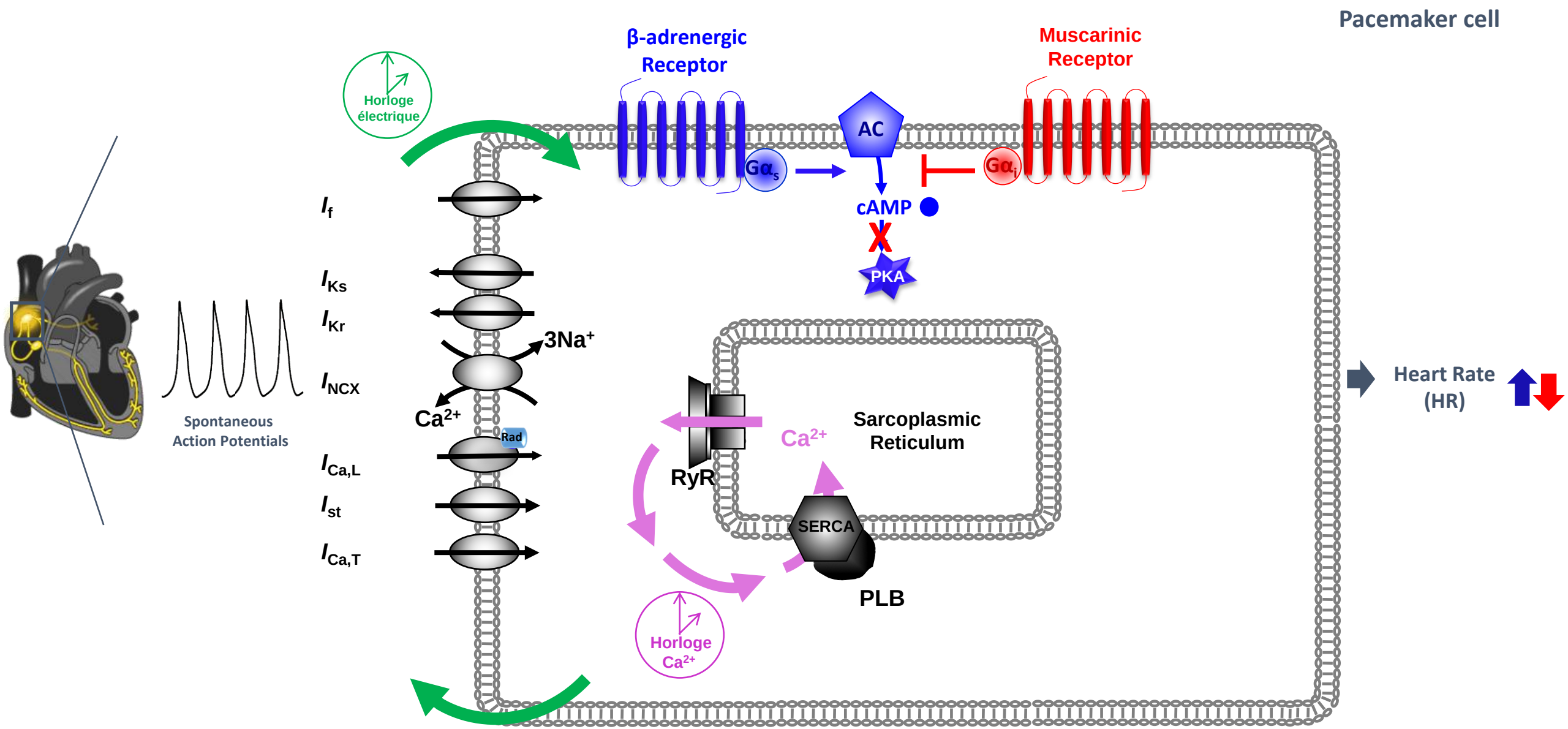
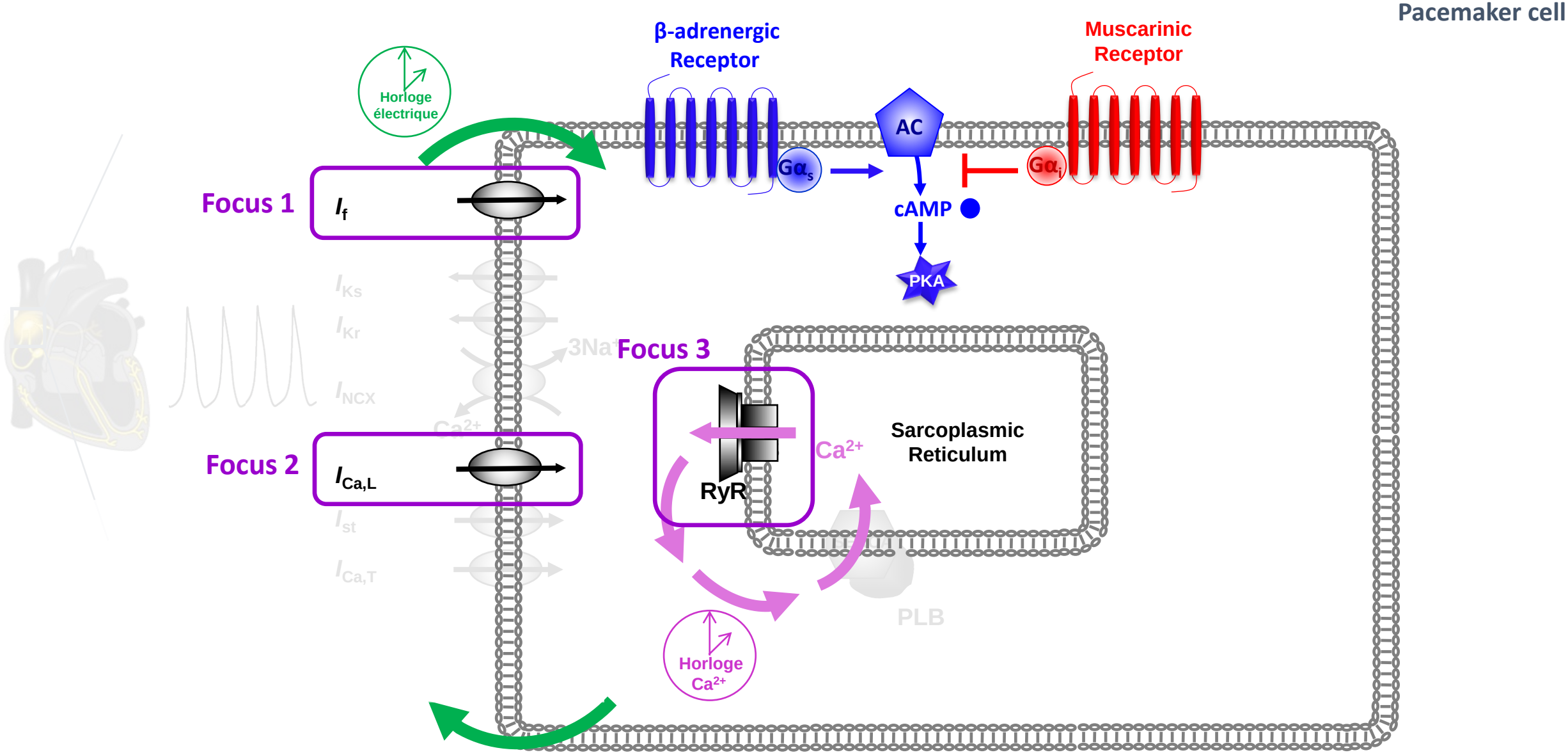


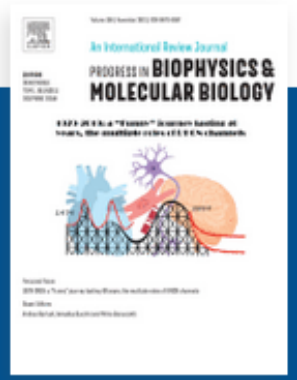
Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

Generation of pacemaker activity



Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

Focus 1 — *'funny'* current I_f – HCN channels



Special Issue in Honor of Dario DiFrancesco (Nov 2021)

Progress in Biophysics and Molecular Biology

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1979-2019: a "Funny" journey lasting 40 years, the multiple roles of f/HCN channels

Edited by Andrea Barbuti, Annalisa Bucchi, Mirko Baruscotti

Volume 166,

Pages 1-204 (November 2021)

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MINI REVIEW

published: 22 January 2020

doi: 10.3389/fphys.2019.01599

A Brief History of Pacemaking

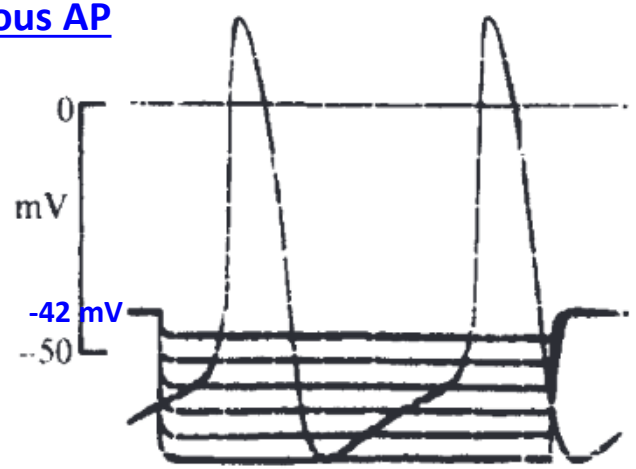
*Dario DiFrancesco**

Department of Biosciences, University of Milano, IBF-CNR University of Milano Unit, Milan, Italy

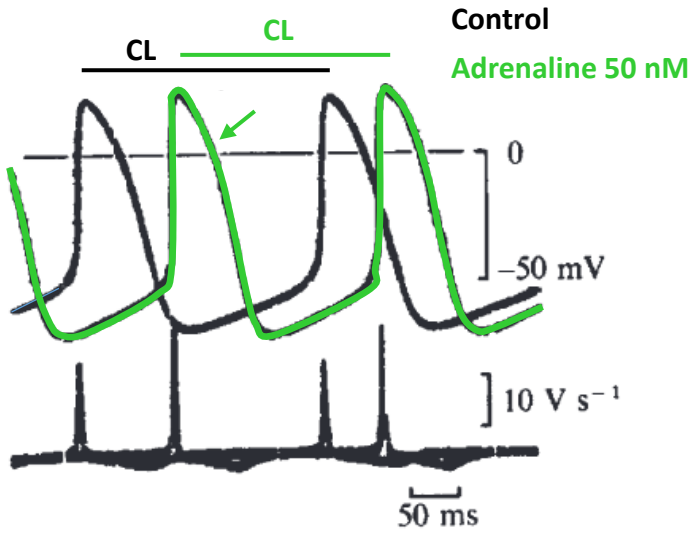
1979: discovery of the '*funny*' current I_f

Brown H. F., DiFrancesco D., Noble S. J. (1979). Nature

Rabbit SA node
Spontaneous AP



CL=Cycle Length



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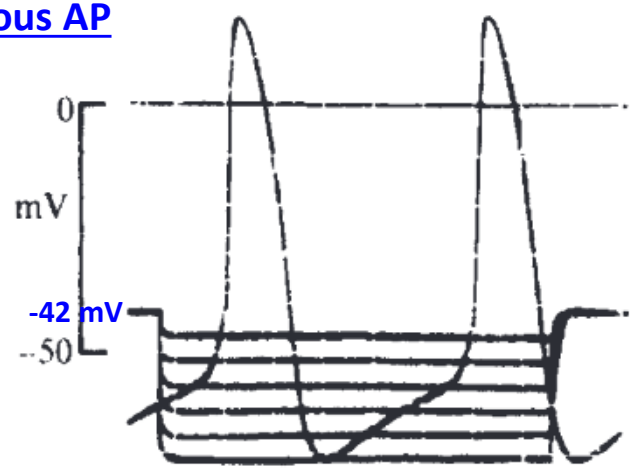

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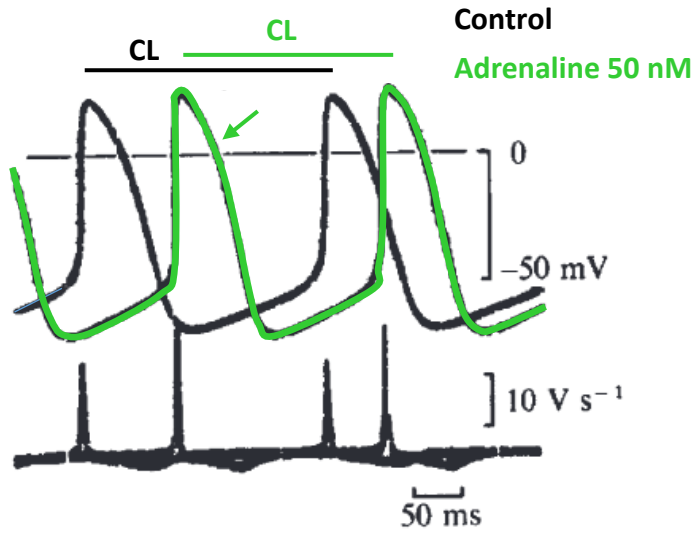
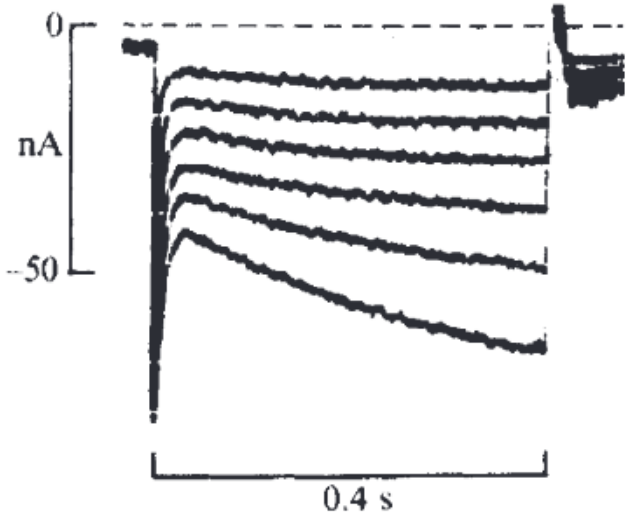
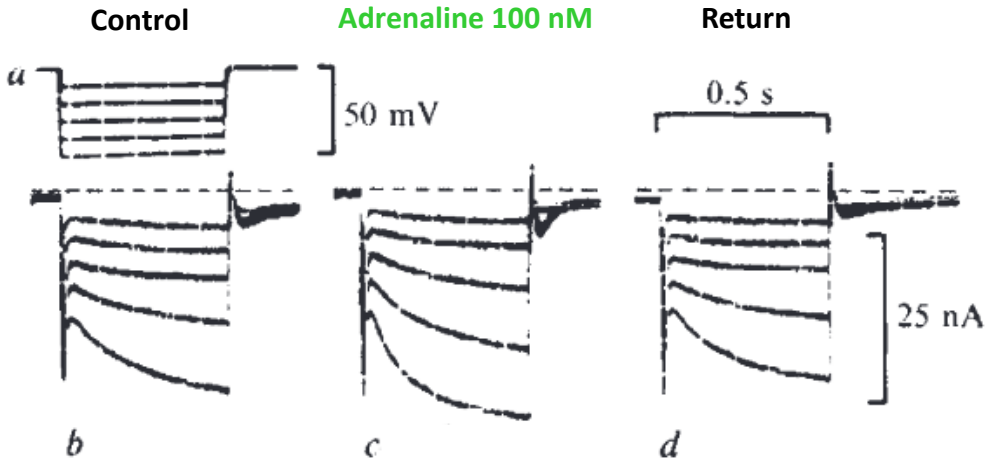


I_f current recordings





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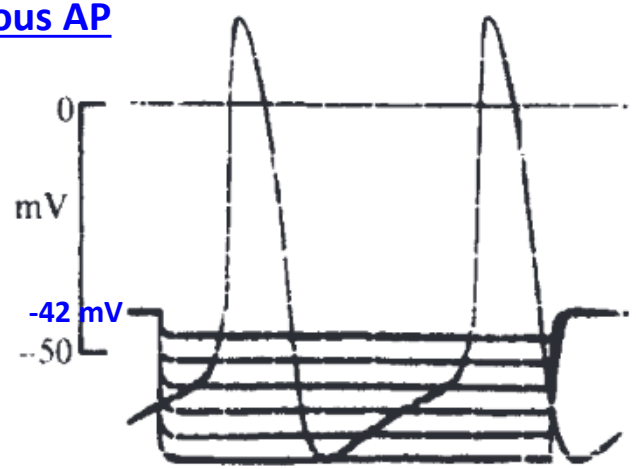

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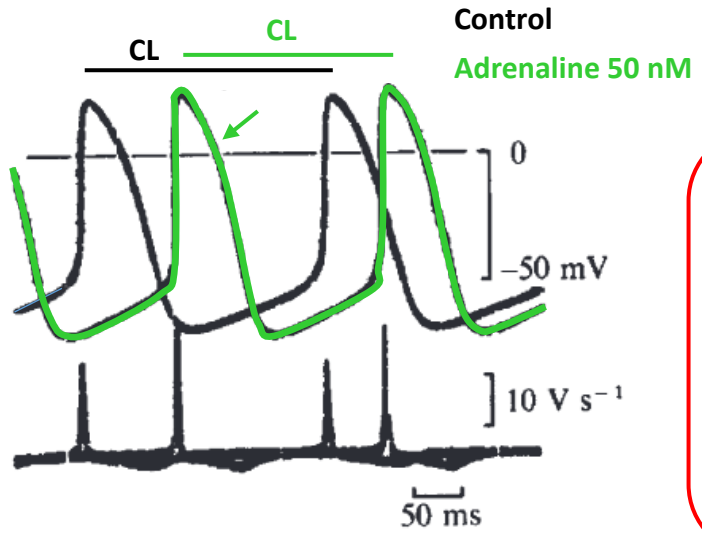
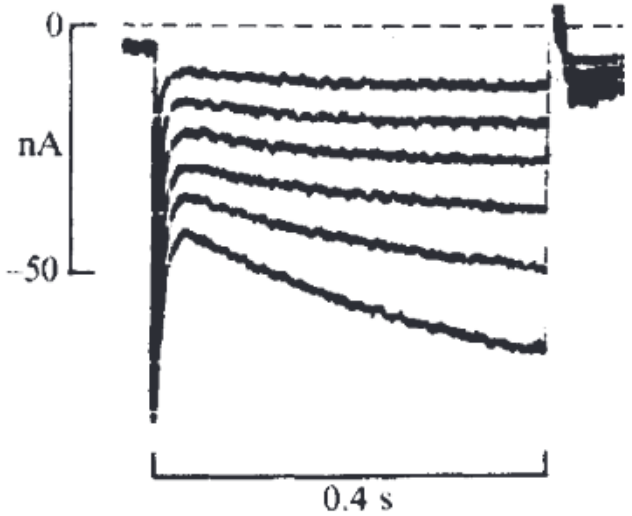
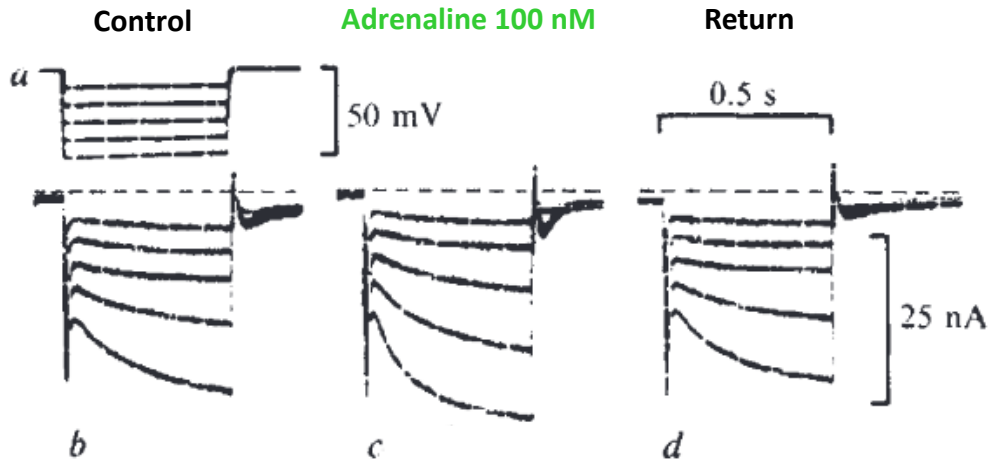


"funny" = unusual properties
inward current activated on *hyperpolarization*
mixed Na^+ and K^+ current

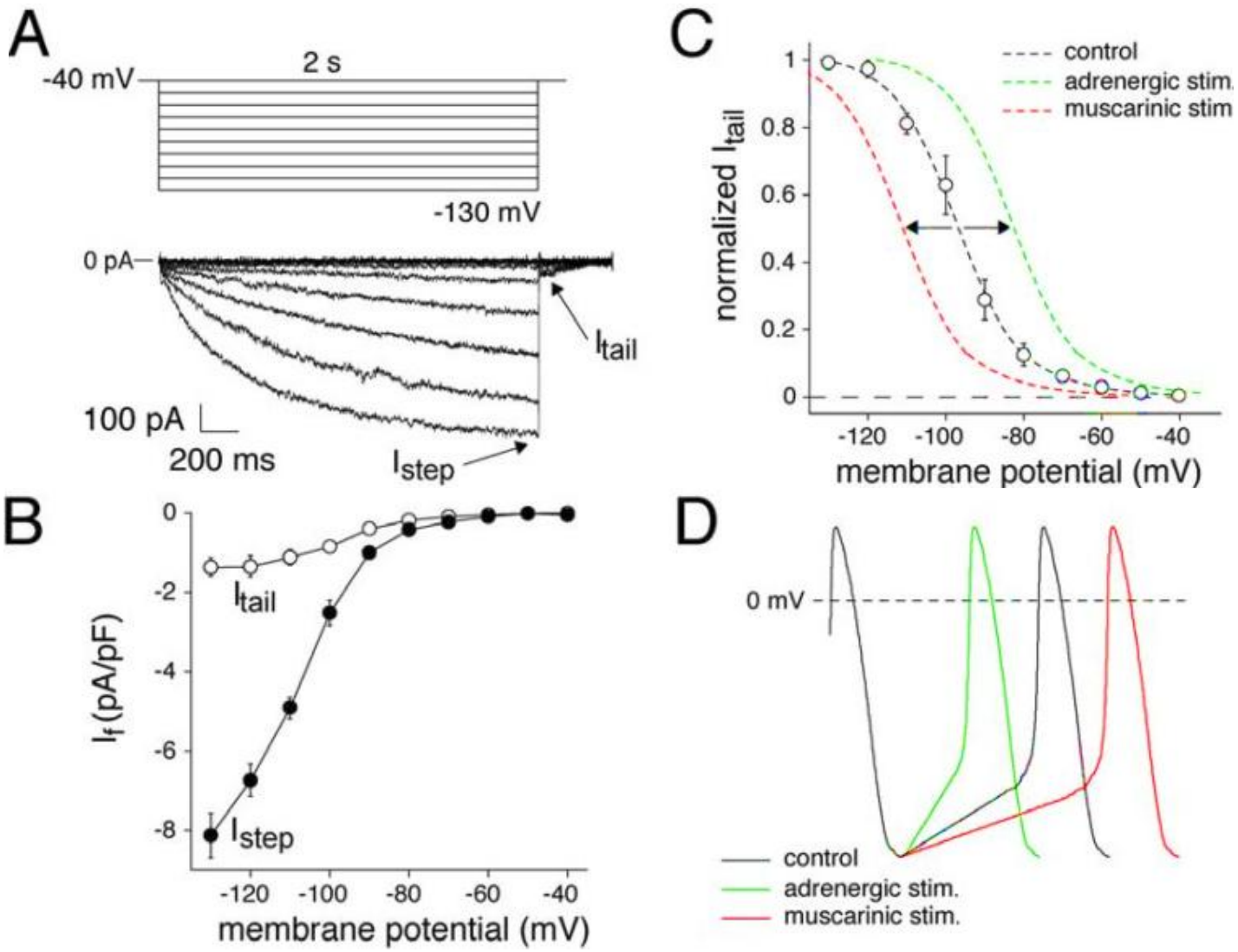
Increased in adrenaline

I_f current recordings



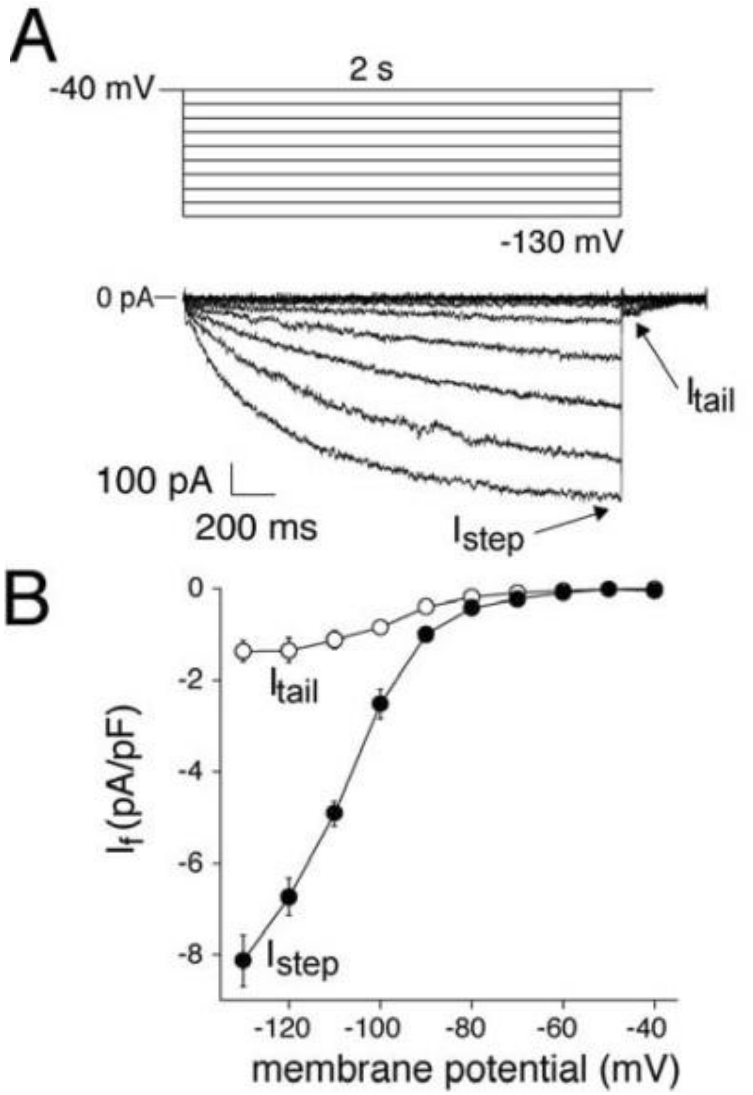


'*funny*' current I_f in a human SAN cell



Papaioannou, et al. *CurrCardiolReviews* 9(1), 2012

'*funny*' current I_f in a human SAN cell

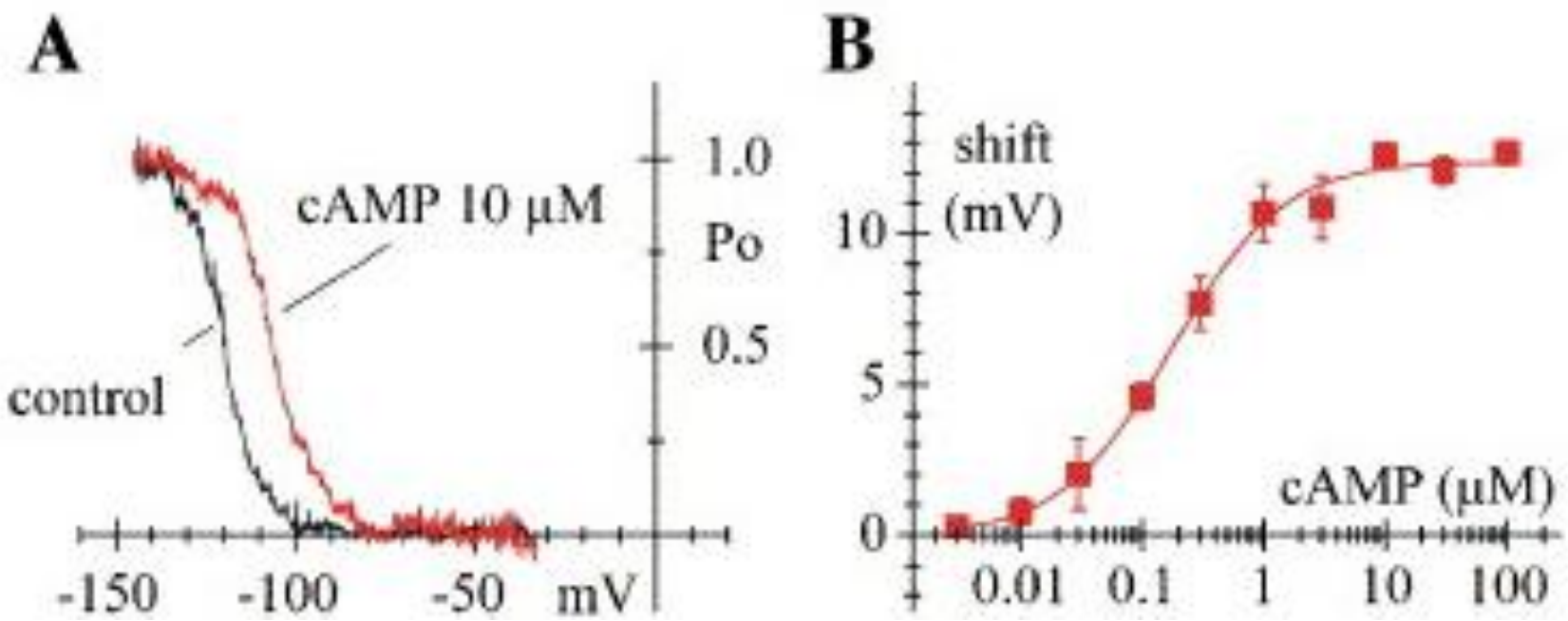


I_f is increased by adrenergic stimulation

I_f is decreased by muscarinic stimulation

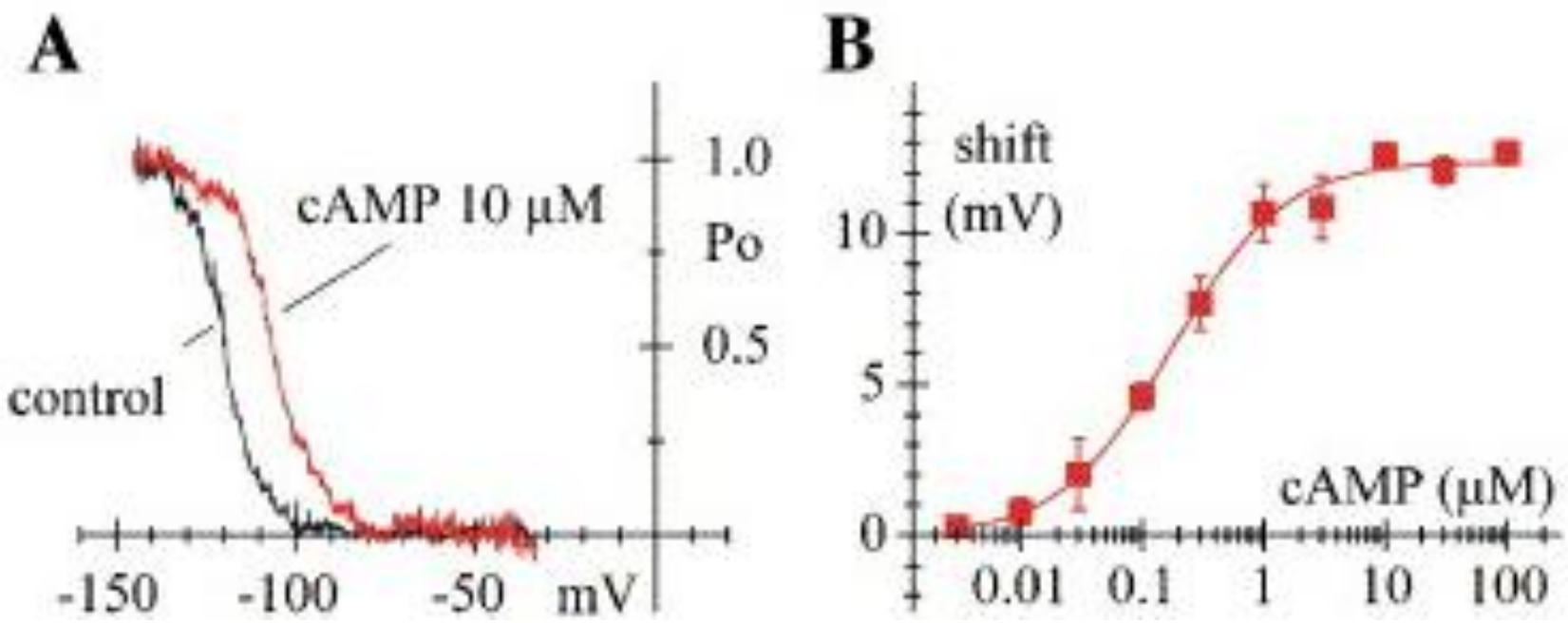
Papaioannou, et al. *CurrCardiolReviews* 9(1), 2012

Activation of I_f current by cAMP



Baruscotti et al. *Pharmacol& Therapeut*107 (2005) 59–79

Activation of I_f current by cAMP

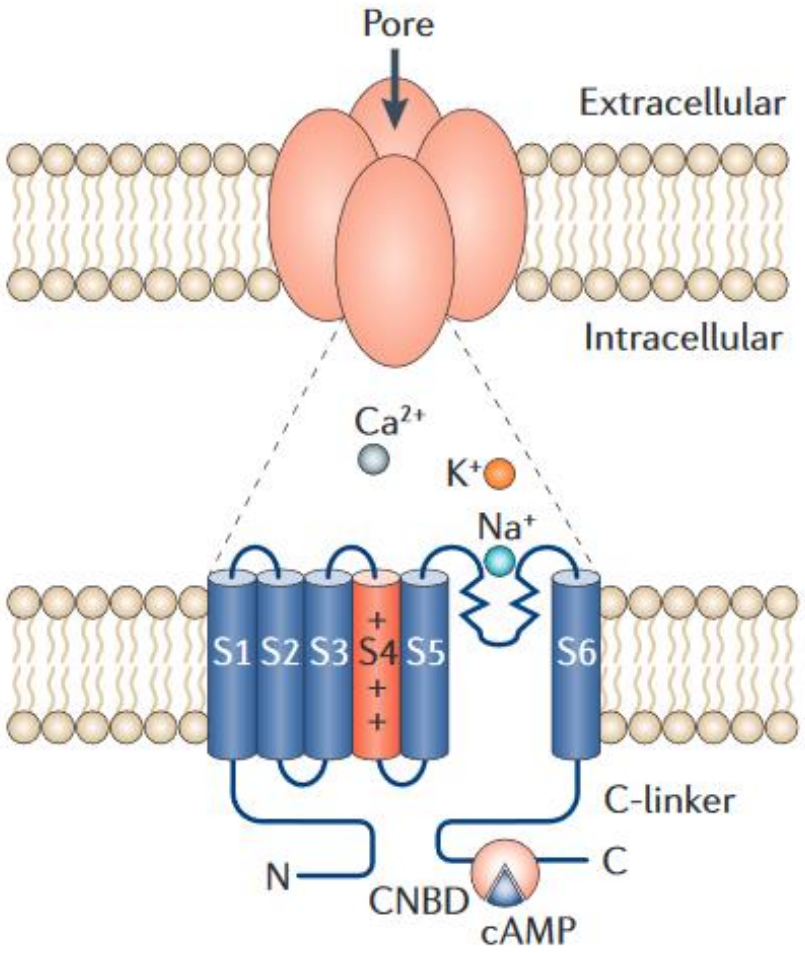


cAMP directly binds HCN channels, shifting the open probability to more positive voltages and increasing I_f current

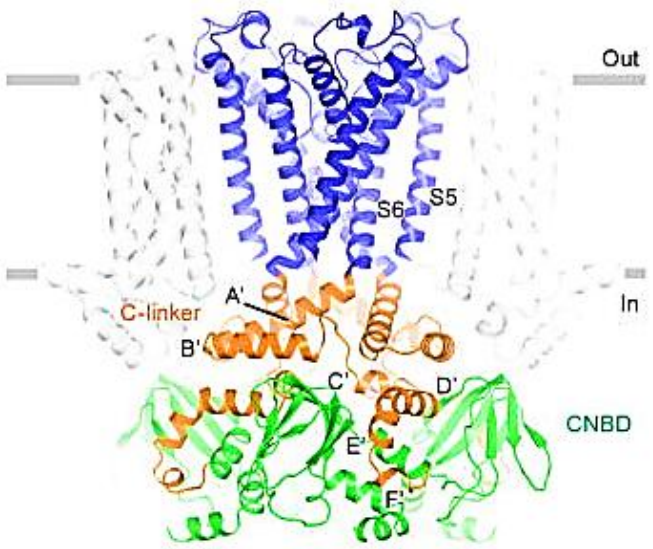
Baruscotti et al. *Pharmacol& Therapeut*107 (2005) 59–79

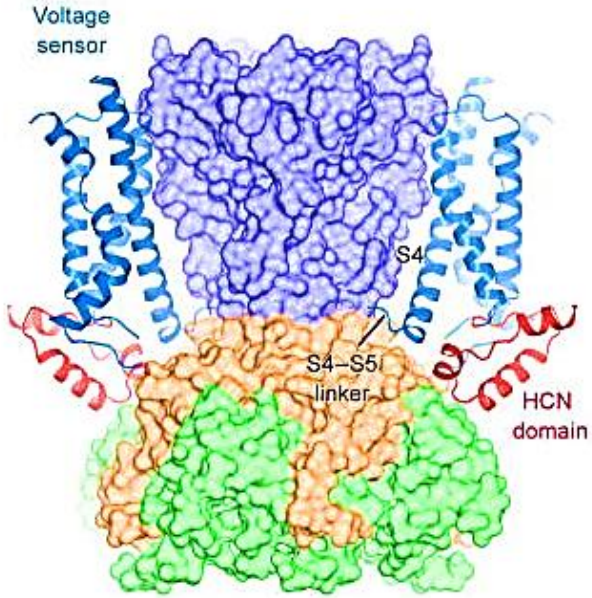
'*funny*' current I_f – HCN channels

Hyperpolarization-activated
Cyclic Nucleotid-gated channels



Postea & Biel Nat Rev Drug Discov 2011



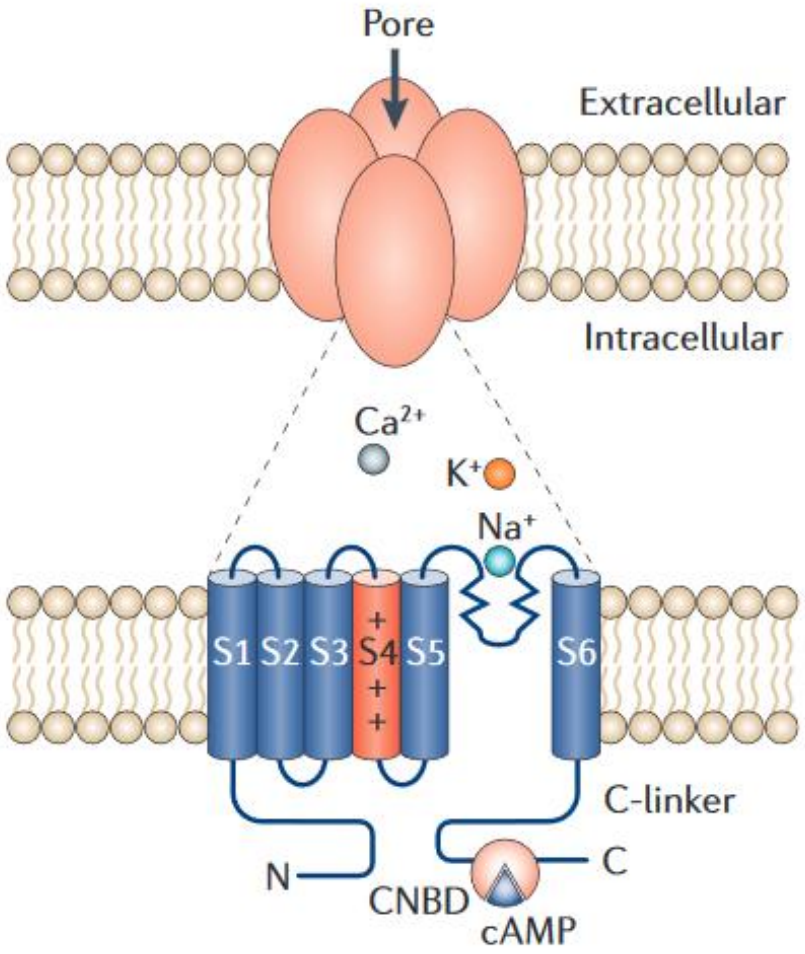


Lee C. H., MacKinnon R. (2017). Structures of the Human HCN1 Hyperpolarization-Activated Channel. Cell

4 subunits around a central pore
Each subunit = 6 transmembrane segments (S1-S6)
1 P-loop
Intracellular N and C-term regions
S6*4 (around the pore) and P-loop: ion conduction
positively-charged S4 helix: voltage sensor
CNBD = cyclic nucleotides binding domain

'*funny*' current I_f – HCN channels

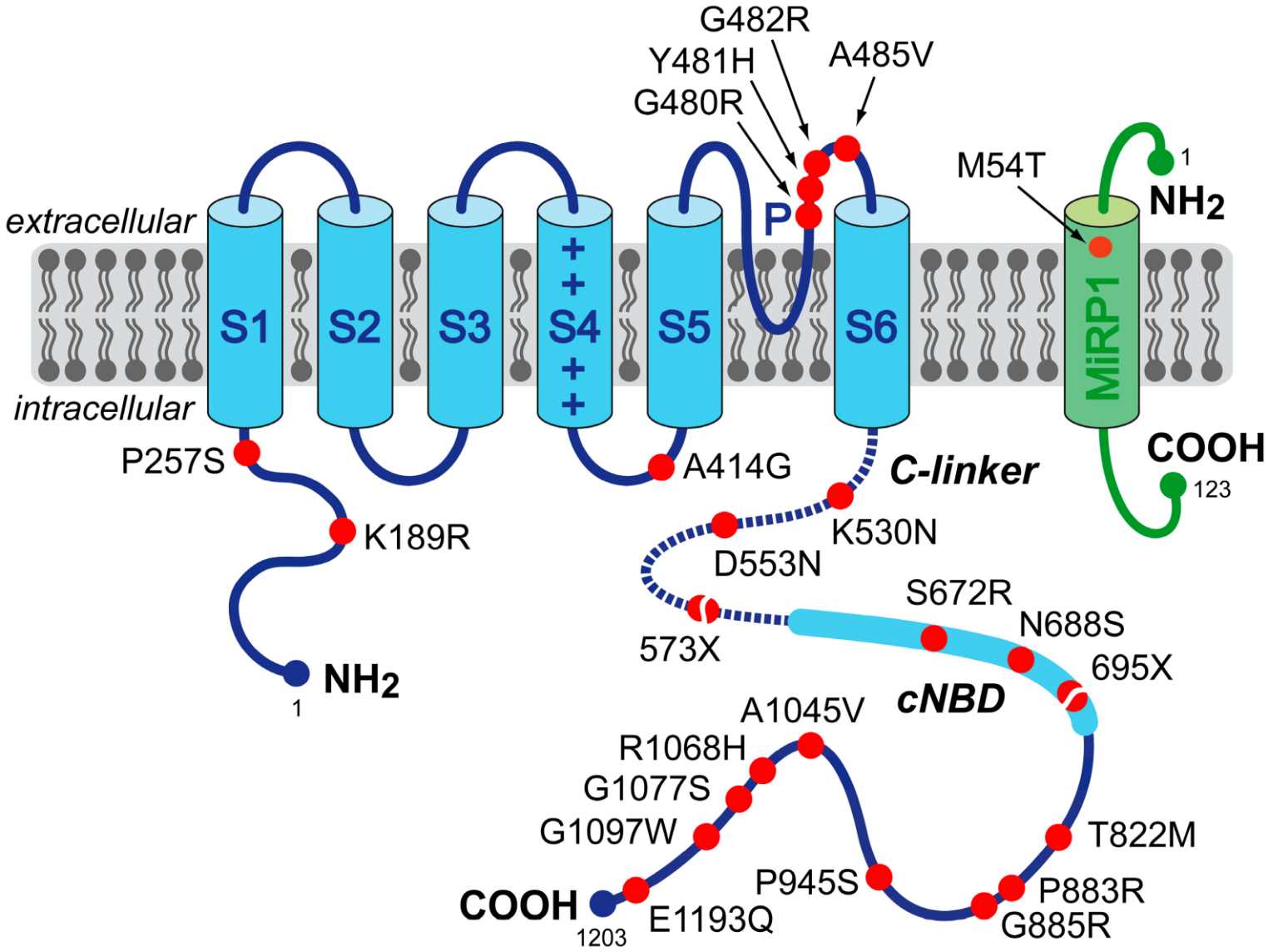
Hyperpolarization-activated
Cyclic Nucleotid-gated channels



Postea & Biel Nat Rev Drug Discov 2011

Isoform	Expression sites	Phenotypes
Nervous system		
HCN1	Neocortex, hippocampus, brain stem[19,28,30,31]	• Mouse knockout: impaired motor learning, enhanced hippocampal-dependent learning and memory, less cold allodynia, epilepsy and altered vision[116,128,141]
HCN2	Ubiquitous[28,30,31]	• Mouse knockout: ataxia, absence epilepsy[41], as well as reduced inflammatory and neuropathic pain[127]
HCN3	Olfactory bulb, hypothalamic nuclei, retinal cone pedicles[2,28,28,31,34]	• Mouse knockout: not reported
HCN4	Thalamic nuclei, basal ganglia, olfactory bulb[28,30,31]	• Mouse knockout: not reported
Heart		
HCN1	Sinoatrial and atrioventricular node[20, 142,145]	• Not reported
HCN2	Ubiquitous[20,32]	• Mouse knockout: sinus arrhythmia[41]
HCN3	Heart muscle[33]	• Mouse knockout: increased T wave amplitude in the electrocardiogram at basal heart rate[33]
HCN4	Sinoatrial and atrioventricular node, Purkinje fibres[20,32,39,145]	• Mouse knockout: embryonic lethal phenotype, bradycardia, nonfunctional pacemaker cells[39] • Conditional mouse knockout: repetitive sinus pauses[80,81], deep bradycardia, heart block[40] (a disease in the electrical system of the heart) • Human mutation: bradycardia, QT prolongation syncope and conduction disturbances in the sinoatrial and atrioventricular node[73–76]

Mutations in HCN4 are associated with sinus sick syndrome and bradycardia

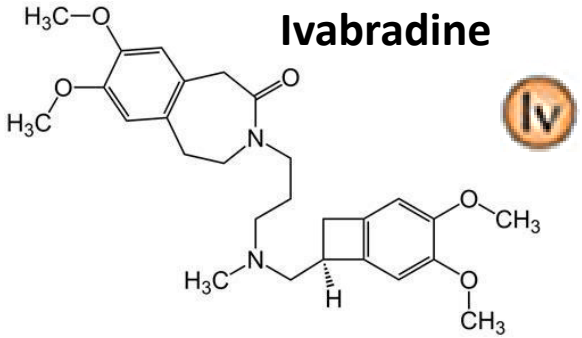


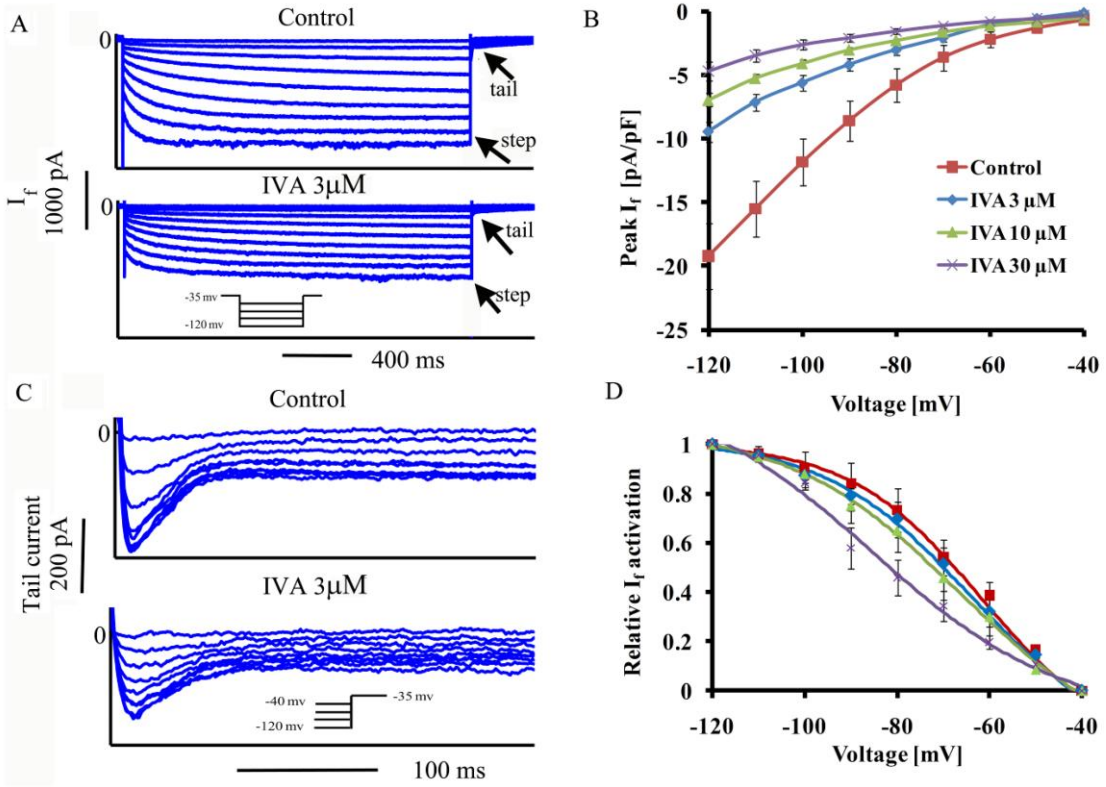
- Mutations associated with SSS
- Non-sense truncated mutations

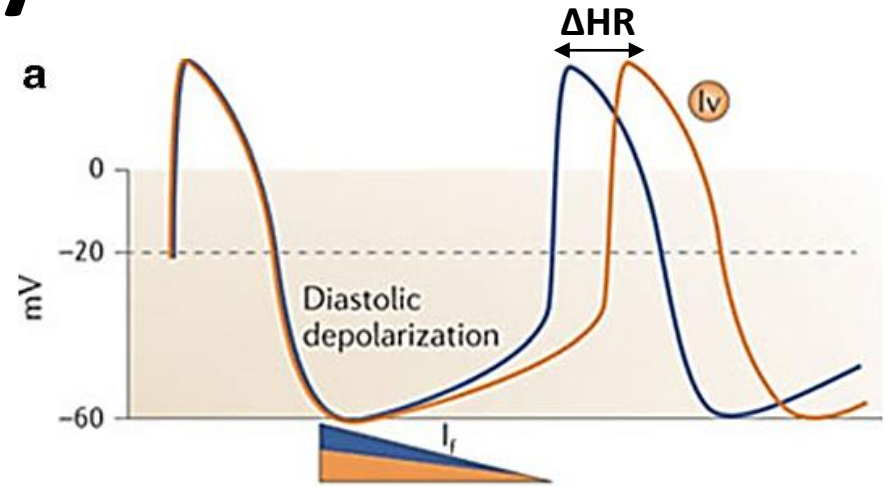
MiRP1=β-subunit

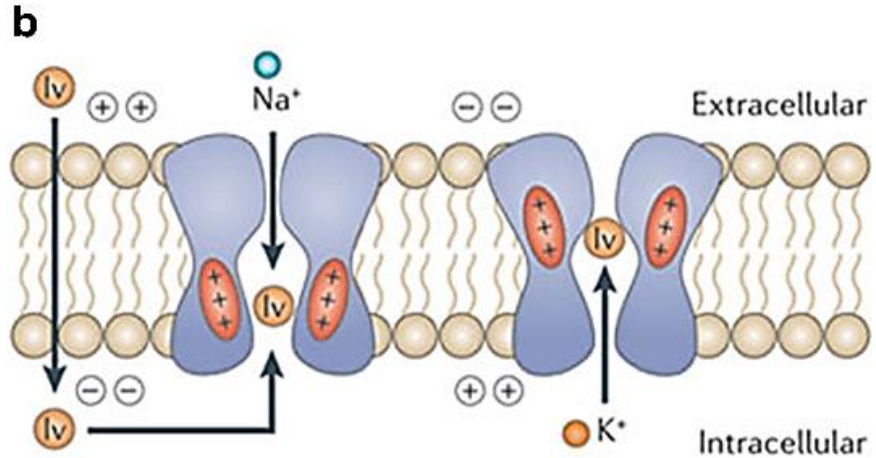
Verkerk & Wilders Int. J. Mol. Sci. 2015

Blockade of HCN4 by Ivabradine







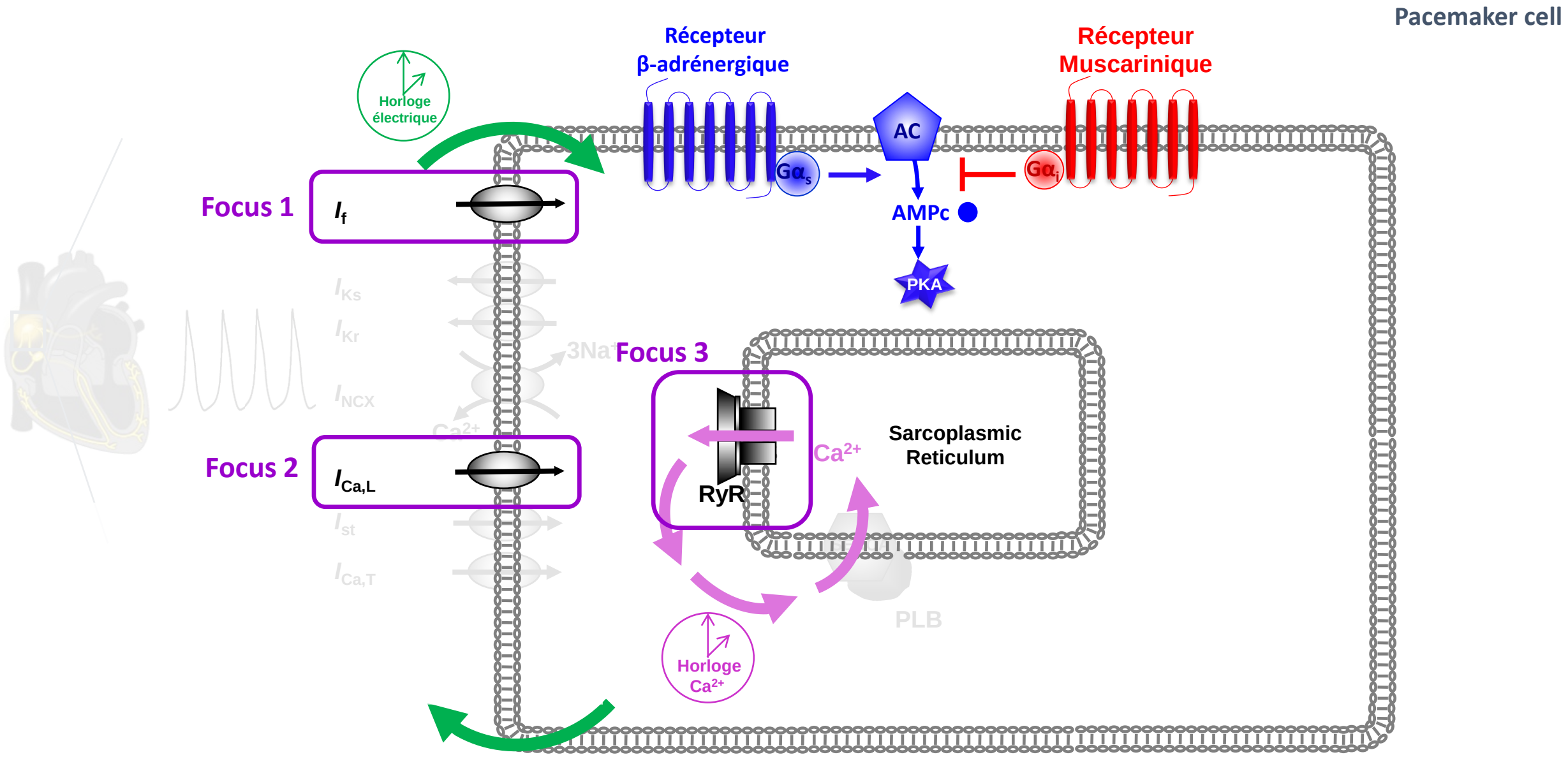


IVA reduces the slow diastolic depolarization
➔ pure HR reduction (without inotropic effect)

Yaniv et al. Molecules 2012

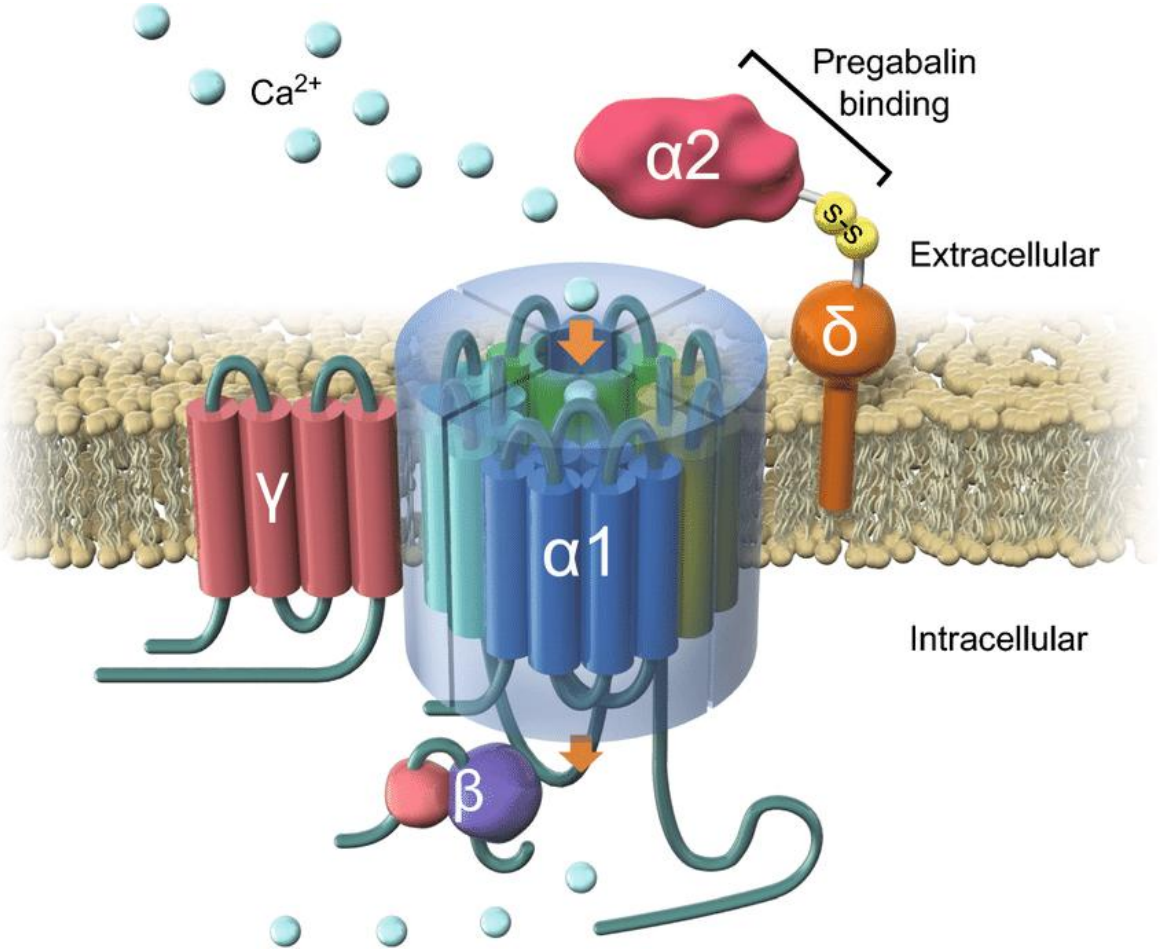
Orasanu et al. Curr Heart Fail Rep 2016

Régulation de l'activité pacemaker par le système nerveux autonome



Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

Focus 2 L-Type Ca^{2+} Channels

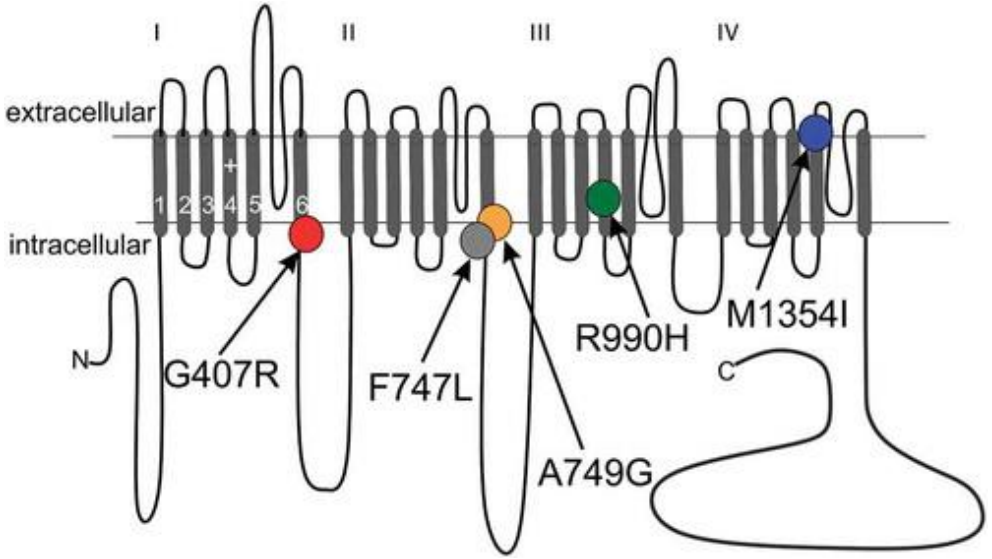


Ninomiya et al. Doc Ophtal 2020

- Voltage-gated calcium channels
- Sensible to dihydropyridines (DHPR, nifedipine)

- Activation: -50 to -30 mV
- Voltage and Ca^{2+}-dependent inactivation

- $Ca_V1.3$ expression: **NSA, NAV,** Brain, Pancreas, Cochlea



Pinggera et al. Channels 2018

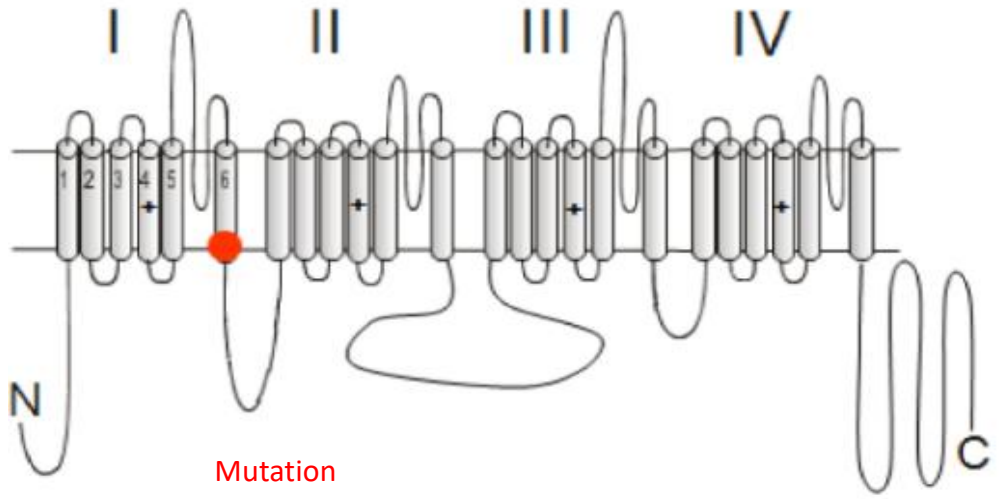
nature neuroscience

Loss of $Ca_v1.3$ (*CACNA1D*) function in a human channelopathy with bradycardia and congenital deafness

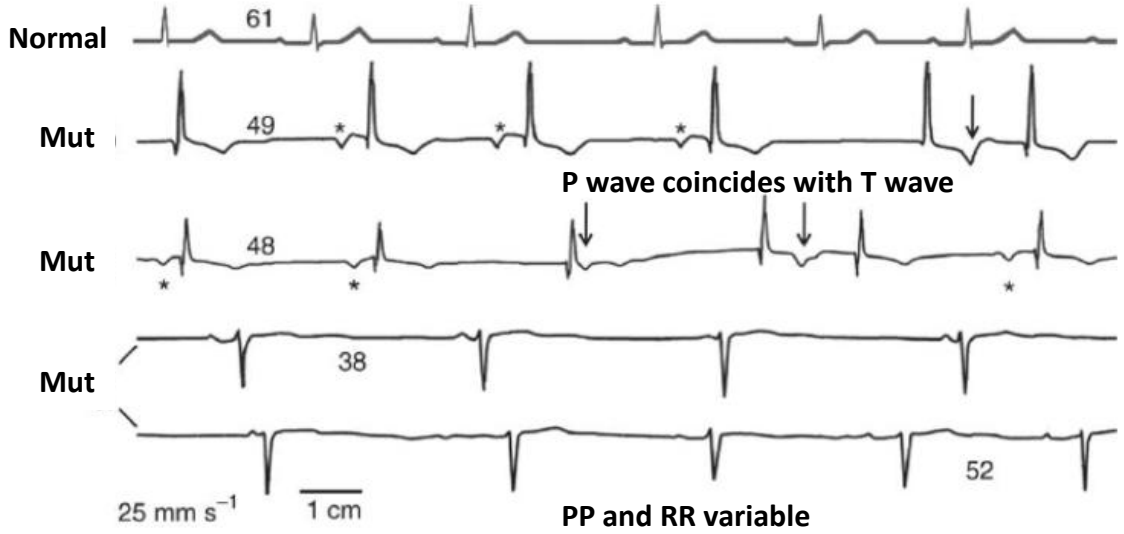
Shahid M Baig[1,11], Alexandra Koschak[2,11], Andreas Lieb[2], Mathias Gebhart[2], Claudia Dafinger[3], Gudrun Nürnberg[4], Amjad Ali[1], Ilyas Ahmad[1], Martina J Sinnegger-Brauns[2], Niels Brandt[5,6], Jutta Engel[5,6], Matteo E Mangoni[7], Muhammad Farooq[1], Habib U Khan[8], Peter Nürnberg[4,9], Jörg Striessnig[2] & Hanno J Bolz[3,10]

SANDD patients (sinoatrial node dysfunction and deafness)

α1 subunit



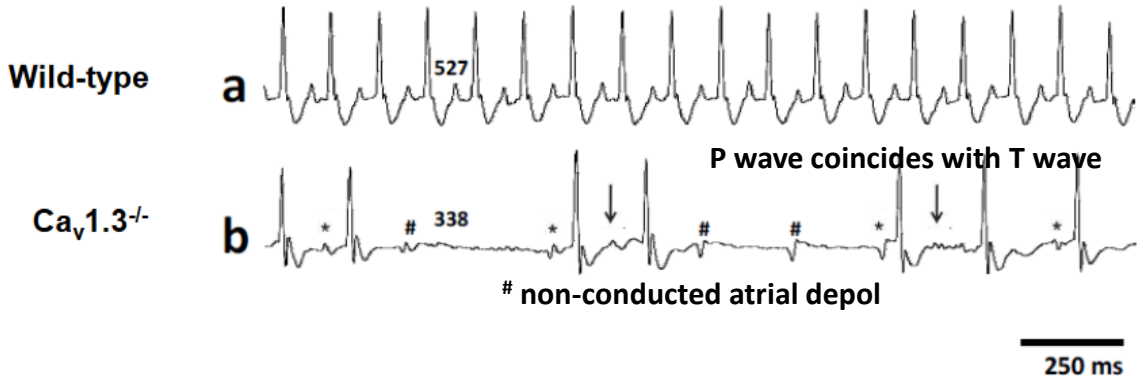
Mutation
p.403-404InsGly *CACNA1D*



Non-conducting calcium channels - abnormal voltage-dependent gating.
All deaf subjects showed pronounced SAN dysfunction at rest.

Baig et al. Nat Neurosci, 2011

Focus 2 $Ca_V1.3$ - L-Type Ca^{2+} Channels



Torrente et al. Cardiovasc Res, 2016

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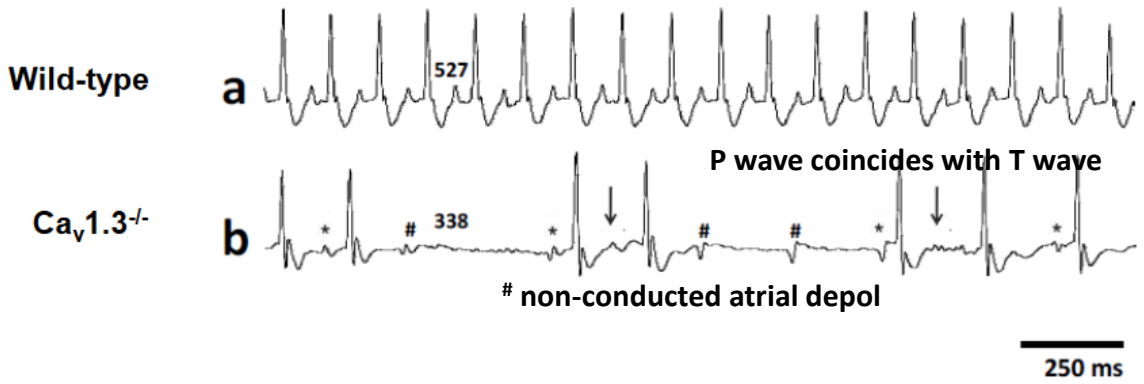
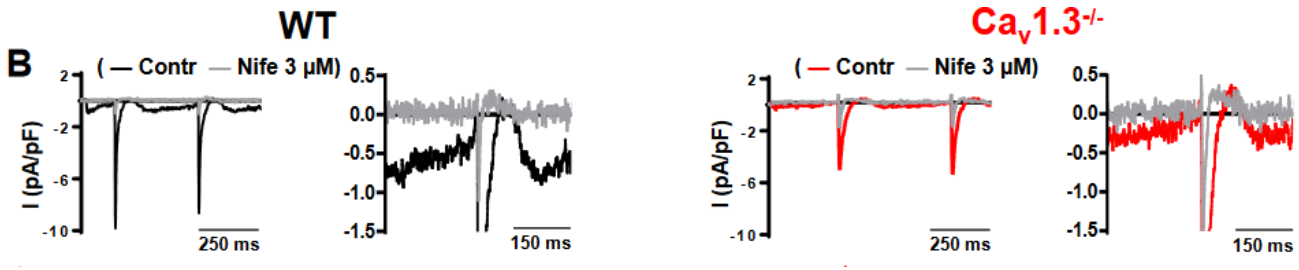


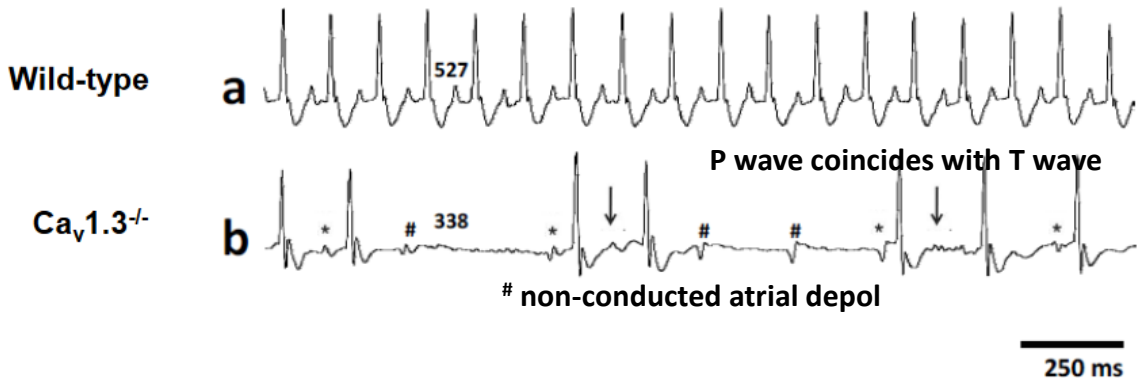
SA node cells



Strong decrease of $I_{Ca,L}$ in Cav1.3$^{-/-}$ cells

Torrente et al. Cardiovasc Res, 2016

Focus 2 $Ca_V1.3$ - L-Type Ca^{2+} Channels



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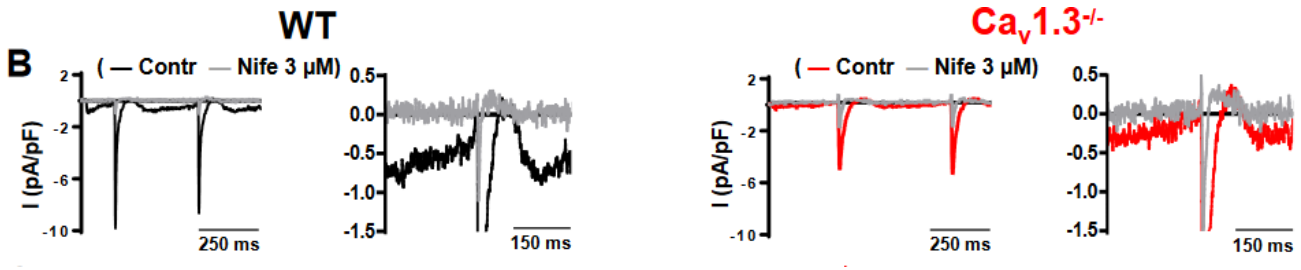

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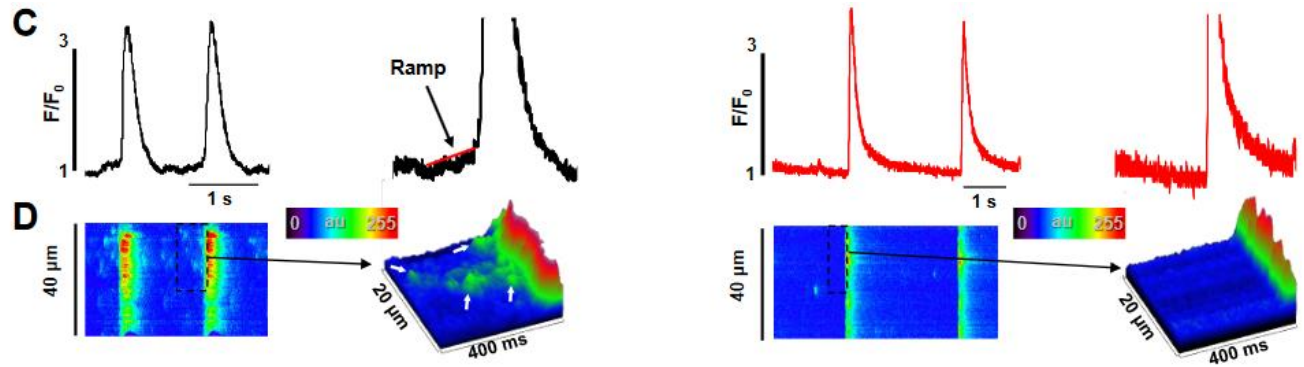
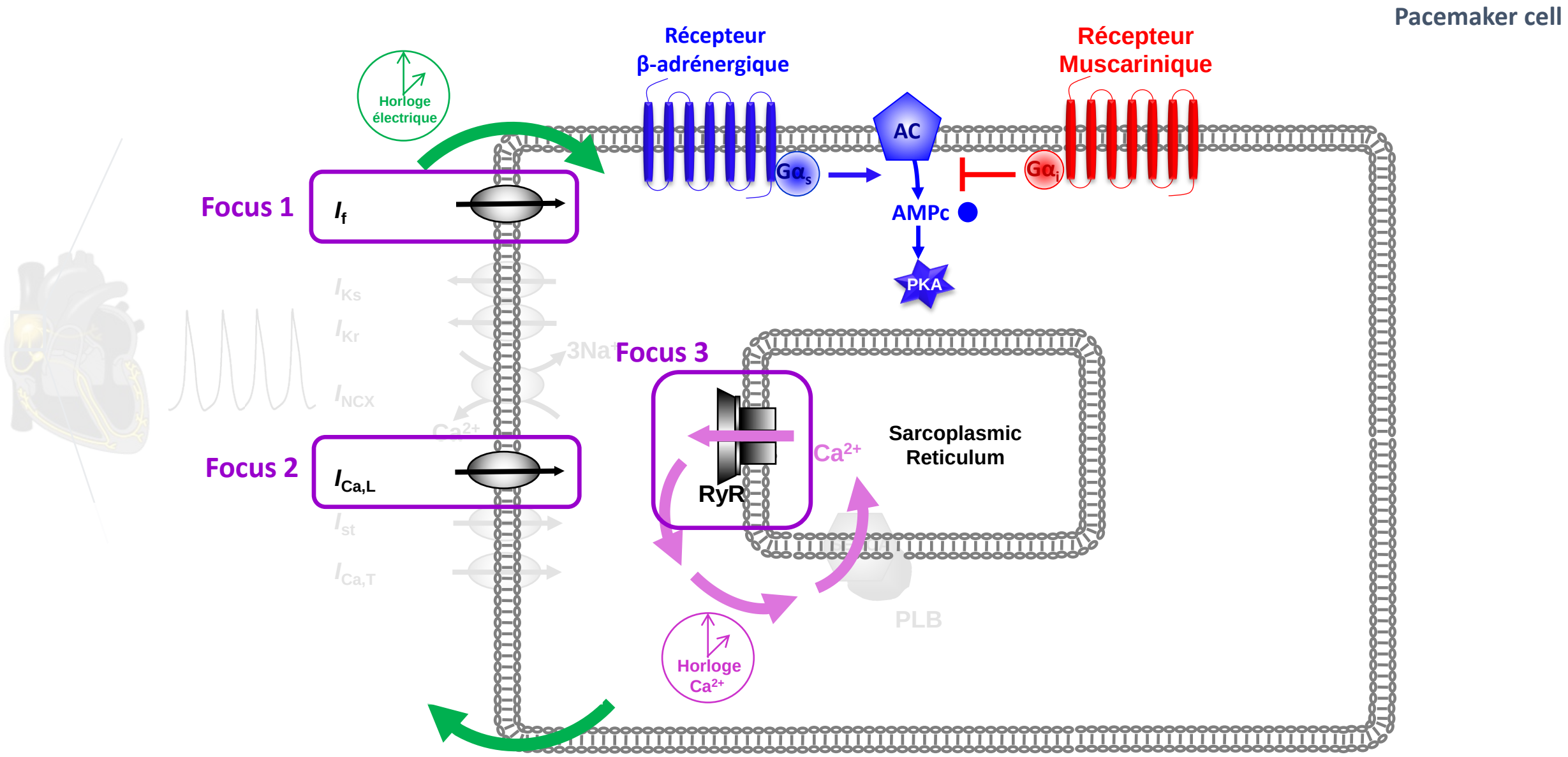


Strong decrease of LDCRs and CL in Cav1.3$^{-/-}$ cells
=> Ca^{2+} entry *via* $Ca_V1.3$ triggers LDCRs

Torrente et al. Cardiovasc Res, 2016

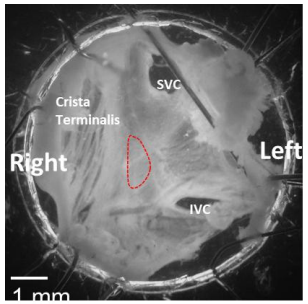
Régulation de l'activité pacemaker par le système nerveux autonome



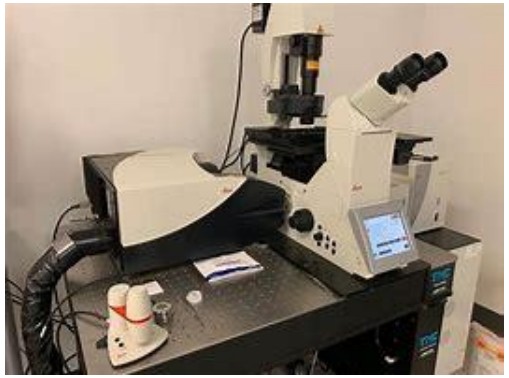
Modifié de Mika & Fischmeister, Prog Biophys Mol Biol., 2021

Measurements of Ca^{2+} homeostasis in SA node

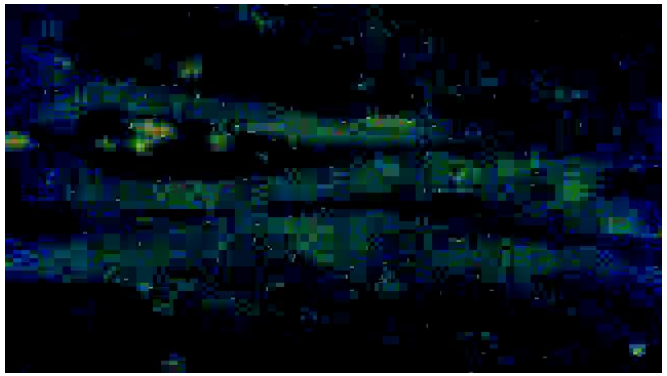
Mouse SA Node

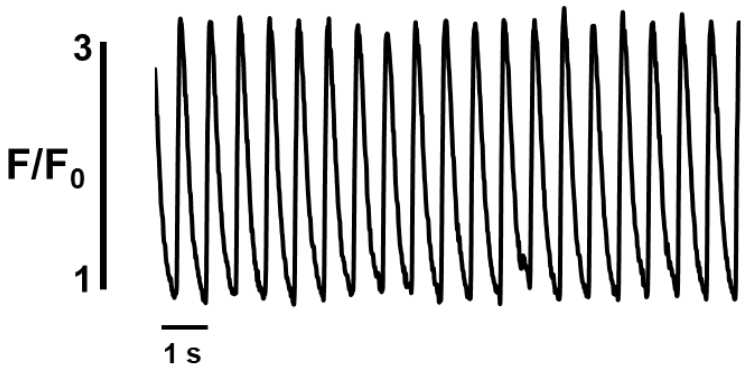


Confocal microscopy
Ca^{2+} Probe: Fluo-4 AM

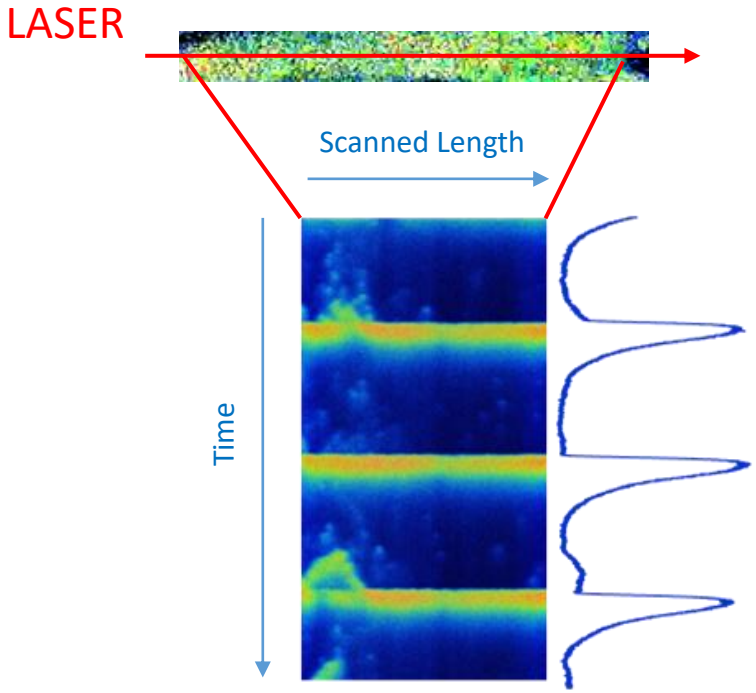


2D Movie: Ca^{2+} oscillations



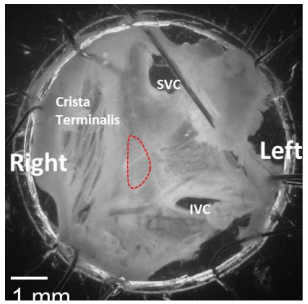


Line-scan configuration mode

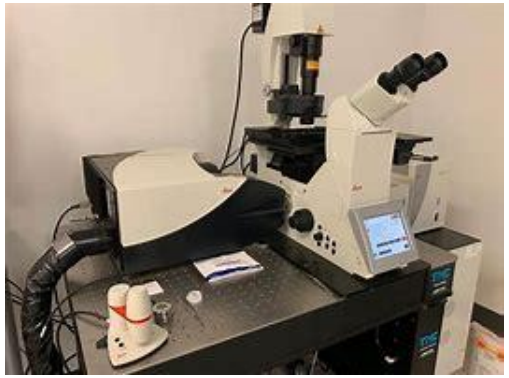


Measurements of Ca^{2+} homeostasis in SA node

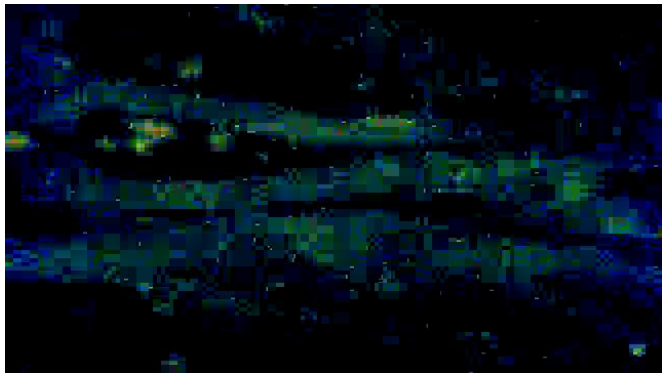
Mouse SA Node

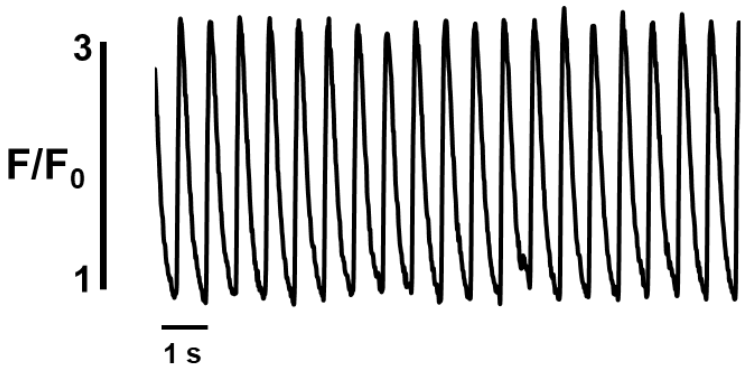


Confocal microscopy
Ca^{2+} Probe: Fluo-4 AM

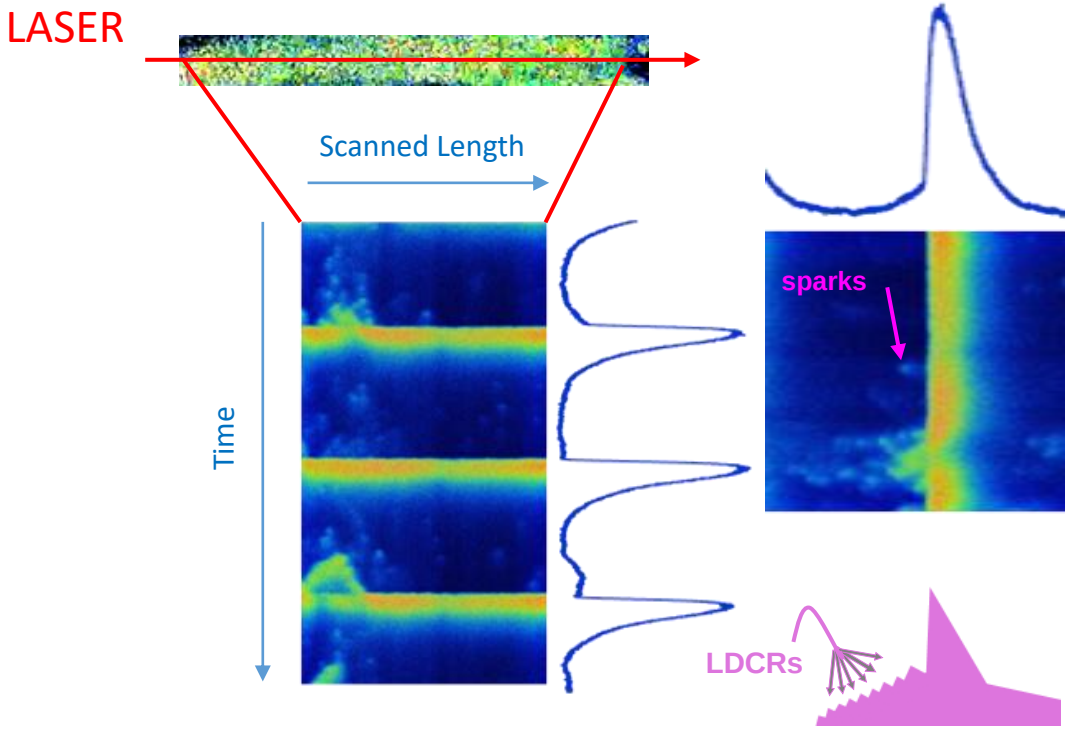


2D Movie: Ca^{2+} oscillations

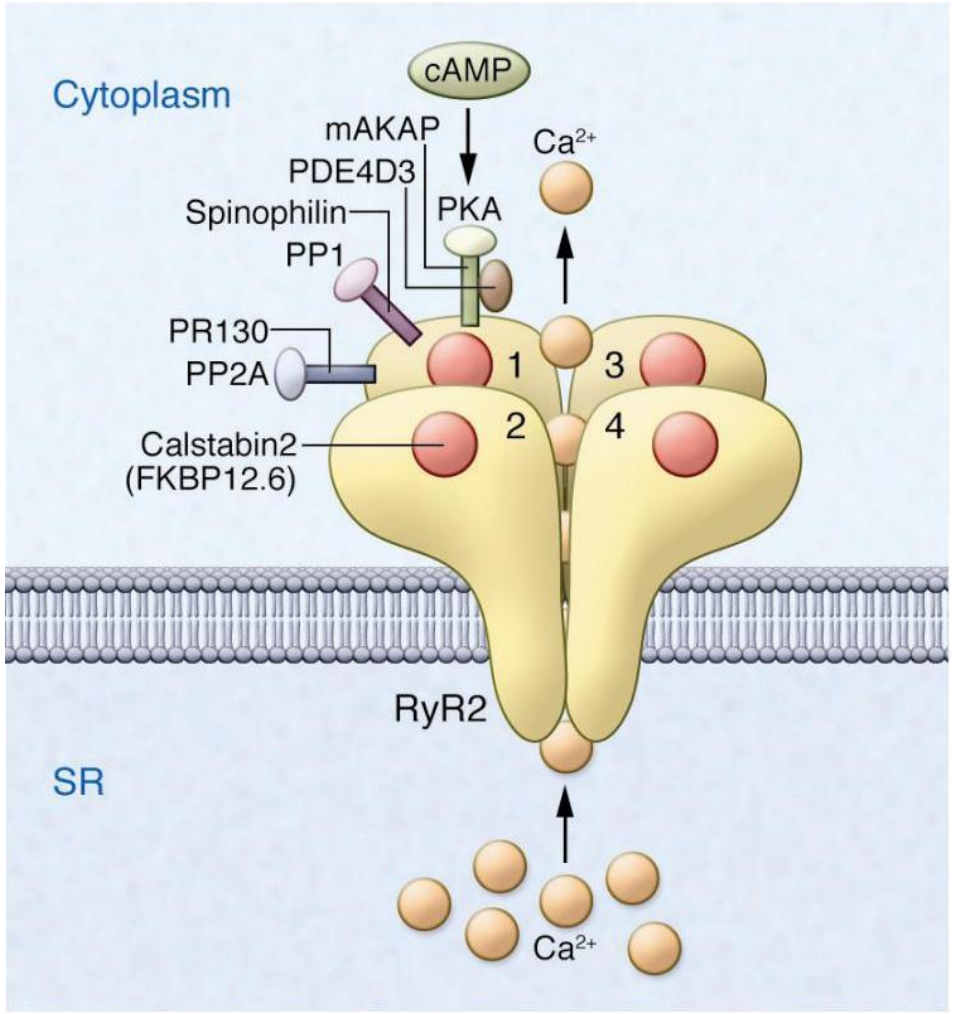




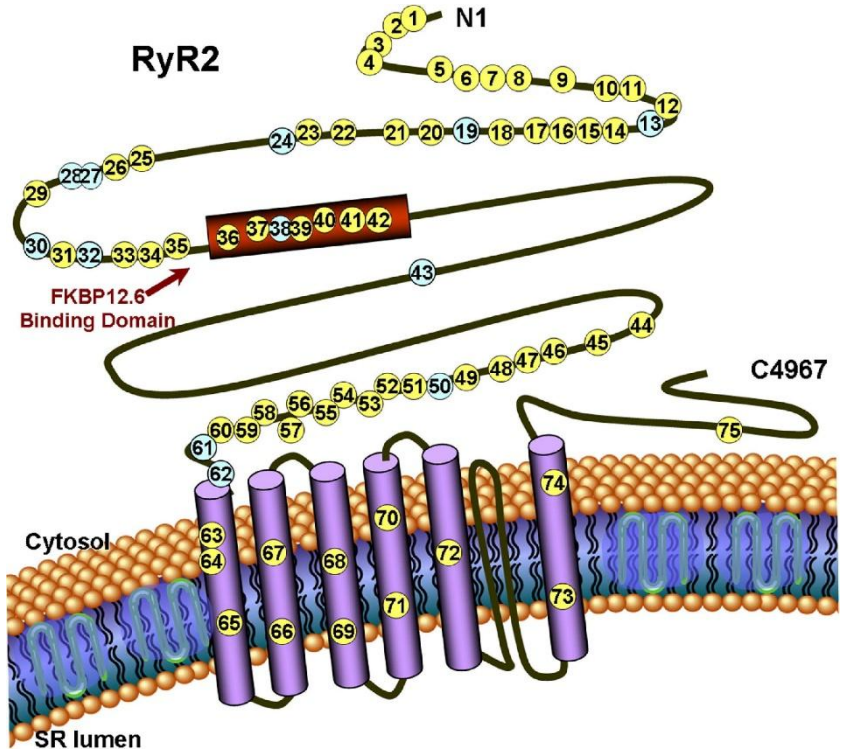
Line-scan configuration mode



Focus 3 Ryanodine Receptor (RyR2)



- Type 2 Ryanodine Receptor expressed in the heart
- Homo-tetramer (4 monomers of 565 kDa)
- Trans-membrane channel sensible to ryanodine



Medeiros-Domingo et al. JACC, 2009

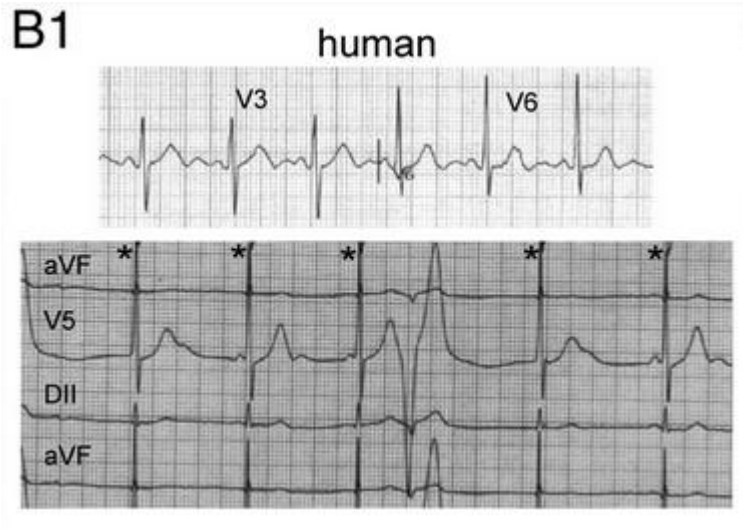
Focus 3 Ryanodine Receptor (RyR2)

CPVT patients

Mutation RyR^{R4496C}

At rest

After exercice



Isolated ventricular extrasystoles
sudden decrease in sinus rate overcome by a junctional escape rhythm (*)

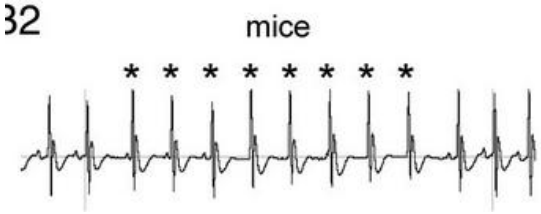
Neco et al Circulation 2012

Focus 3 Ryanodine Receptor (RyR2)

CPVT patients

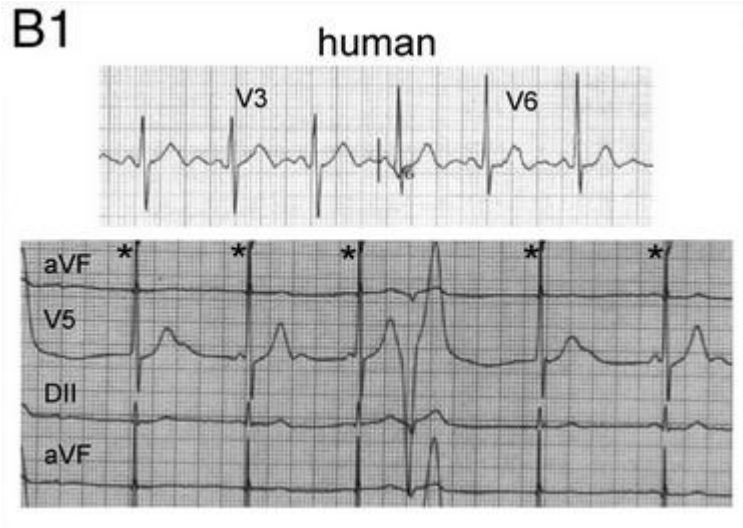
Mutation RyR^{R4496C}

Under ISO



At rest

After exercice



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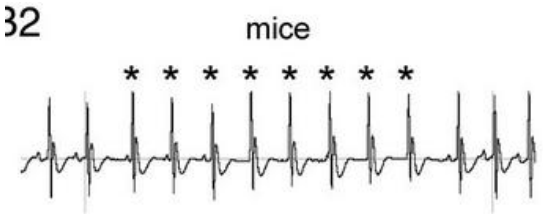
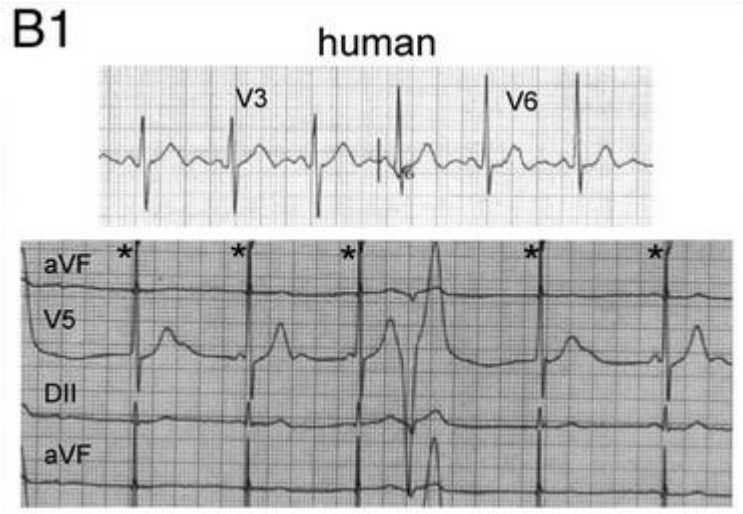
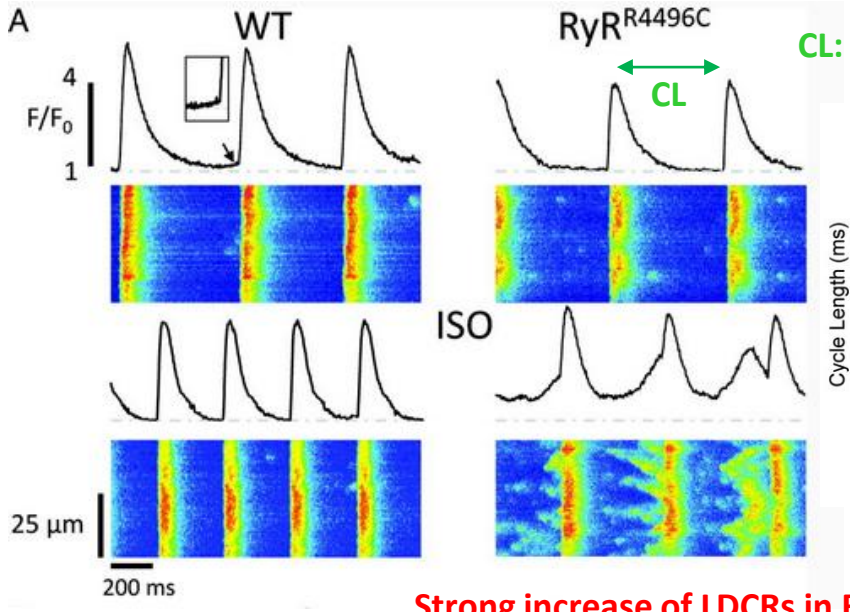


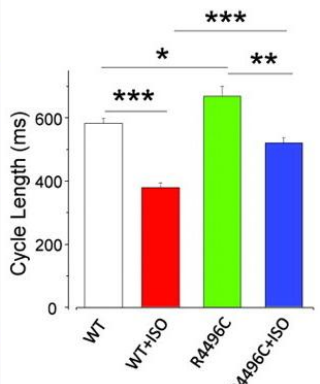
At rest

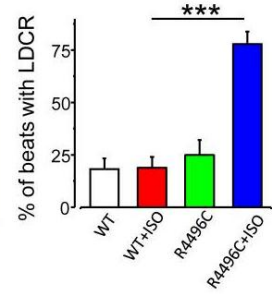
After exercice





CL: cycle length





Strong increase of LDCRs in RyR^{R4496C} mice under ISO

Isolated ventricular extrasystoles
sudden decrease in sinus rate overcome by a junctional escape rhythm (*)

Neco et al Circulation 2012

Focus 3 Ryanodine Receptor (RyR2)

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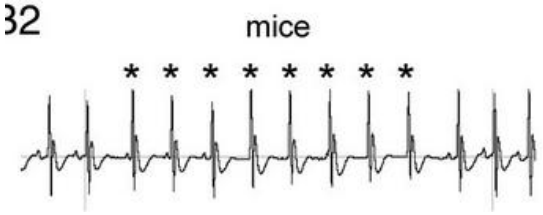
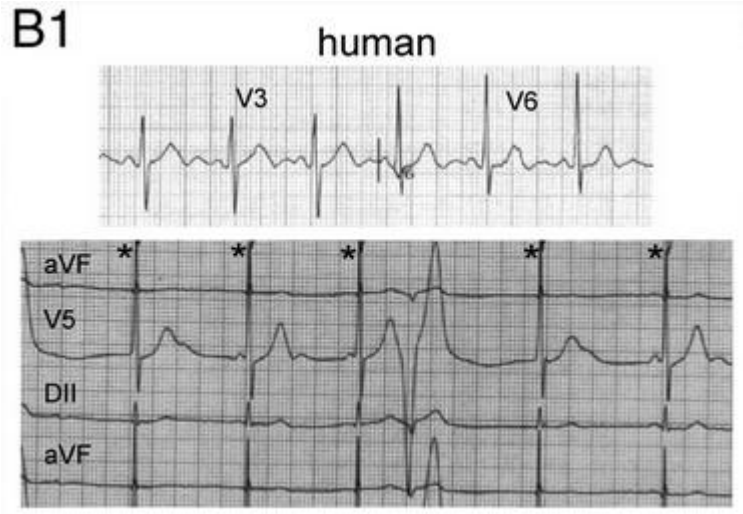
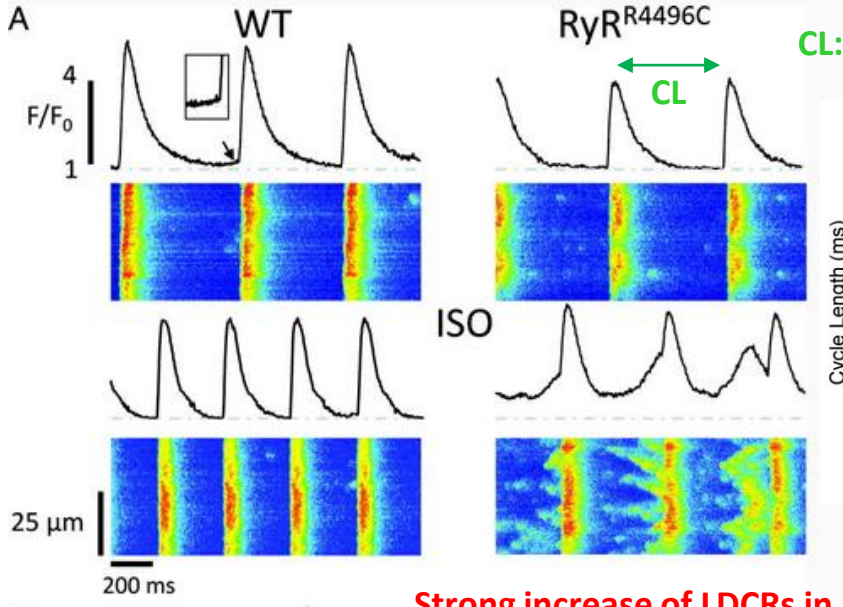


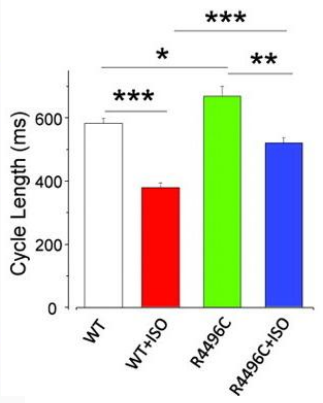
At rest

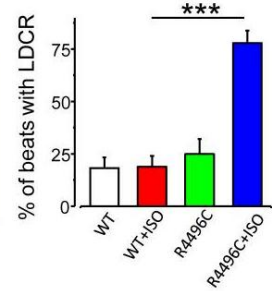
After exercice





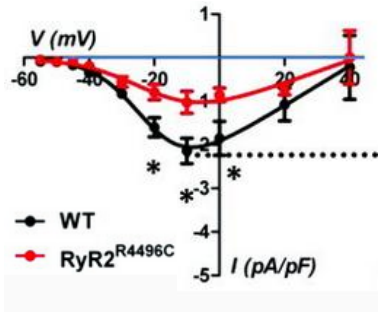
CL: cycle length

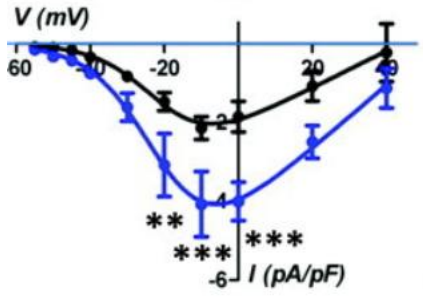


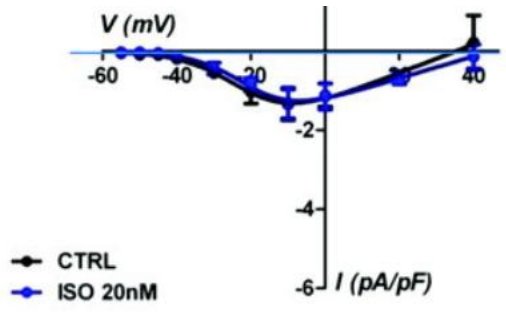


Strong increase of LDCRs in RyR^{R4496C} mice under ISO

Control ***WT*** ***RyR^{R4496C}***







Increased SR Ca2+ release in RyR2^{R4496C} partially inactivates LTCC, leading to slowing of pacemaker activity

Neco et al Circulation 2012

THANK YOU

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