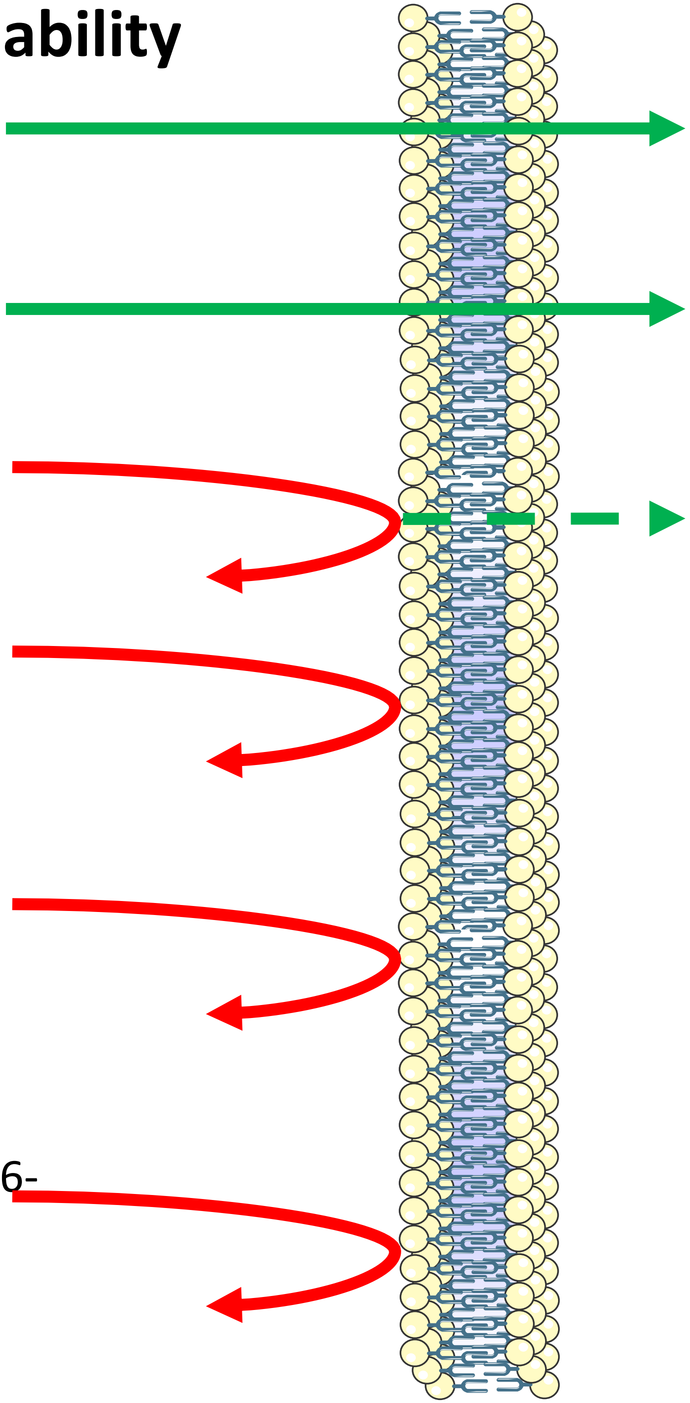
Cardiac electrophysiology

Romain Perrier
MCU physiologie
INSERM UMR-S1180 Signalisation et physiopathologie cardiovasculaire
HM1 – 3rd floor
romain.perrier@universite-paris-saclay.fr

Genesis of membrane potential

Lipids bilayer permeability



Gaz	CO_2, O_2, N_2
Small uncharged polar molecules	Ethanol, urea, stéroids , glycerol
Water	H_2O
Ions	Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}
Large uncharged polar molecules	Glucose, fructose,…
Charged polar molecules	Amino acid, ATP, glucose 6-P, proteins, nucleic acids

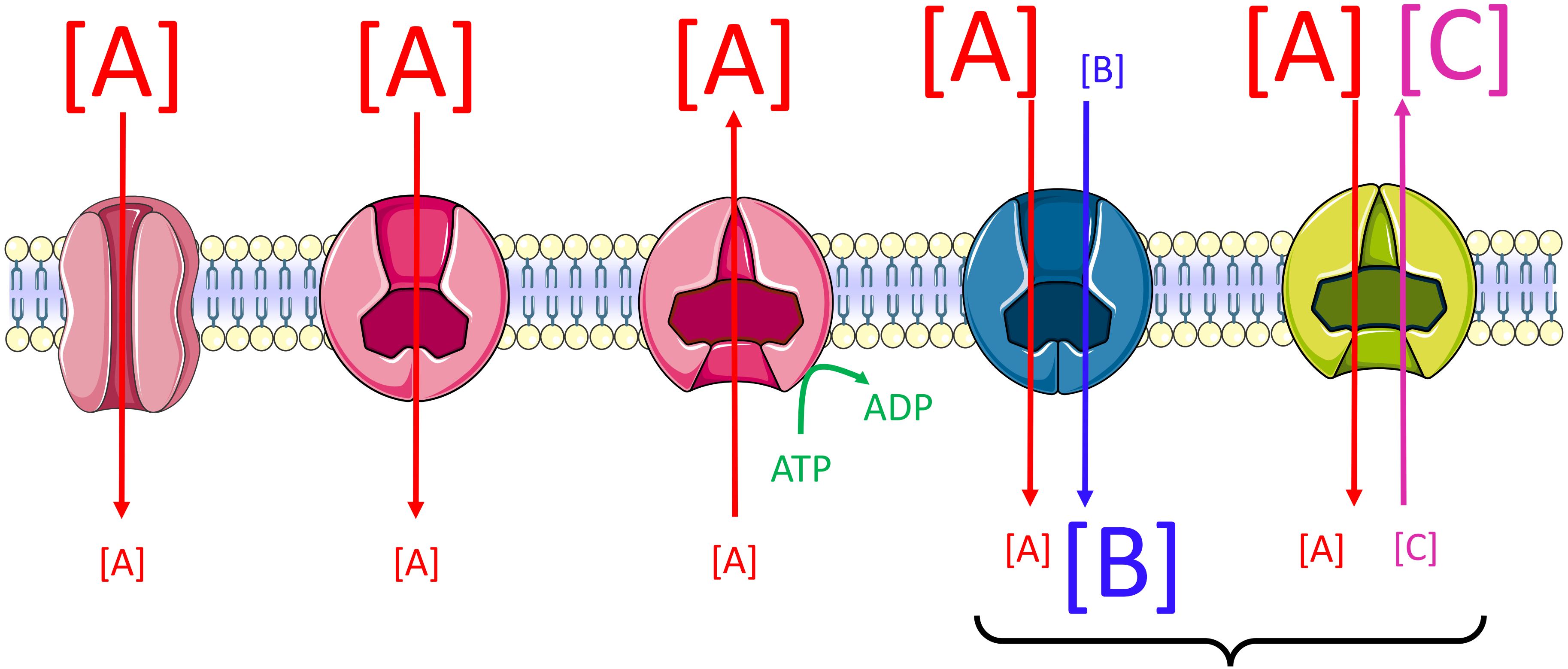
Channels
Pores

Transporters

ATPase pump
ATP-dependant
transporters

Symporters

Antiporters



Facilitated
diffusion

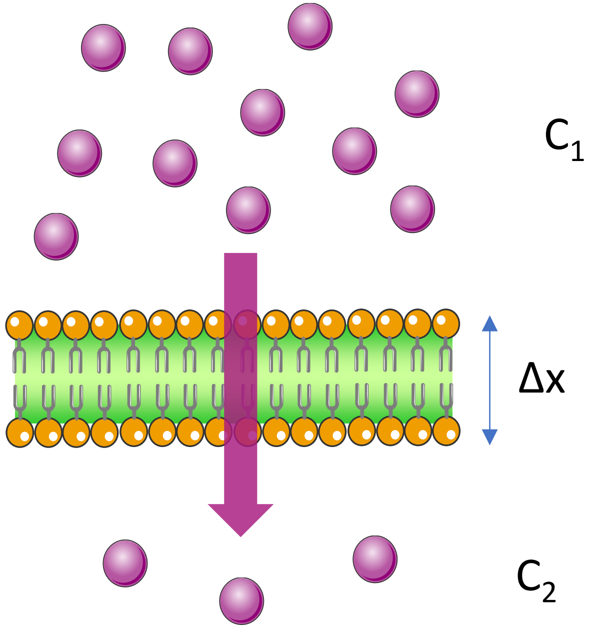
Passive
transport

Primary
active
transport

Secondary
active
transport

Fick's laws of diffusion

$$J_d = \frac{\mathrm{d}n}{\mathrm{d}t} = -D \cdot \frac{\mathrm{d}C_P}{\mathrm{d}x}$$



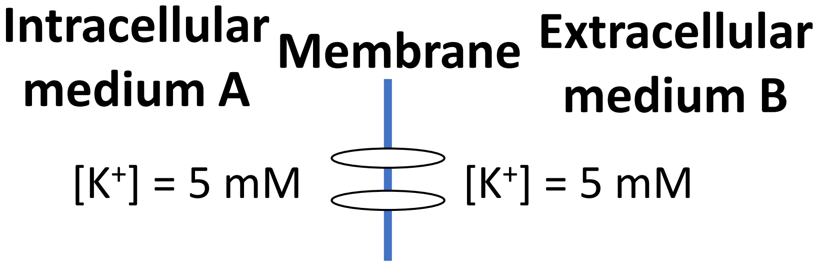
Jd : diffusion flux

D : diffusion coefficient

For an **uncharged** molecule, diffusion occures from the most concentrated compartment to the least concentrated

Equilibrium : $C_1 = C_2$

Reversal potential



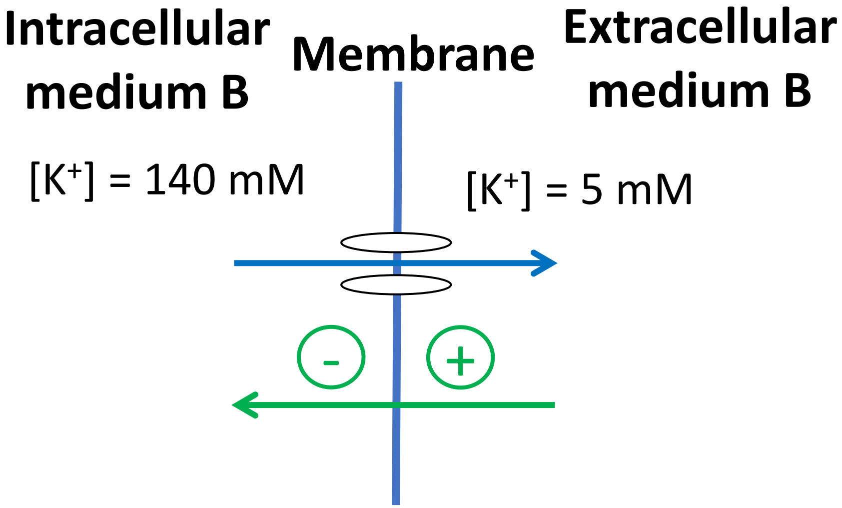
Intracellular medium A

Membrane

Extracellular medium B

$[K^+] = 5$ mM

$[K^+] = 5$ mM



Intracellular medium B

Membrane

Extracellular medium B

$[K^+] = 140$ mM

$[K^+] = 5$ mM

$[K^+]_{int} = [K^+]_{ext}$

- No electric potential difference (0 mV)

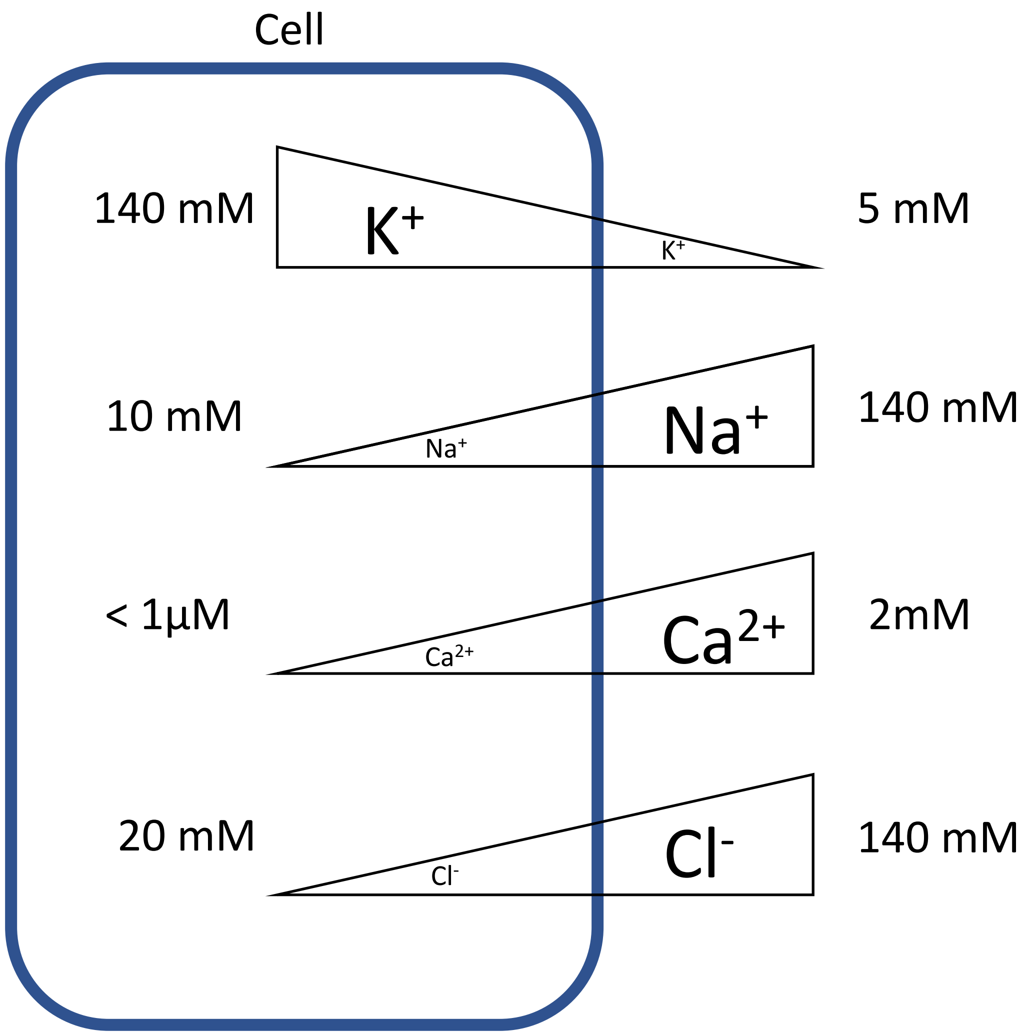
$[K^+]_{int} > [K^+]_{ext}$

- K^+ ions move from A to B regarding **concentration gradient** with a movement of positive charges
- creation of an **electric gradient** (electric potential of medium A: negative / milieu B) which opposes the persistence of K^+ flux

- **Stopping of K^+ flux**: perfect equality of two opposing forces, the concentration gradient (K^+ from A to B) and the electric gradient (K^+ from B to A)

☛ **Reversal (equilibrium) potential of** K^+ ions

Reversal potential



Reversal potential for an ion : membrane potential at which there is no net ion flux

Nernst equation :

$$E_{ion} = \frac{RT}{zF} \ln \frac{c_e}{c_i}$$

R: Ideal gas constant: $8.314\ \mathrm{J.K^{-1}.mol^{-1}}$

T: Temperature in Kelvin

z: Valence

F: Faraday constant: $96\,485\ \mathrm{C.mol^{-1}}$

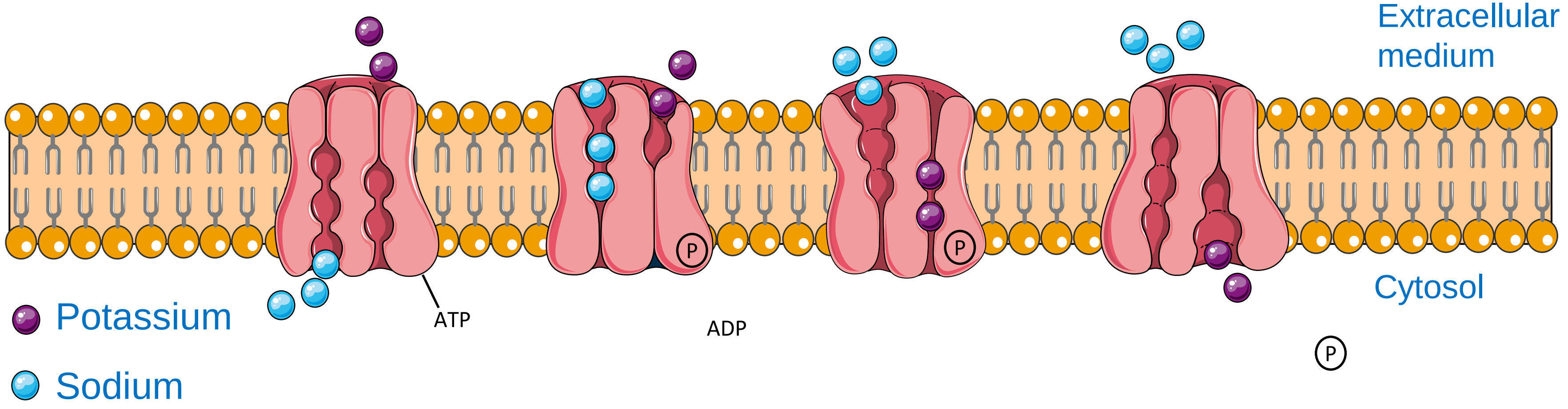
$E_{Na} = + 60$ mV

$E_K = - 100$ mV

$E_{Ca} = + 100$ mV

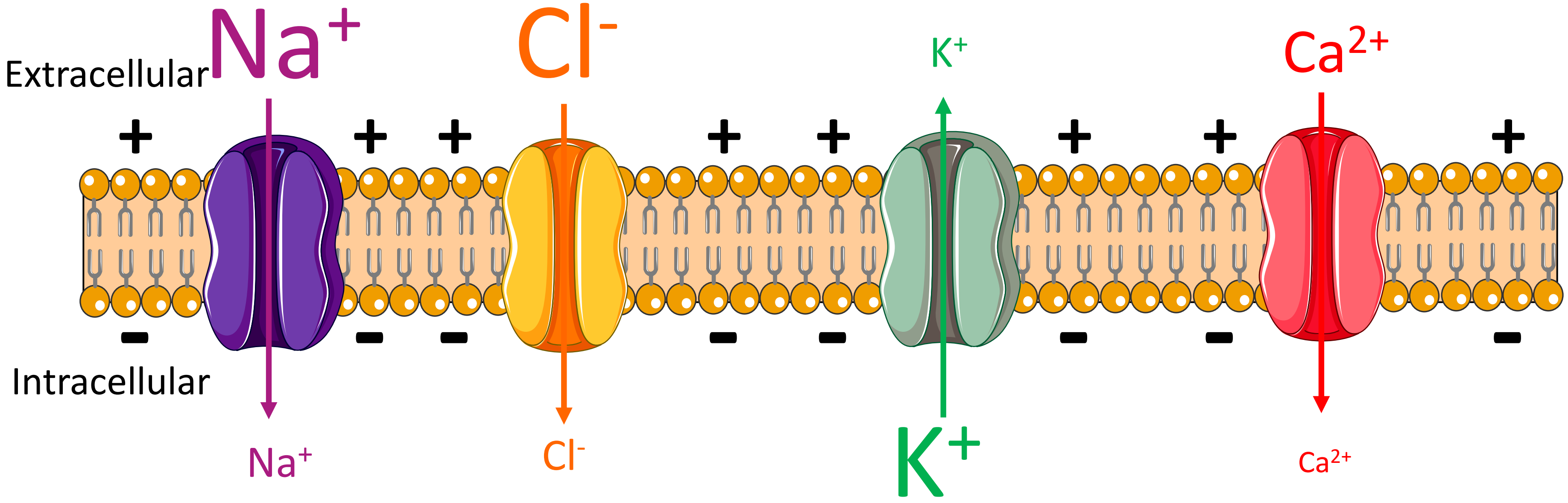
$E_{Cl} = - 50$ mV

Na/K ATPase pump



Maintenance of the electrochemical gradient: essential for the electrical activity of excitable cells

Membrane potential

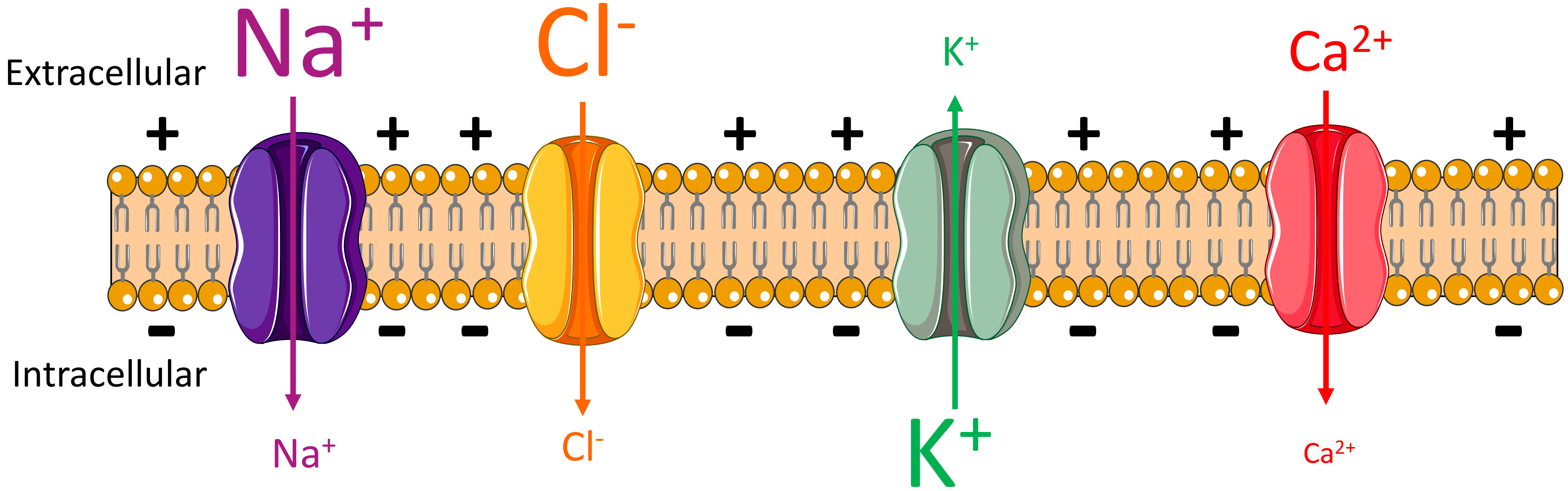


Membrane potential: potential difference between intracellular and extracellular compartment

Goldman-Hodgkin-Katz voltage equation (for monovalent ions)

$$E_m = \frac{RT}{F} \ln \frac{P_{Na}[Na^+]_{out} + PK[K^+]_{out} + PCl[Cl^-]_{in}}{P_{Na}[Na^+]_{in} + PK[K^+]_{in} + PCl[Na^-]_{out}}$$

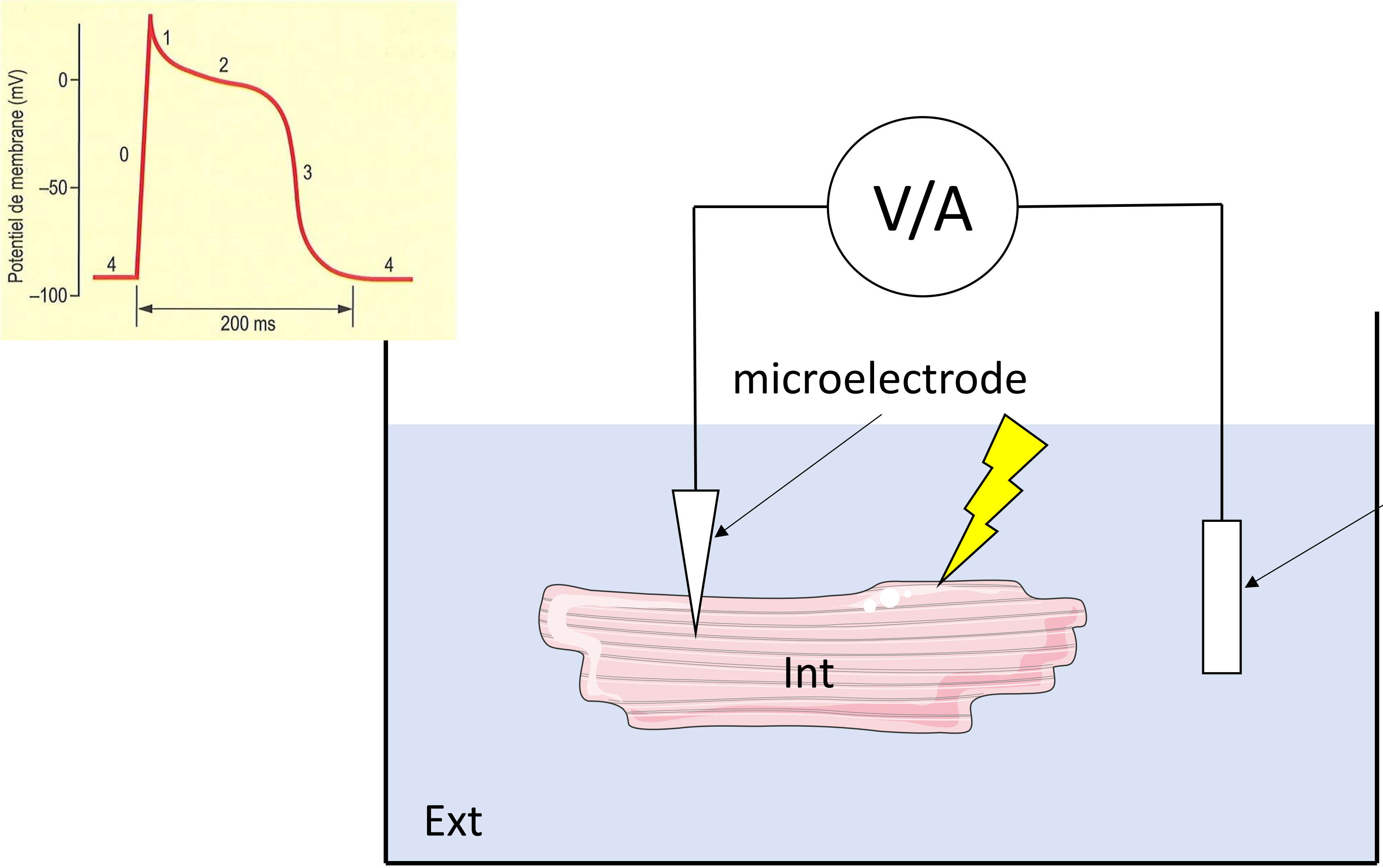
Membrane potential



$$E_m = \frac{G_K.EK + GNa.ENa + GCl.ECl + GCa.ECa}{G_K + GNa + GCl + GCa}$$

Ohm’s law : U = R.I
Conductance G = 1/R

Membrane potential recording



V/A

microelectrode

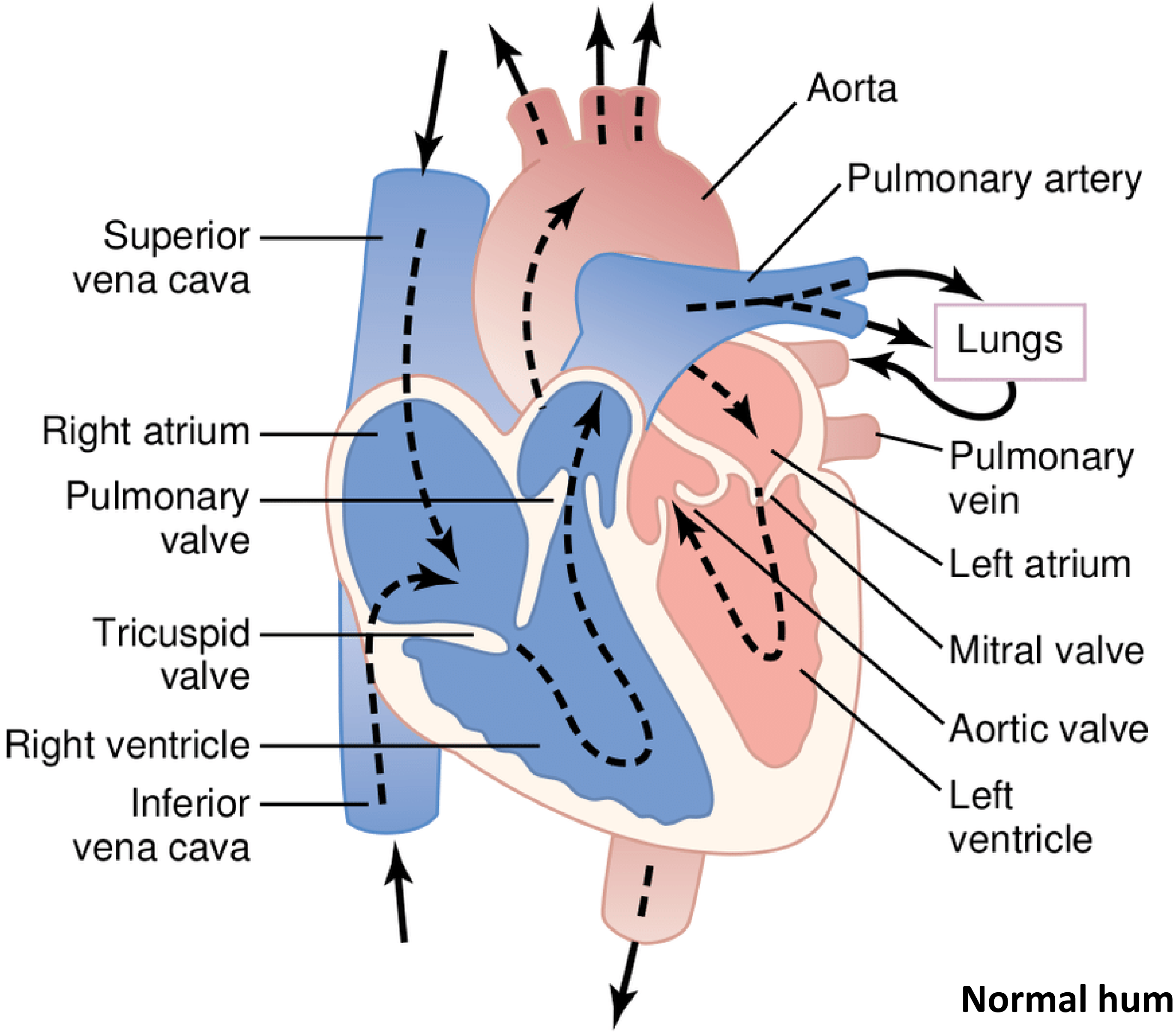
Reference electrode

Int

Ext

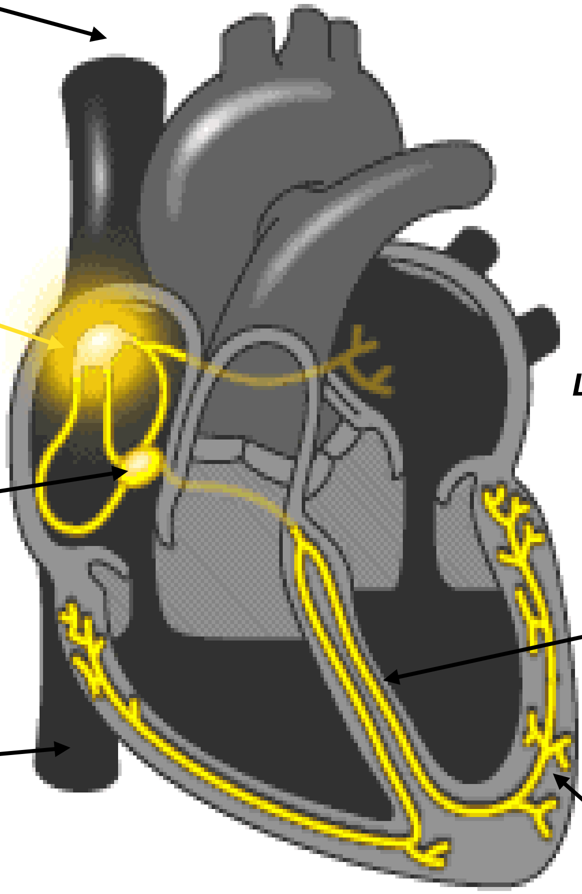
Cardiac electrophysiology

Blood Flow Through the Heart



Normal human heart: 60-100 cycles /min

Cardiac Conduction System



Superior vena cava

Sinoatrial Node
= primary pacemaker

Right Atria

Left Atria

Auriculo-ventricular Node

Bundle of His

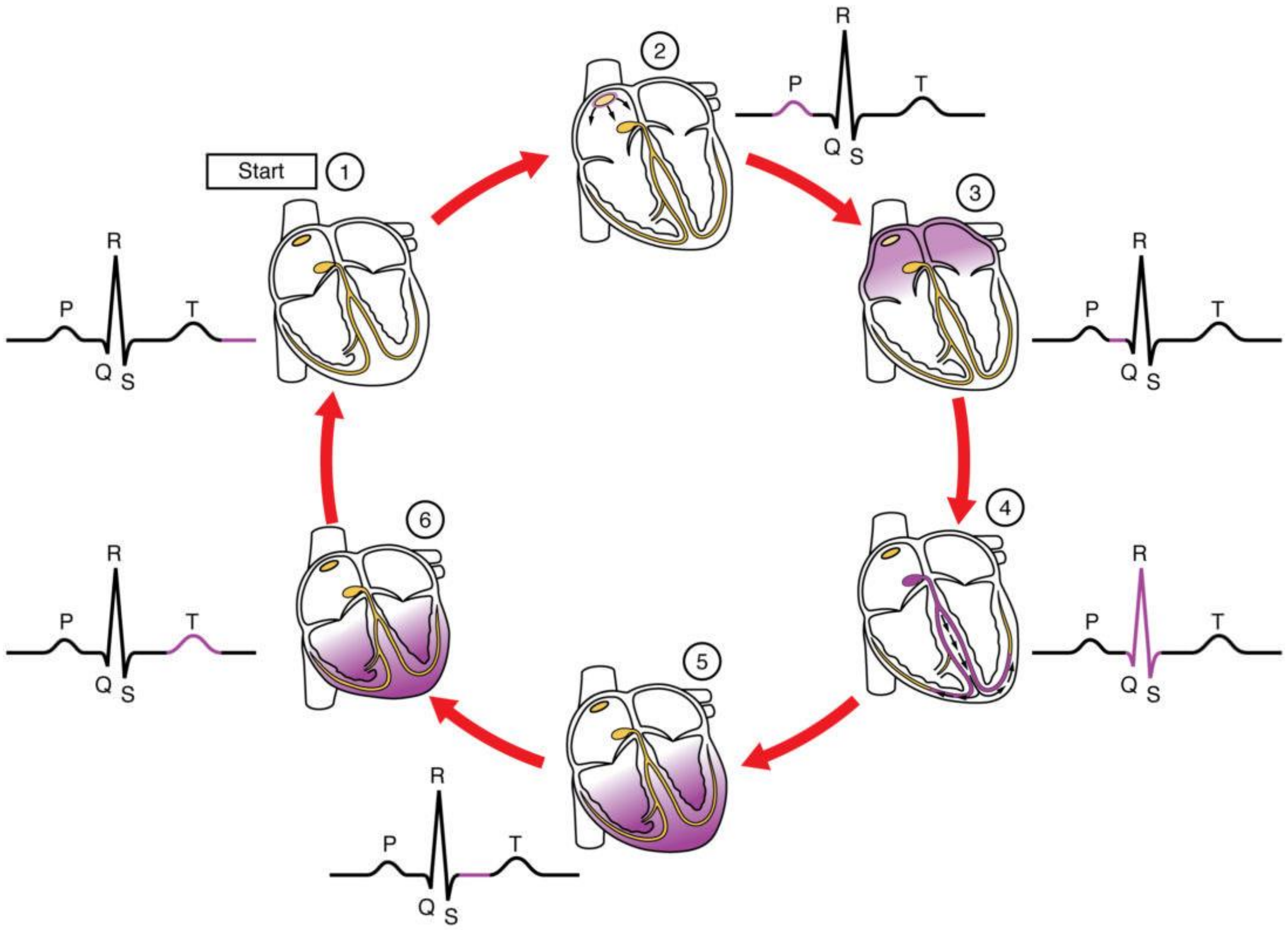
Left Ventricle

Inferior vena cava

Right Ventricle

Purkinje fibers

Electrocardiogram (ECG)



P = Atrial depolarisation

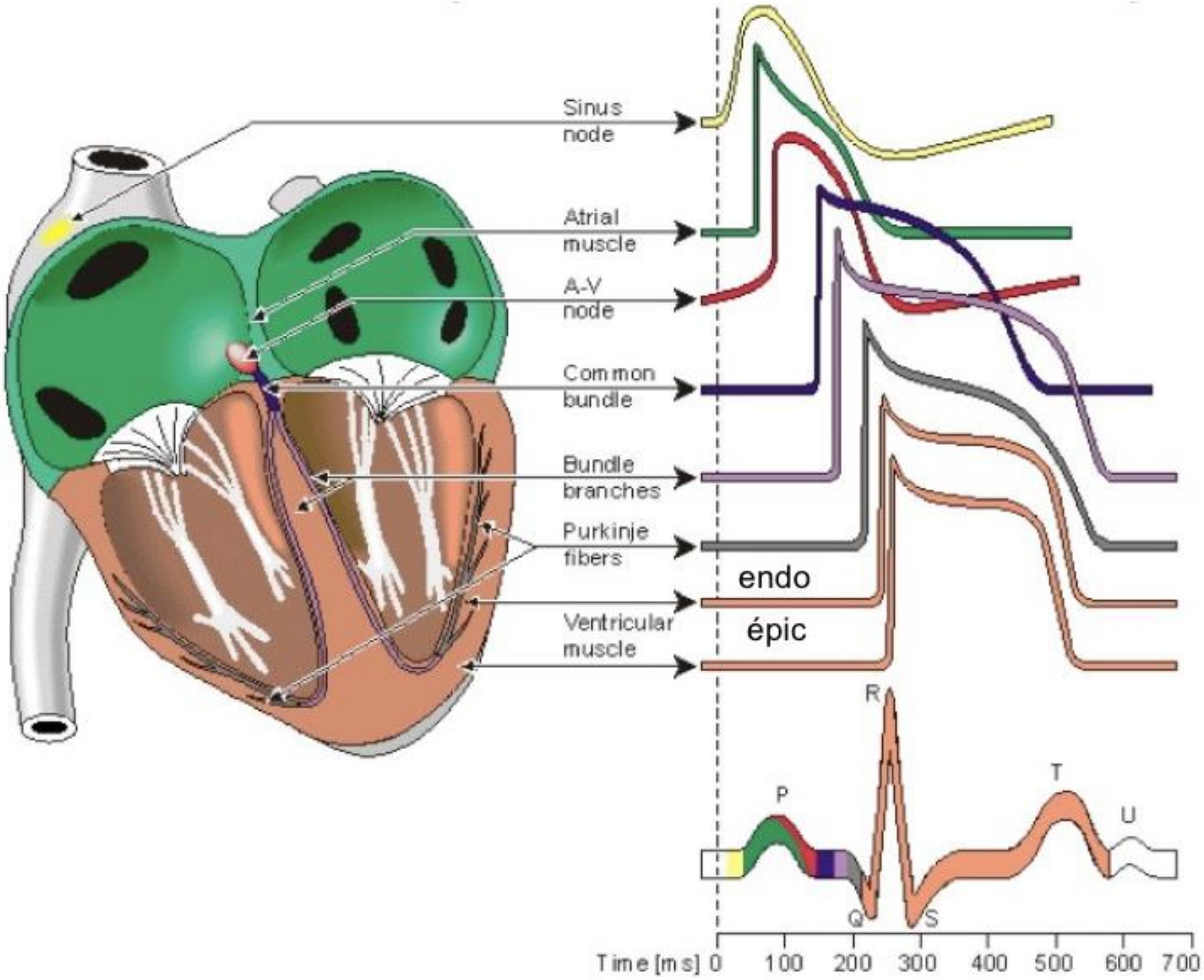
PQ = propagation from SA node to AV node

QRS = ventricular depolarisation
Q = interventricular septum depolarisation
R = main mass ventricular depolarisation
S = last phase of V depolarisation (base)
Atrial repolarisation

ST = plateau of the AP – contraction of the V

T = ventricular repolarisation

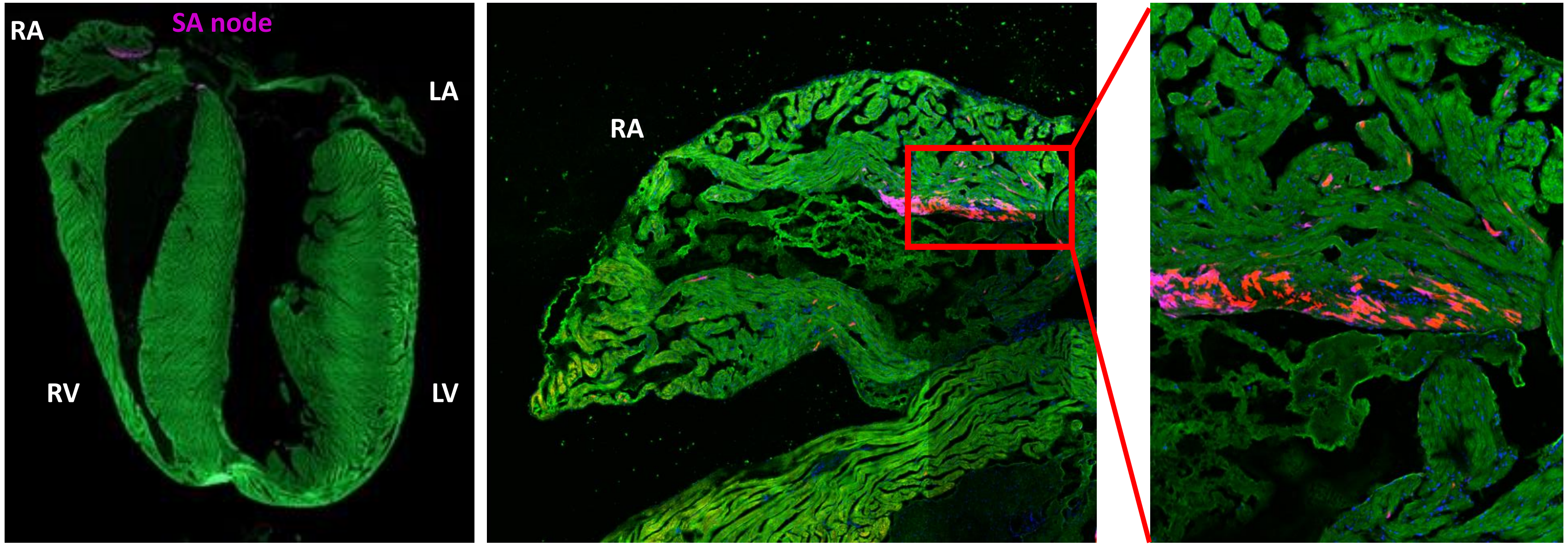
Cardiac Action Potentials



Sinoatrial Node (SA Node): Description

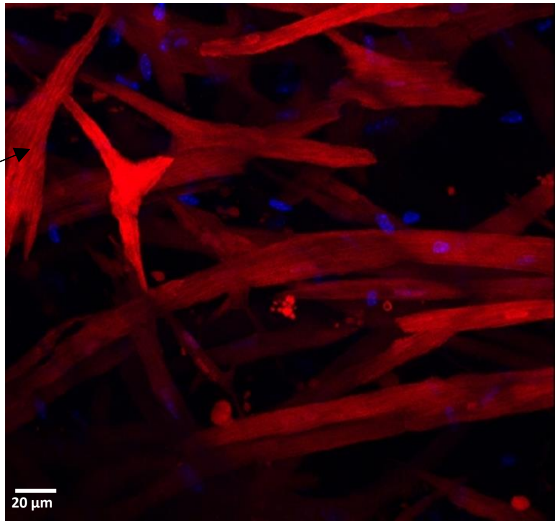
(Mezzano et al. Cardiovasc Res, 2016)

HCN4Cre-ERT2-tomato mice



Pacemaker cells within the tissue

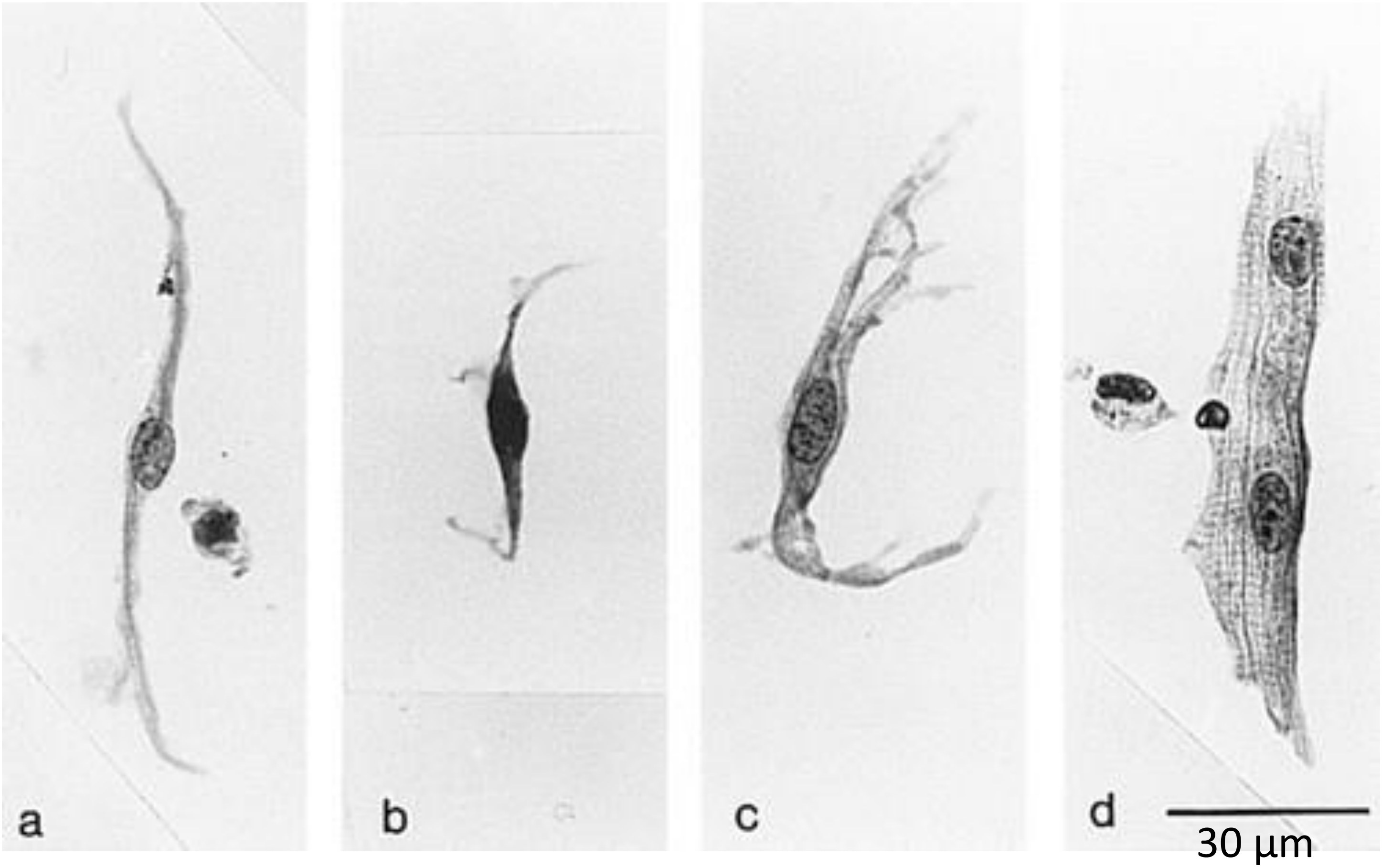
Confocal Imaging



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

D Mika (Châtenay-Malabry), F Rochais (Marseille)

Cell types in the rabbit sinus node



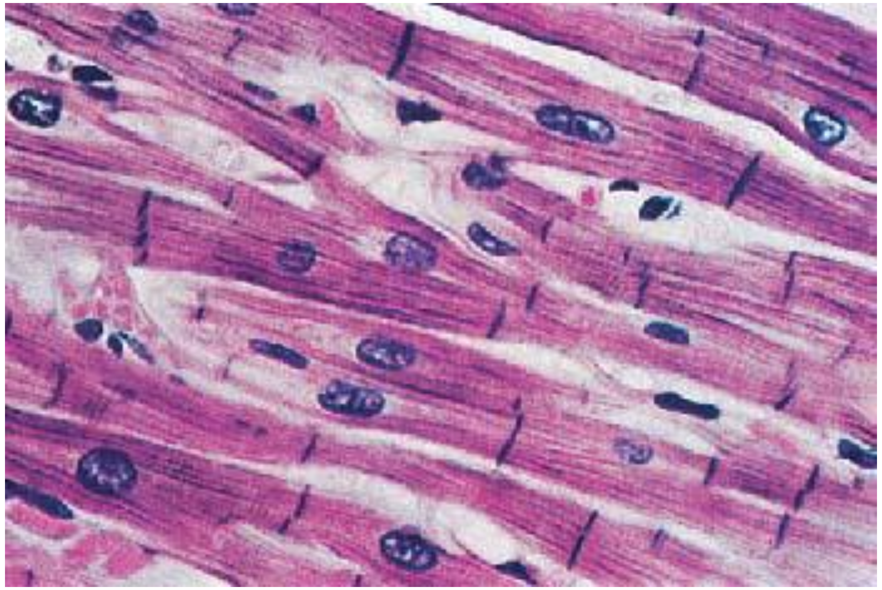
Elongated spindle cell **Spindle cell** **Spider cell** **Atrial cell**

Verheijck et al. Circulation 1998

Ventricular action potential

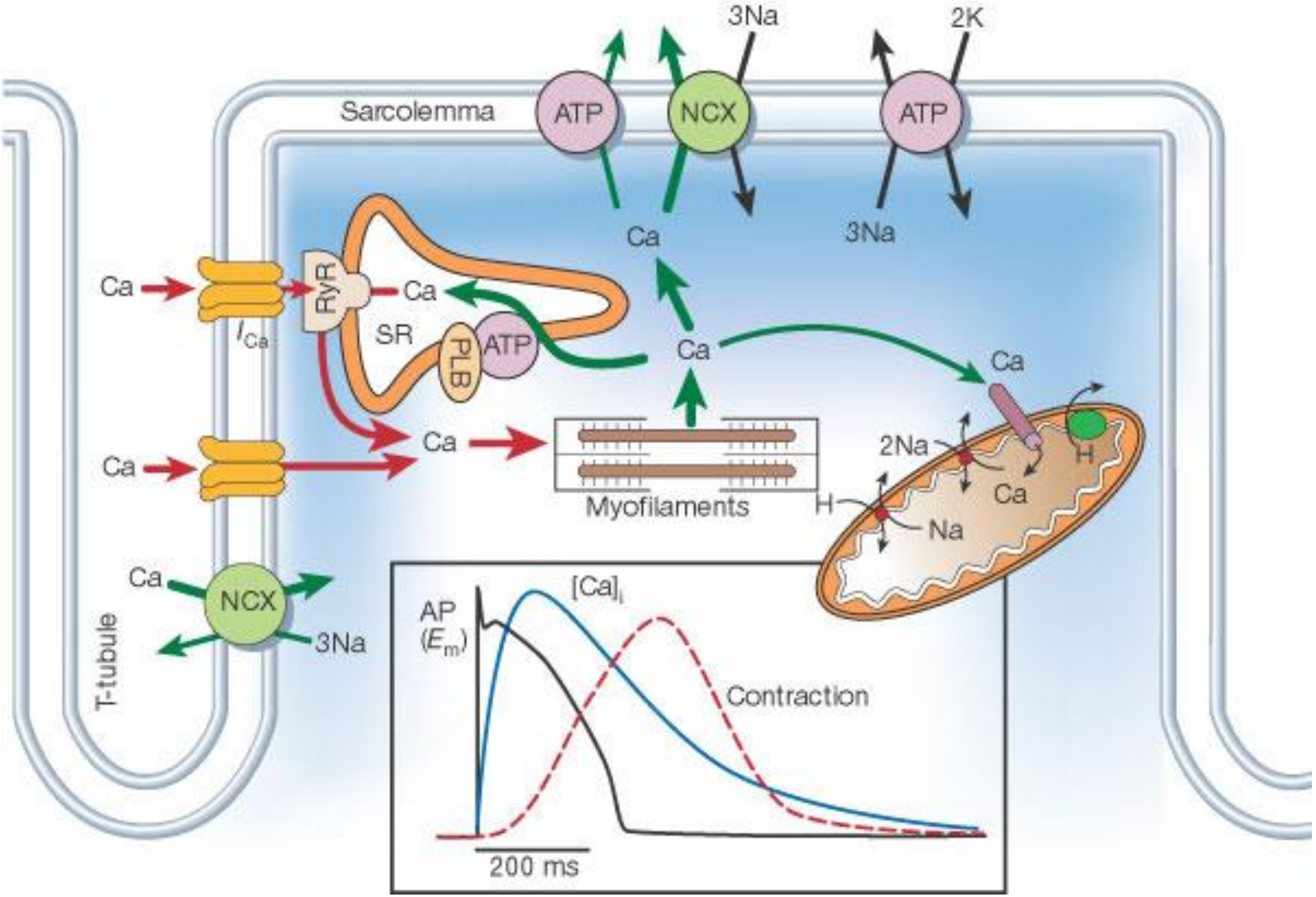
Ventricular cell properties

- Stick shape with ramifications
- Width~25 μm, length~100 μm, thickness <20 μm
- Striated
- Single central nucleus (sometimes 2)
- **Excitables**
- **Contractiles**
- **Conductives**

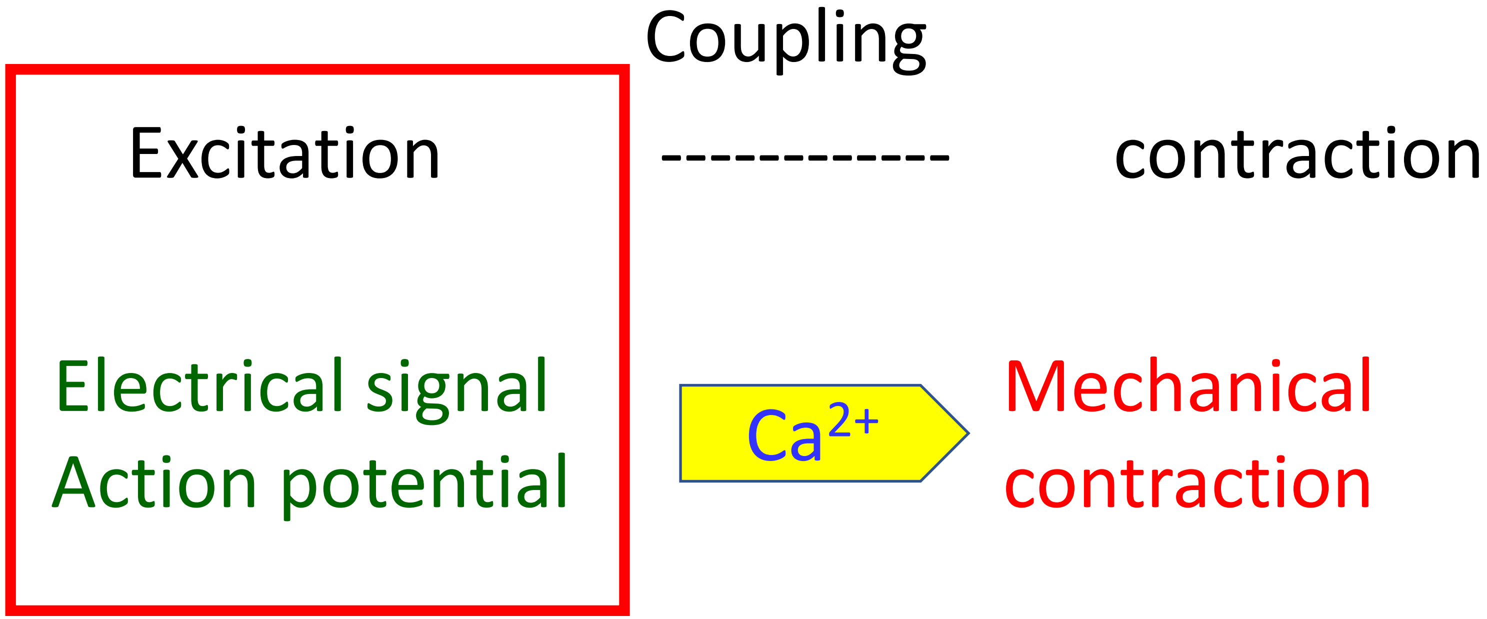


Cf Pf Veksler: Bases of cardiac physiology

Excitation-contraction coupling



Laetitia Pereira 05/12/2024



Coupling

Excitation ------------ contraction

Electrical signal
Action potential

Ca^{2+}

Mechanical
contraction

Patch-Clamp

The Patch-Clamp technique allows to electrically isolate a fragment of membrane or an entire cell in order to apply a current (current clamp) or a potential (voltage clamp) to it and record the response.

Developped by Neher and Sakmann in 1978, and improved in 1981.

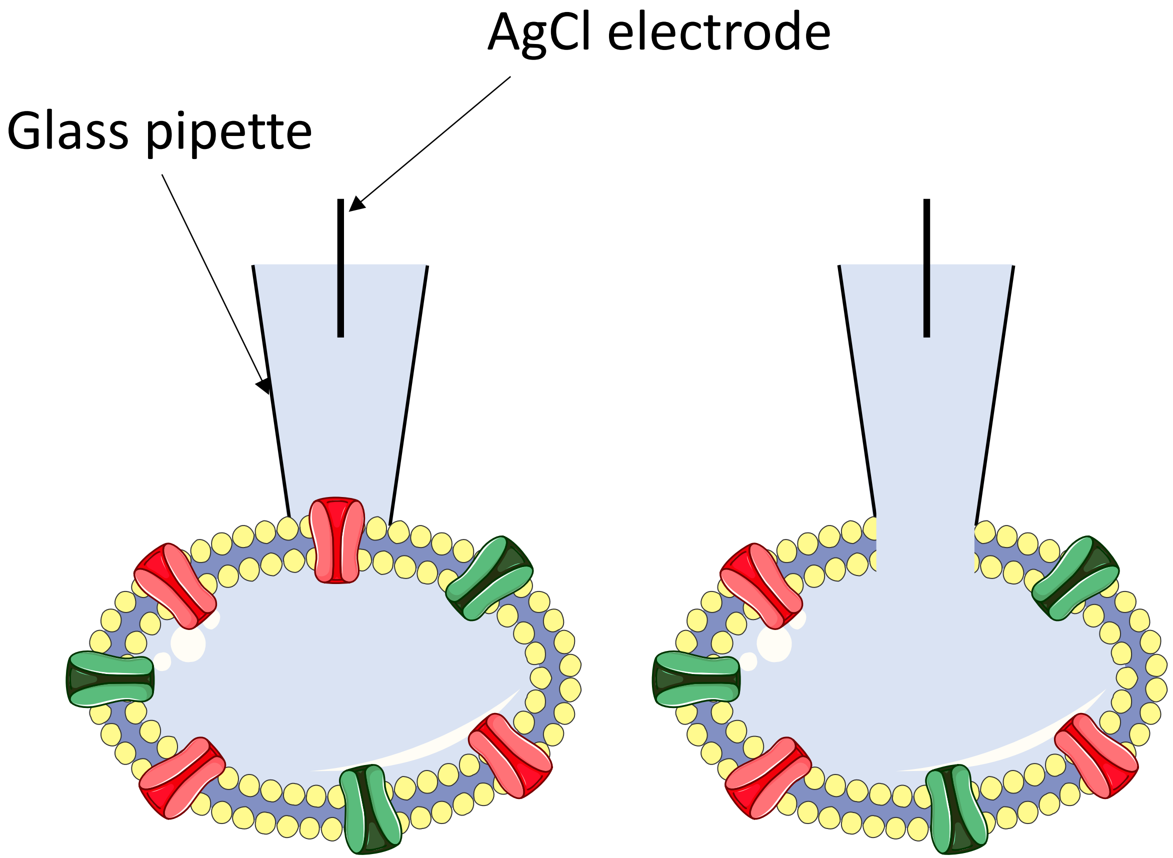
The resistance between the pipette and the membrane is very high (GigaOhm)

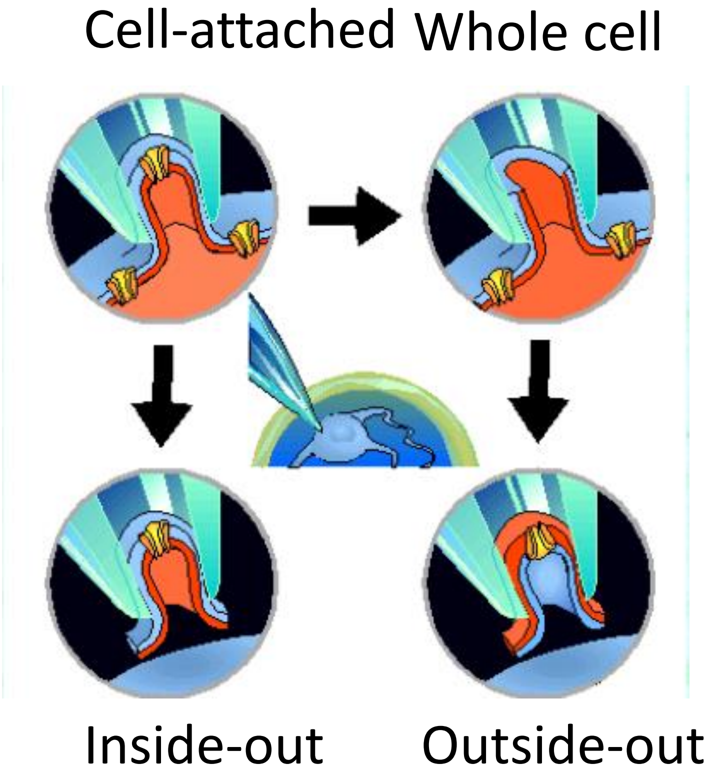
Erwin Neher

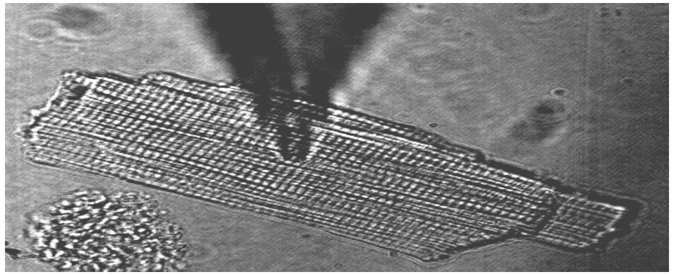
Bert Sakmann

Nobel Prize in Medecine 1991

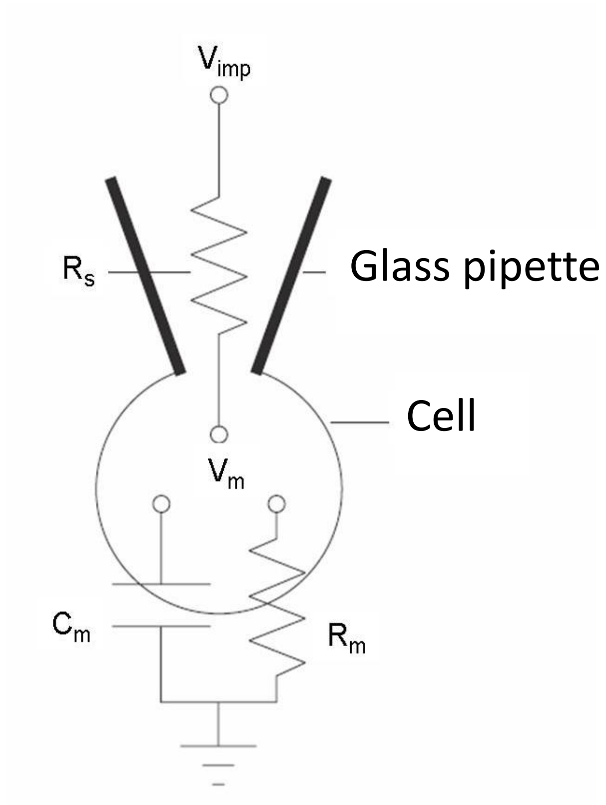
Patch-Clamp


AgCl electrode
Glass pipette


Cell-attached
Whole cell
Inside-out
Outside-out



Patch-Clamp: whole-cell configuration



V_{imp}: imposed potential

V_m: membrane potential

R_S: series resistance

R_m: membrane resistance (ion channels)

C_m: membrane capacitance (lipid bilayer)

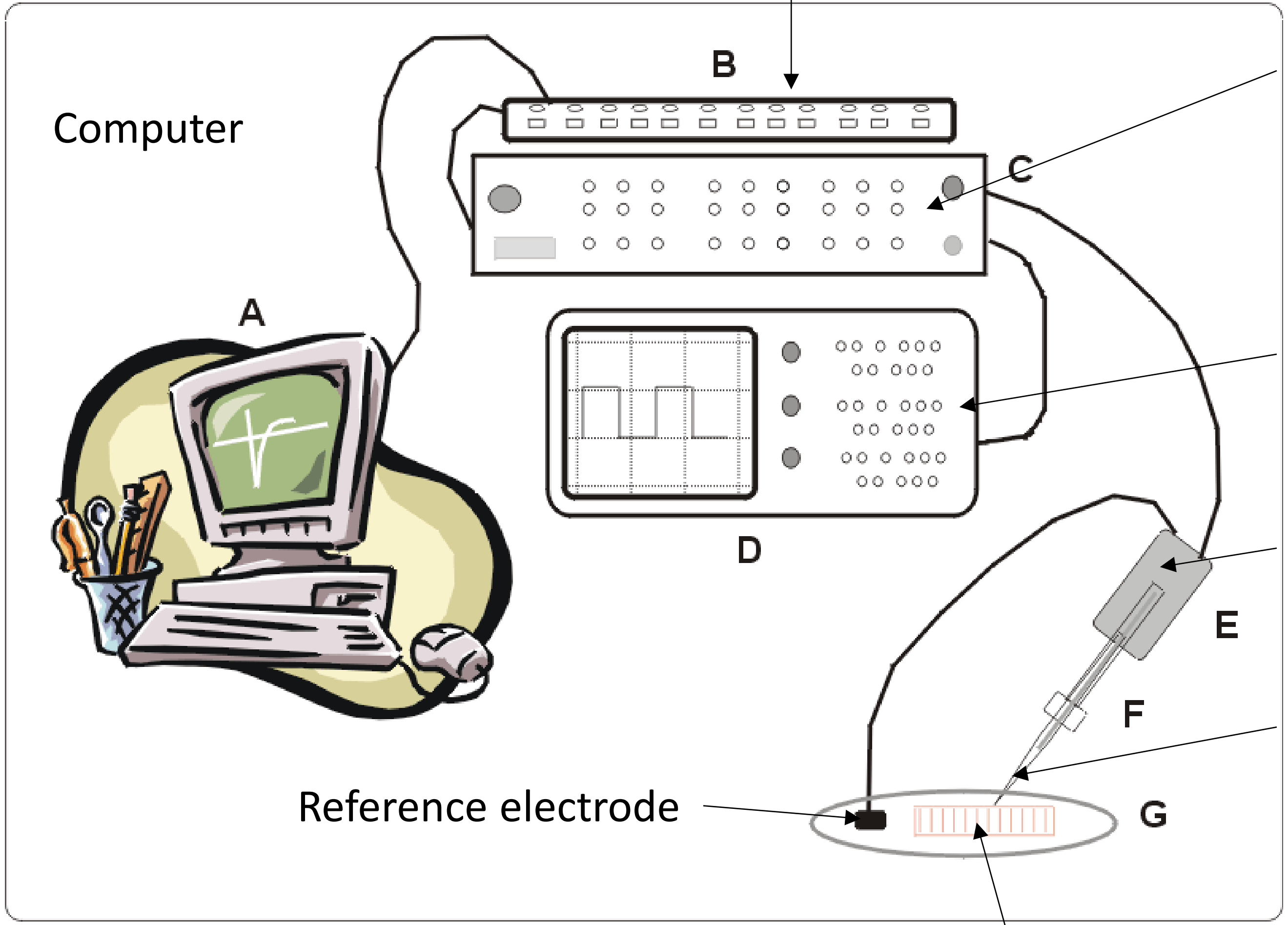
$V_m = R_m \times I$

Voltage-clamp: imposed potential to the membrane → current ($I = N.P_o.i$) recording

N:number of channel, P_o: open probability of the channel, i: single channel current

Current-clamp: Imposed current → variation of membrane potential recording

Patch-Clamp set-up



Analog-to-digital converter

Computer

Amplifier

Oscilloscope

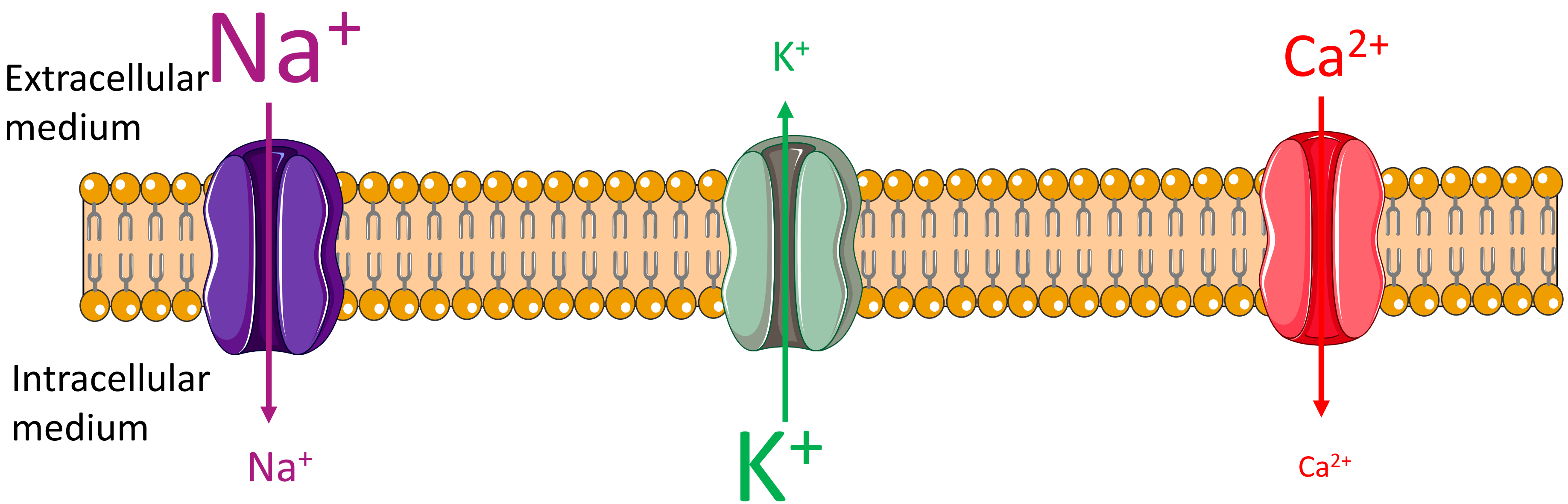
Pre-amplifier

Glass pipette

Reference electrode

Perfusion chamber

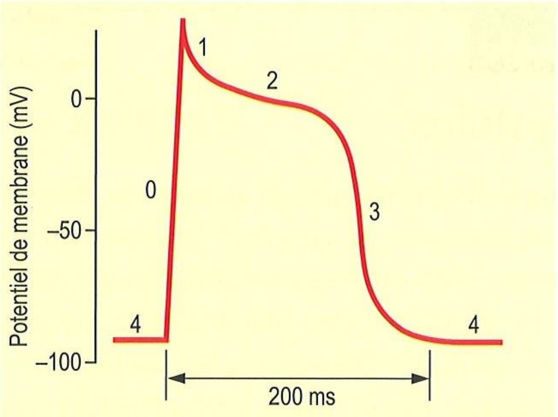
Voltage-gated channels and ventricular action potential



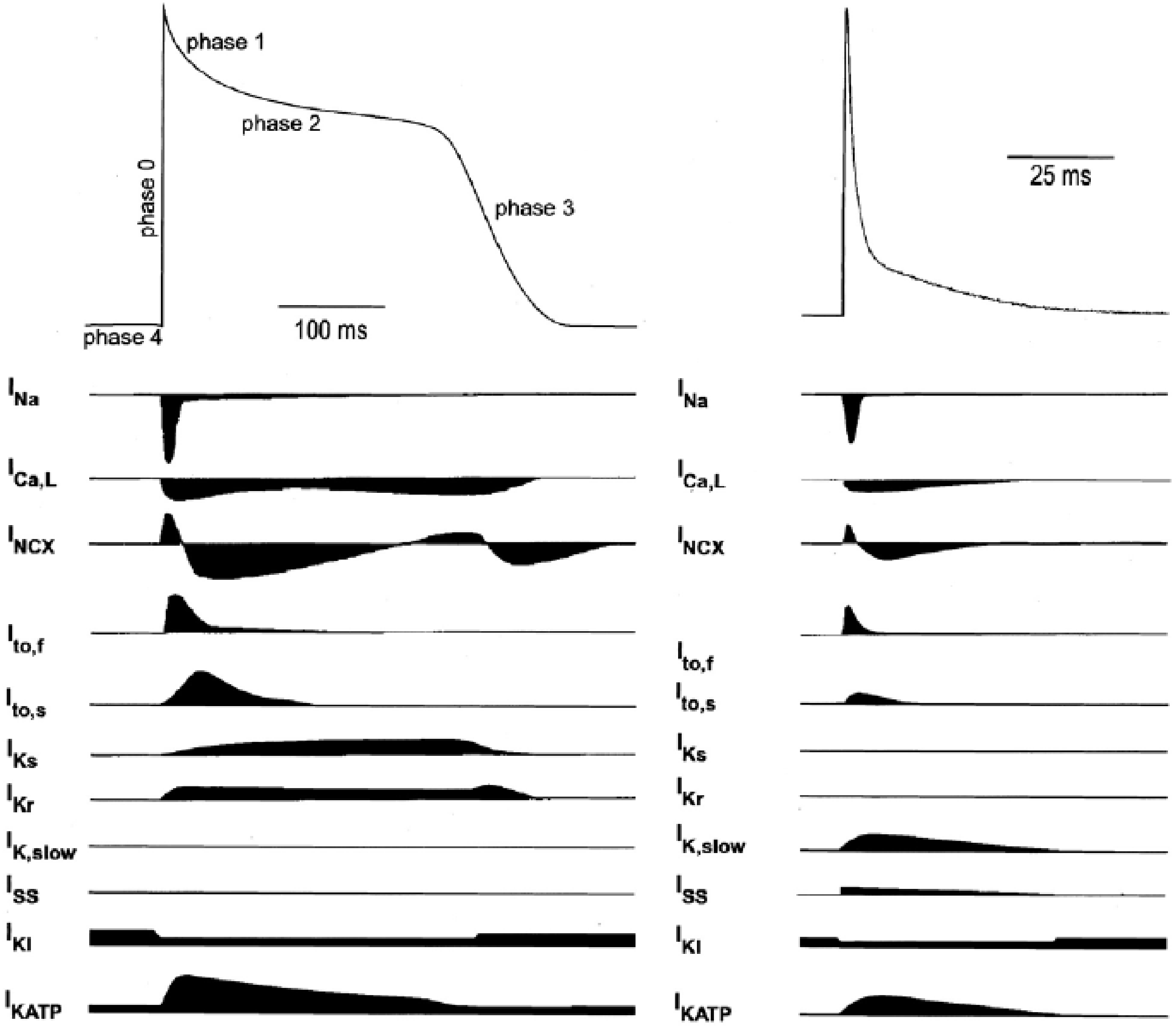
Voltage-gated sodium channels: depolarisation

Voltage-gated potassium channels: repolarisation

Volta-gated calcium channels: depolarisation



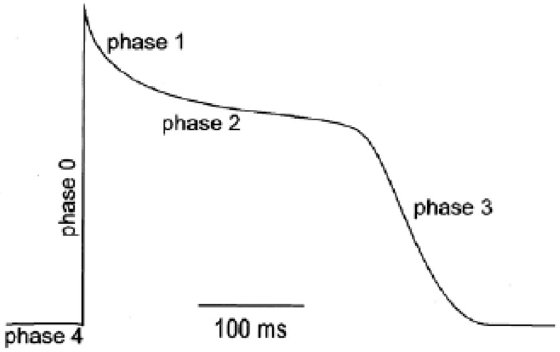
Ventricular cardiomyocytes Action Potential

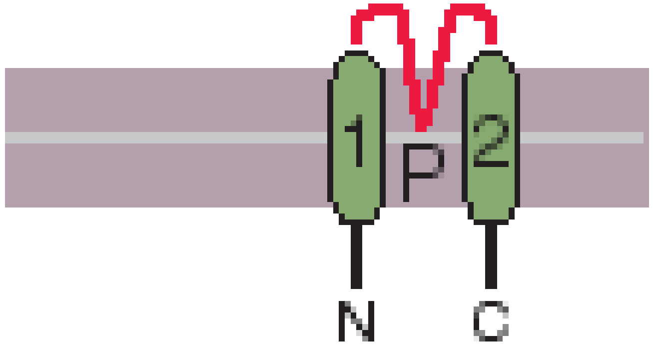


Human

Mice

Membrane resting potential: I_{K1}





Inward rectifier K^+ channel is responsible of membrane resting potential

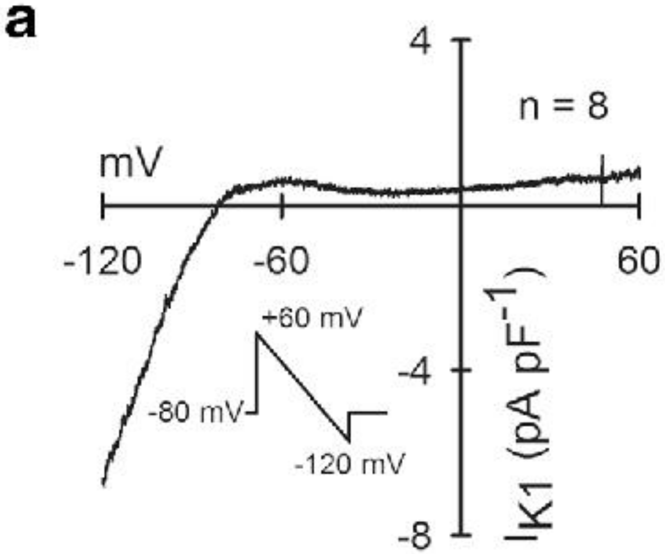
Heterotetramer: Kir2.1, Kir2.2

Responsible of I_{K1} current

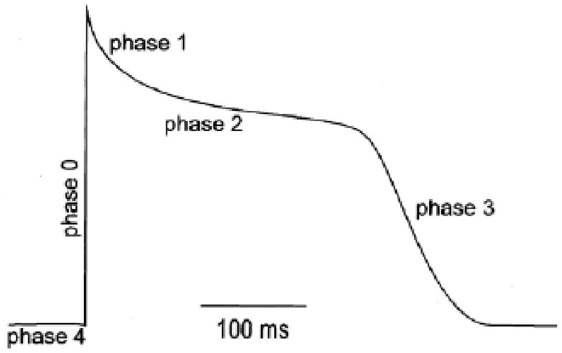
I_{K1} current maintains the membrane potential at -80mV

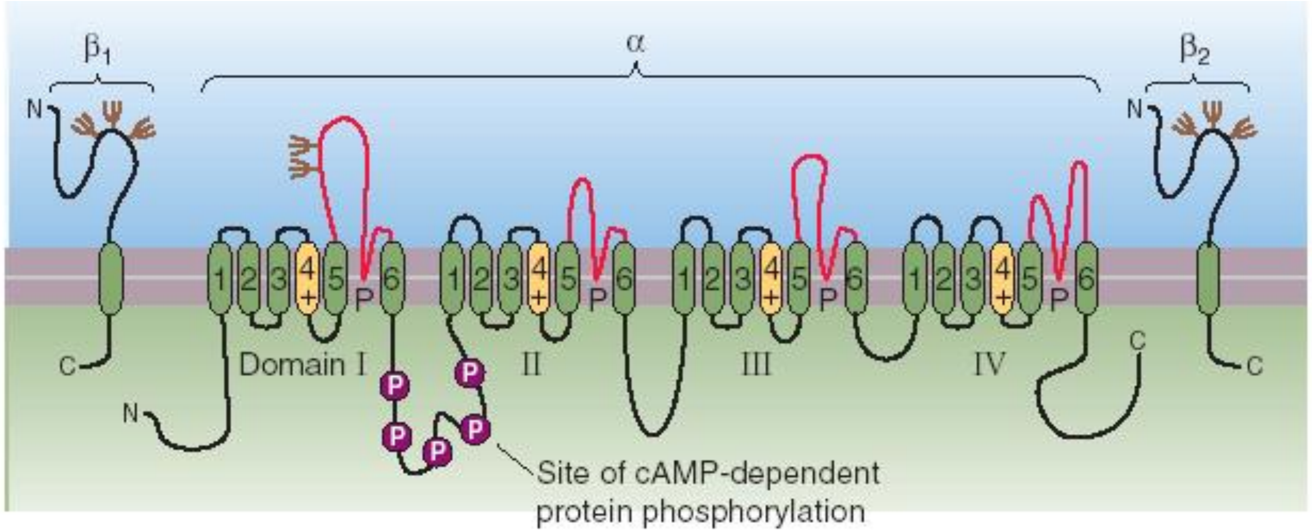
Always open

Blocked by cesium or barium



Voltage-gated sodium channels





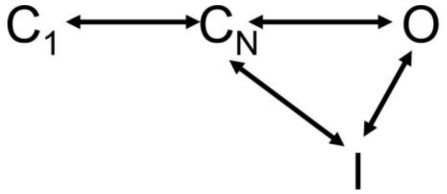
Site of cAMP-dependent protein phosphorylation

One pore forming subunit α: 4 x 6 transmembrane domains, S4 voltage sensor

In ventricules α subunit is mainly $Na_v1.5$

Responsible of the upstroke of action potential

3 states: close, open, inactivated

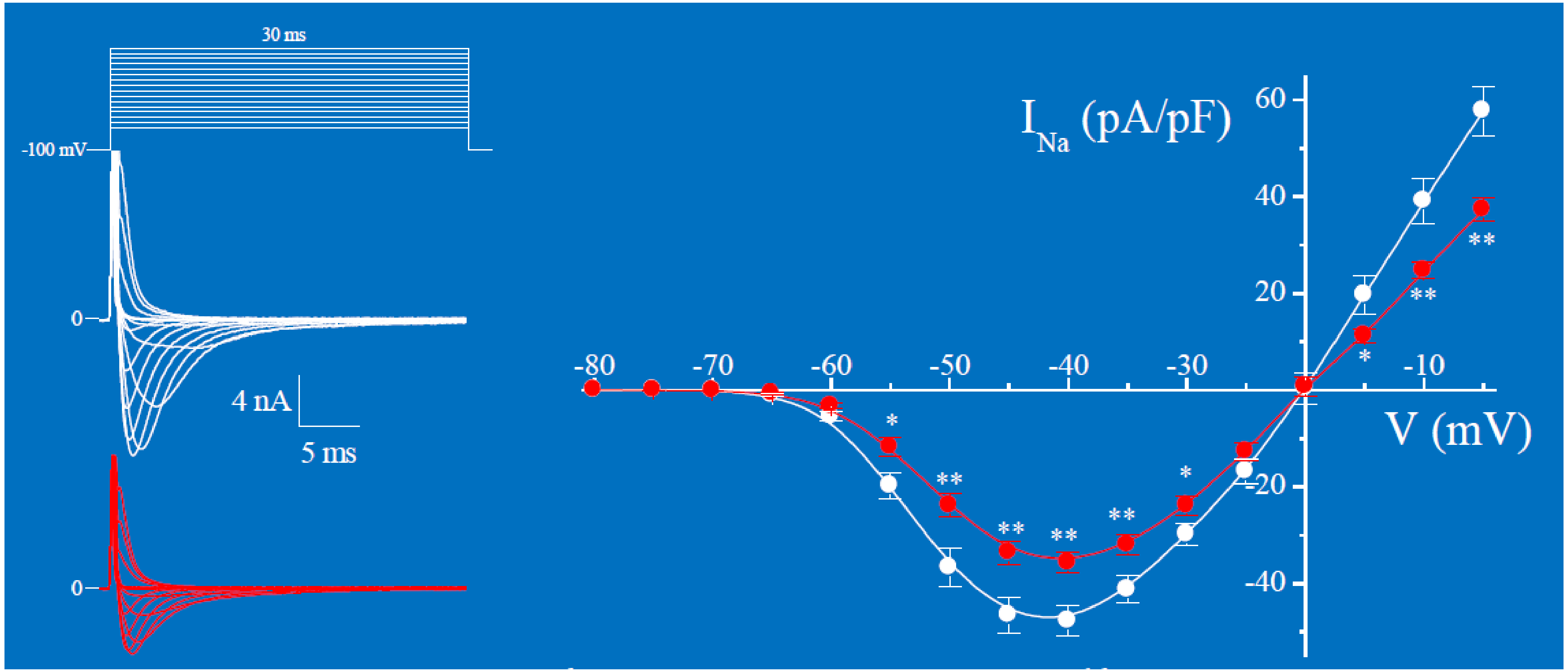


C_1 ≡ Initial closed state
C_N ≡ Closed state before the O state
O ≡ Open state
I ≡ Inactivated state

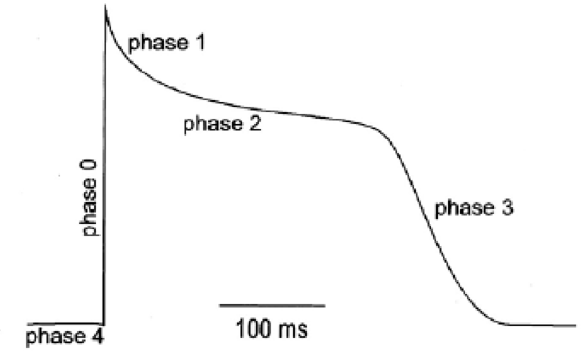
Opening at -70/-60 mV

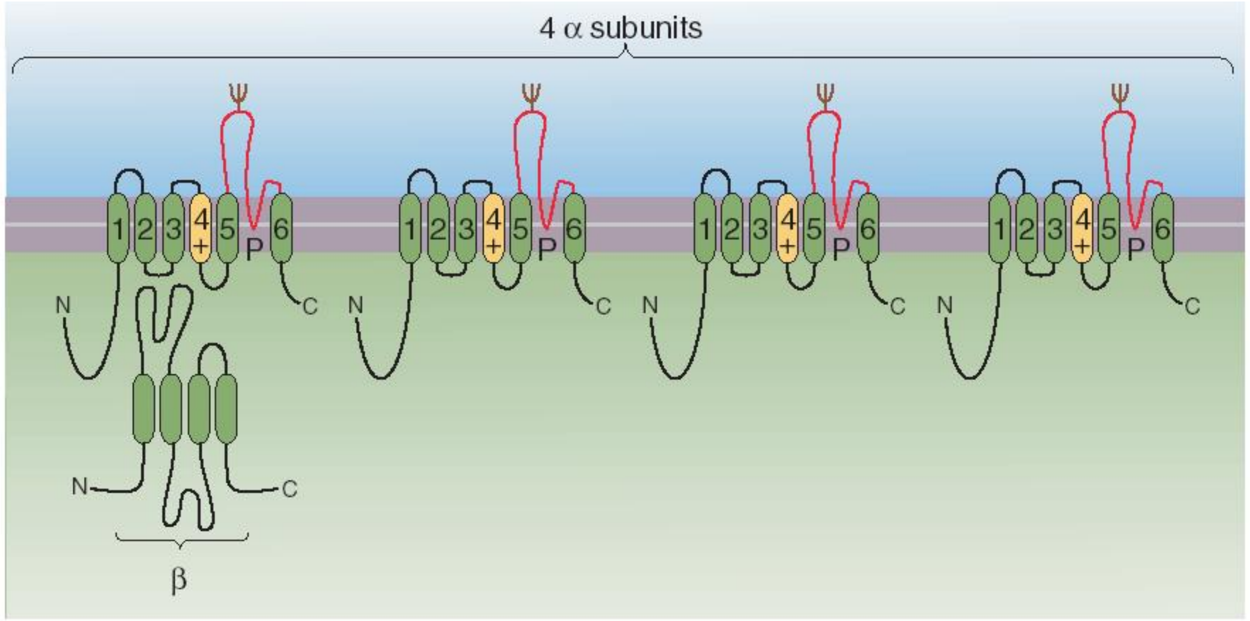
Can be blocked with high dose of tetrodotoxin

Voltage-gated sodium channels



Voltage-gated potassium channels





4 α subunits: 6 transmembrane domains, S4 voltage sensor

Main subunits: K_vx.x

The voltage-gated potassium channels are remarkable for their diversity. They include 40 different channels that are classified into 12 distinct groups based on their amino acid sequence homology (K_v1– K_v12)

Involved in cell repolarization

Voltage-gated potassium channels: I_{to}

Transient outward potassium current (I_{to}) involved in the early phase of repolarization

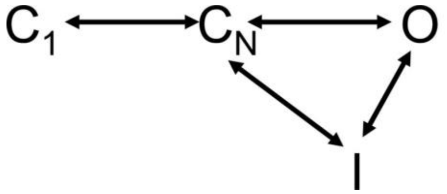
In human homotetramere of $K_v4.3$ and 1 regulatory subunit KChIP

In rodent heterotetramere of $K_v4.2/K_v4.3$

3 states: close, open, inactivated

Fast inactivation

Blocked by 4-aminopyridine



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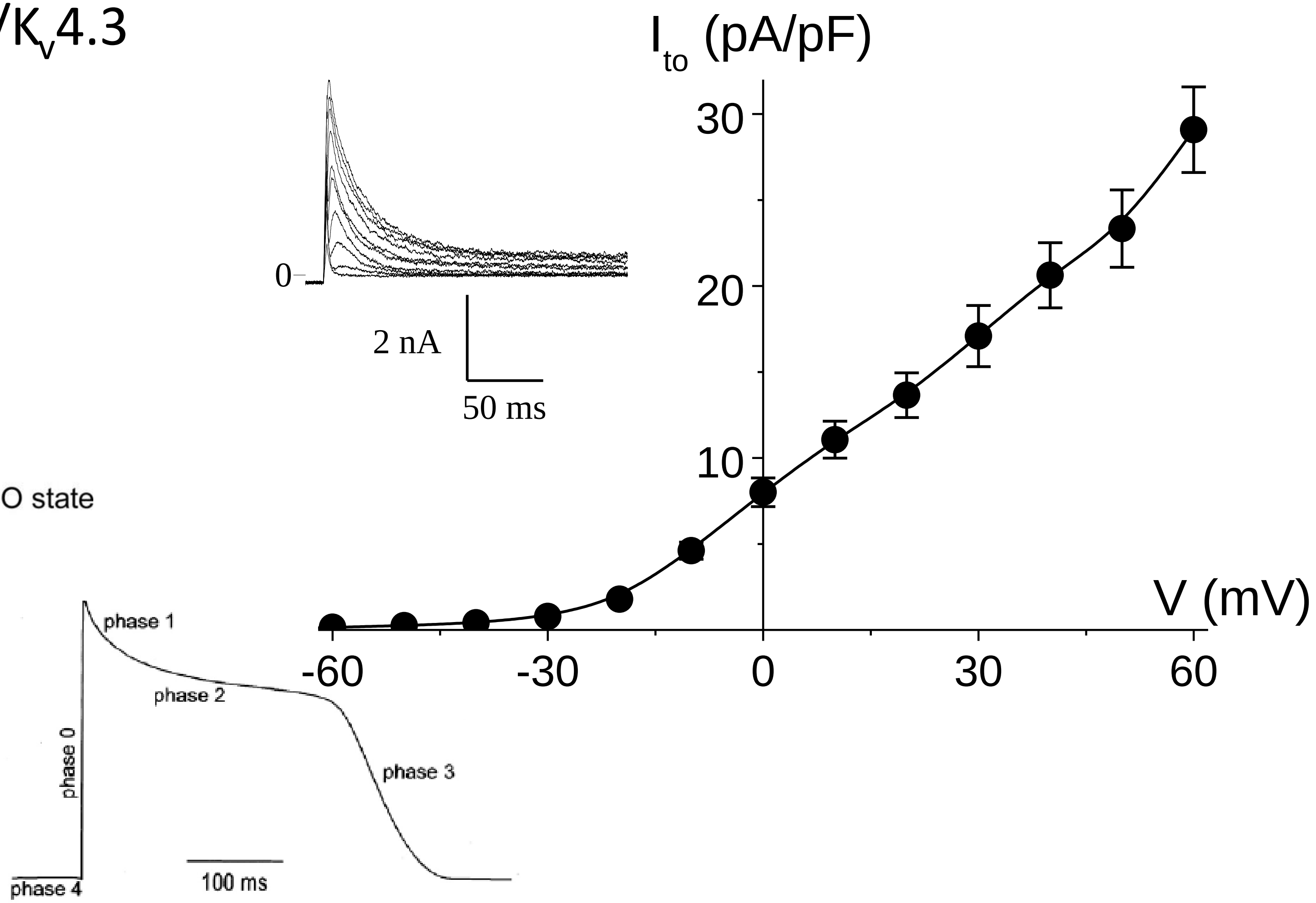


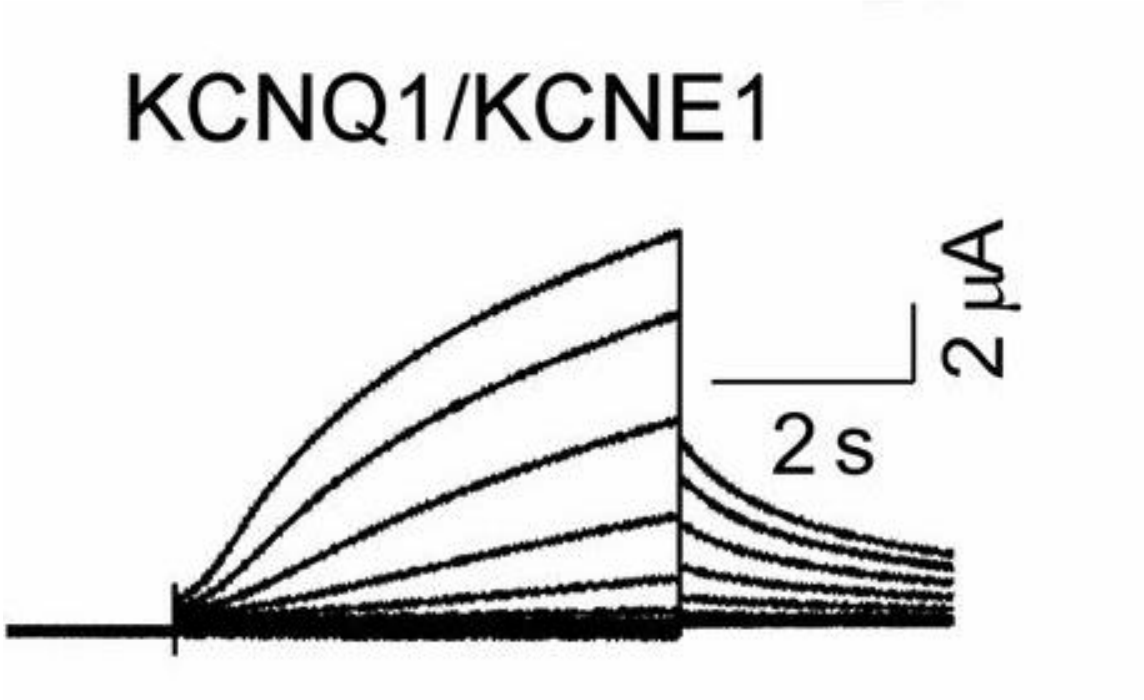
Voltage-gated potassium channels: I_{Ks}

I_{Ks} (slow) is a delayed potassium current: slow activation

Main subunit K_v7.1 (K_vLQT1), encoded by *KCNQ1* gene, associated with KCNE1 regulatory subunit

Involved in the plateau phase of action potential

Blocked by indapamine



Voltage-gated potassium channels: I_{KR}

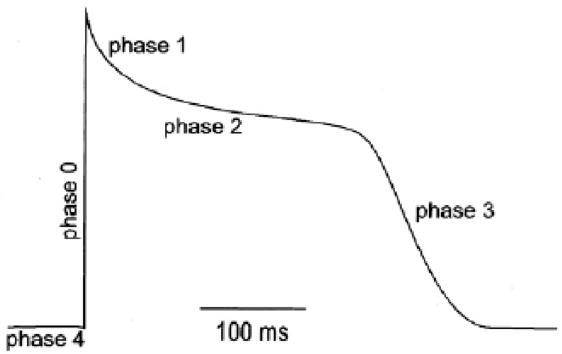
I_{KR} (slow) is a current wich activates rapidly and for more negative potential than I_{Ks}

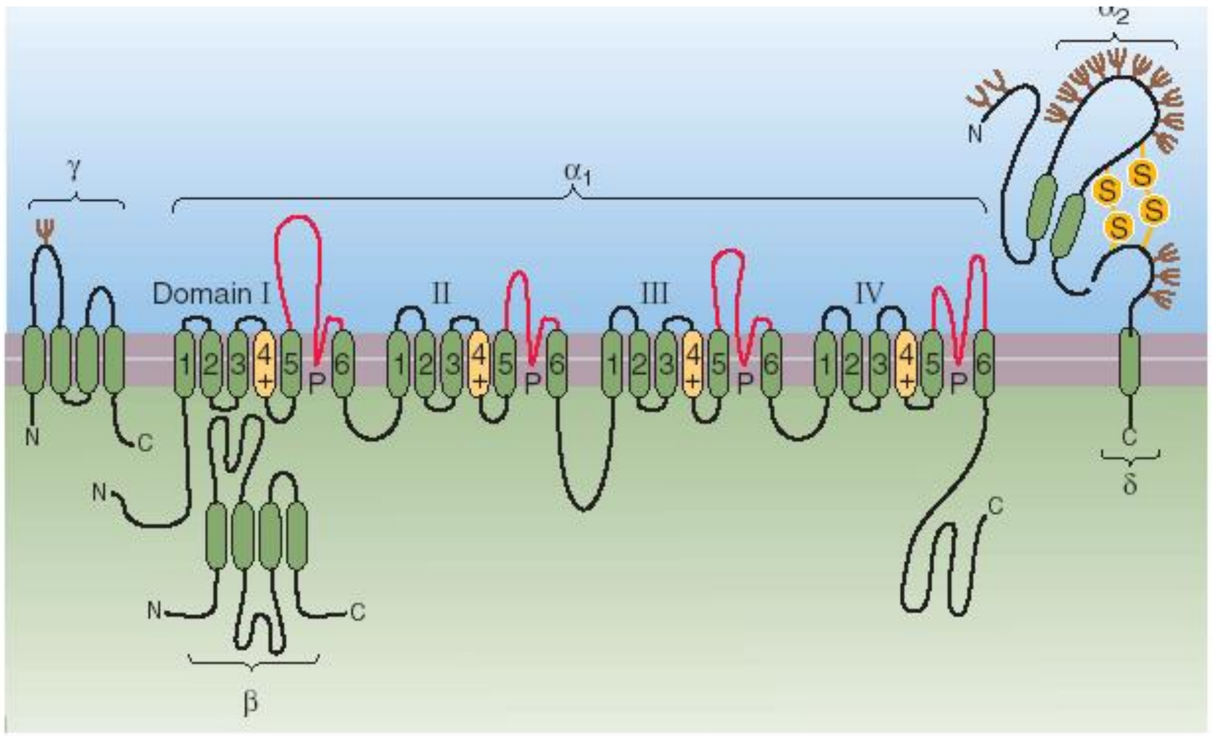
Main subunit K_v11.1 (hERG), encoded by *KCNH2* gene, probably associated with KCNE2 regulatory subunit

Involved in early phase of repolarization

Blocked by a large number of drugs → risk of deaths caused by long QT syndrome-induced torsades de pointes

Voltage-gated Calcium channels: I_{CaL}





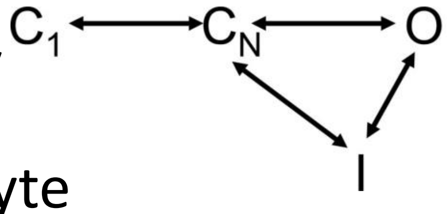
One pore forming subunit α: 4 x 6 transmembrane domains, S4 voltage sensor

In ventricules α subunit is mainly $Ca_v1.2$ for L-type calcium channel

3 states: closed, opened, inactivated. Opening at -40 mV

Responsible of the main entry of Ca^{2+} in the cardiomyocyte

Key player of excitation contraction coupling



C_1 ≡ Initial closed state
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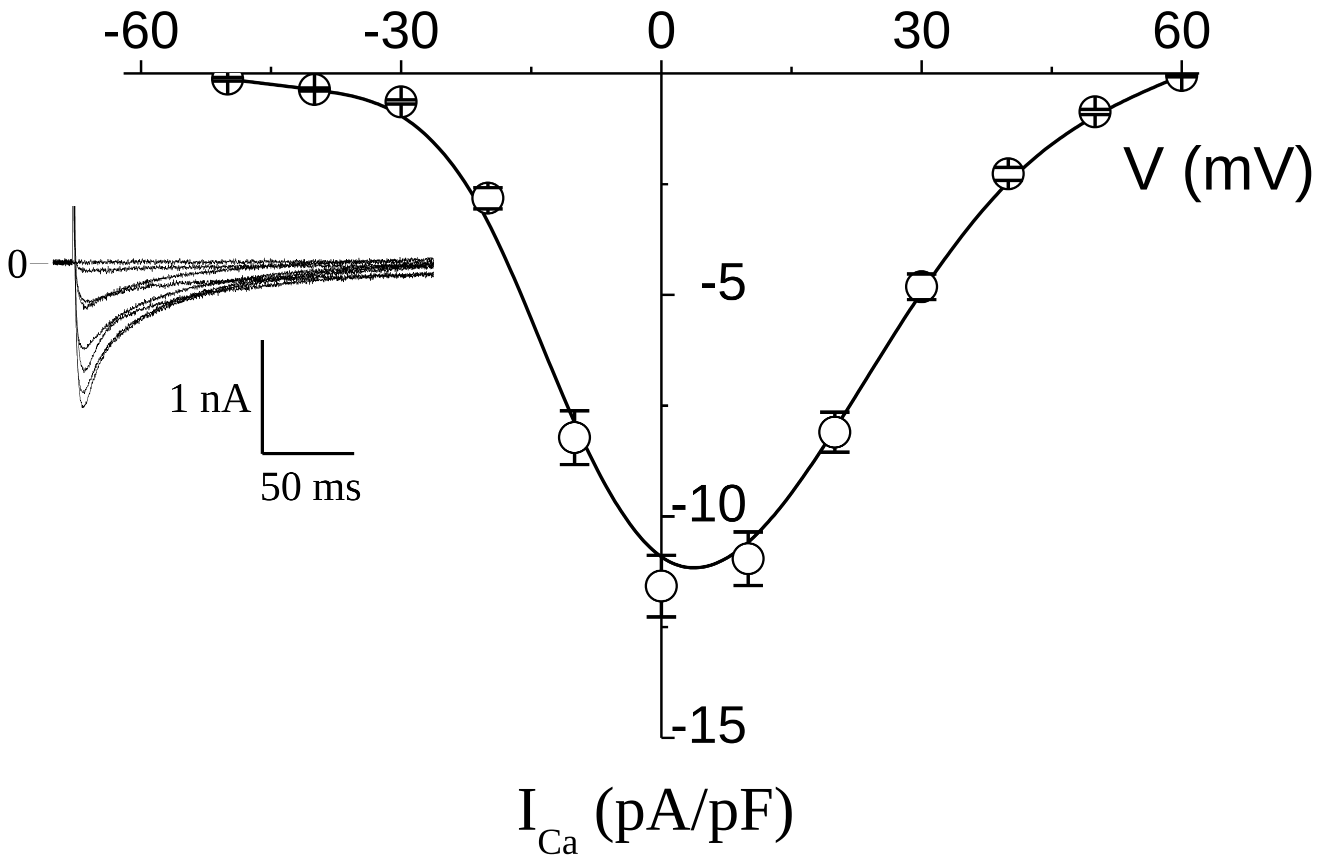
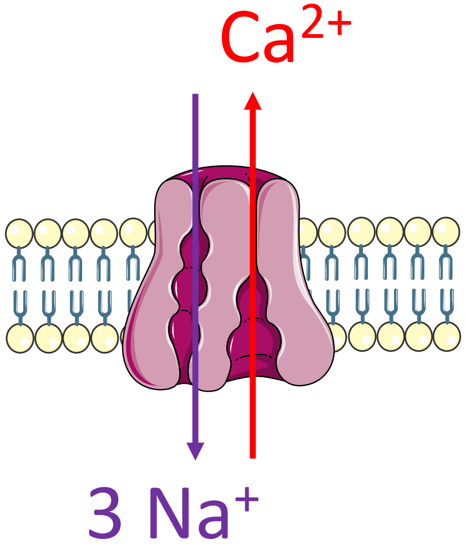
I ≡ Inactivated state

Voltage-gated Calcium channels: I_{Ca}



Blocked by dihydropyridine, verapamil

Sodium/calcium exchanger: NCX



Main isoforme in cardiomycytes : NCX1 encoded by SLC8A1 gene

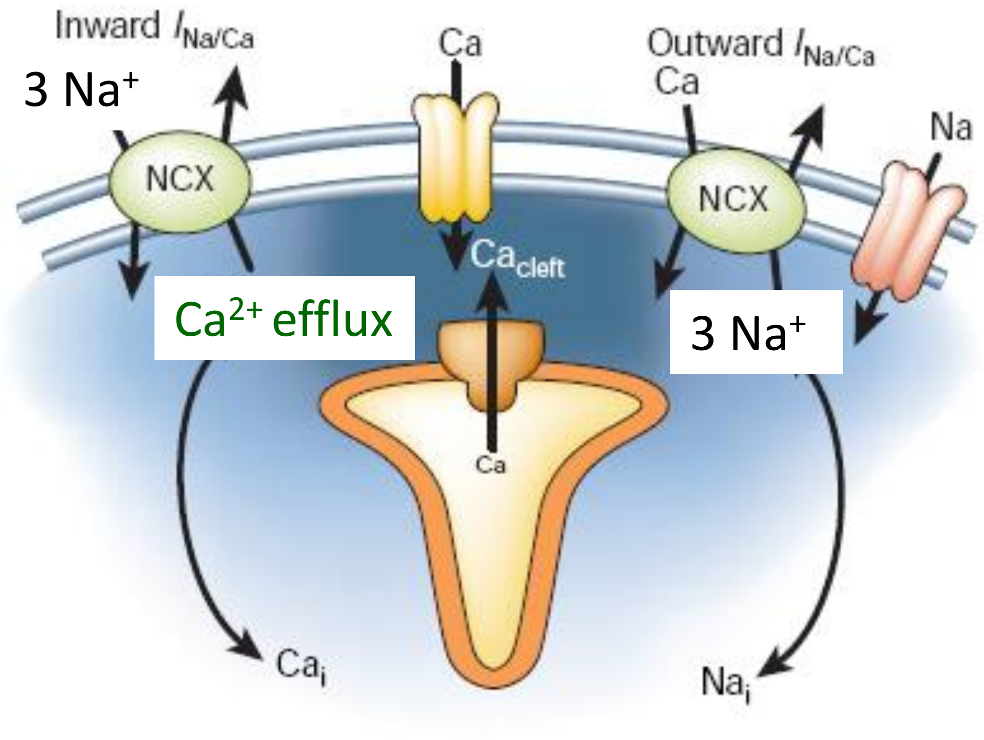
In normal mode responsible of calcium extrusion : 1 Ca^{2+} out / 3 Na^+ in

Electrogenic: it induces depolarisation

Na^+/Ca^{2+} exchanger: NCX

Ca^{2+} influx

3 Na^+

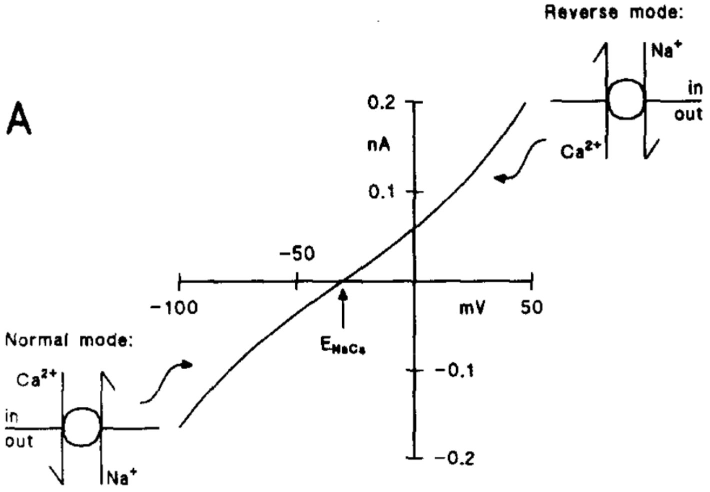


Ca^{2+} efflux

3 Na^+

Na^+/Ca^{2+} exchanger

Outward: Ca^{2+} entre

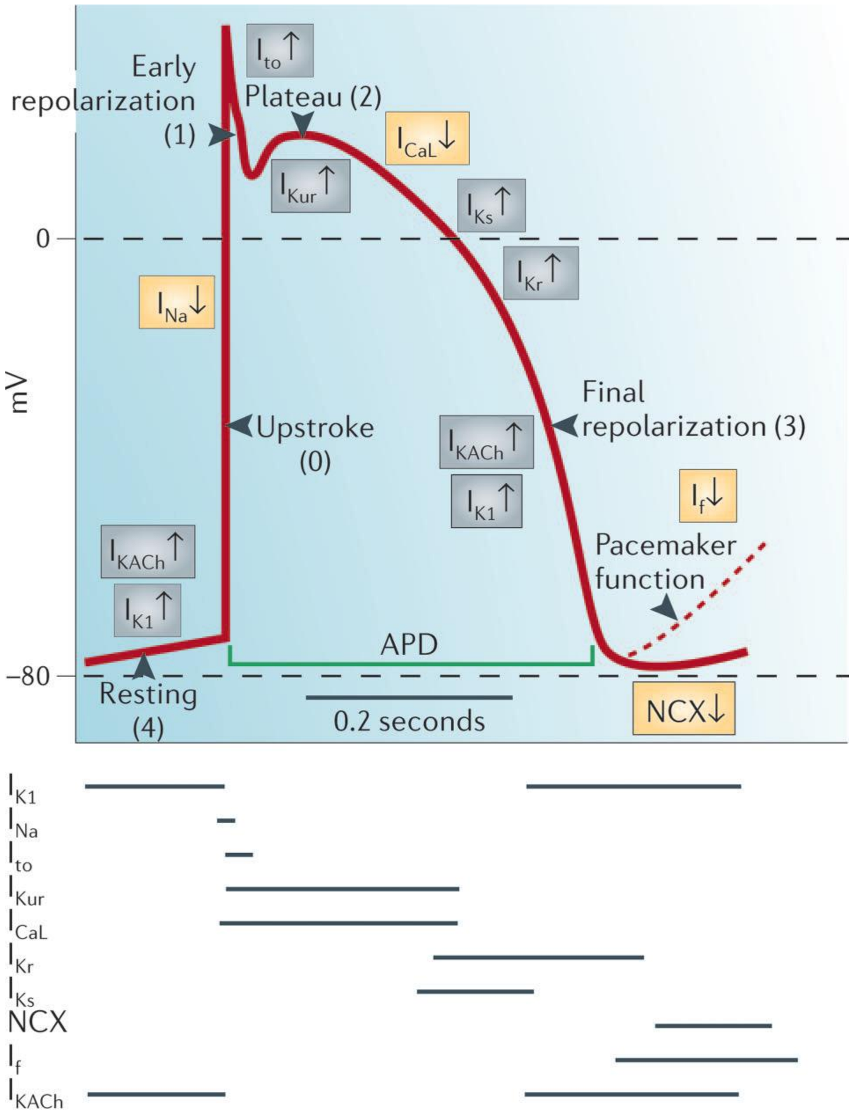


Inward: Ca^{2+} sort

Janvier et al. Cardiovascular Research 1996

Bers DM . Nature 2002

Summary



Grant et al., 2009

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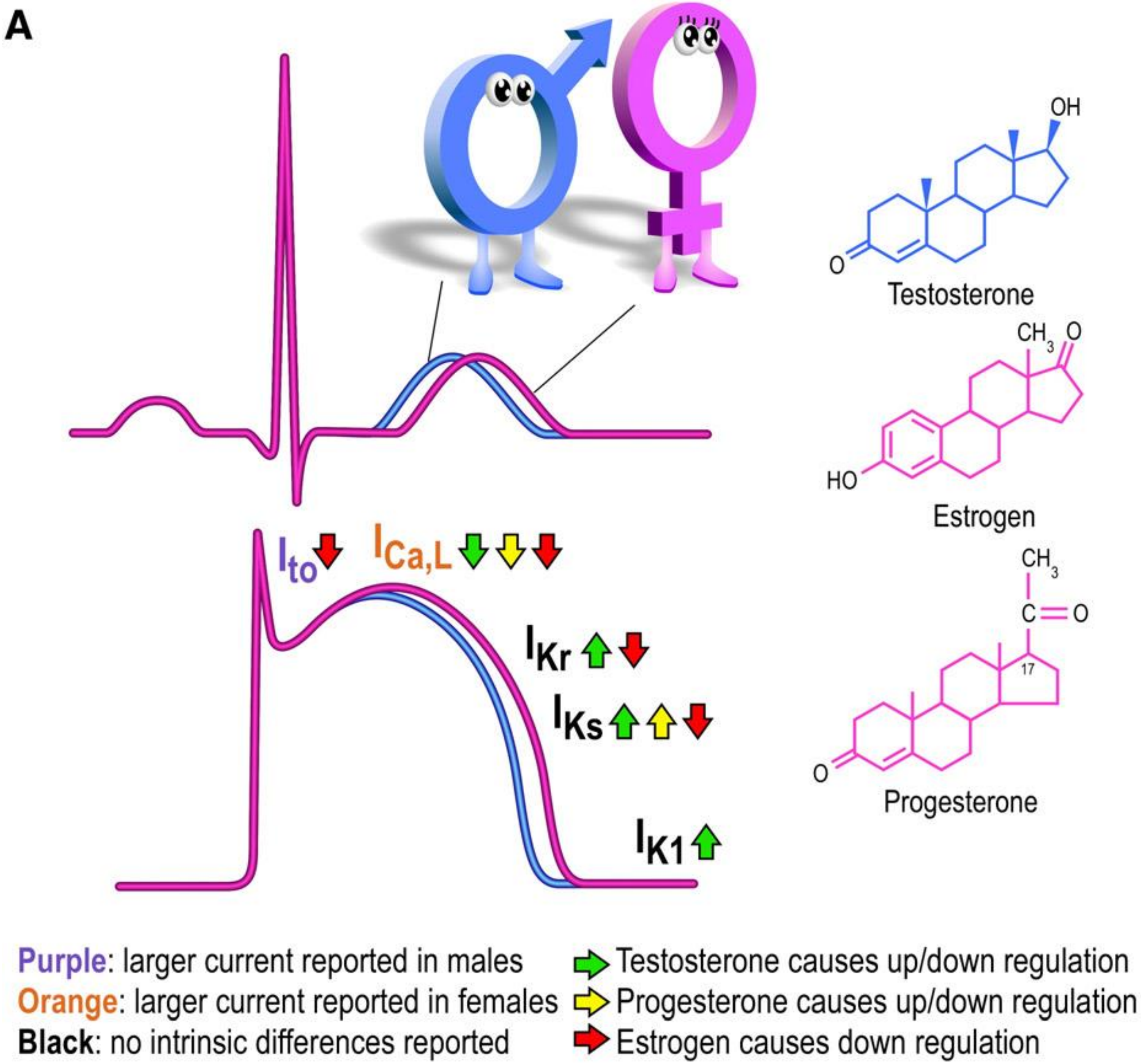
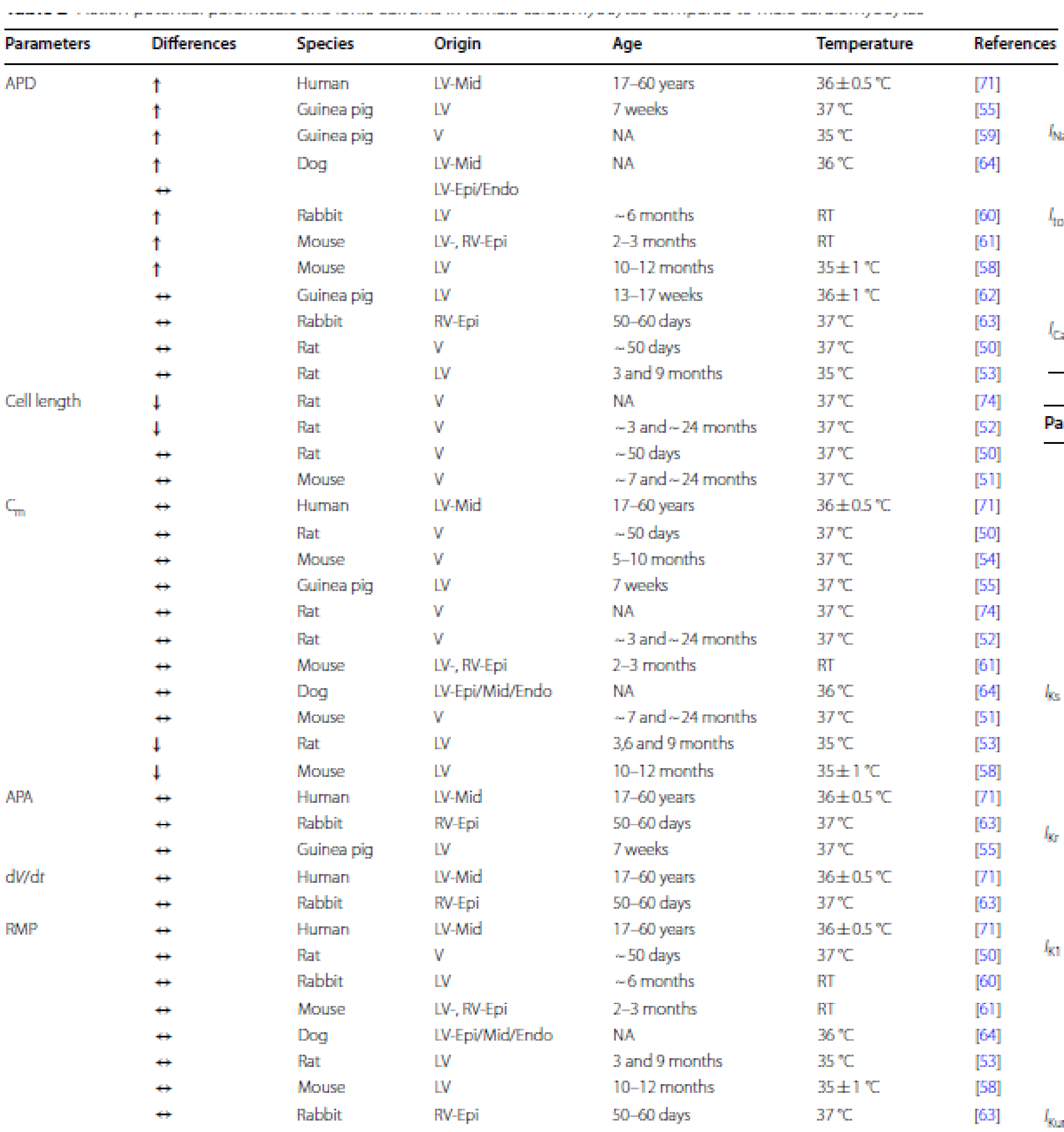
Gender differences in ventricular repolarization



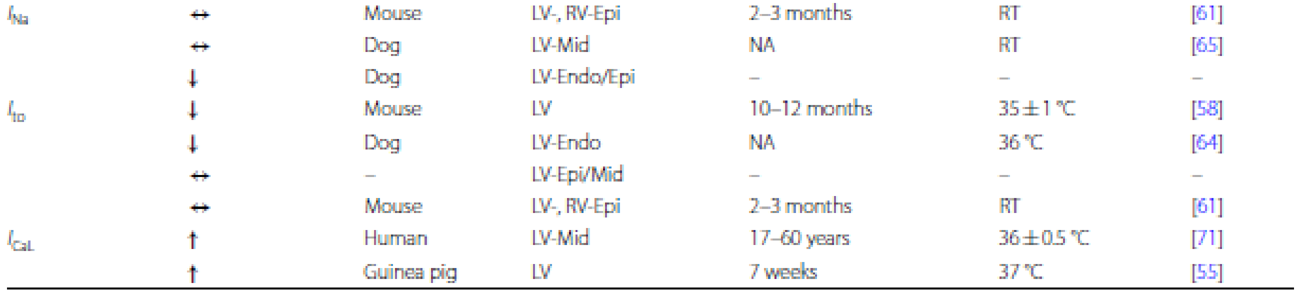
Jonnson et al., 2010

Gender differences in ventricular repolarization

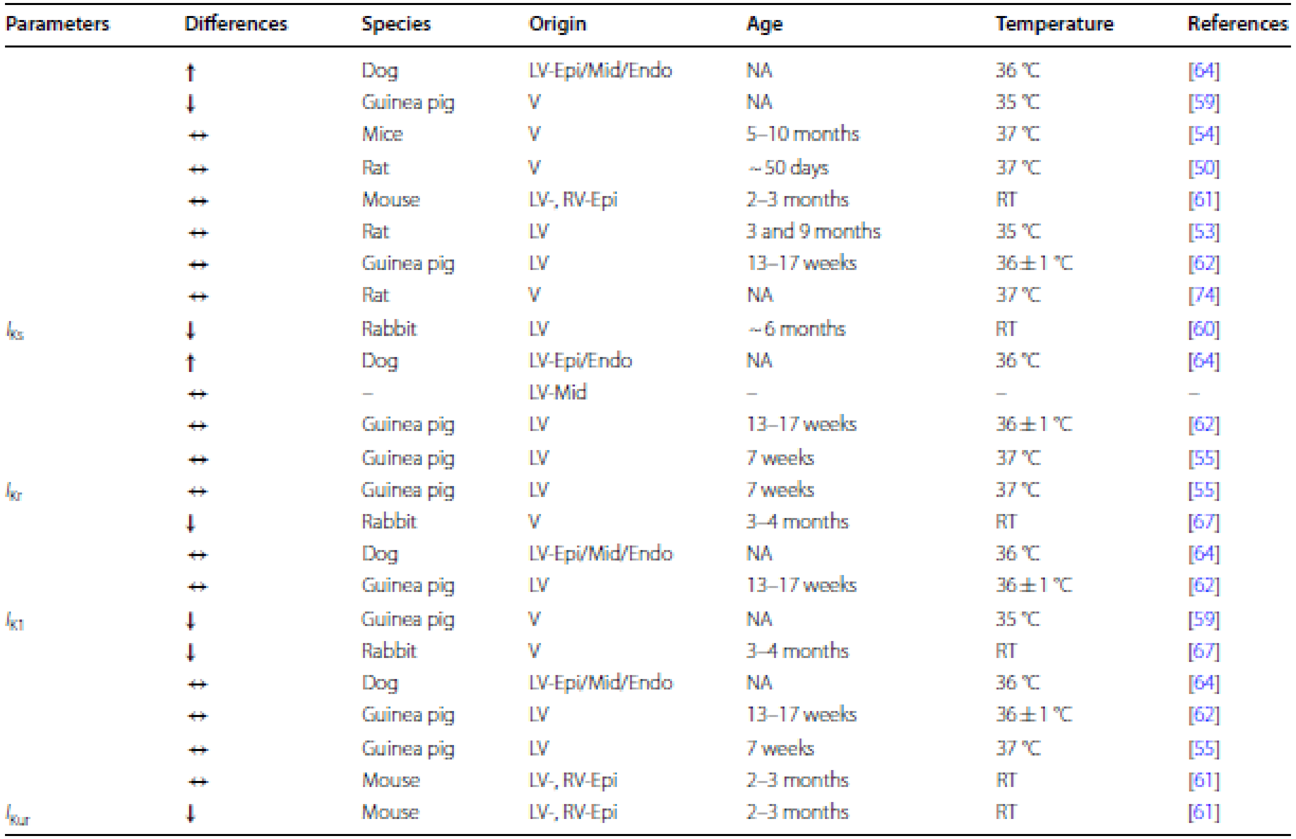
Prajapati et al., 2022



Parameters	Differences	Species	Origin	Age	Temperature	References
APD	↑	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↑	Guinea pig	LV	7 weeks	37 °C	[55]
	↑	Guinea pig	V	NA	35 °C	[59]
	↑	Dog	LV-Mid	NA	36 °C	[64]
	↔		LV-Epi/Endo			
	↑	Rabbit	LV	~6 months	RT	[60]
	↑	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
	↑	Mouse	LV	10–12 months	35 ± 1 °C	[58]
	↔	Guinea pig	LV	13–17 weeks	36 ± 1 °C	[62]
	↔	Rabbit	RV-Epi	50–60 days	37 °C	[63]
	↔	Rat	V	~50 days	37 °C	[50]
	↔	Rat	LV	3 and 9 months	35 °C	[53]
Cell length	↓	Rat	V	NA	37 °C	[74]
	↓	Rat	V	~3 and ~24 months	37 °C	[52]
	↔	Rat	V	~50 days	37 °C	[50]
	↔	Mouse	V	~7 and ~24 months	37 °C	[51]
C_m	↔	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↔	Rat	V	~50 days	37 °C	[50]
	↔	Mouse	V	5–10 months	37 °C	[54]
	↔	Guinea pig	LV	7 weeks	37 °C	[55]
	↔	Rat	V	NA	37 °C	[74]
	↔	Rat	V	~3 and ~24 months	37 °C	[52]
	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
	↔	Dog	LV-Epi/Mid/Endo	NA	36 °C	[64]
	↔	Mouse	V	~7 and ~24 months	37 °C	[51]
	↓	Rat	LV	3,6 and 9 months	35 °C	[53]
	↓	Mouse	LV	10–12 months	35 ± 1 °C	[58]
APA	↔	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↔	Rabbit	RV-Epi	50–60 days	37 °C	[63]
	↔	Guinea pig	LV	7 weeks	37 °C	[55]
dV/dt	↔	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↔	Rabbit	RV-Epi	50–60 days	37 °C	[63]
RMP	↔	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↔	Rat	V	~50 days	37 °C	[50]
	↔	Rabbit	LV	~6 months	RT	[60]
	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
	↔	Dog	LV-Epi/Mid/Endo	NA	36 °C	[64]
	↔	Rat	LV	3 and 9 months	35 °C	[53]
	↔	Mouse	LV	10–12 months	35 ± 1 °C	[58]
	↔	Rabbit	RV-Epi	50–60 days	37 °C	[63]

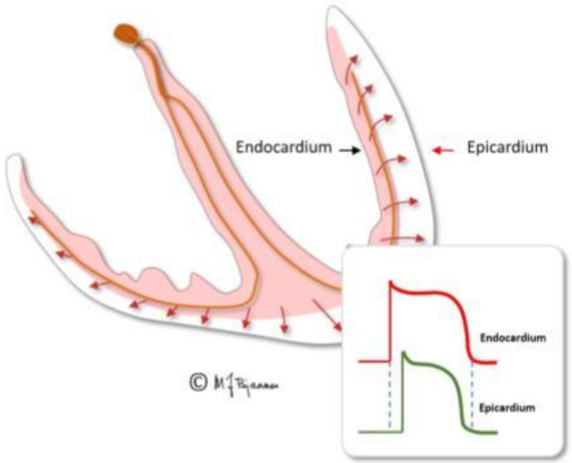


I_{Na}	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
	↔	Dog	LV-Mid	NA	RT	[65]
	↓	Dog	LV-Endo/Epi	–	–	–
I_{to}	↓	Mouse	LV	10–12 months	35 ± 1 °C	[58]
	↓	Dog	LV-Endo	NA	36 °C	[64]
	↔	–	LV-Epi/Mid	–	–	–
	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
I_{CaL}	↑	Human	LV-Mid	17–60 years	36 ± 0.5 °C	[71]
	↑	Guinea pig	LV	7 weeks	37 °C	[55]

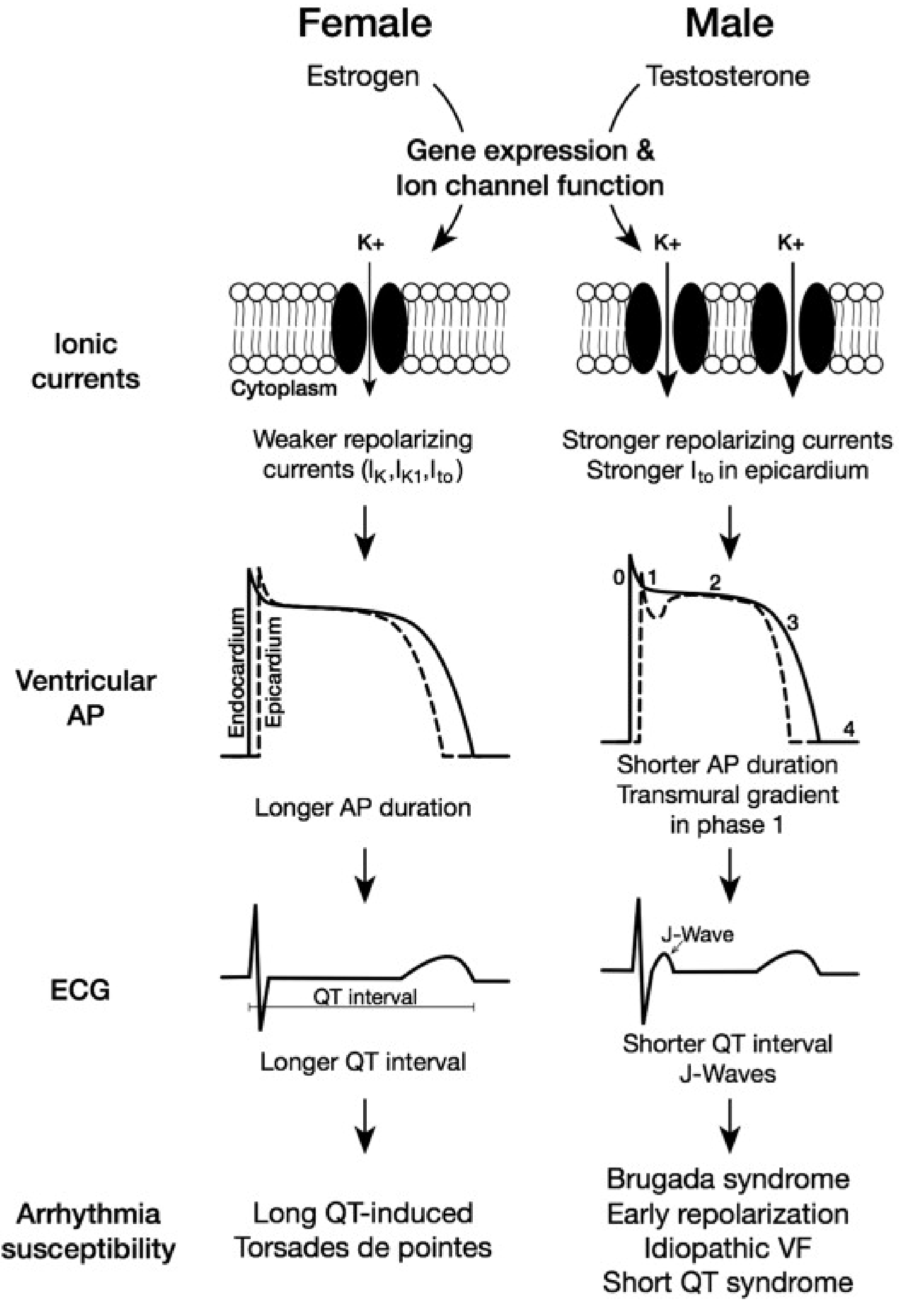


Parameters	Differences	Species	Origin	Age	Temperature	References
	↑	Dog	LV-Epi/Mid/Endo	NA	36 °C	[64]
	↓	Guinea pig	V	NA	35 °C	[59]
	↔	Mice	V	5–10 months	37 °C	[54]
	↔	Rat	V	~50 days	37 °C	[50]
	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
	↔	Rat	LV	3 and 9 months	35 °C	[53]
	↔	Guinea pig	LV	13–17 weeks	36 ± 1 °C	[62]
	↔	Rat	V	NA	37 °C	[74]
I_{Ks}	↓	Rabbit	LV	~6 months	RT	[60]
	↑	Dog	LV-Epi/Endo	NA	36 °C	[64]
	↔	–	LV-Mid	–	–	–
	↔	Guinea pig	LV	13–17 weeks	36 ± 1 °C	[62]
	↔	Guinea pig	LV	7 weeks	37 °C	[55]
I_{Kr}	↔	Guinea pig	LV	7 weeks	37 °C	[55]
	↓	Rabbit	V	3–4 months	RT	[67]
	↔	Dog	LV-Epi/Mid/Endo	NA	36 °C	[64]
	↔	Guinea pig	LV	13–17 weeks	36 ± 1 °C	[62]
I_{K1}	↓	Guinea pig	V	NA	35 °C	[59]
	↓	Rabbit	V	3–4 months	RT	[67]
	↔	Dog	LV-Epi/Mid/Endo	NA	36 °C	[64]
	↔	Guinea pig	LV	13–17 weeks	36 ± 1 °C	[62]
	↔	Guinea pig	LV	7 weeks	37 °C	[55]
	↔	Mouse	LV-, RV-Epi	2–3 months	RT	[61]
I_{Kur}	↓	Mouse	LV-, RV-Epi	2–3 months	RT	[61]

Transmural gradient of cardiac repolarization and gender differences



https://studmed.uio.no/elaring/fag/hjertesykdommer/en/ecg/basal_elfys.html



Female

Estrogen

Male

Testosterone

Gene expression &
Ion channel function

Ionic currents

Female: Weaker repolarizing currents (I_K, I_{K1}, I_{to})

Male: Stronger repolarizing currents
Stronger I_{to} in epicardium

Ventricular AP

Female: Longer AP duration

Male: Shorter AP duration
Transmural gradient
in phase 1

ECG

Female: Longer QT interval

Male: Shorter QT interval
J-Waves

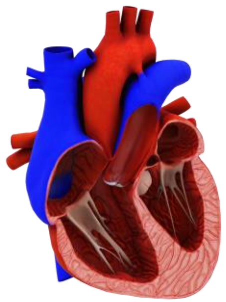
Arrhythmia susceptibility

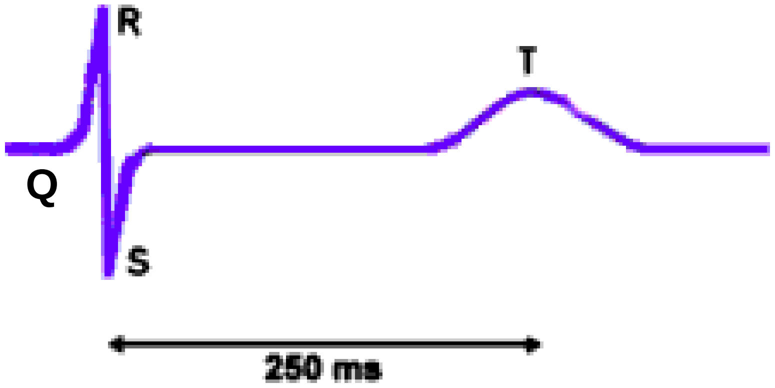
Female: Long QT-induced
Torsades de pointes

Male: Brugada syndrome
Early repolarization
Idiopathic VF
Short QT syndrome

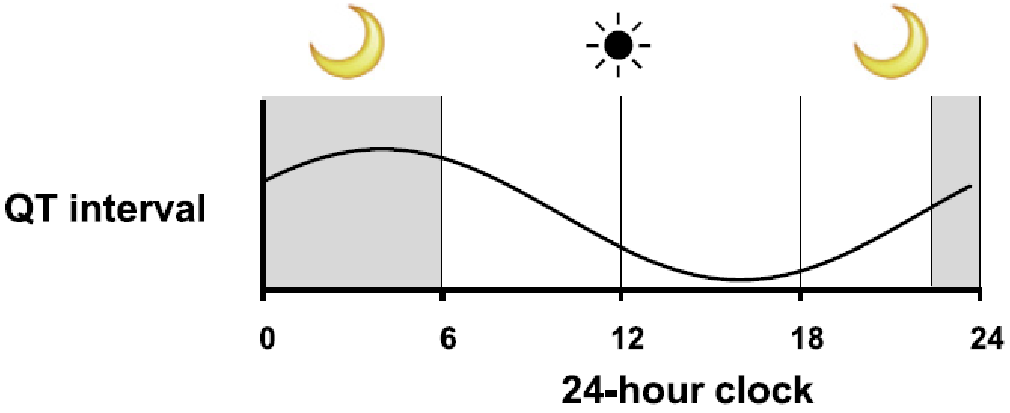
Tadros et al., 2014

Circadian ventricular electrical activity





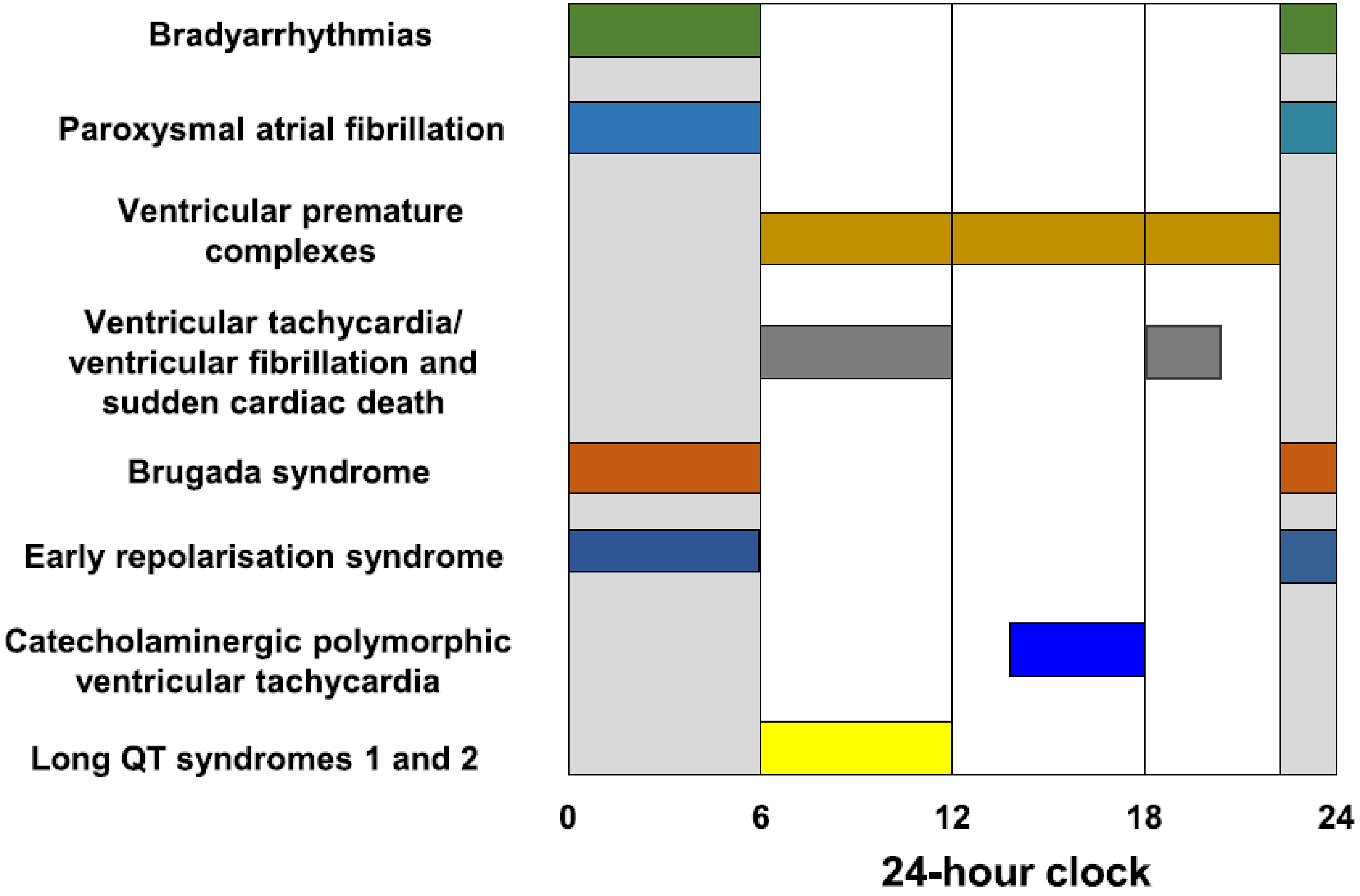
Circadian regulation of ventricular repolarisation



K^+ channels responsible of circadian rhythm of ventricular electrical activity

Kcnd2 (Kv4.2)

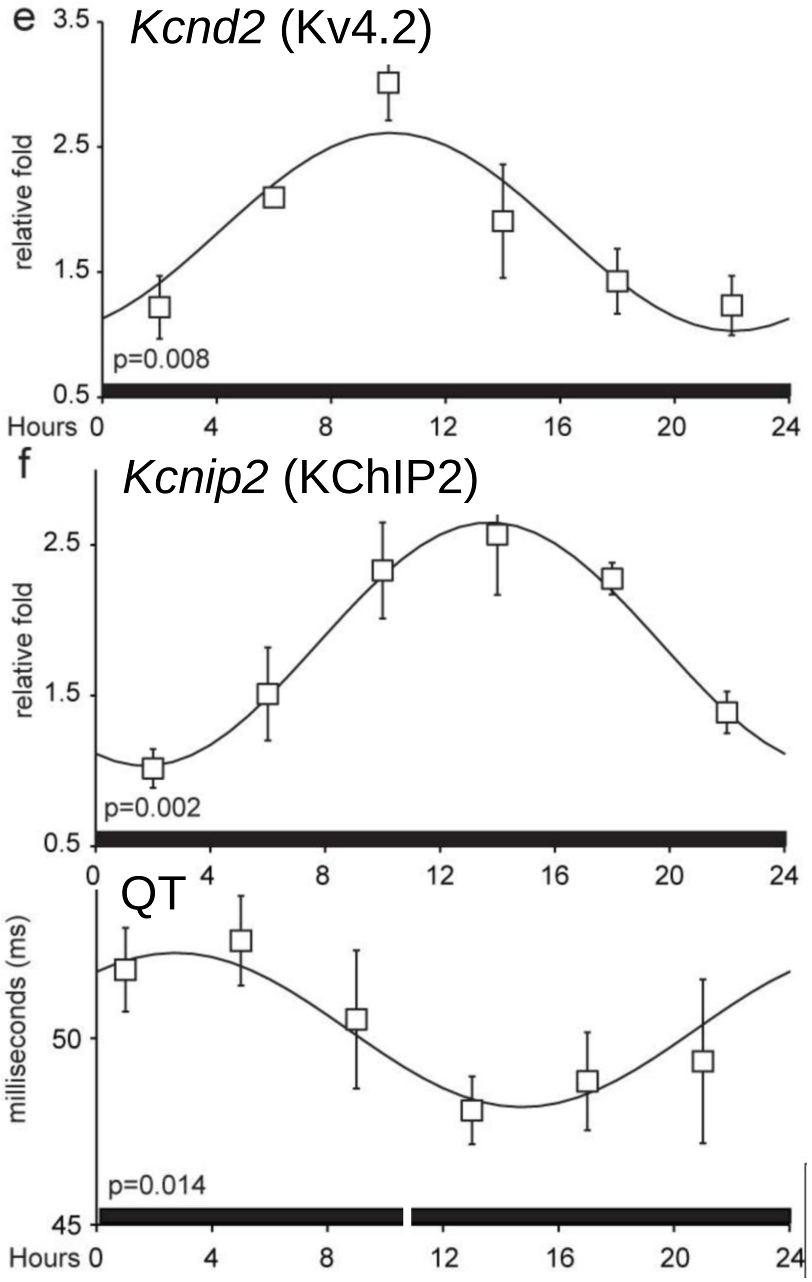
Time dependence of arrhythmias prevalence



Black et al., Heart Rhythm 2019

Circadian ventricular electrical activity

K⁺ channels responsible of circadian rhythm of ventricular electrical activity



Jeyaraj et al., Nature 2012

Electrical Coupling of Myocytes: Gap Junctions

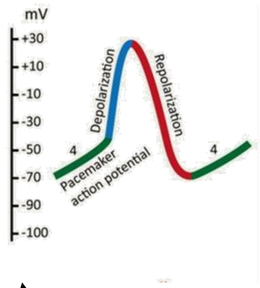
Gap Junctions

➔ Large-conductance pores permeable for ions and small molecules

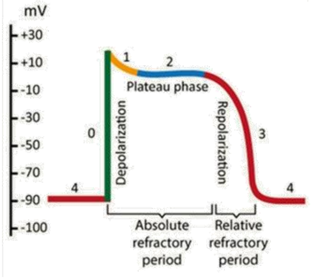
➔2 sets of 6 subunits = connexons

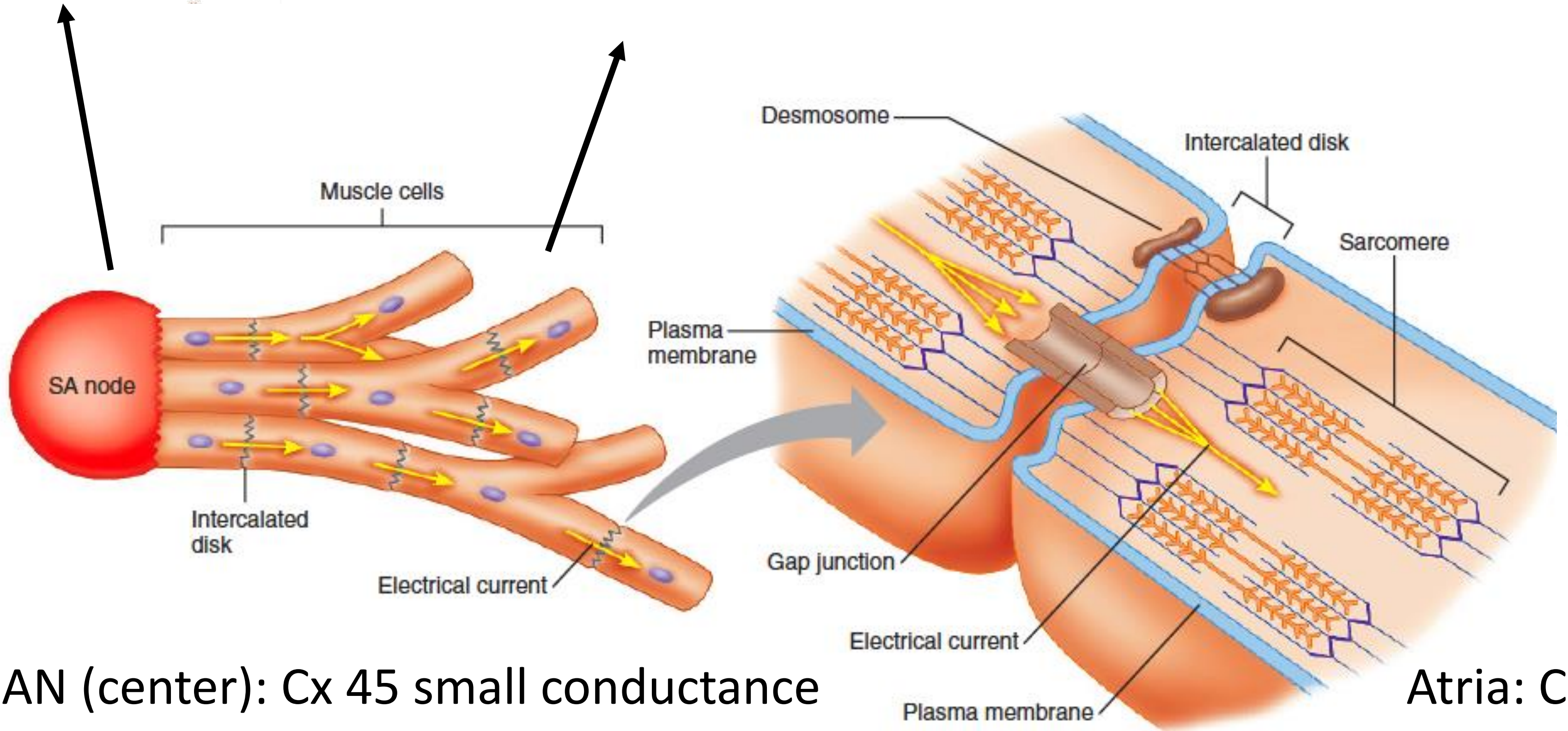
➔ Electrical coupling of myocytes

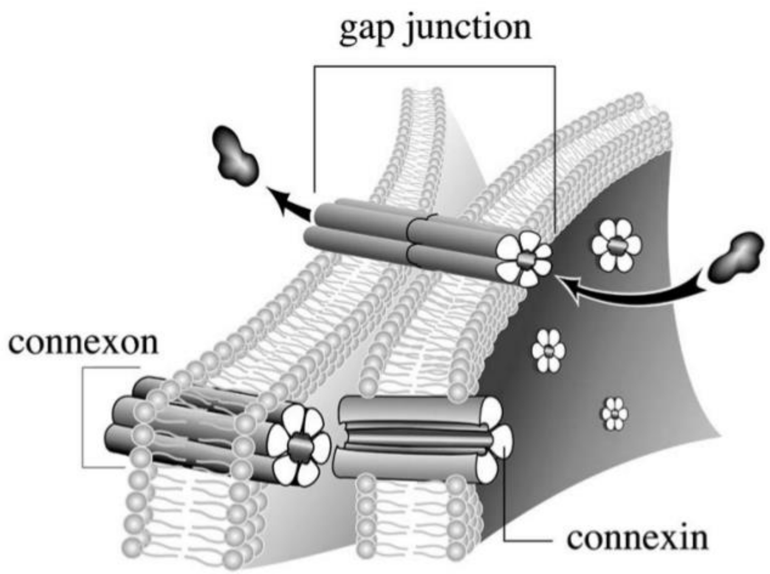
SA node



Contractile Myocardium



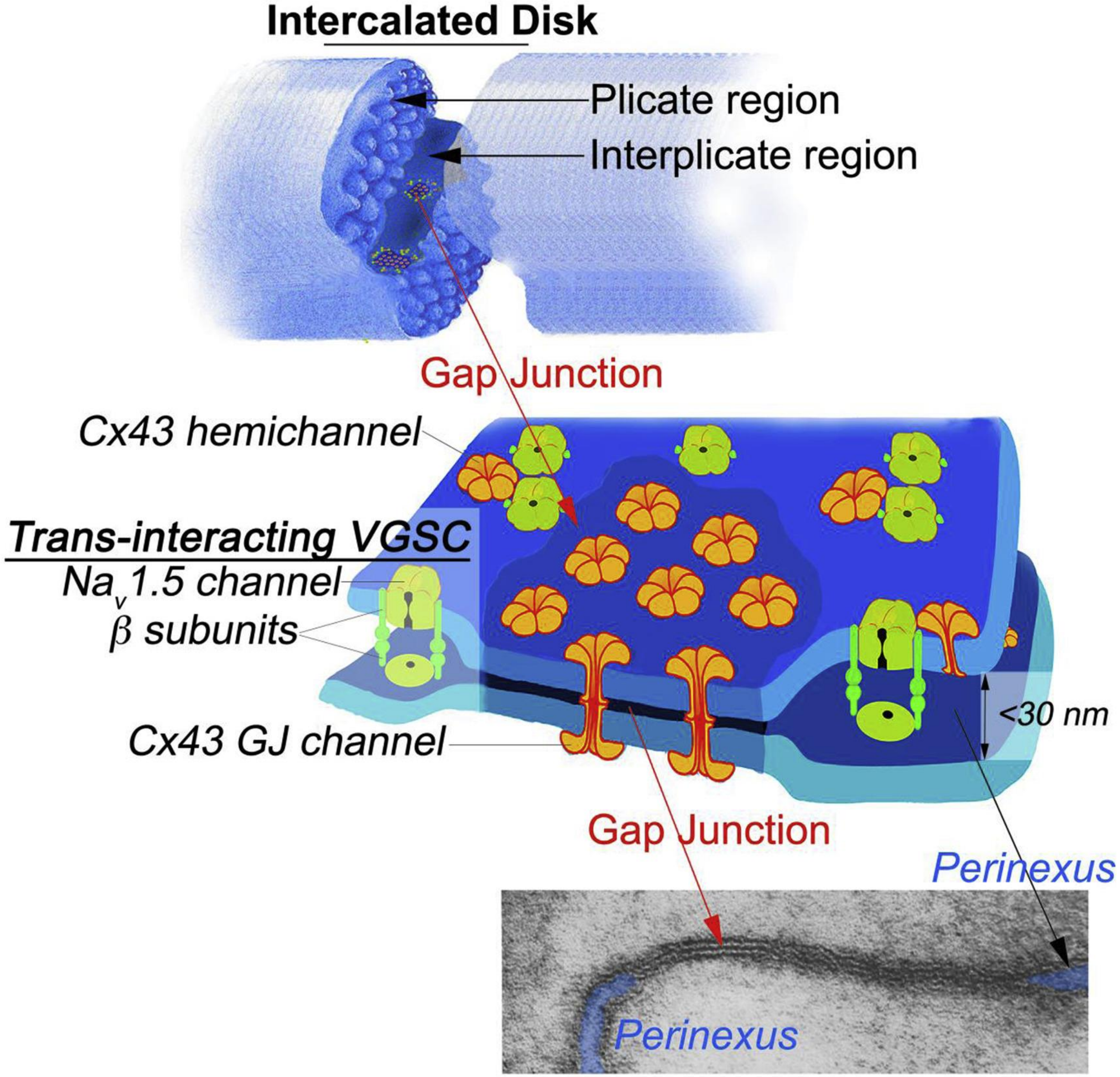




SAN (center): Cx 45 small conductance
SAN (periphery): Cx 45 and Cx 43

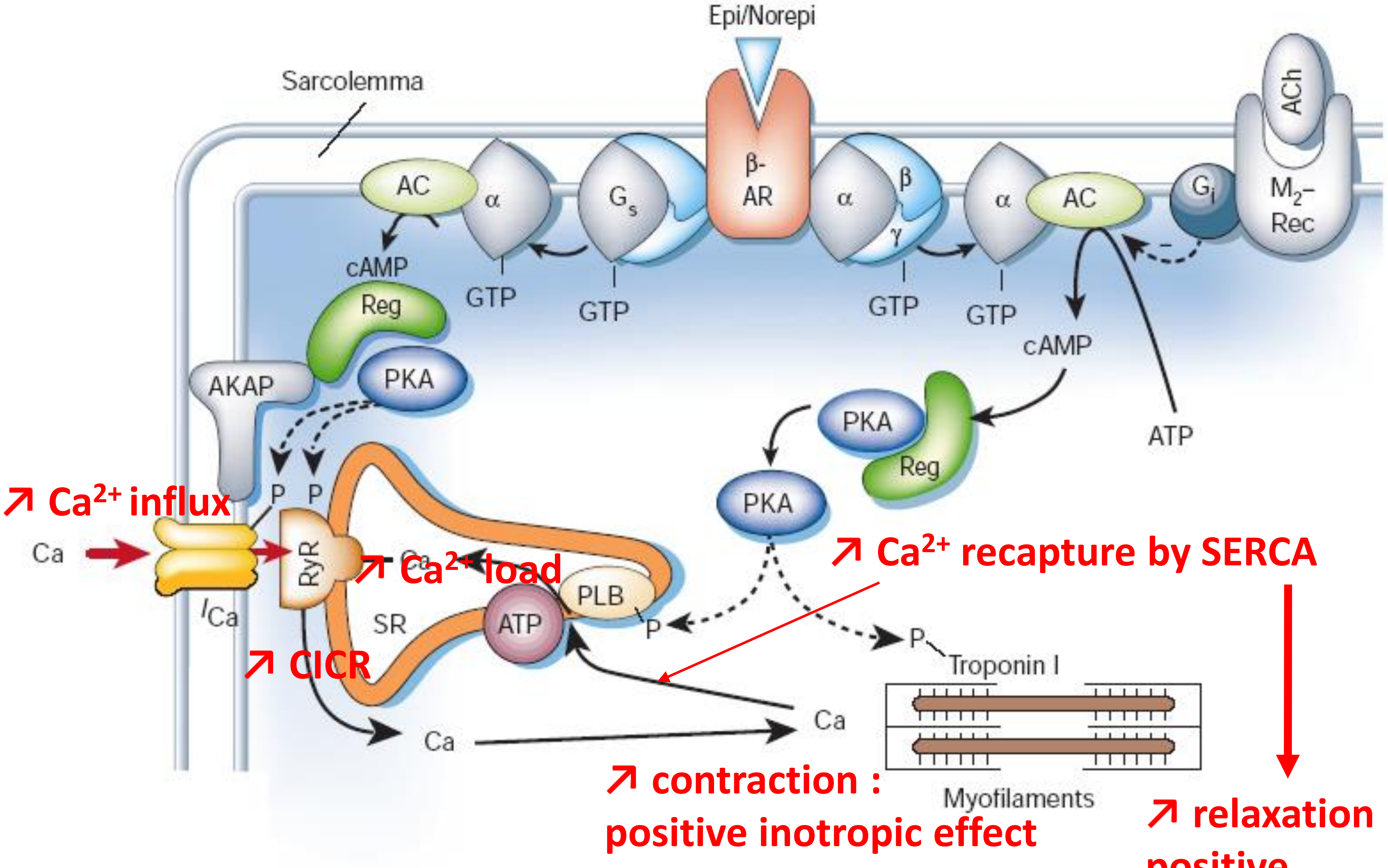
Atria: Cx 40 and Cx 43 large conductance
Ventricles: Cx 43

Electrical Coupling of Myocytes: colocalization of Cx43 and $Na_v1.5$ in perinexus



Hoagland *et al.*, 2019

β-adrenergic stimulation and excitation-contraction coupling



↗ Ca^{2+} influx

↗ Ca^{2+} load

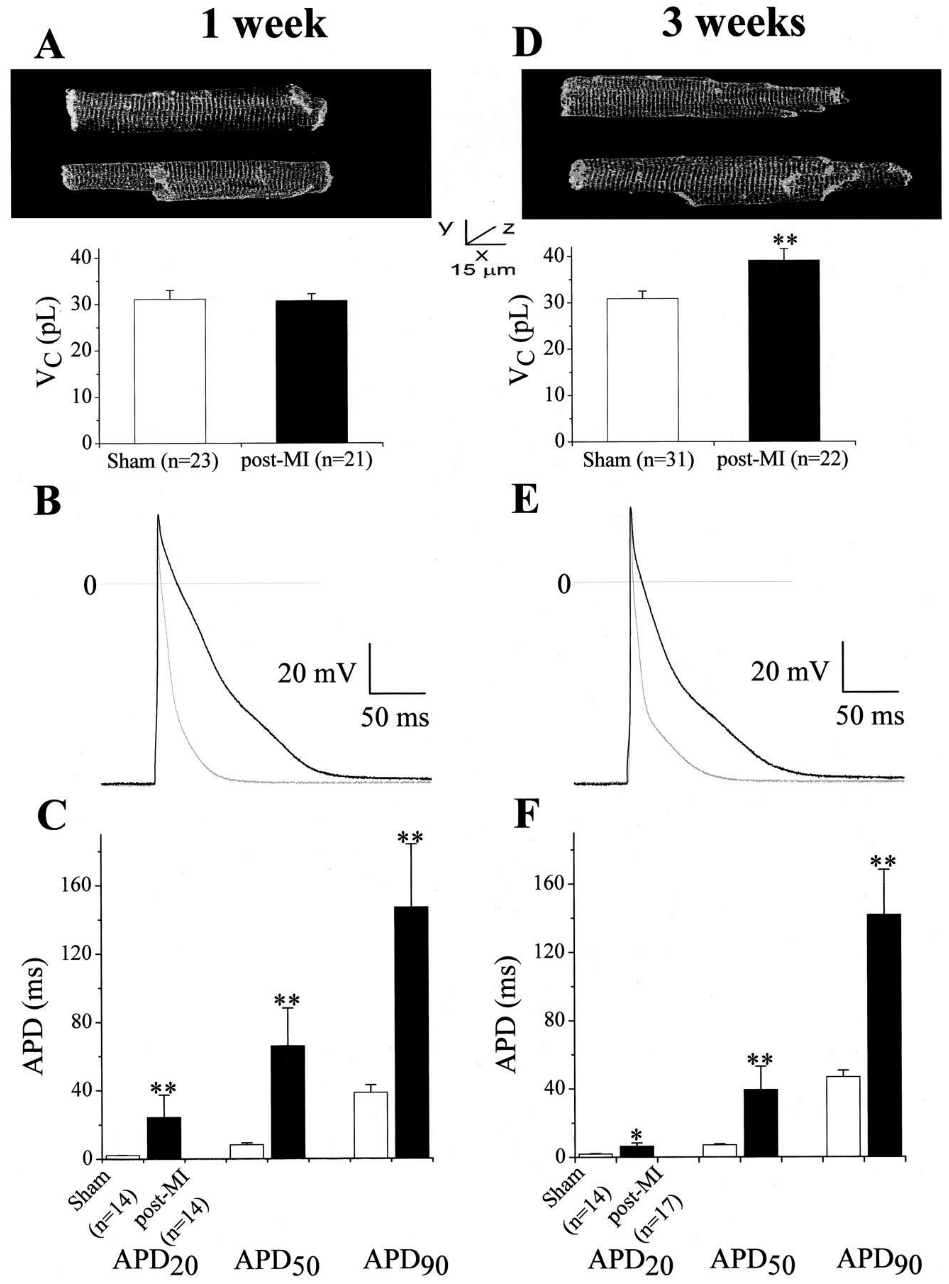
↗ CICR

↗ Ca^{2+} recapture by SERCA

↗ contraction :
positive inotropic effect

↗ relaxation :
positive
lusitropic effect

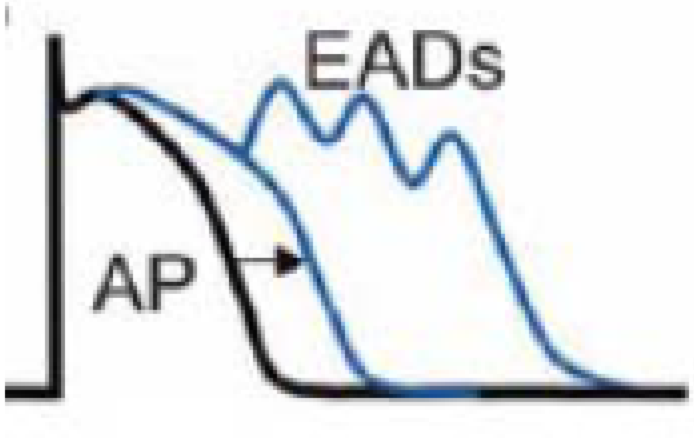
Electrophysiological remodelling during cardiac hypertrophy



Rats with myocardal infarction

Cardiomyocytes hypertrophy

Increased action potential duration

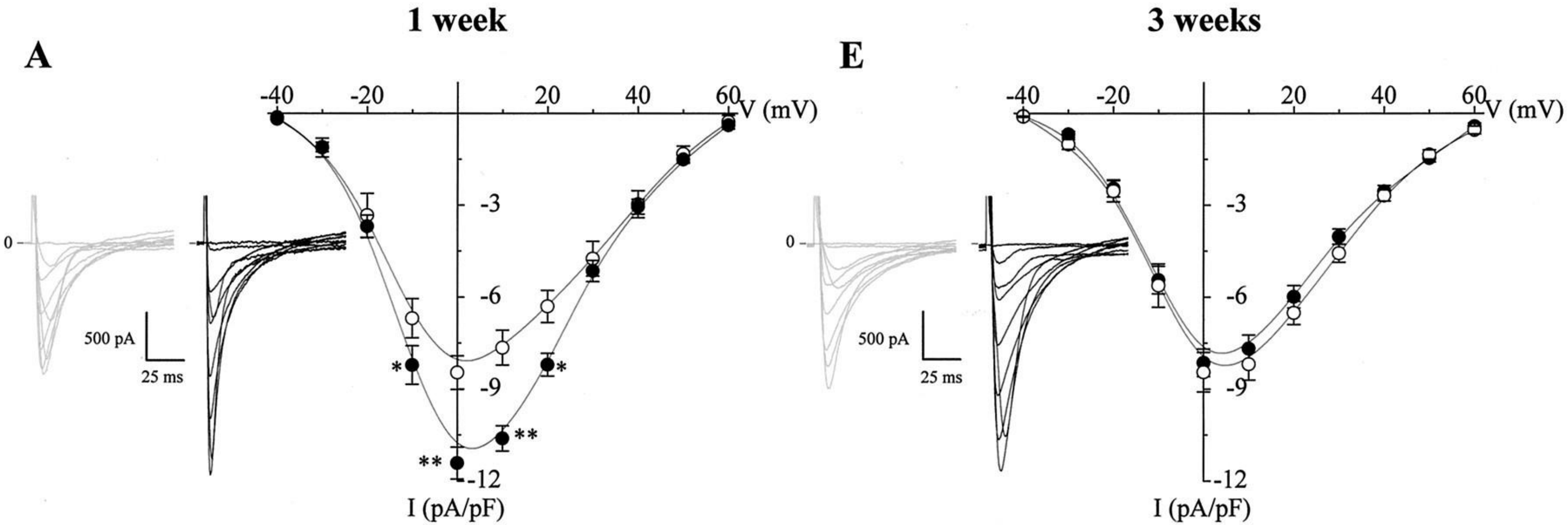


Reactivation of I_{CaL}

Perrier et al, Circulation, 2004

Electrophysiological remodelling during cardiac hypertrophy

Rats with myocardal infarction

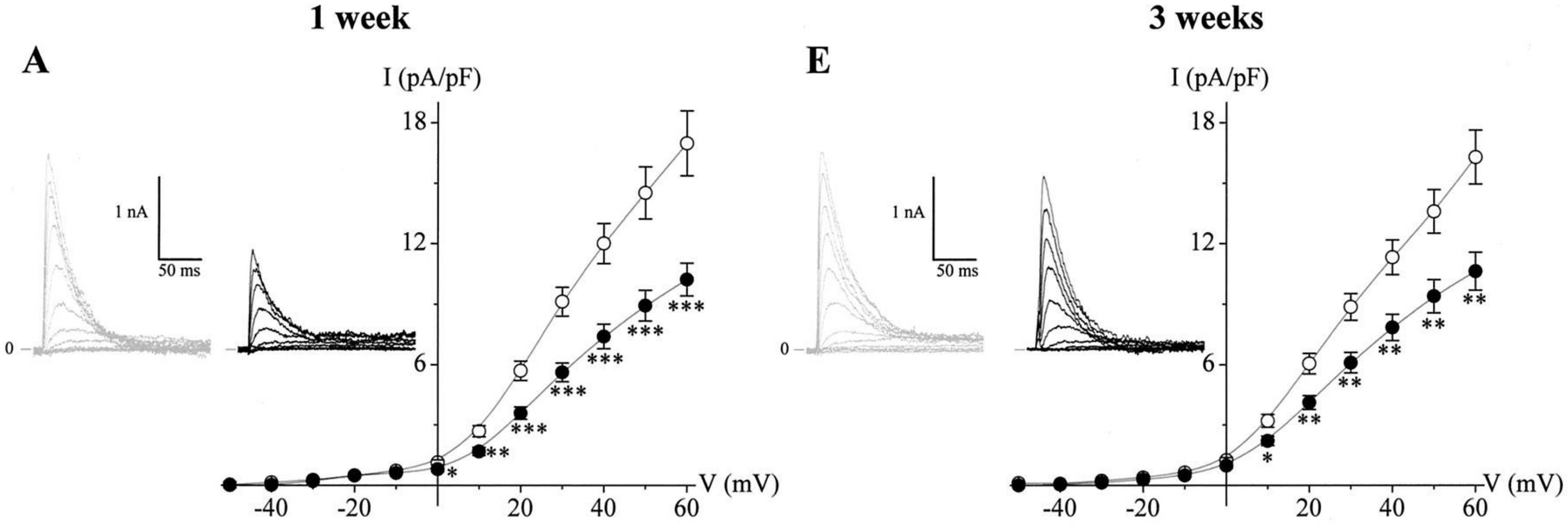


Increased L-type calcium current → increased action potential duration

Perrier et al, Circulation, 2004

Electrophysiological remodelling during cardiac hypertrophy

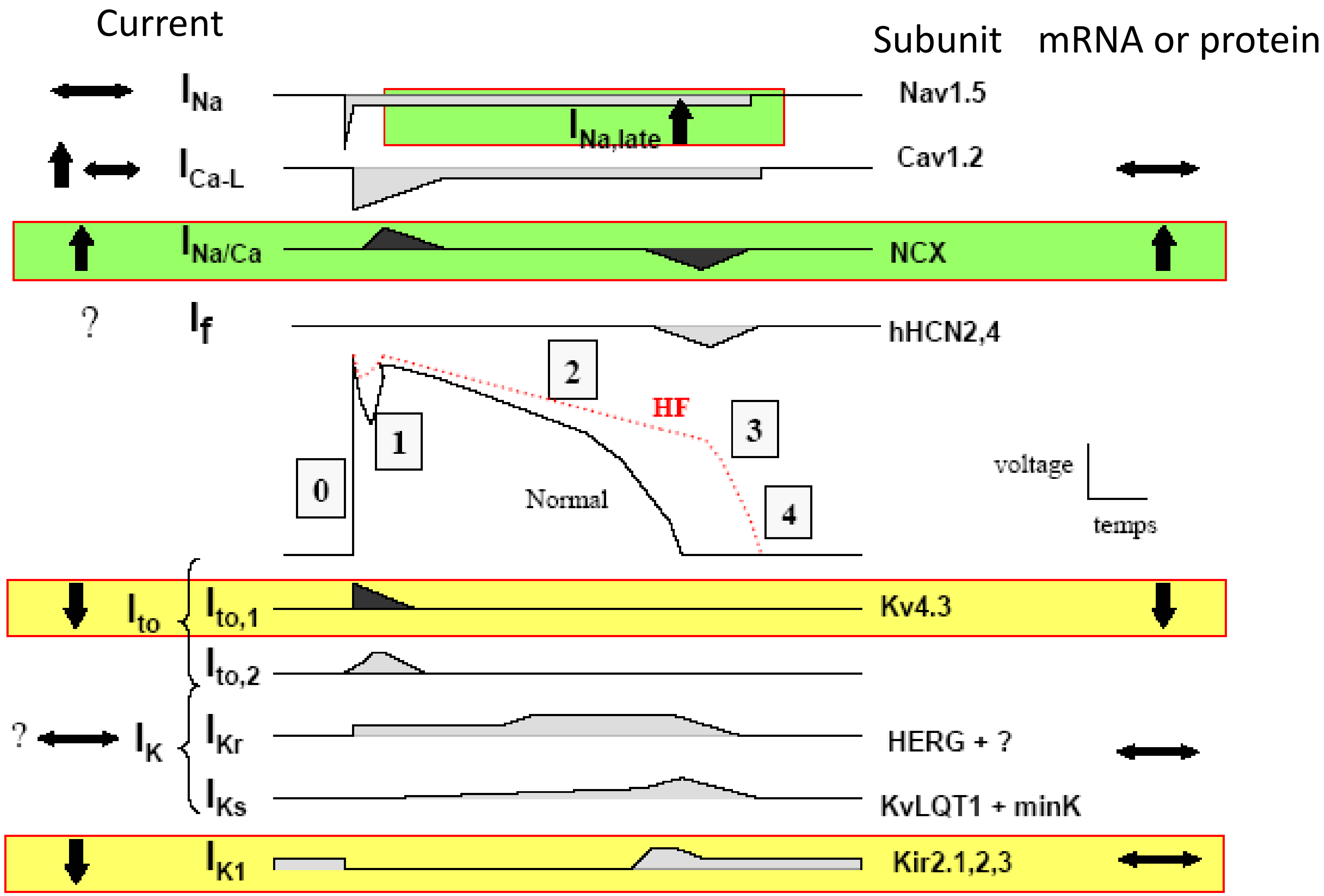
Rats with myocardal infarction



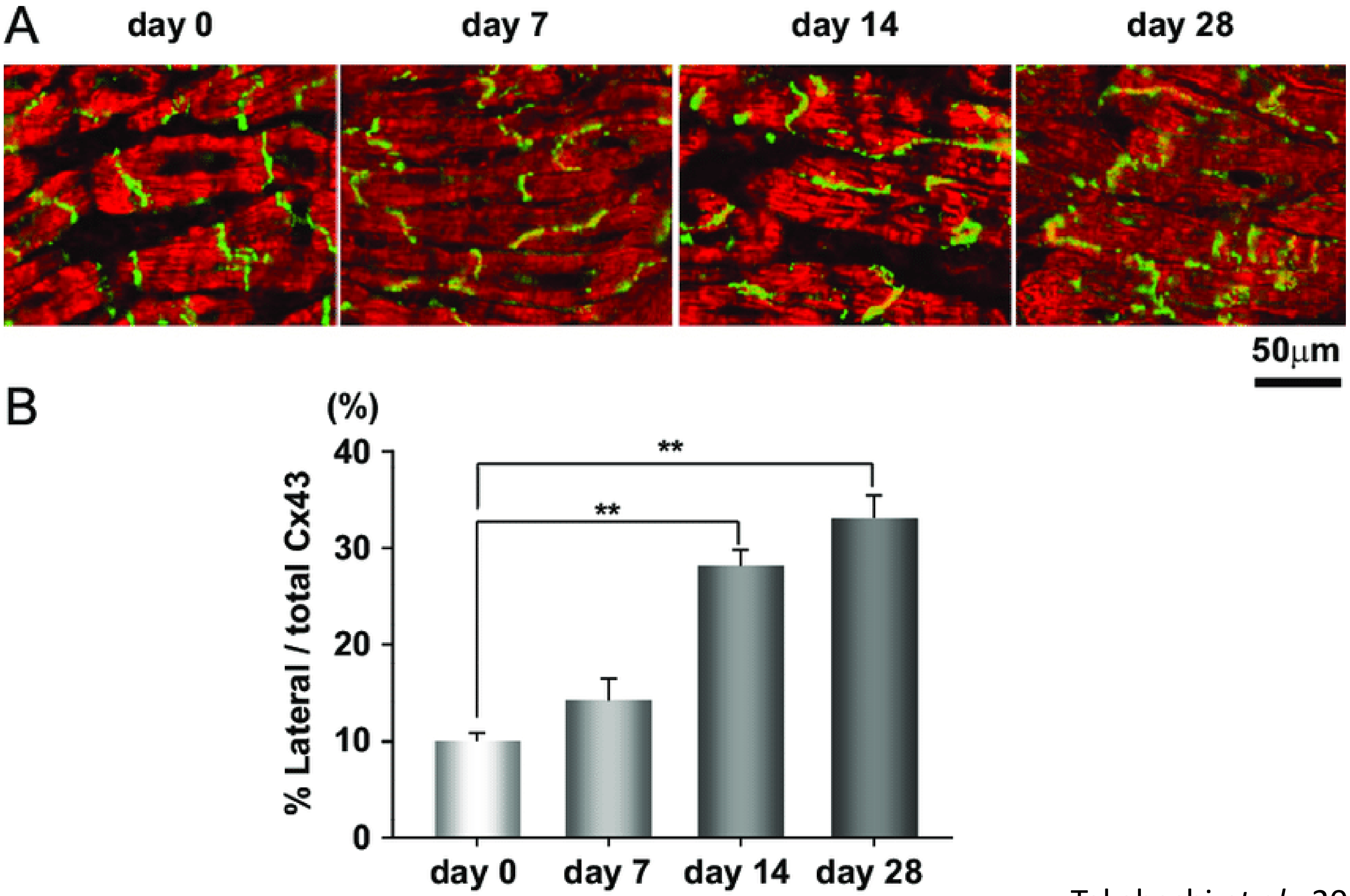
Decreased potassium current (I_{to}) → increased action potential duration

Perrier et al, Circulation, 2004

Electrophysiological remodelling during heart failure

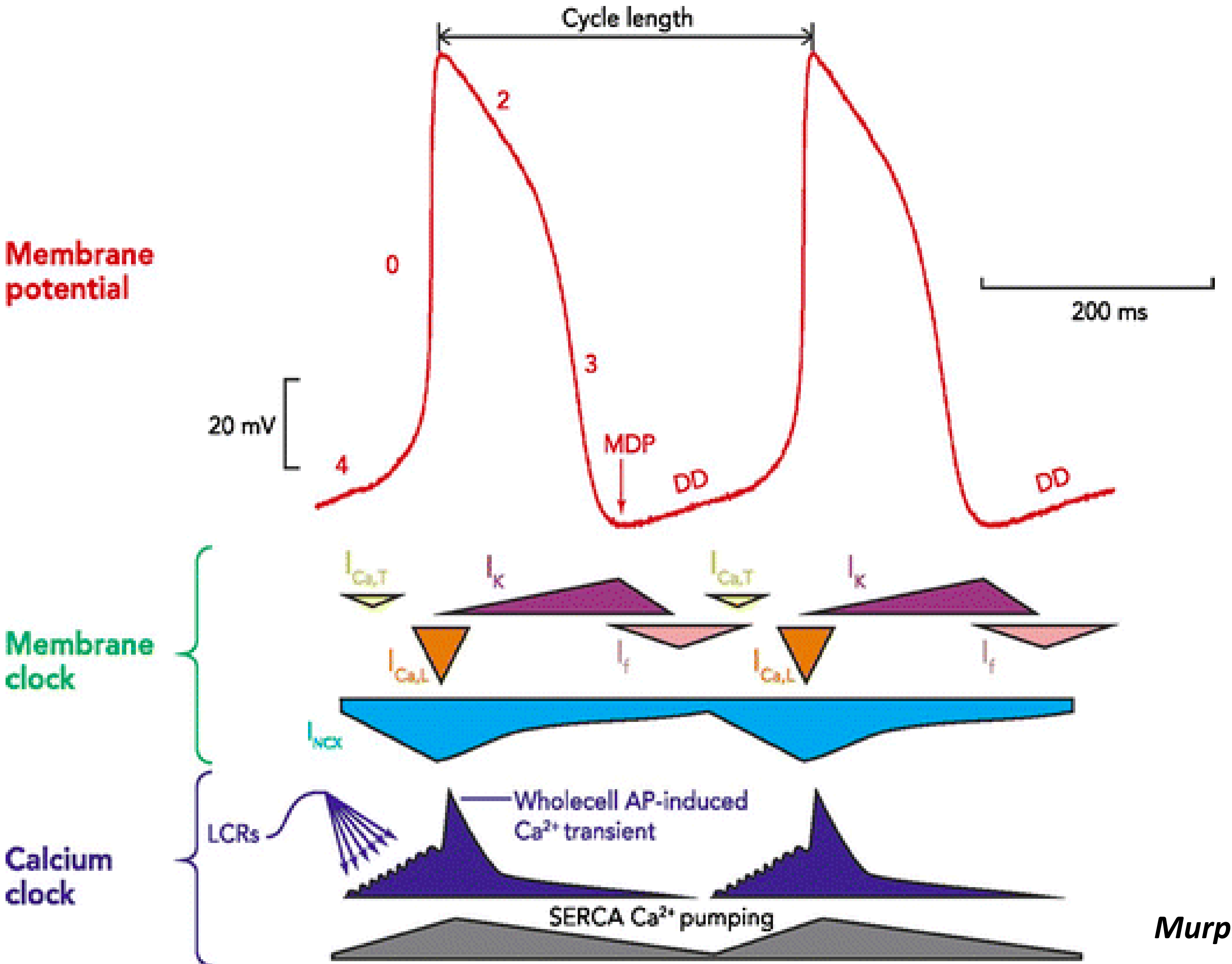


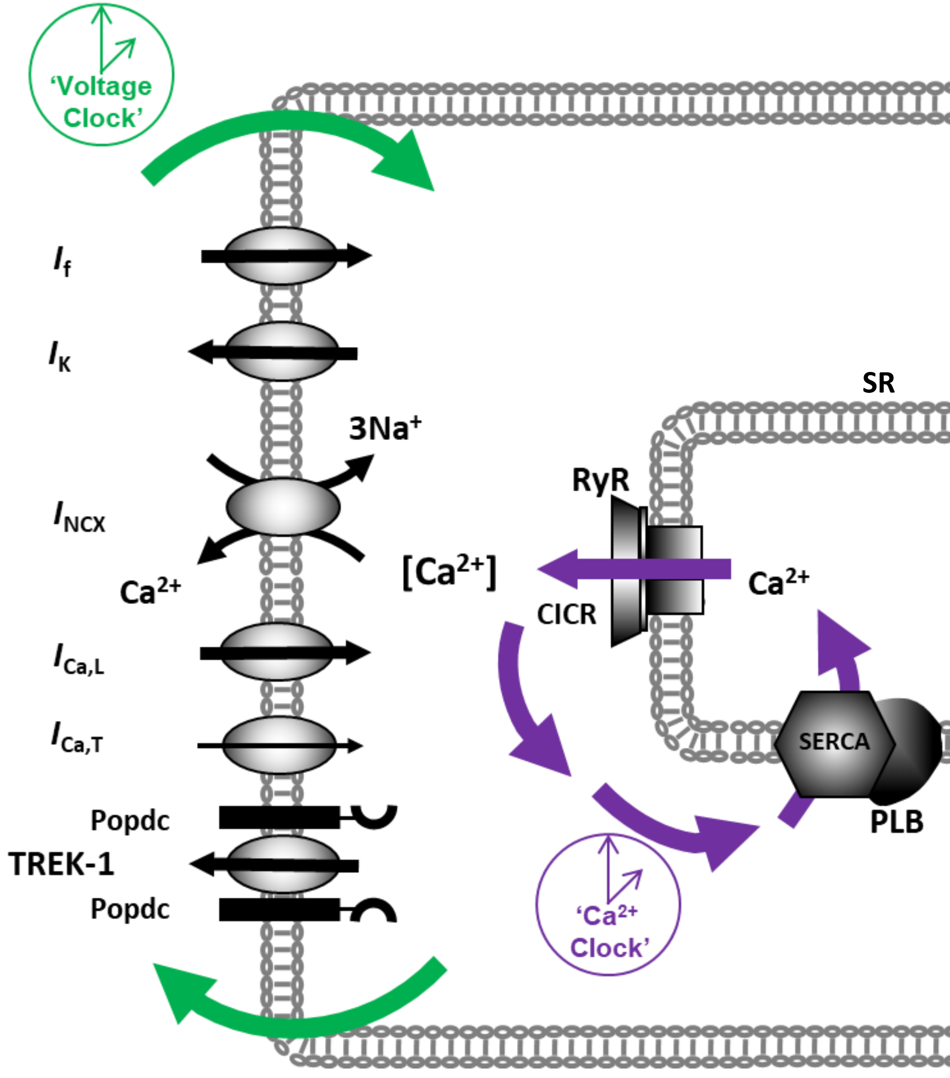
Lateralization of Cx43 abdominal aortic constriction



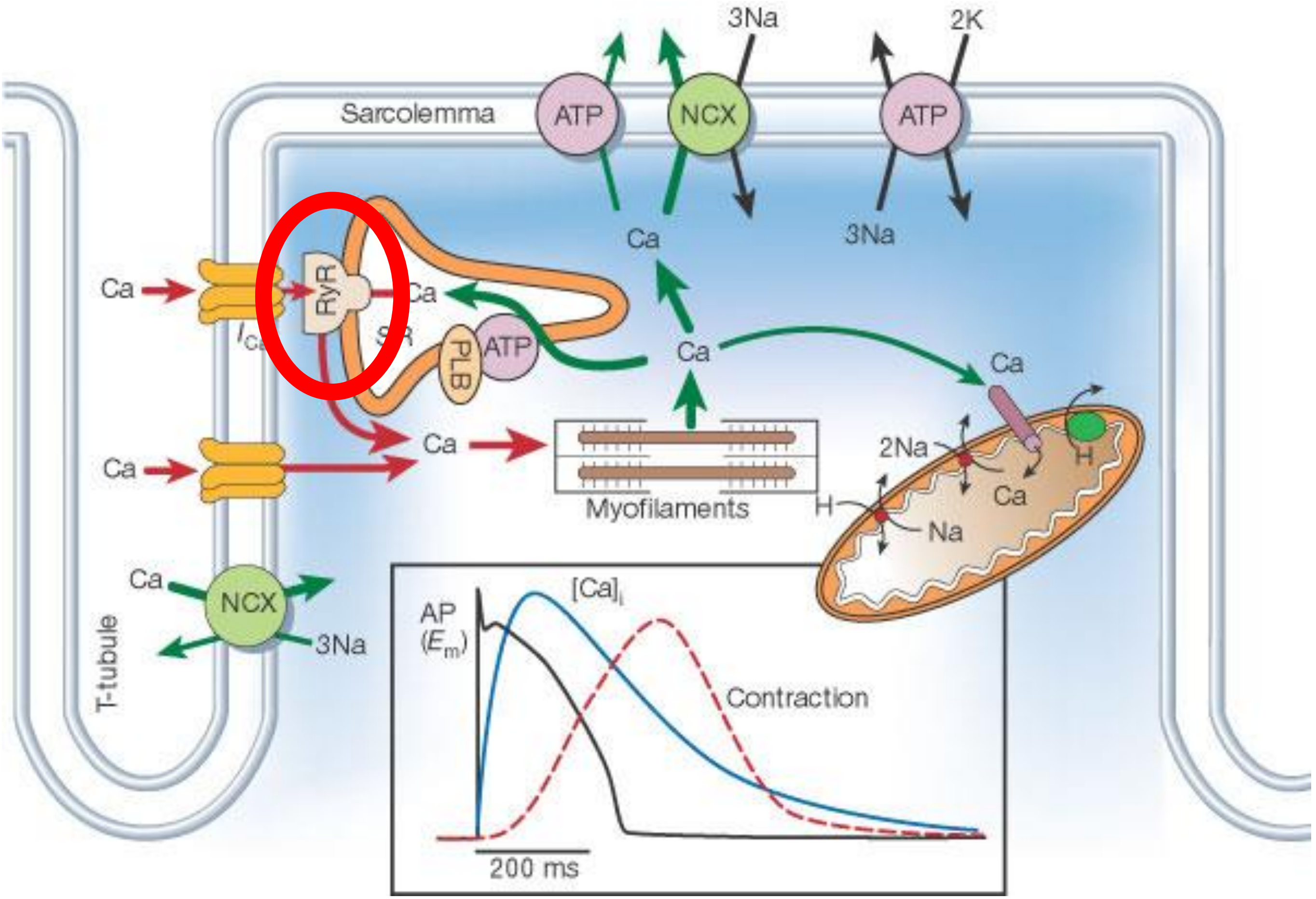
Takahashi *et al.*, 2012

Coupled-Clock System in the SA Node Cell





Murphy & Lazzara J Interv Card Electrophysiol 2016



Bers D.M. Nature. 2002