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# Cardiac action potential and underlying ionic currents

Methods, physiology and selected pathologies

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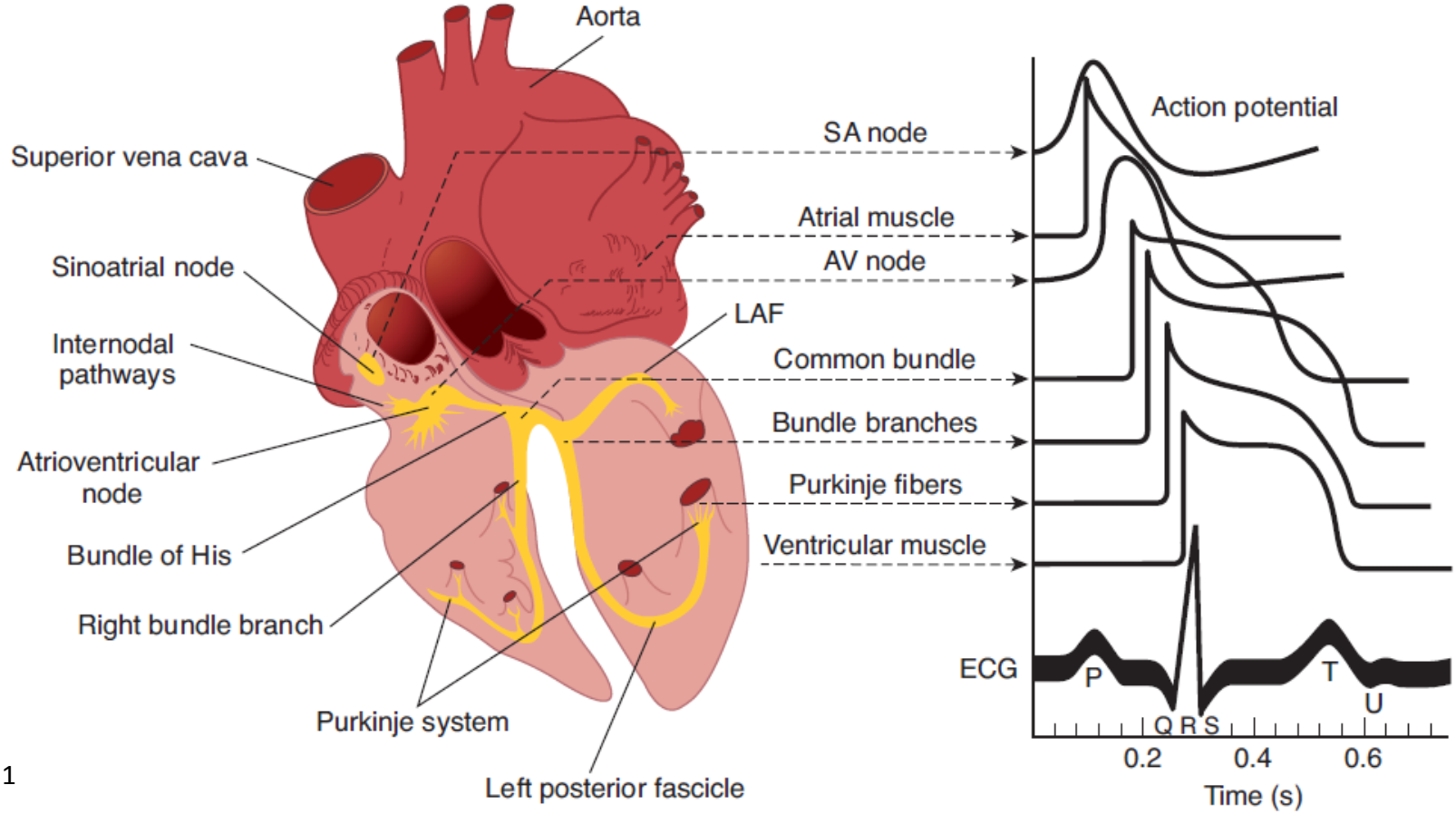
*The presentation was created with the support of the FRMU project „Modernization of teaching of cardiac cellular electrophysiology“, MUNI/FR/1490/2018.*

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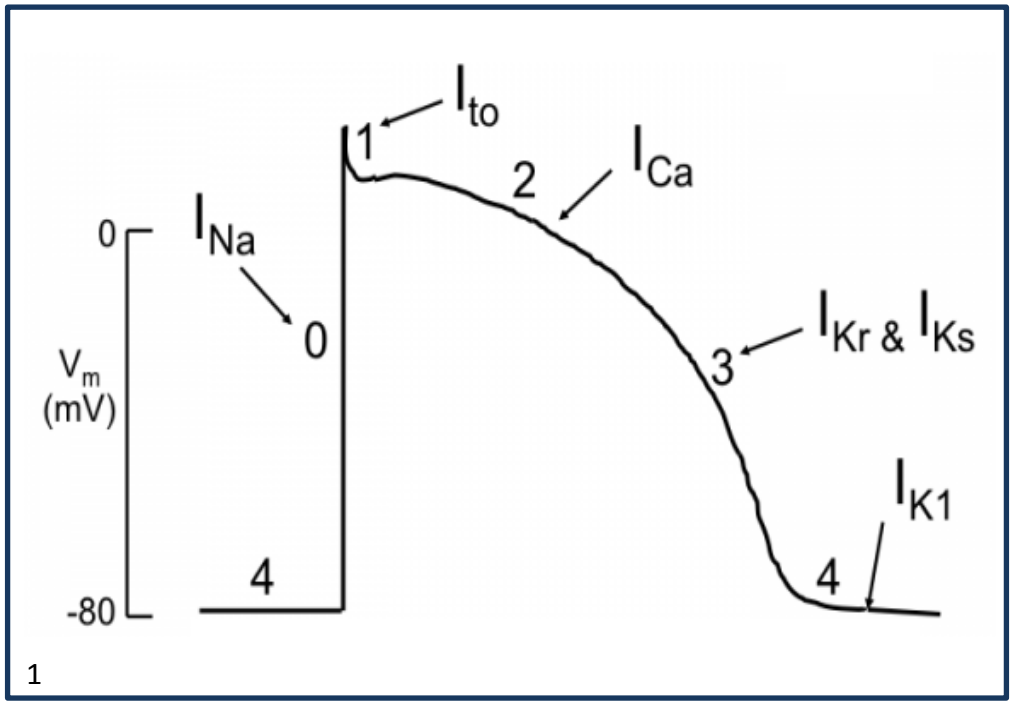
1:Ganong´s Review of Medical Physiology, 23rd edition

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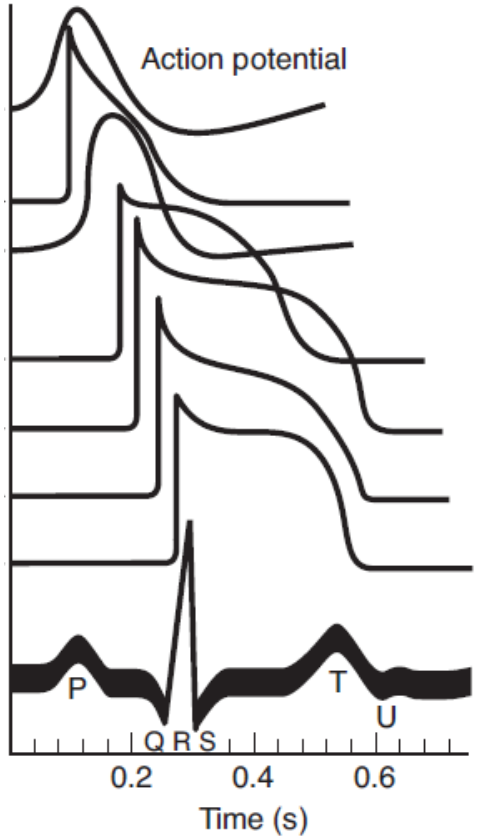
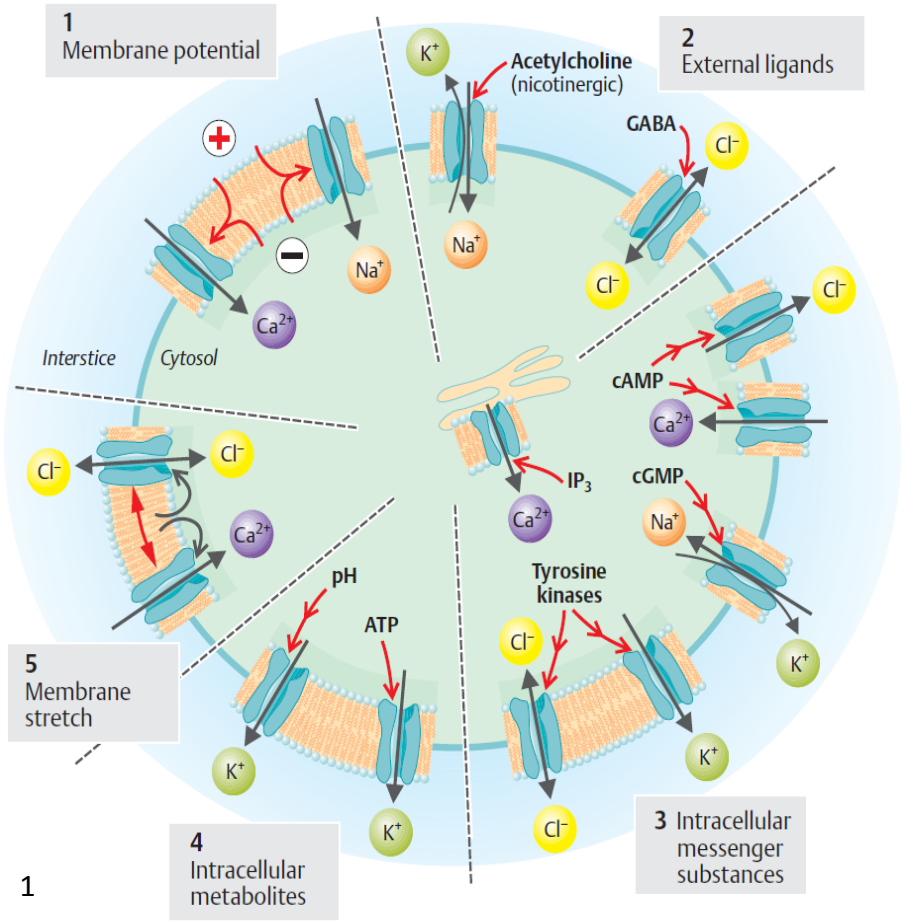



1: http://tmedweb.tulane.edu/tmedwiki/doku.php/intro_to_the_heart_cardiac_electrophysiology

# Ionic Channels

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1:Despopoulos, Color Atlas of Physiology, 2003

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# Impact of Knowledge on Electrical Properties of Cardiac Cells for Clinical Medicine

- → **Inherited Arrhythmogenic Syndromes**
- → **Acquired Arrhythmogenic Syndromes**
  - On a base of other primary cardiac diseases
  - Side effects of drugs
  - Effects of other substances including addictive drugs
- → **Sudden Cardiac Death**
- → **Mechanisms of Action of Antiarrhythmic Drugs**

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# Electrophysiological Methods in Cardiology

**Measurements could be performed:**

1. On the level of whole organism
   **Example:** *ECG*
2. On the level of the heart
   **Example:** *Intracardiac ECG*
3. On the  isolated heart
   **Example:** *Langendorff heart perfusion*
4. On the multicellular cardiac samples
5. On the isolated cardiomyocytes
   **Example:** *Whole cell patch clamp*
6. On the single membrane channels
   **Example:** *Single channel patch clamp*

# Electrophysiological Methods in Cardiology

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3. On the isolated heart
   **Example:** *Langendorff heart perfusion*

**Basic principle of methods:**

→ We measure the potential difference between two points of a volume conductor

→ Measured quantity **voltage**.

→ Recorded signals represent a sum of contributions of electrical activities of individual cells of the organ during propagation of excitation.

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# Electrophysiological Methods in Cardiology

**Measurements could be performed:**

4. On the multicellular cardiac samples
5. On the isolated cardiomyocytes
   **Example:** *Whole cell patch clamp*
6. On the single membrane channels
   **Example:** *Single channel patch clamp)*

**Basic principle of methods:**

→ We measure **membrane voltage,** which is the difference between the extracellular and intracellular medium, or **membrane current**.

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# Basic principle of measurements on various levels of organism

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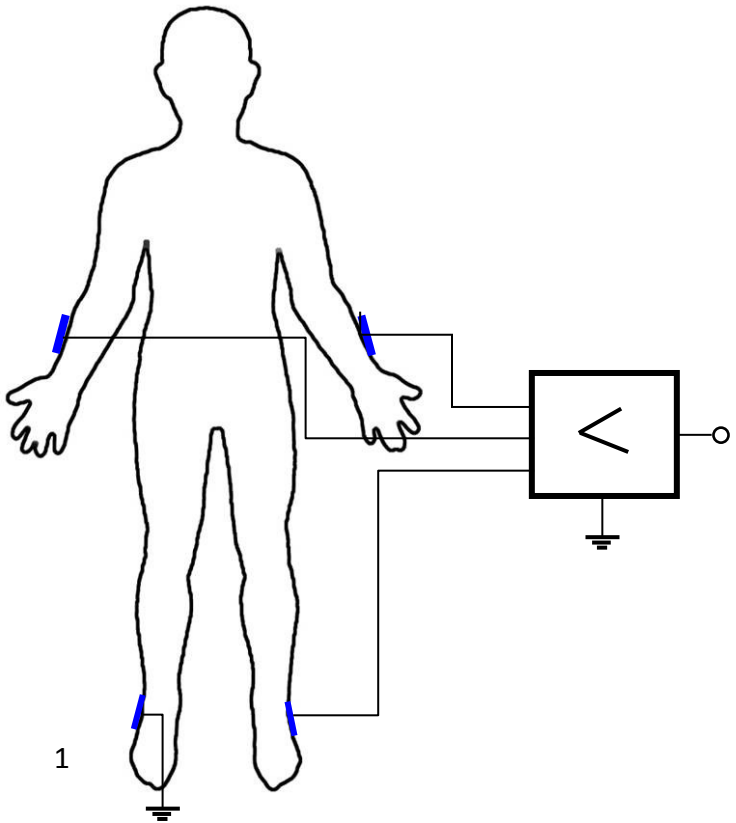
# Electrocardiography



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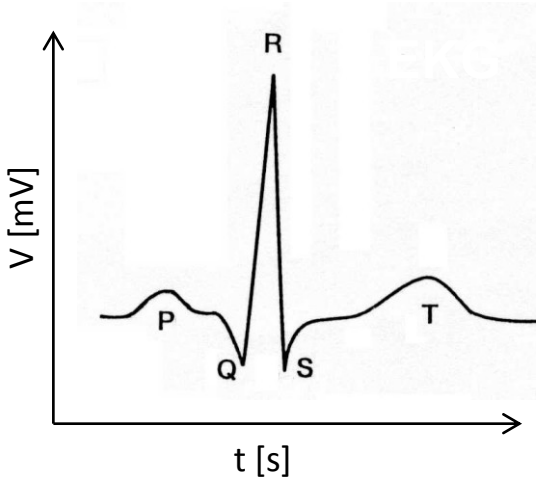
We record **voltage** and
its changes over the **time.**




1:http://www.cndajin.com/group/human-figure-outline/#photo_2

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# Electrocardiography

Mechanism of impulse creation:

Adopted from Malmivuo, J., Plonsey, R., 1995. Bioelectromagnetism: principles and applications of bioelectric and biomagnetic fields. Oxford University Press, New York

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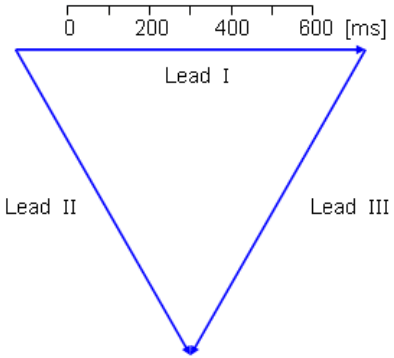
# Electrocardiography

Mechanism of impulse creation:


Adopted from Malmivuo, J., Plonsey, R., 1995. Bioelectromagnetism: principles and applications of bioelectric and biomagnetic fields. Oxford University Press, New York

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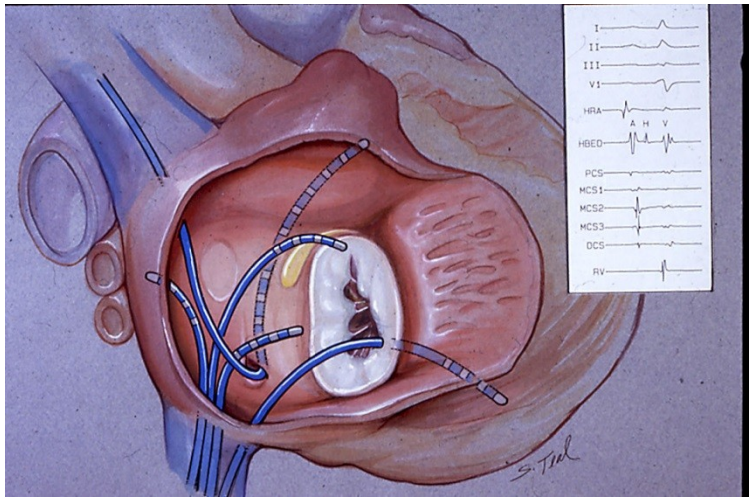
# Intracardiac electrocardiography

- An invasive method, routinely used in clinical practice
- **Basic principle:**
  A multipolar catheter is inserted into the heart. It is positioned in close proximity along the conduction system. The signal is registered from the tip of the catheter.

1. Multipolar catheter




2. Intracardial position of the catheter with the example of recorded signal

1: http://www.medicalexpo.com/prod/osypka/product-111576-879783.html; 2: http://www.drjohnm.dreamhosters.com/wp-content/uploads/2010/12/EPS-Teal_3.jpg

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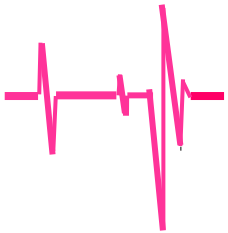
# Intracardiac electrocardiography

**We can measure:**

- → Function of sinus node
- → Conduction thorugh the atrial wall
- → Conduction in atrioventricular node
- → Conduction through Hiss bundle
- → Conduction through Purkinje fibers



Hiss bundle electrogram

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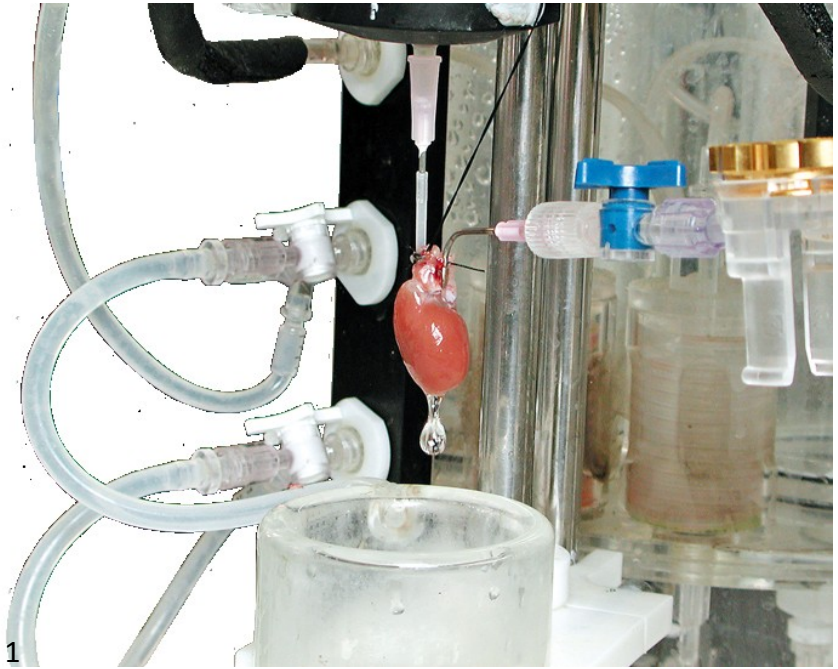
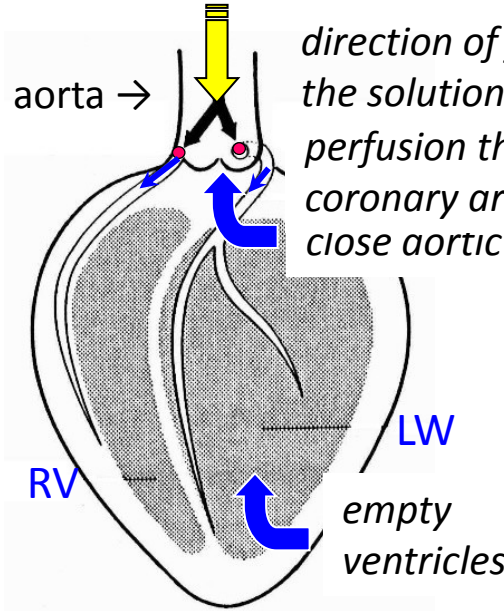
# Measuremt on the isolated heart

- An experimental methos
- The heart is placed in the Langendorff perfusion set:



Principle of the retrogarde perfusion




1: https://www.adinstruments.com/research/animal/cardiovascular/isolated-heart-systems/langendorff

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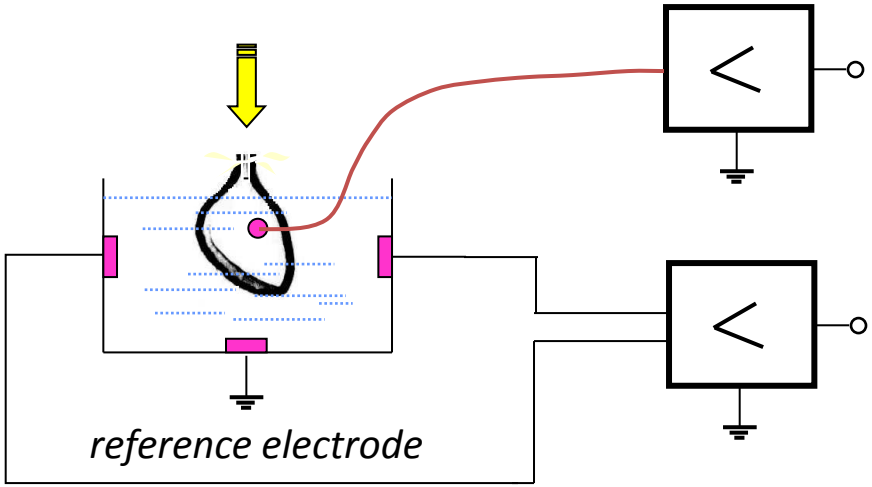
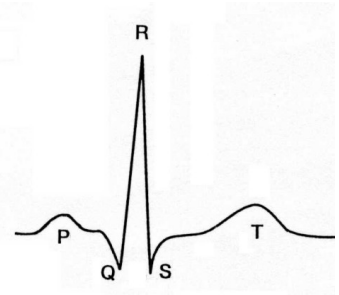
# Measuremt on the isolated heart

**Measurement is performed by:**

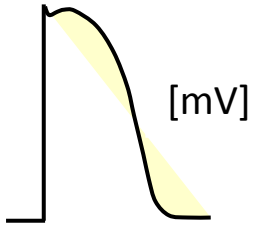
1. electrodes embedded in walls of the bath




2. Epicardial suction electrode




Electrogram




Monophasic action potential

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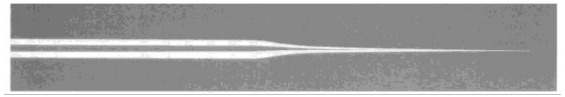
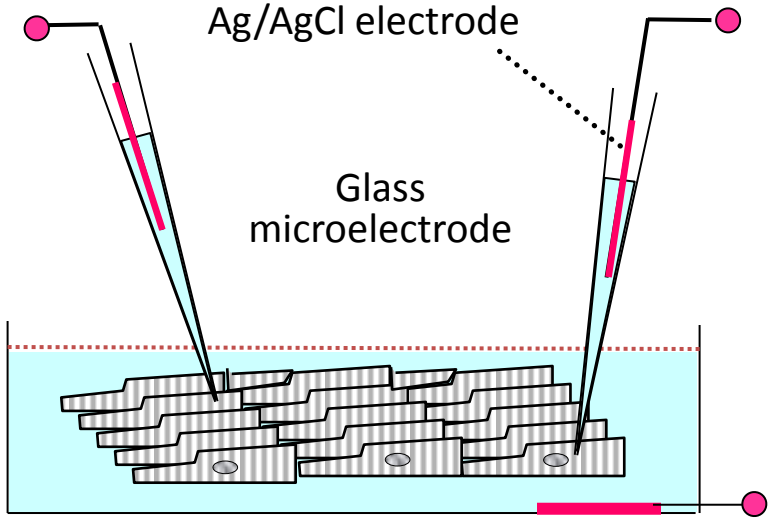
# Measurement on multicullular cardiac samples

Set-up



Glass microelectrode

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# Measurement on multicullular cardiac samples

→ A **stimulation of the sample** is needed for the measurement

→ It could be performed in two modes:

| | **current clamp mode** | **voltage clamp mode** |
|---|---|---|
| Stimulation | | |
| Recorded signal | | |

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# Patch-clamp

→ Both **whole cell patch-clamp** and **single channel patch-clamp** require an isolated cardiac cells.

→ By the successful dissociation, we obtain a sufficient fraction of viable, funtionally undamaged cells responding to electrical stimulation by:

- **Contraction**
- **Characteristic electrical activity** (action potential and membrane currents)

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# Patch-clamp

**Enzymatic isolation of cardiac cells:**

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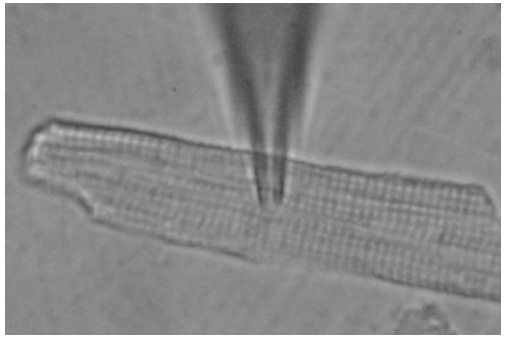
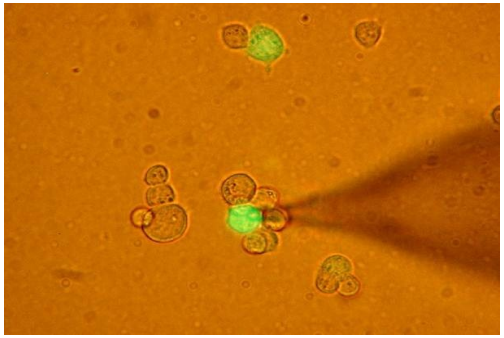
# Possibilities of using patch-clamp

**Measurements could be performed on:**

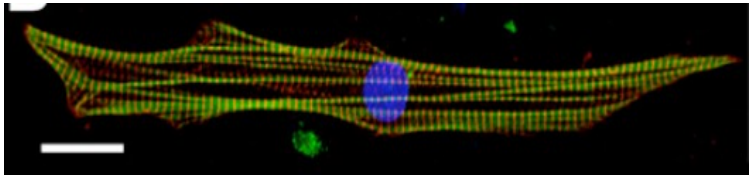
1. Isolated cardiac cells



2. Cell lines transiently expressing human ionic channels



3. Induced pluripotent stem cell-derived cardiac cells (*iPSC-CM*)



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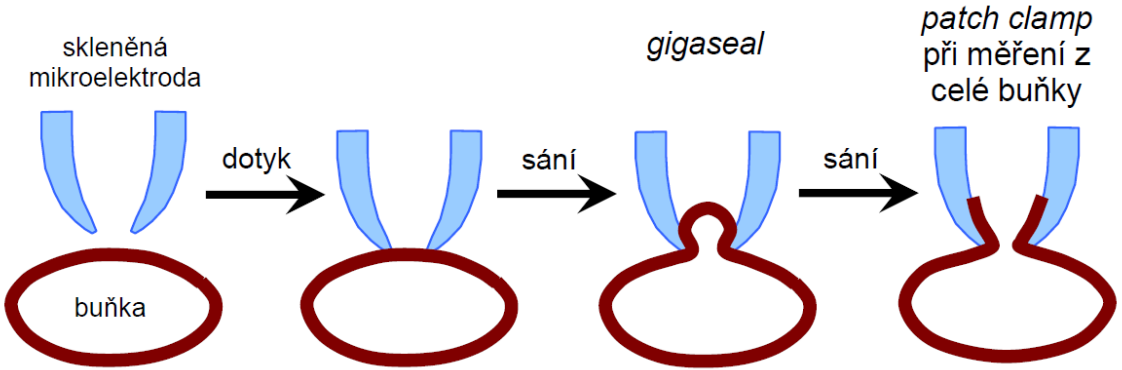
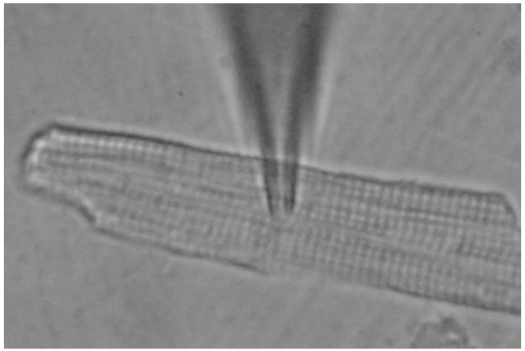
# Patch-clamp

## Whole cell patch-clamp

1. Establish a contact with a cell



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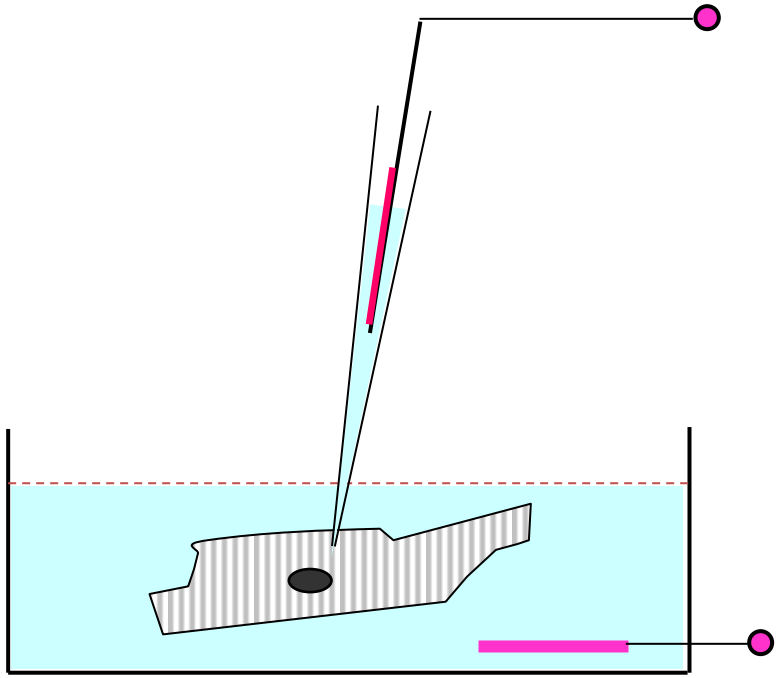
# Patch-clamp

## Whole cell patch-clamp

2. Measurement on a single cell



Two mode of measurement:

- **Voltage clamp**
- **Current clamp**

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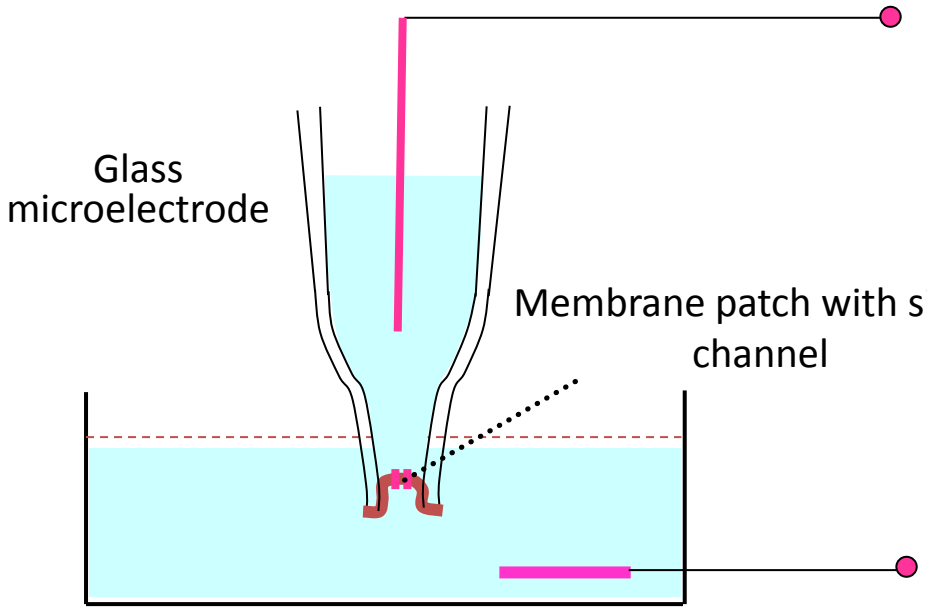
# Patch-clamp

## Single channel patch-clamp





Current flowing through single channel in response to imposed voltage pulse

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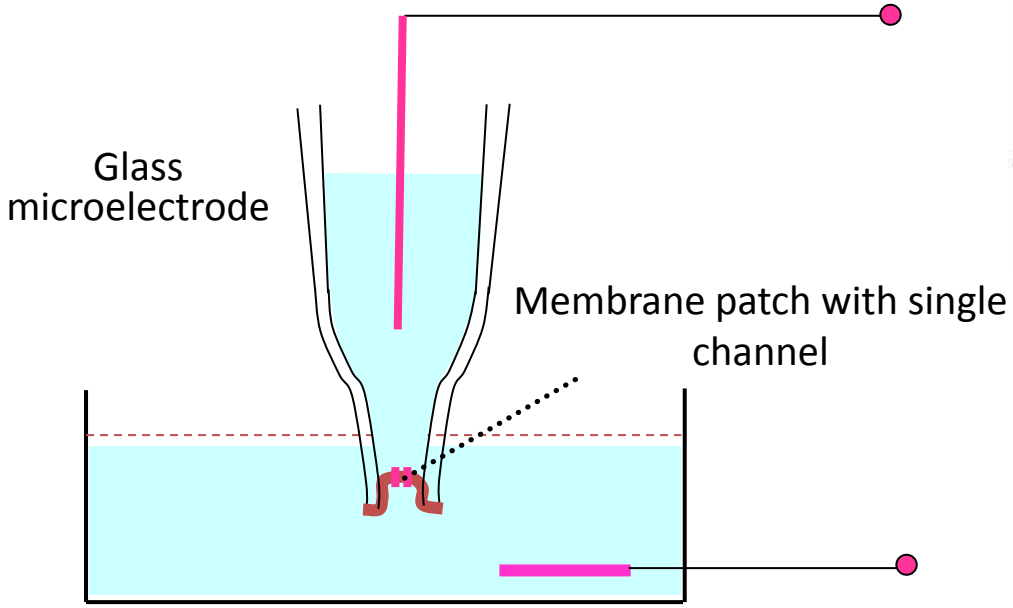
# Patch-clamp

## Single channel patch-clamp





Single channel current at -110 mV induced by acetylcholine at rat neuromuscular junction (nicotinic receptors; Mulrine and Ogden)

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# Possibilities of using patch-clamp

**We can analyse:**

1. Ionic channel gating under physiological and pathological conditions

   **Example:** *Changes of cardiac ionic currents in failing heart*

2. Drug effects on ionic channels

   **Example:** *Describing the pro-arrhythmogenic properties of certain drugs, i.e.:*

   → *Effect of antiarrhythmic drug ajmaline on action potential and on* $I_{K(ATP)}$
   → *Effect of antipsychotic drug perphenazine on* $I_{Na}$ *and* $I_{to}$
   → *Effect of ethanol on* $I_{K1}$
   → *Effect of anidepressant nefazodone on* $I_{Kr}$

3. Impact of a mutation on drug effects on a channel

4. Ionic channel dysfunction caused by a mutation

   **Example:** *Analysis of arrhythmogenic syndromes:*

   → *Long QT syndrome*
   → *Brugada syndrome*

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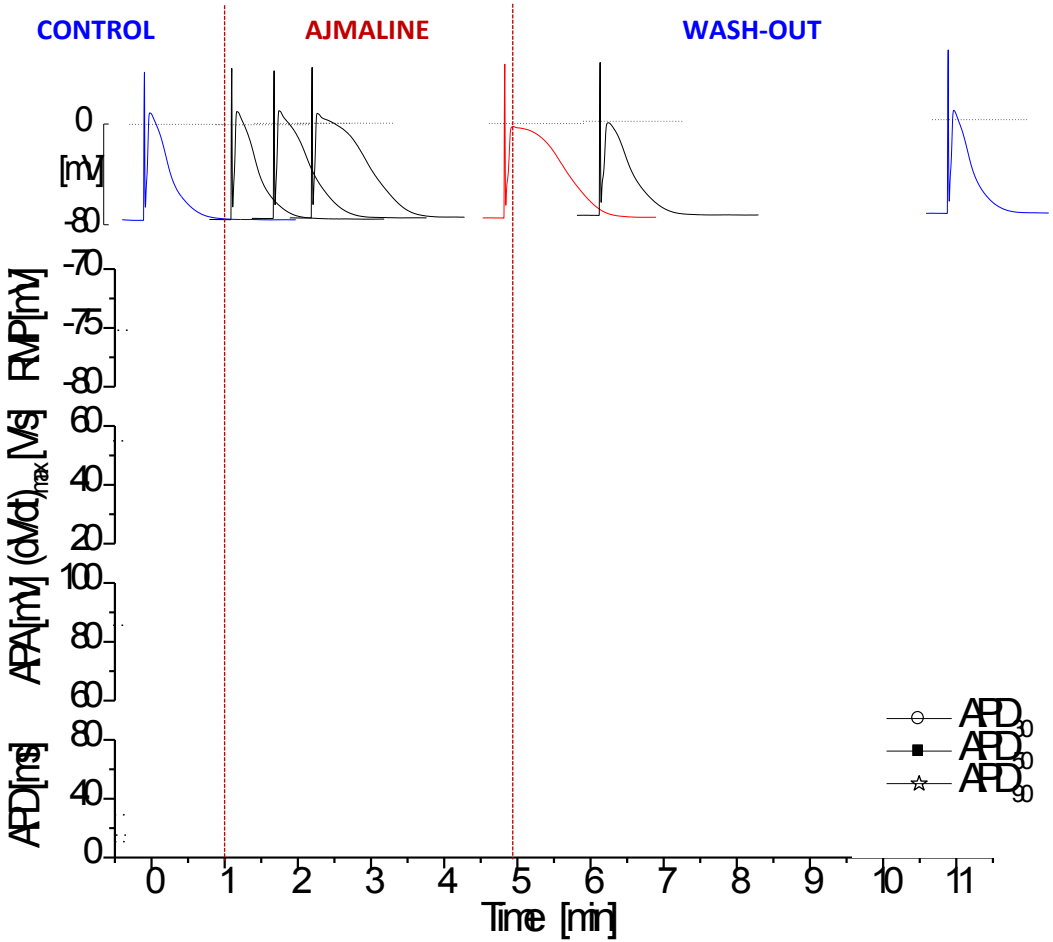
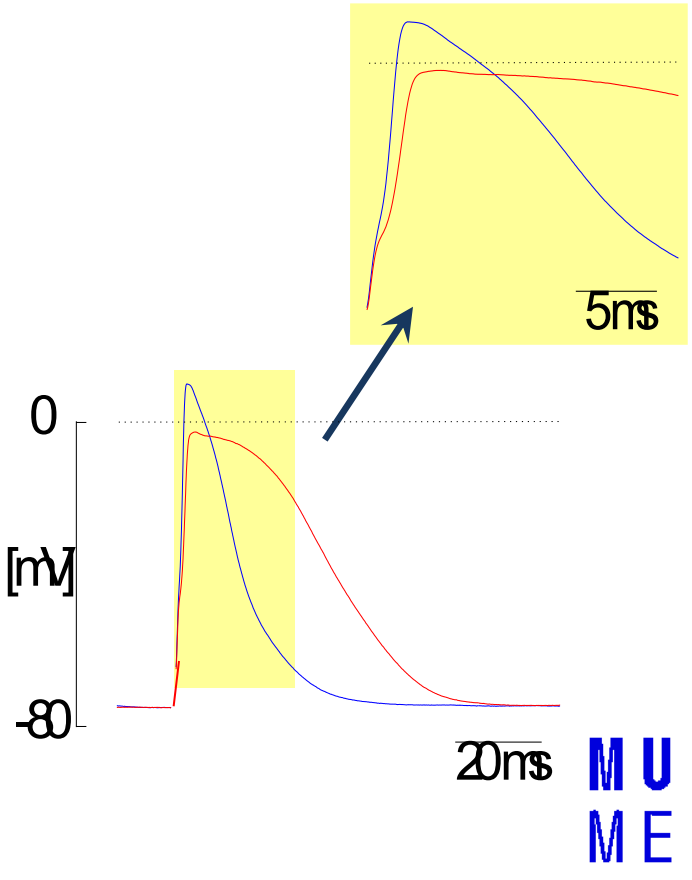
# **Example:** Effect of antiarrhythmic drug ajmaline on action potential

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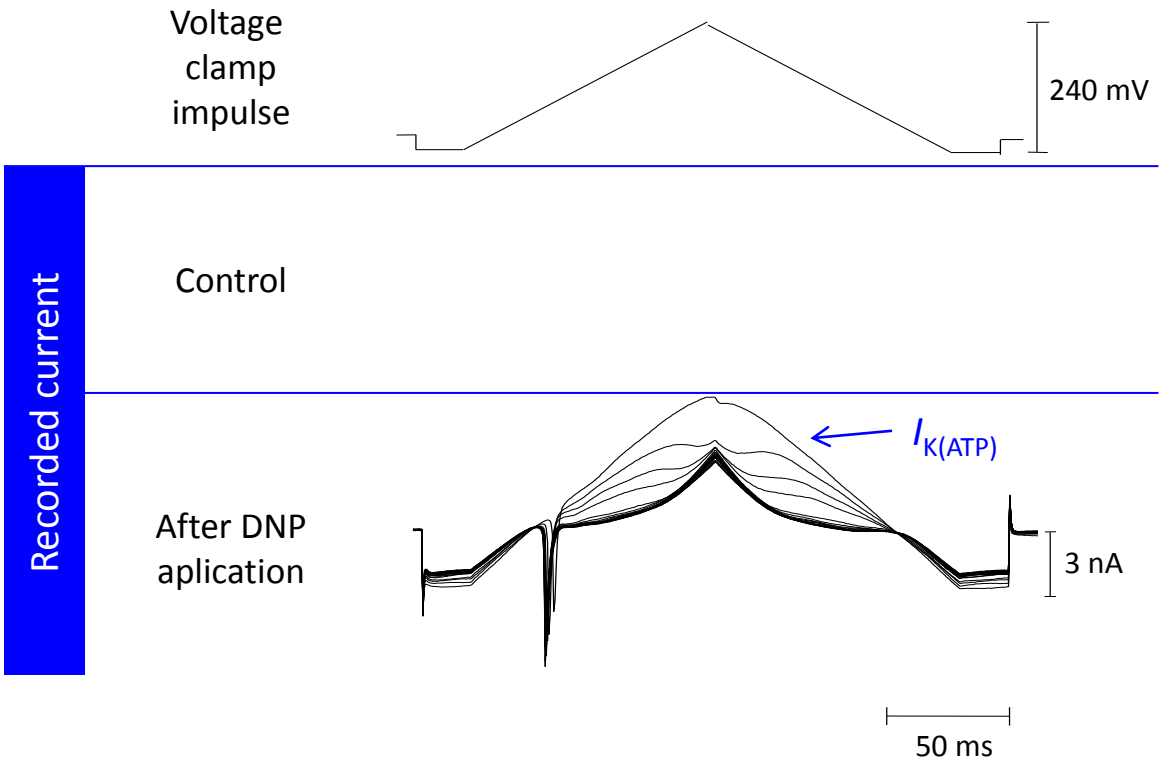
**Příklad:** Vliv antiarytmika ajmalinu na $I_{K(ATP)}$

**Měření $I_{K(ATP)}$**



**Example:** Effect of antiarrhythmic drug ajmaline on $I_{K(ATP)}$

**Measurement of $I_{K(ATP)}$**

Voltage clamp impulse

240 mV

Recorded current

Changes after ajmaline aplication

$I_{K(ATP)}$ *in control*

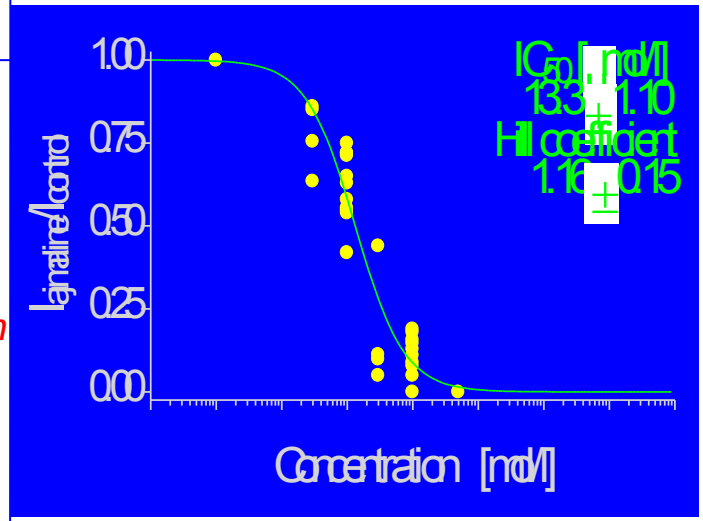
$I_{K(ATP)}$ *after ajm*

+120 0 -120

[mV]

4 nA

50 ms

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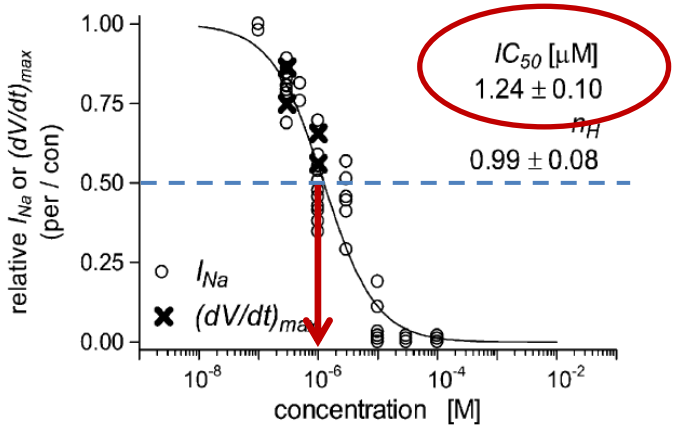
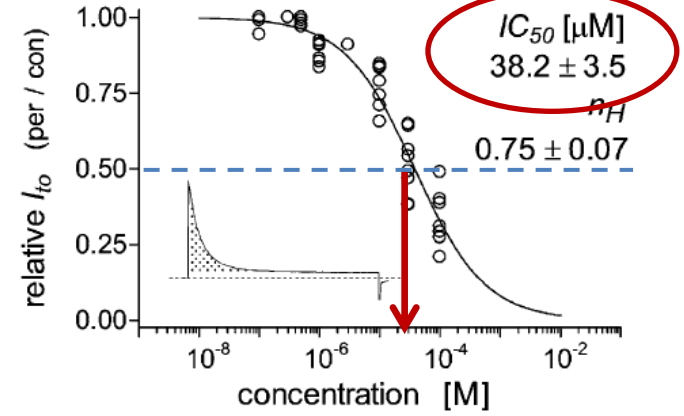
# **Example:** Effect of antipsychotic drug perphenazine on $I_{Na}$ and $I_{to}$

1

$I_{Na}$




$I_{to}$

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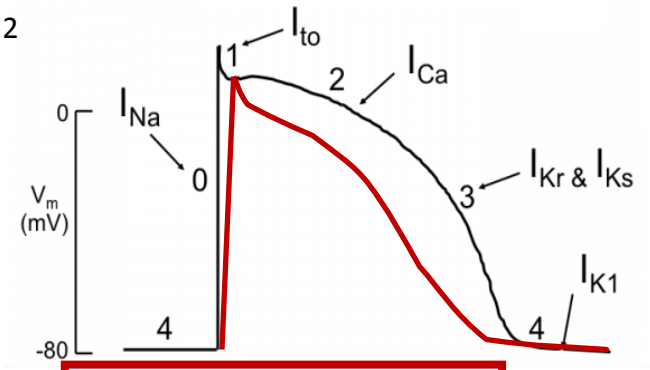


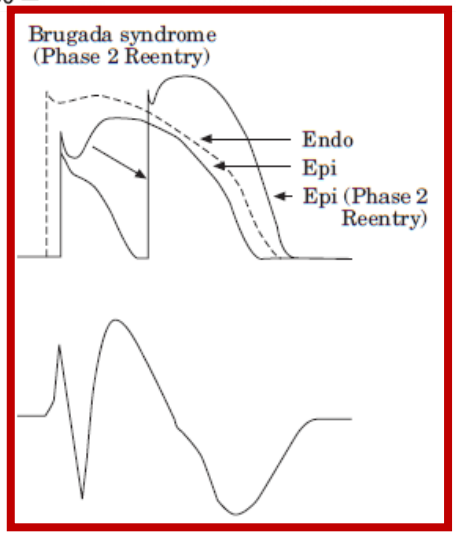


1:Bébarová et al. (Naunyn Schmied Arch Pharmacol 2009; 380:125-133); 2: http://tmedweb.tulane.edu/tmedwiki/doku.php/intro_to_the_heart_cardiac_electrophysiology

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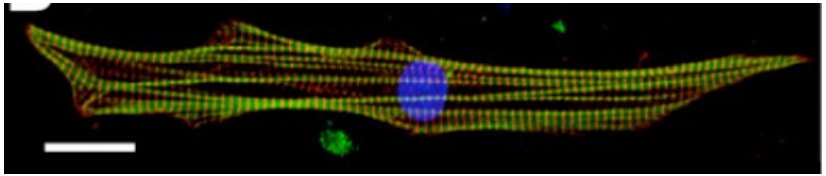
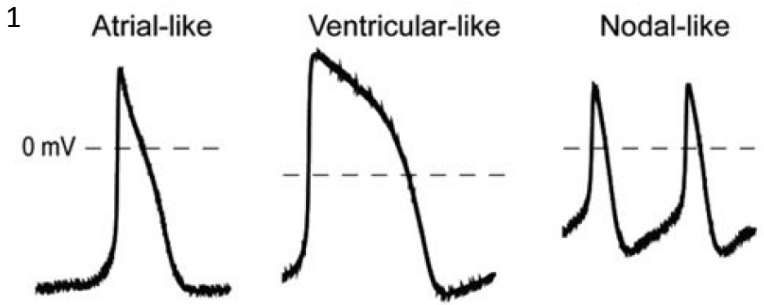
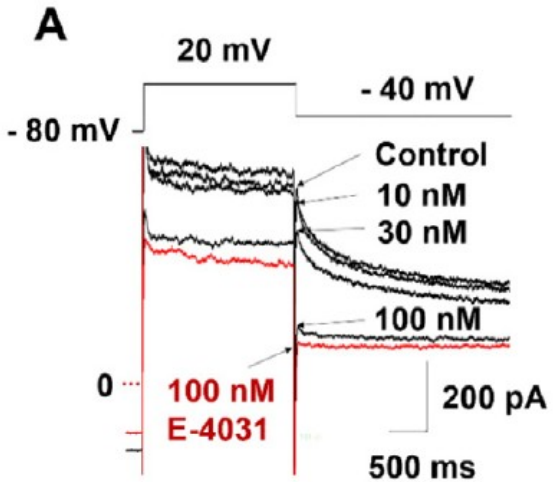
**Example:** Effect of antidepressant nefazodone on $I_{Kr}$

1. Measurement was performed on **Induced pluripotent stem cell-derived cardiac cells**






2. Inhibition of $I_{Kr}$ by nefazodone



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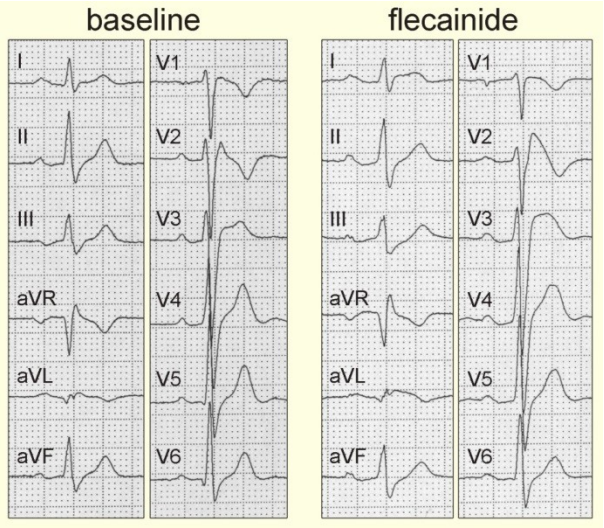
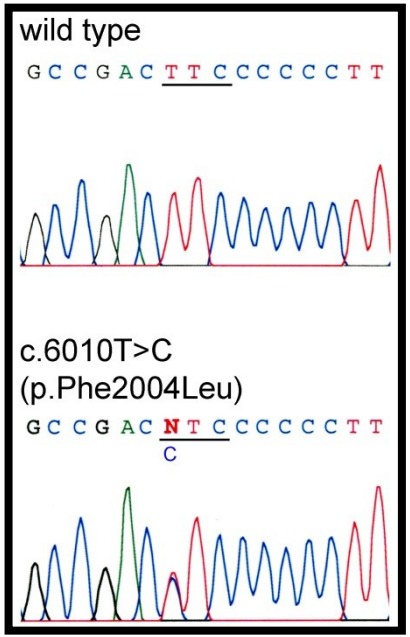
# **Example:** Brugada syndrome

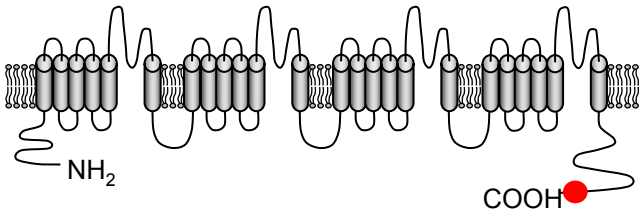



*clinical symptoms:* syncope

*ECG:* ST elevation

| | |
|---|---|
| P wave = 80 ms | (120 ms) |
| QRS complex = 135 ms | (100 ms) |
| QTc interval = 382 ms | (440 ms) |
| AH = 109 ms | (160 ms) |
| HV interval = 56 ms | (50 ms) |




F2004L

Missence mutation in C-terminus $I_{Na}$ channel

Adopted from Bébarová et al. , Am J Physiol Heart Circ Physiol 2008; 295:H48-H58

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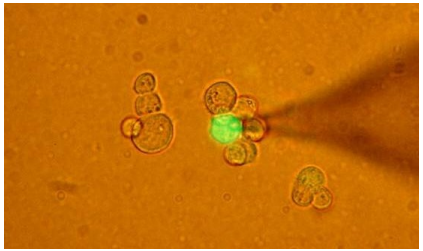
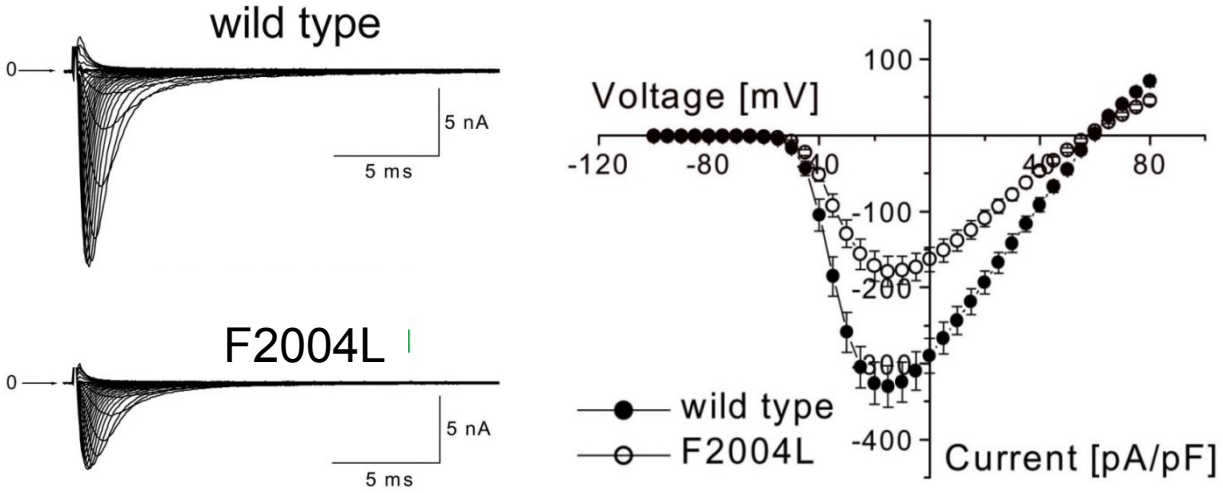
**Example:** Brugada syndrome

1. Measurement was performed on cell line transiently expressing human **wild-type** and **mutated (F2004L) $I_{Na}$ channel**



2. Inhibition of $I_{Na}$ in mutated channel

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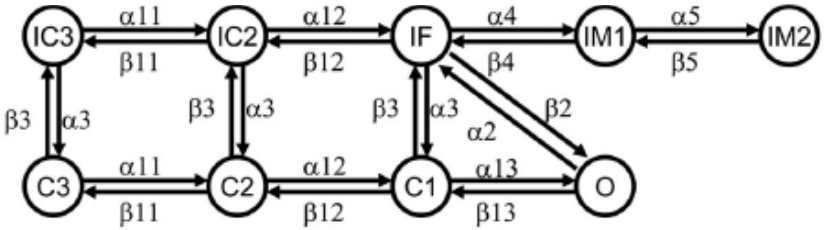
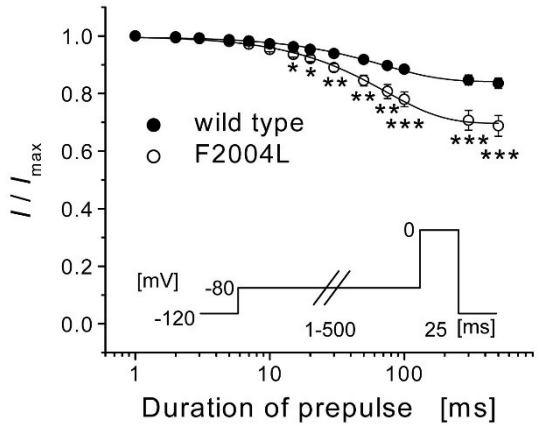
**Example:** Brugada syndrome

1. Kinetic scheme of $I_{Na}$ channel

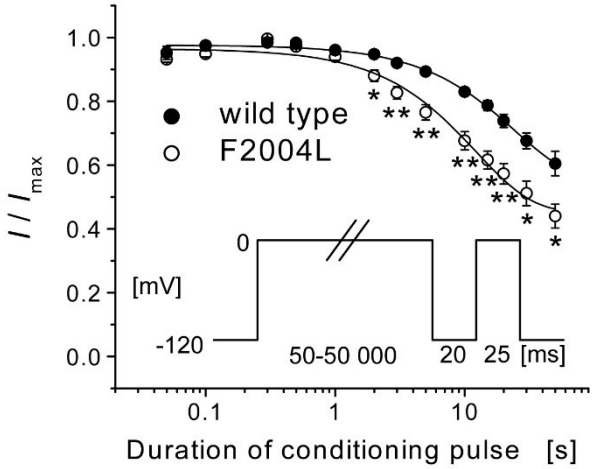



2.

Development of closed state inactivation




Development of slow inactivation




Adopted from Bébarová et al. , Am J Physiol Heart Circ Physiol 2008; 295:H48-H58

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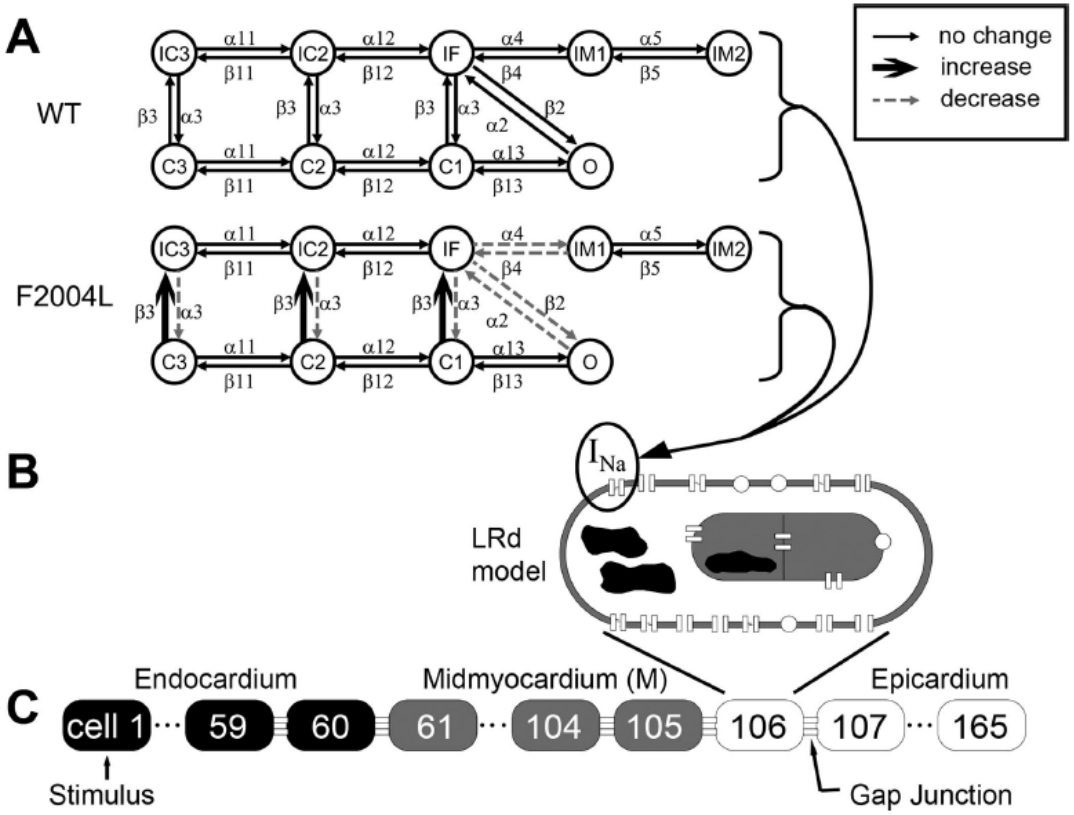
# **Example:** Brugada syndrome

Schematic model




Adopted from Bébarová et al. , Am J Physiol Heart Circ Physiol 2008; 295:H48-H58

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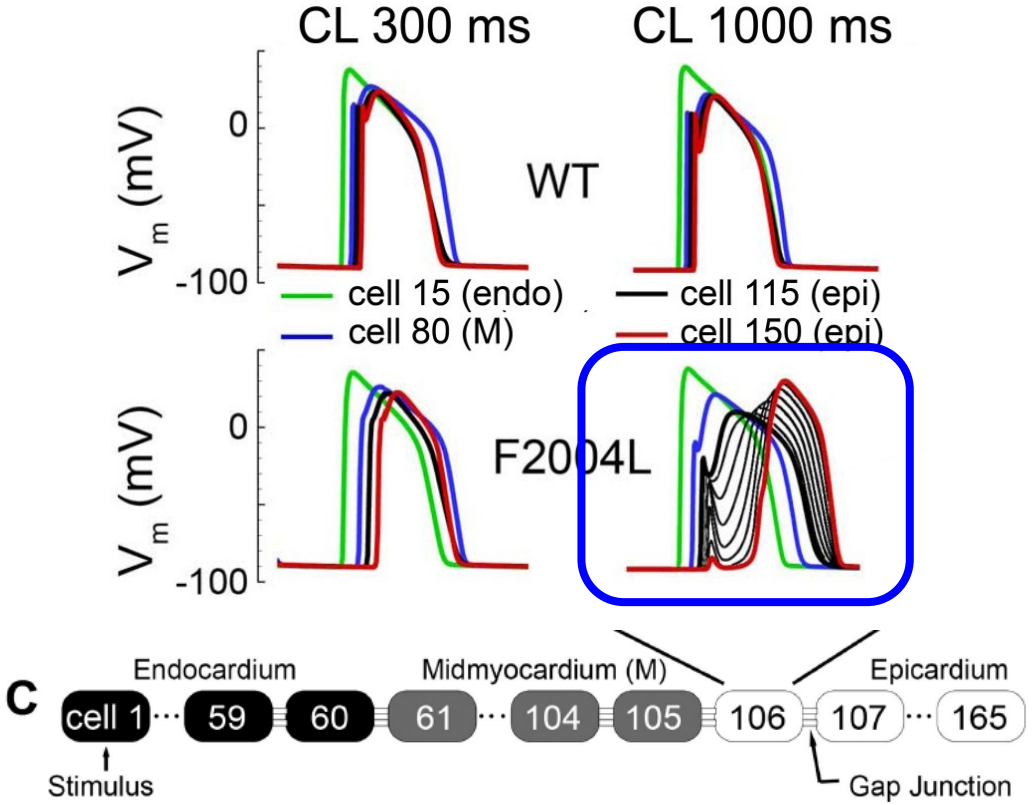
**Example:** Brugada syndrome

Schematic model




Adopted from Bébarová et al. , Am J Physiol Heart Circ Physiol 2008; 295:H48-H58

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