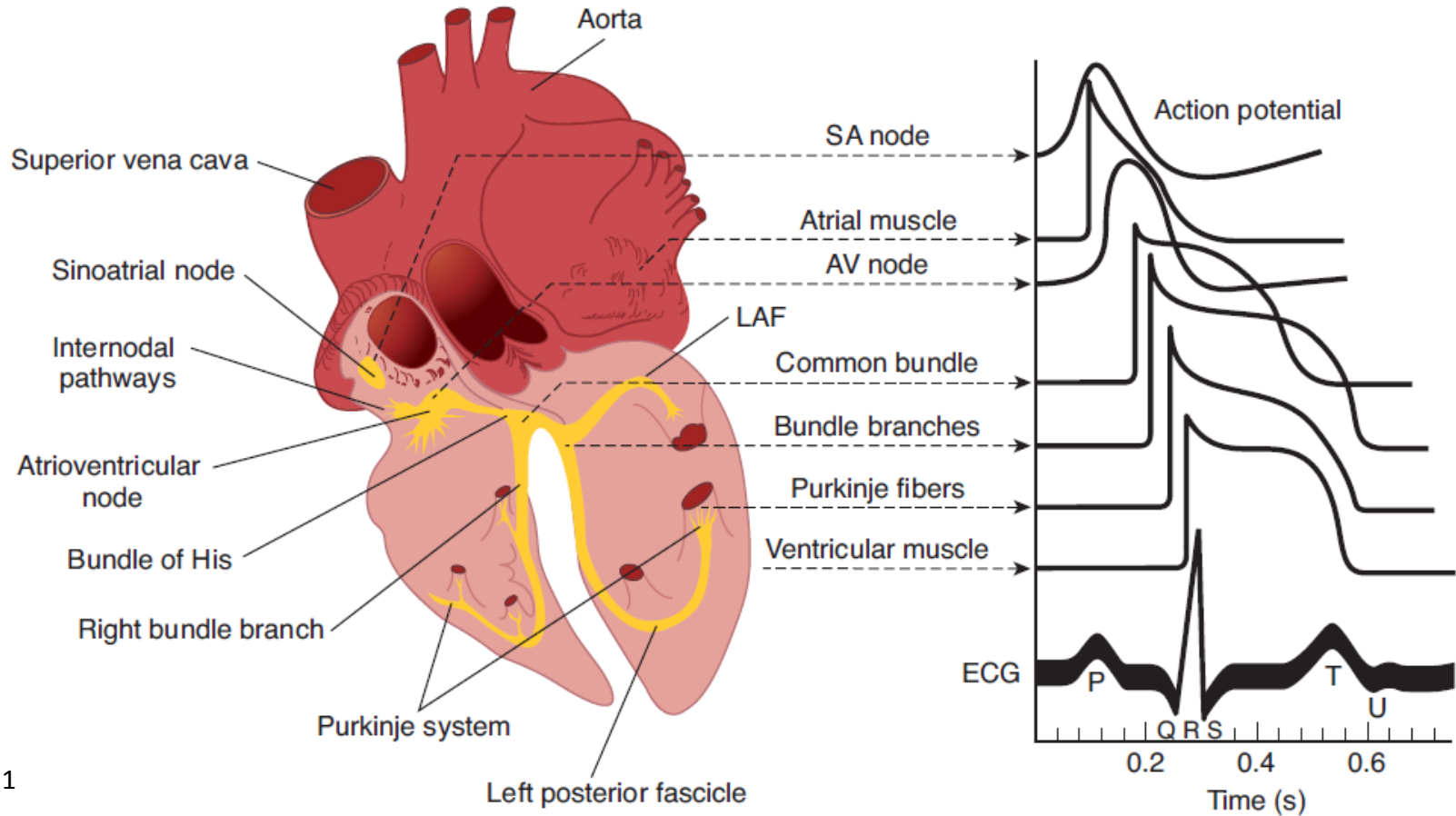


Cardiac action potential and underlying ionic currents

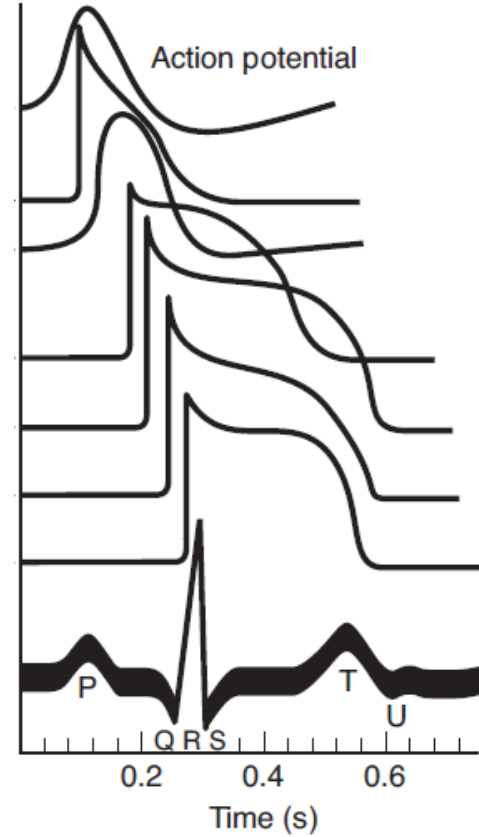
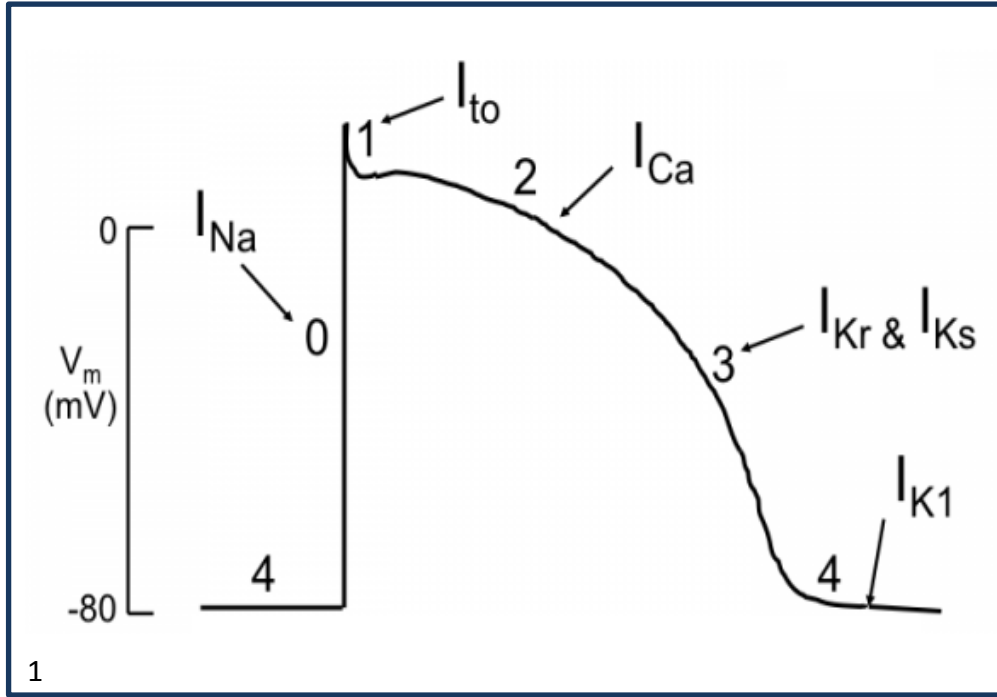
Methods, physiology and selected pathologies

Assoc. Prof. MUDr. Markéta Bébarová, Ph.D.

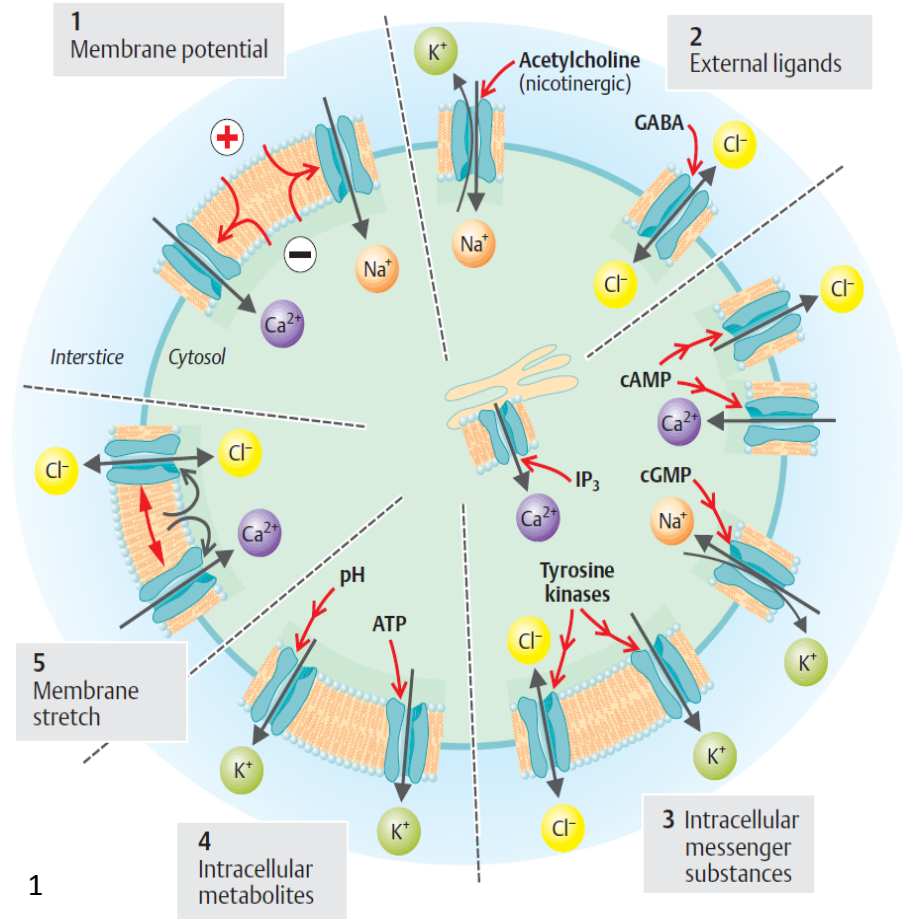
Department of Physiology, Faculty of Medicine, Masaryk University



1



Ionic Channels



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**

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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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*

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.

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*

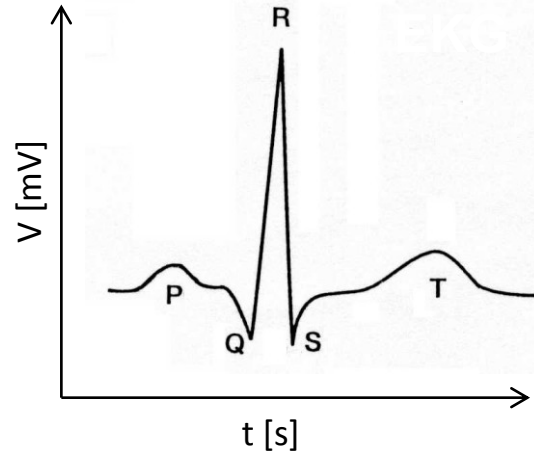
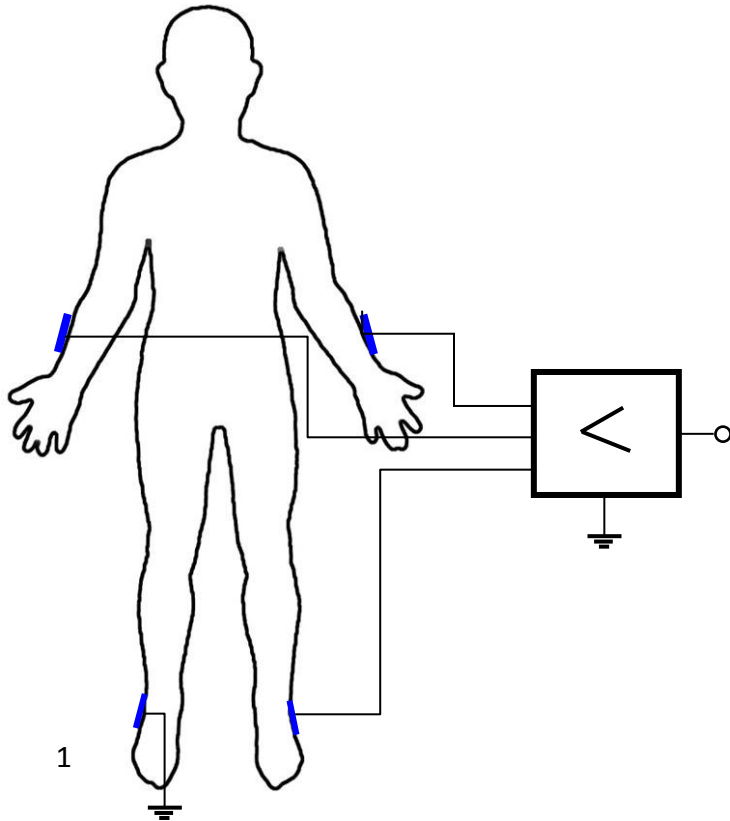
Basic principle of methods:

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

Basic principle of measurements on various levels of organism

Electrocardiography

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

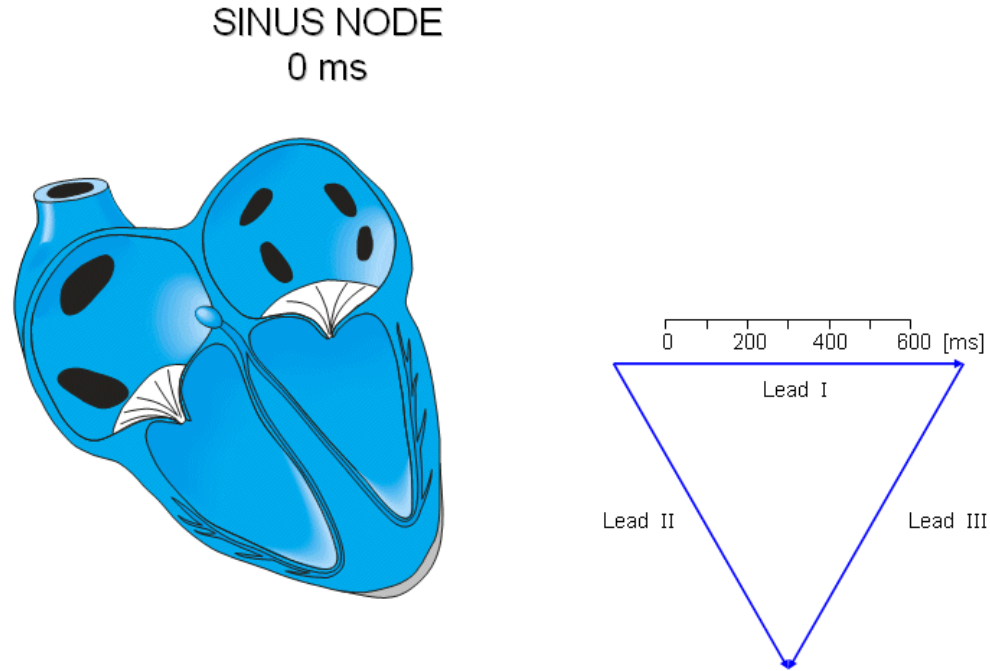


Electrocardiography

Mechanism of impulse creation:

Electrocardiography

Mechanism of impulse creation:



Intracardiac electrocardiography

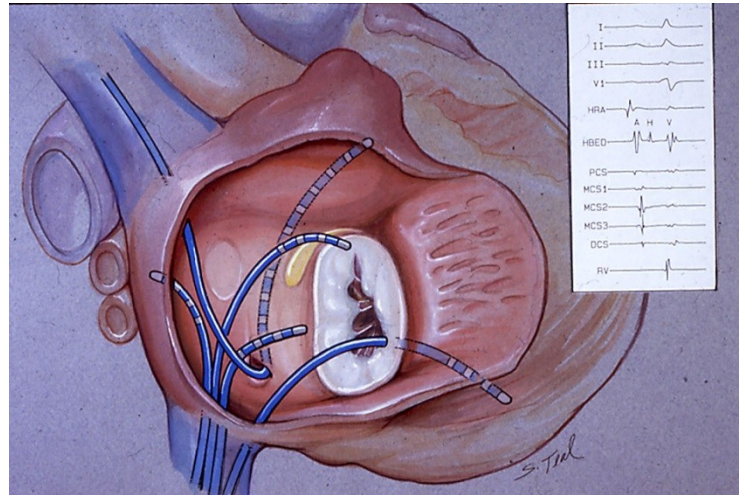
→ An invasive method, routinely used in clinical practice

→ **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

Intracardiac electrocardiography

We can measure:

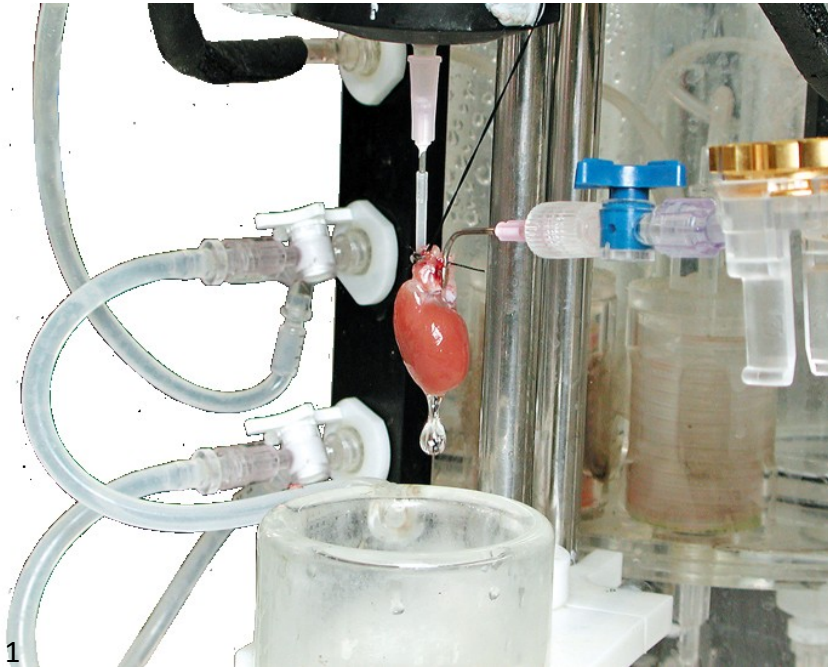
- Function of sinus node
- Conduction through the atrial wall
- Conduction in atrioventricular node
- Conduction through Hiss bundle
- Conduction through Purkinje fibers



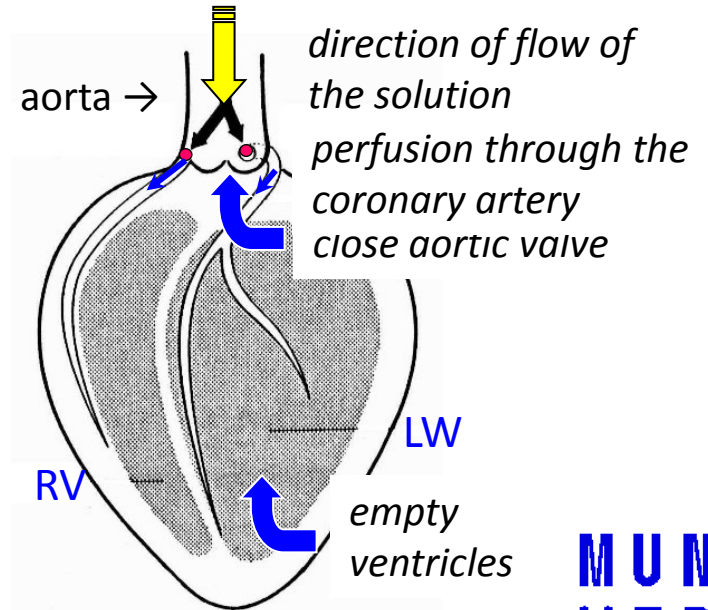
His bundle
electrogram

Measurement on the isolated heart

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



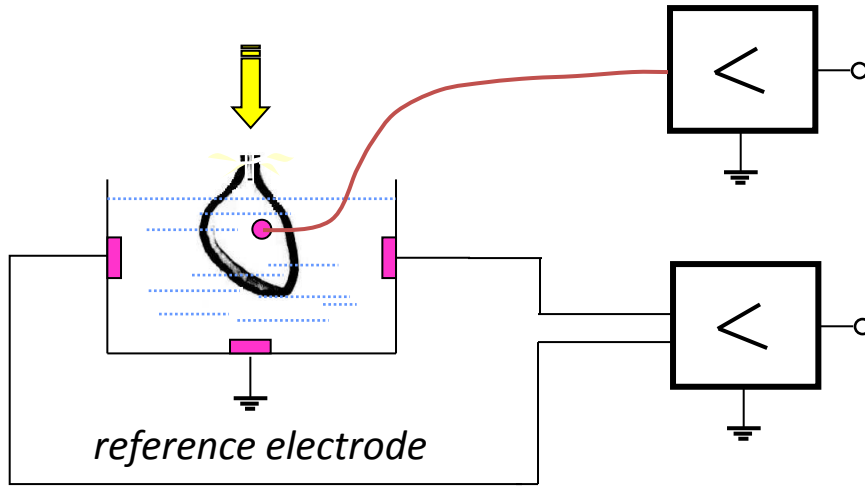
Principle of the retrograde perfusion



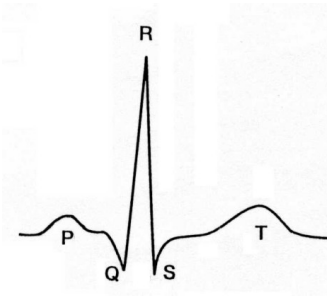
Measurement on the isolated heart

Measurement is performed by:

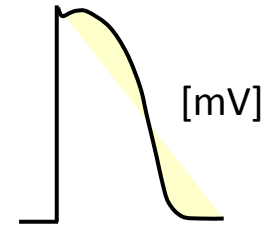
1. electrodes embedded in walls of the bath



2. Epicardial suction electrode



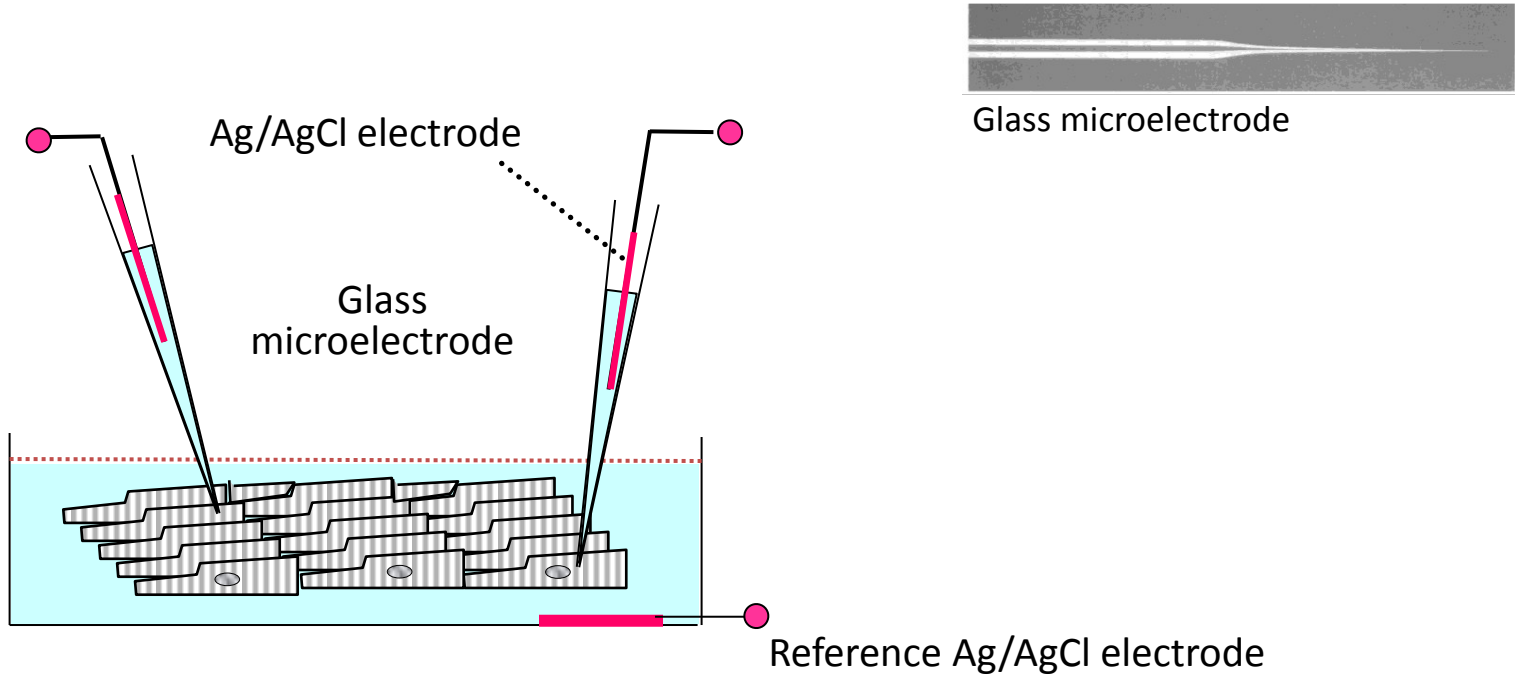
Electrogram



Monophasic action potential

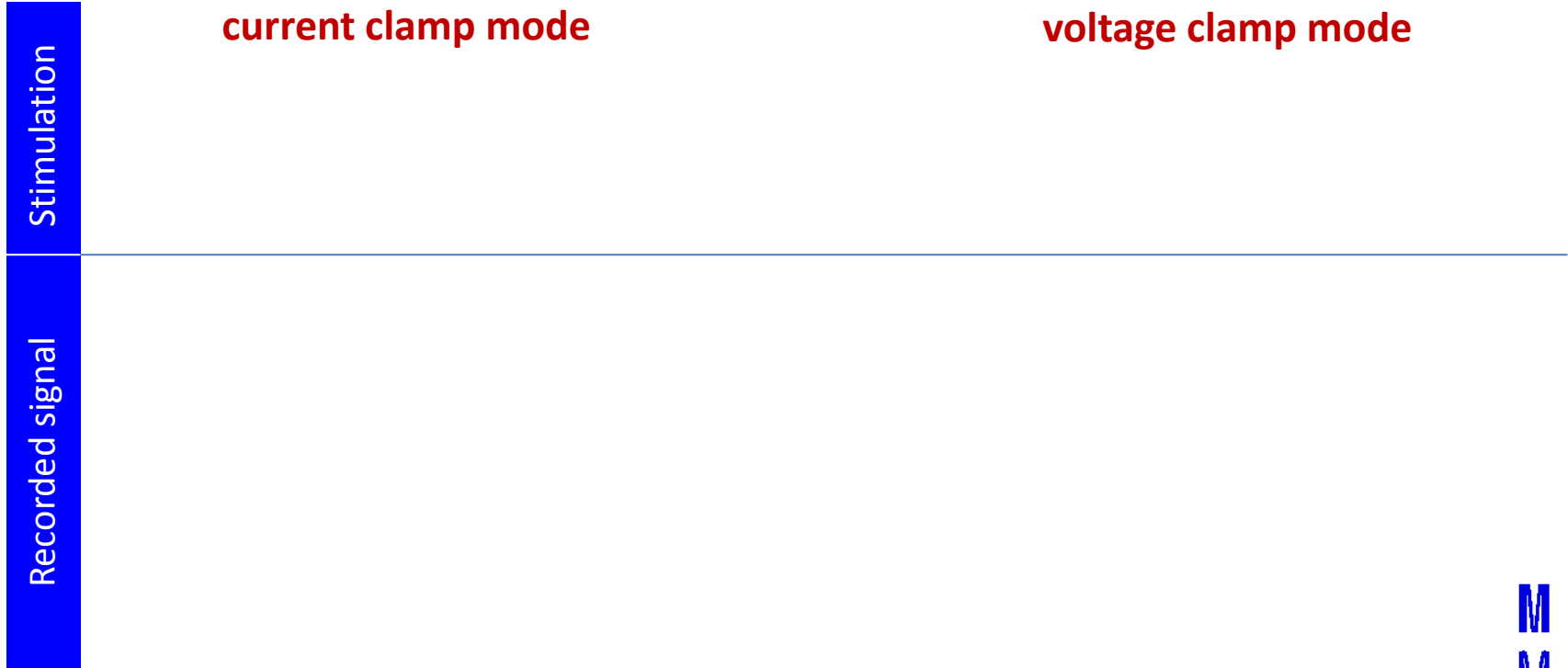
Measurement on multicellular cardiac samples

Set-up



Measurement on multicellular cardiac samples

- A **stimulation of the sample** is needed for the measurement
- It could be performed in two modes:



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, functionally undamaged cells responding to electrical stimulation by:
 - **Contraction**
 - **Characteristic electrical activity** (action potential and membrane currents)

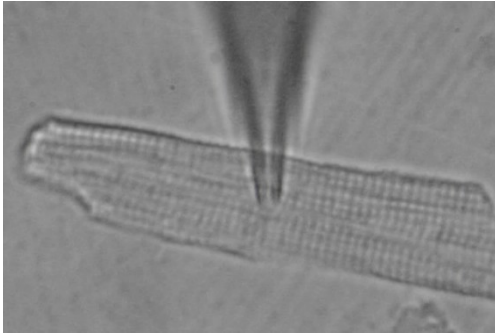
Patch-clamp

Enzymatic isolation of cardiac cells:

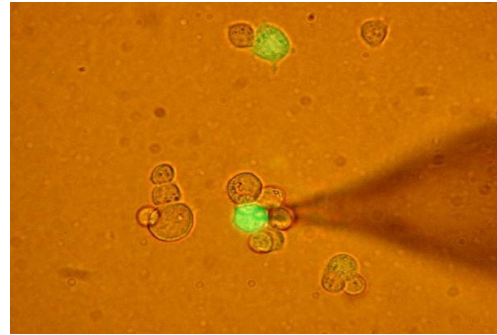
Possibilities of using patch-clamp

Measurements could be performed on:

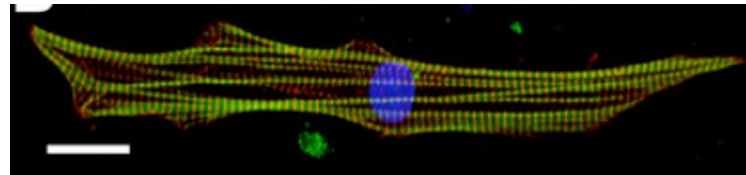
1. Isolated cardiac cells



2. Cell lines transiently expressing human ionic channels



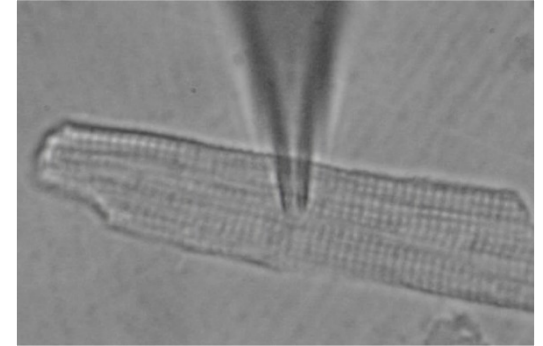
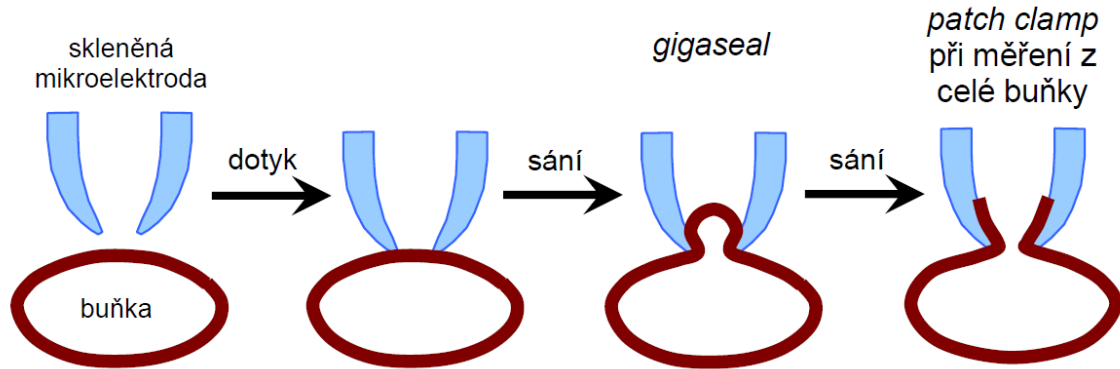
3. Induced pluripotent stem cell-derived cardiac cells (*iPSC-CM*)



Patch-clamp

Whole cell patch-clamp

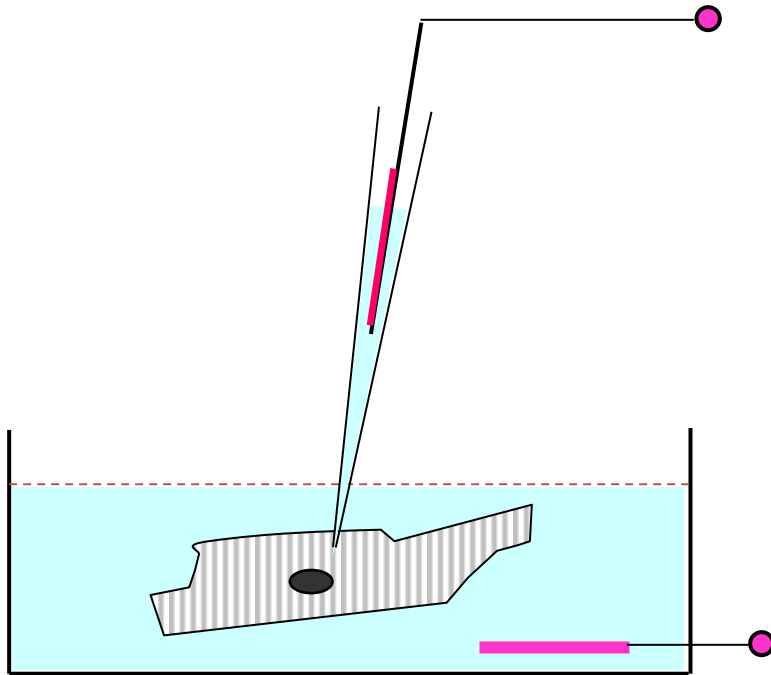
1. Establish a contact with a cell



Patch-clamp

Whole cell patch-clamp

2. Measurement on a single cell

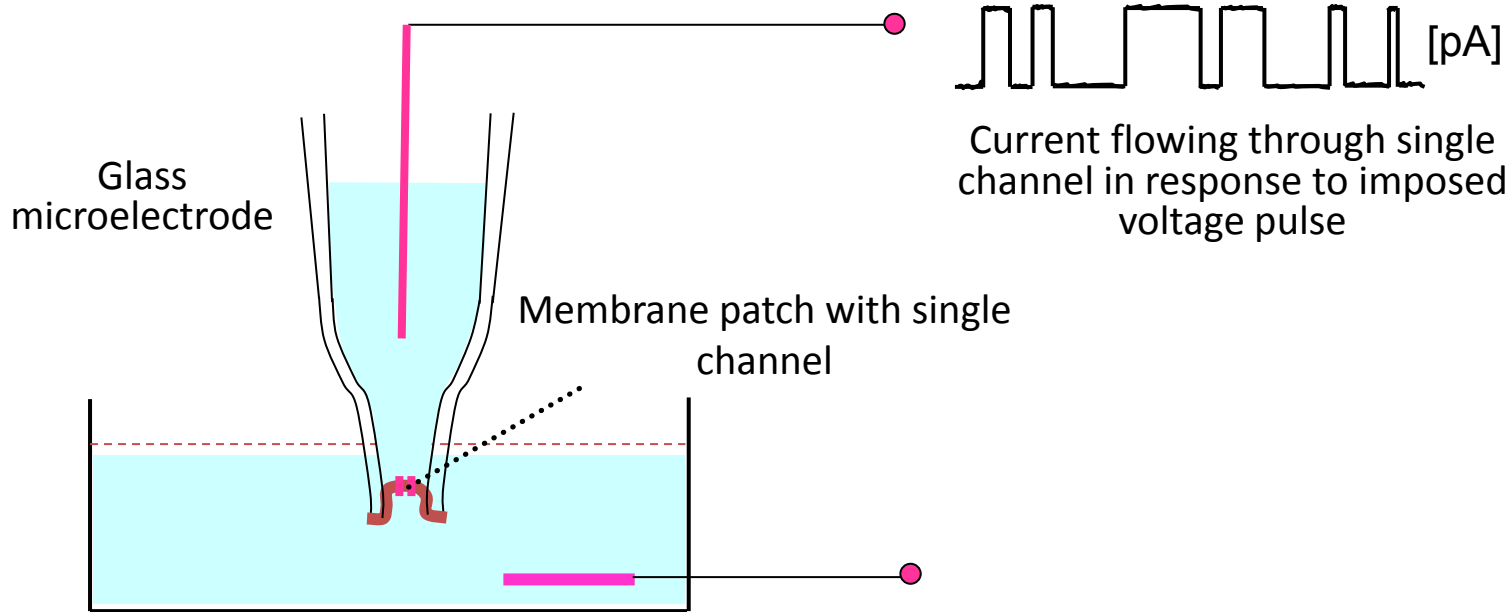


Two mode of measurement:

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

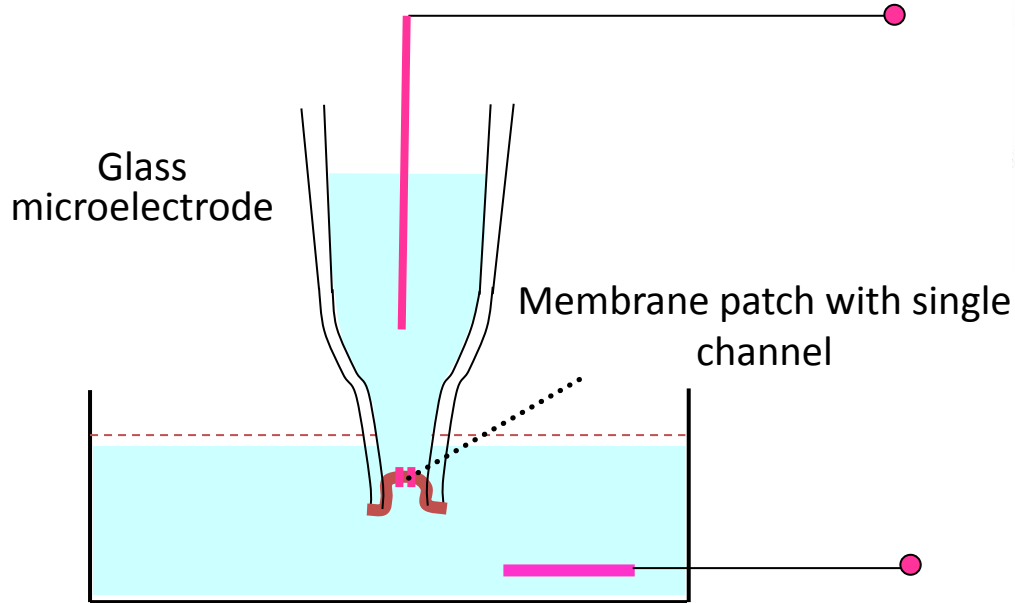
Patch-clamp

Single channel patch-clamp



Patch-clamp

Single channel patch-clamp



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

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 antidepressant nefazodone on I_{Kr}*

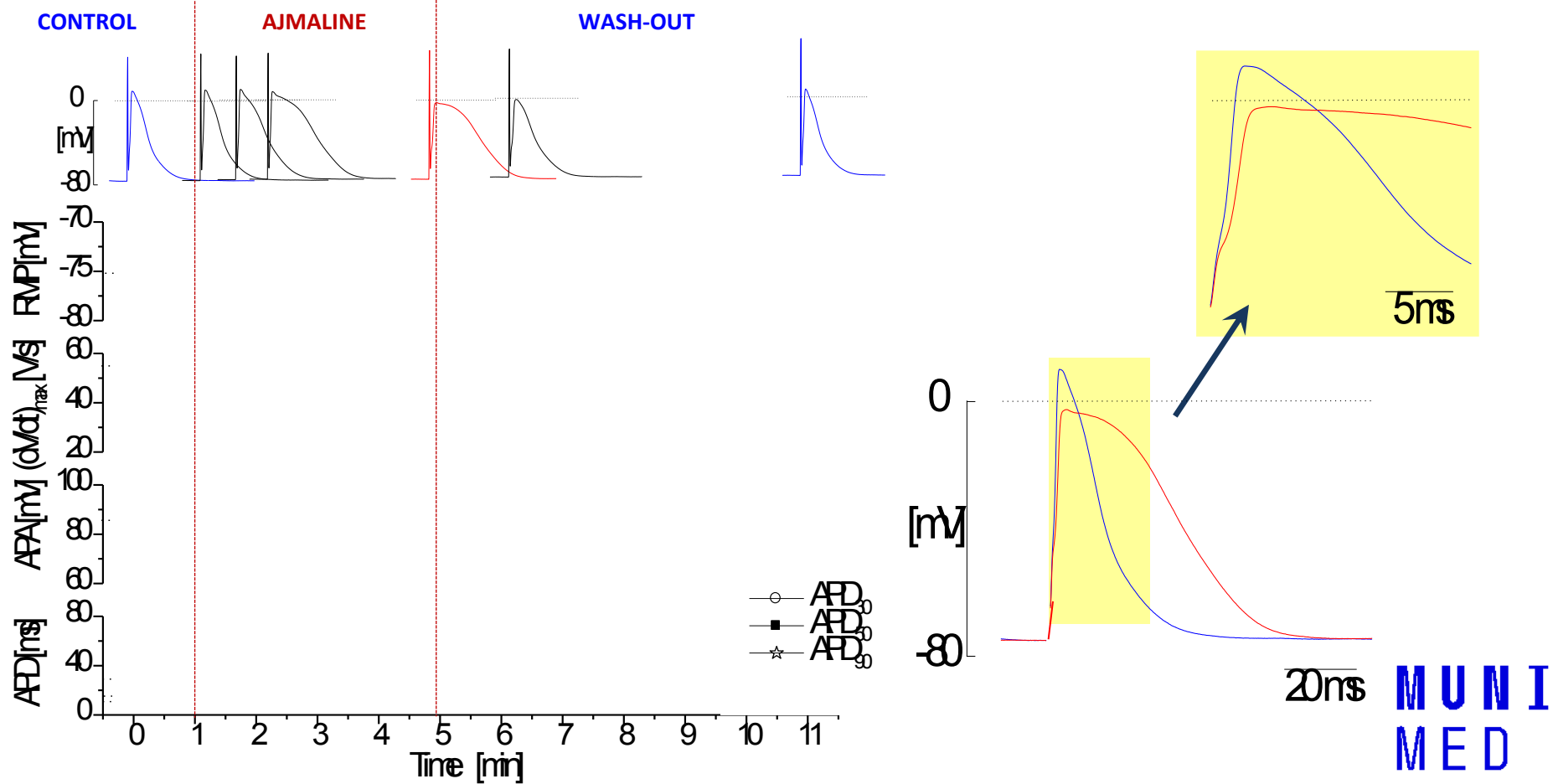
3. Impact of a mutation on drug effects on a channel

4. Ionic channel dysfunction caused by a mutation

Example: *Analysis of arrhythmogenic syndromes:*

- *Long QT syndrome*
- *Brugada syndrome*

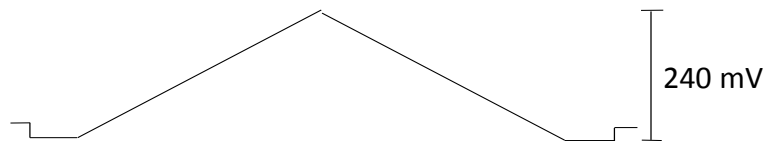
Example: Effect of antiarrhythmic drug ajmaline on action potential



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

Měření $I_{K(ATP)}$

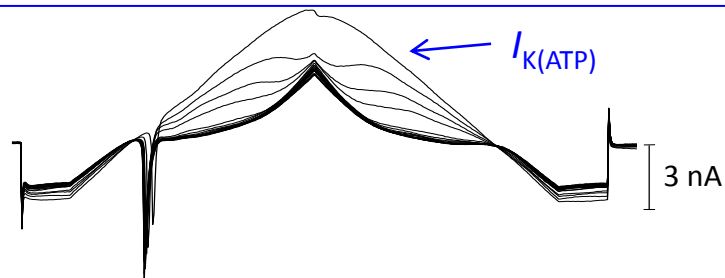
Voltage clamp impulse



Recorded current

Control

After DNP application

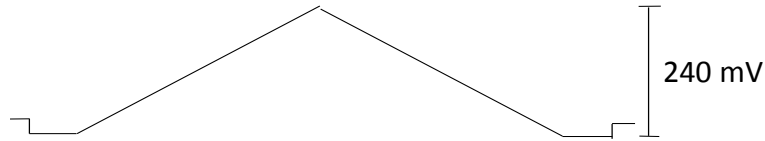


50 ms

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

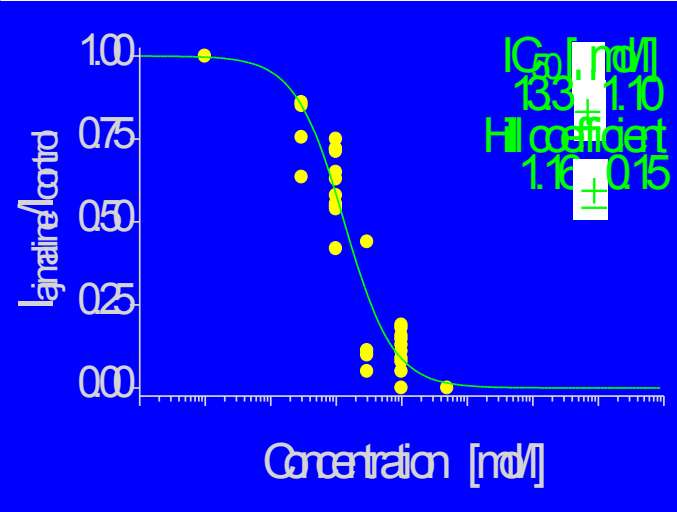
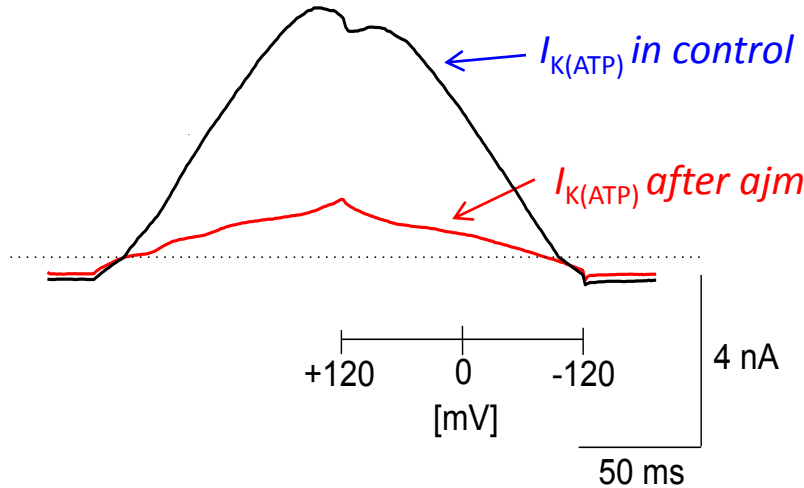
Measurement of $I_{K(ATP)}$

Voltage clamp impulse

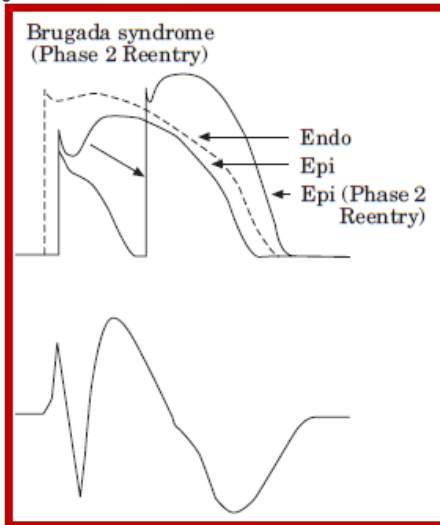
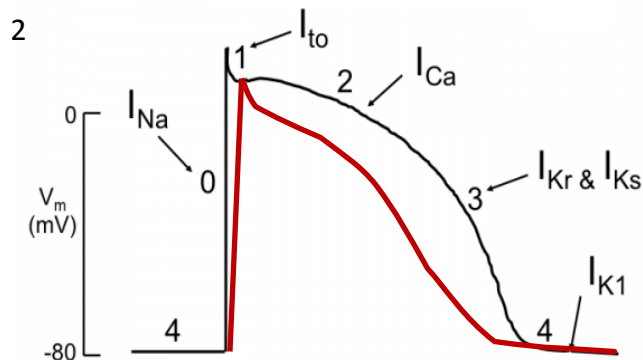
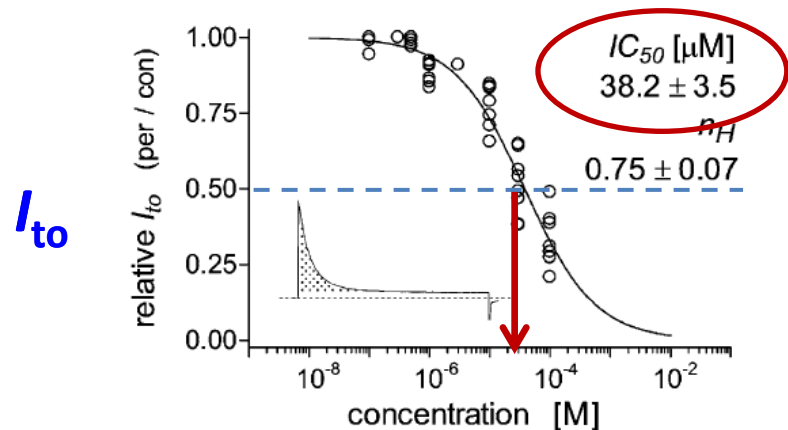
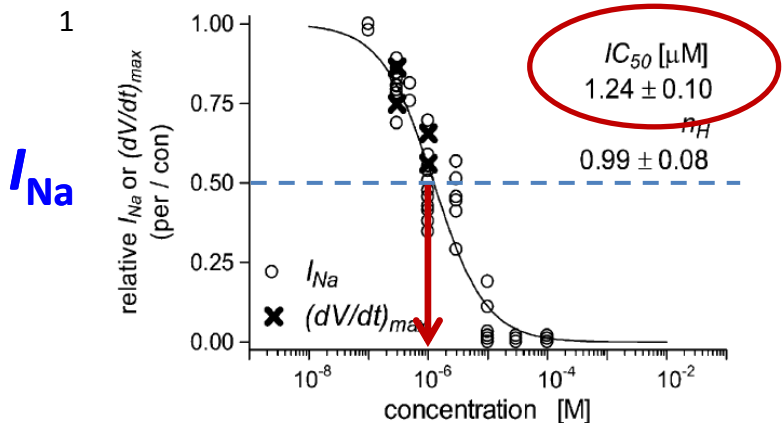


Recorded current

Changes after ajmaline application

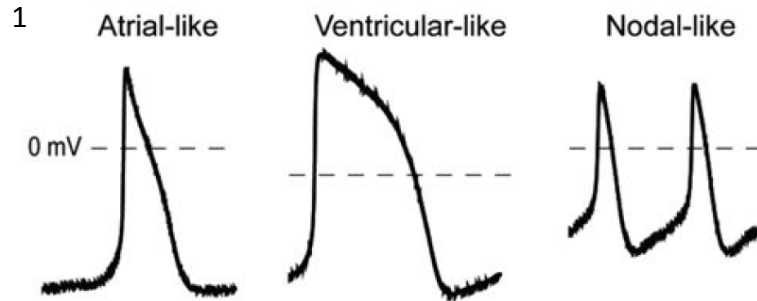
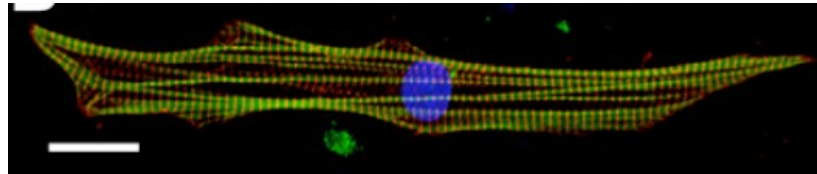


Example: Effect of antipsychotic drug perphenazine on I_{Na} and I_{to}

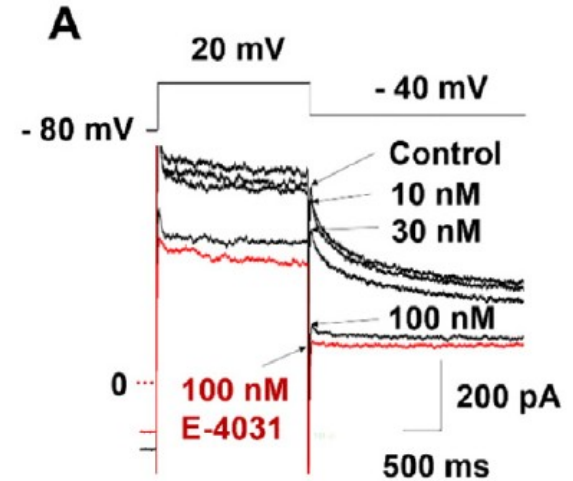


Example: Effect of antidepressant nefazodone on I_{Kr}

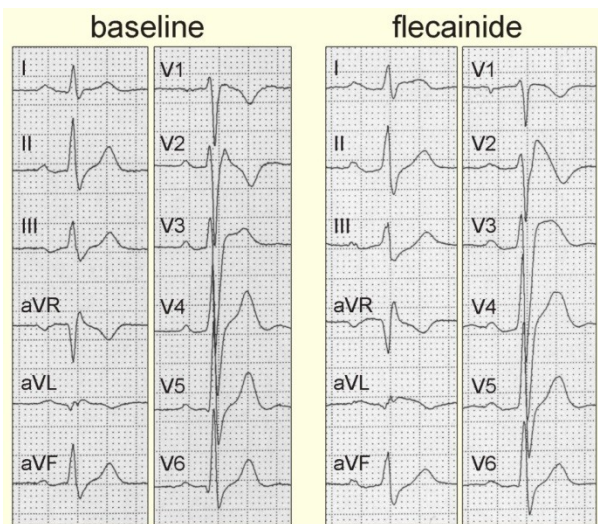
1. Measurement was performed on **Induced pluripotent stem cell-derived cardiac cells**



2. Inhibition of I_{Kr} by nefazodone



Example: Brugada syndrome



clinical symptoms: syncope

ECG: ST elevation

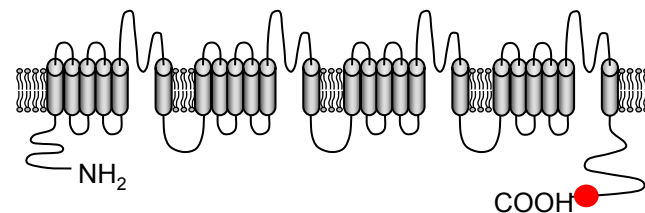
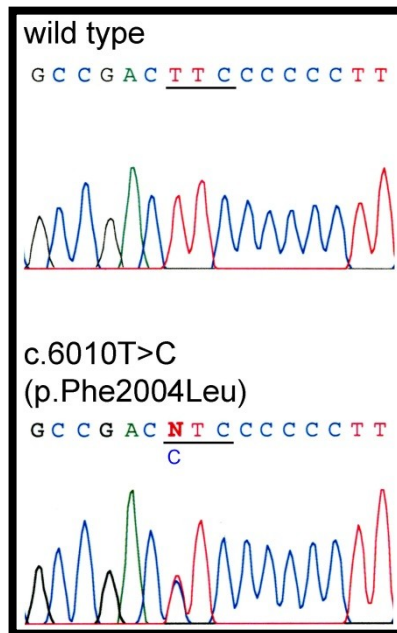
P wave = 80 ms (120 ms)

QRS complex = 135 ms (100 ms)

QTc interval = 382 ms (440 ms)

AH = 109 ms (160 ms)

HV interval = 56 ms (50 ms)

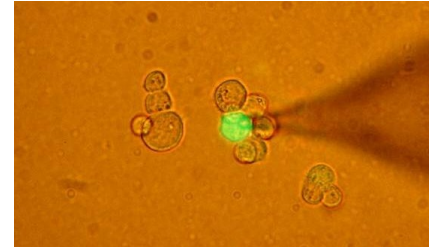


F2004L

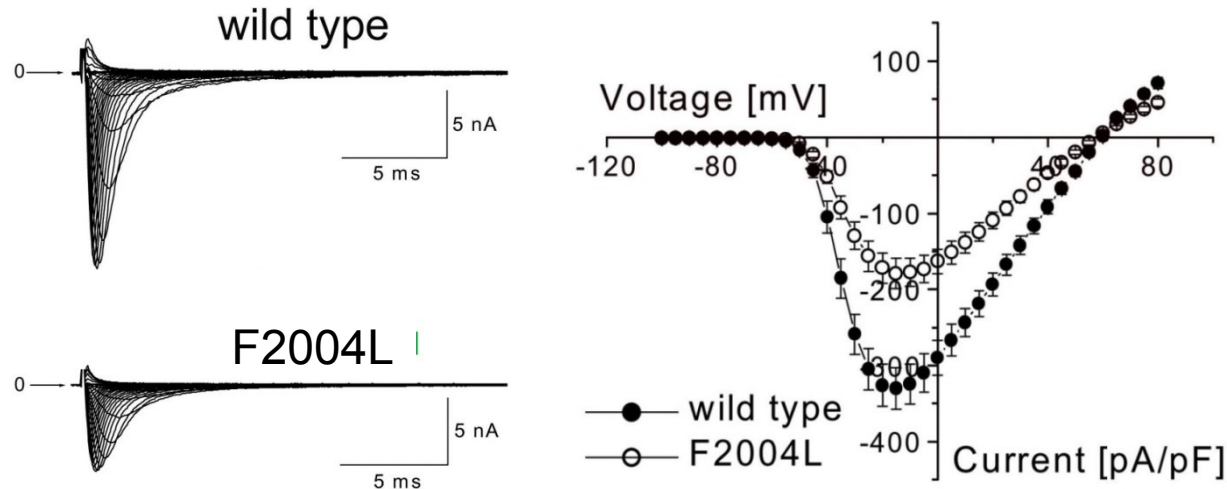
Missense mutation in C-terminus I_{Na} channel

Example: Brugada syndrome

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

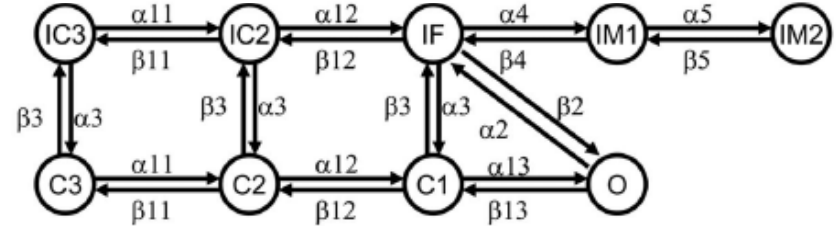


2. Inhibition of I_{Na} in mutated channel



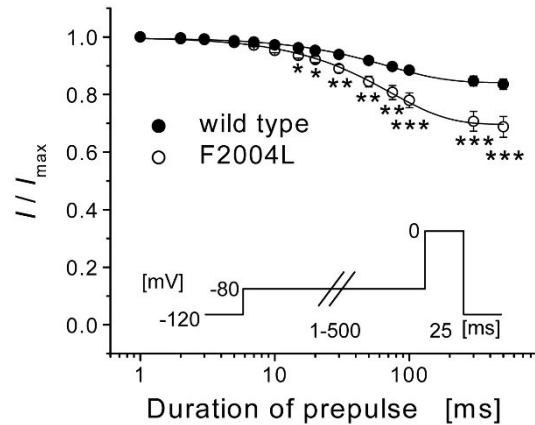
Example: Brugada syndrome

1. Kinetic scheme of I_{Na} channel

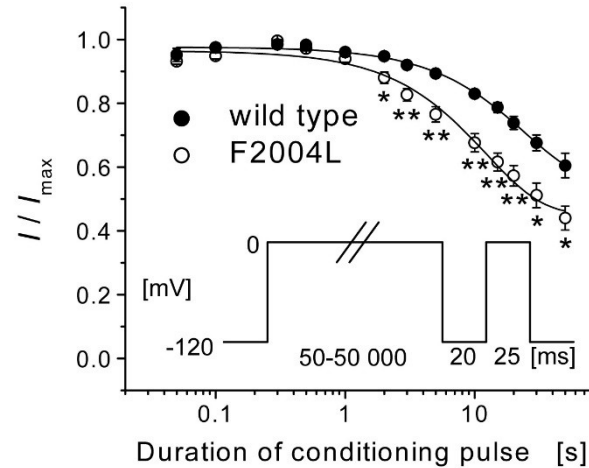


2.

Development of closed state inactivation

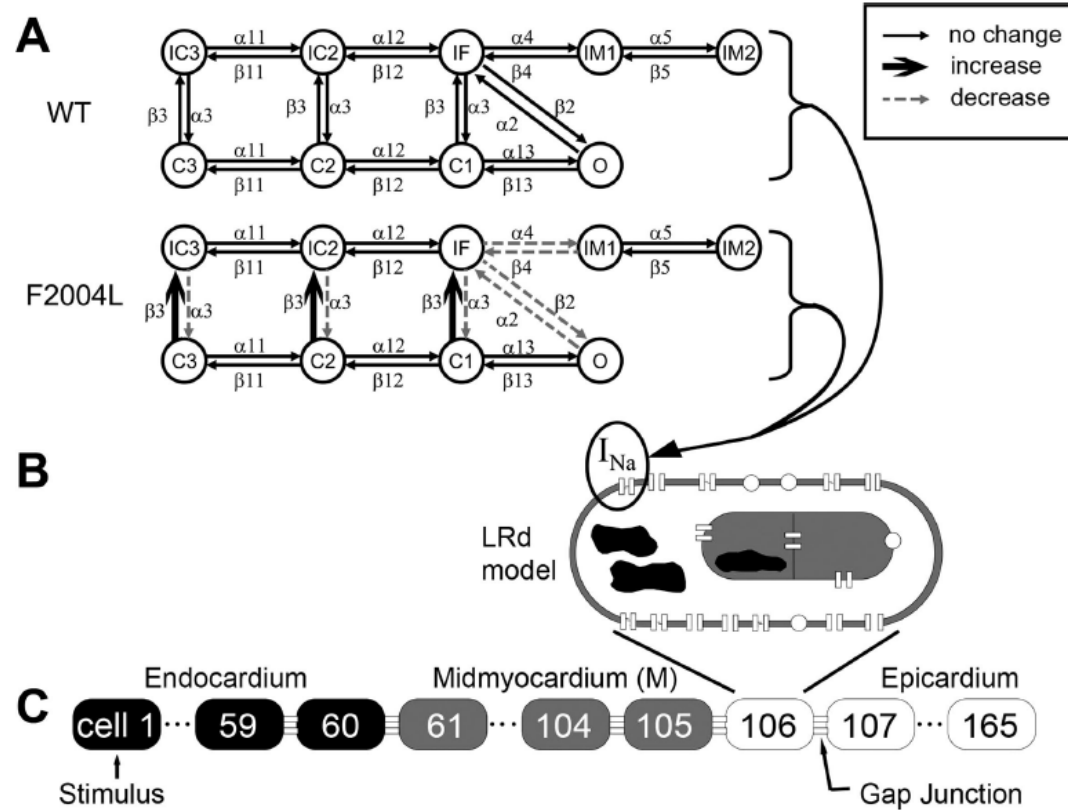


Development of slow inactivation



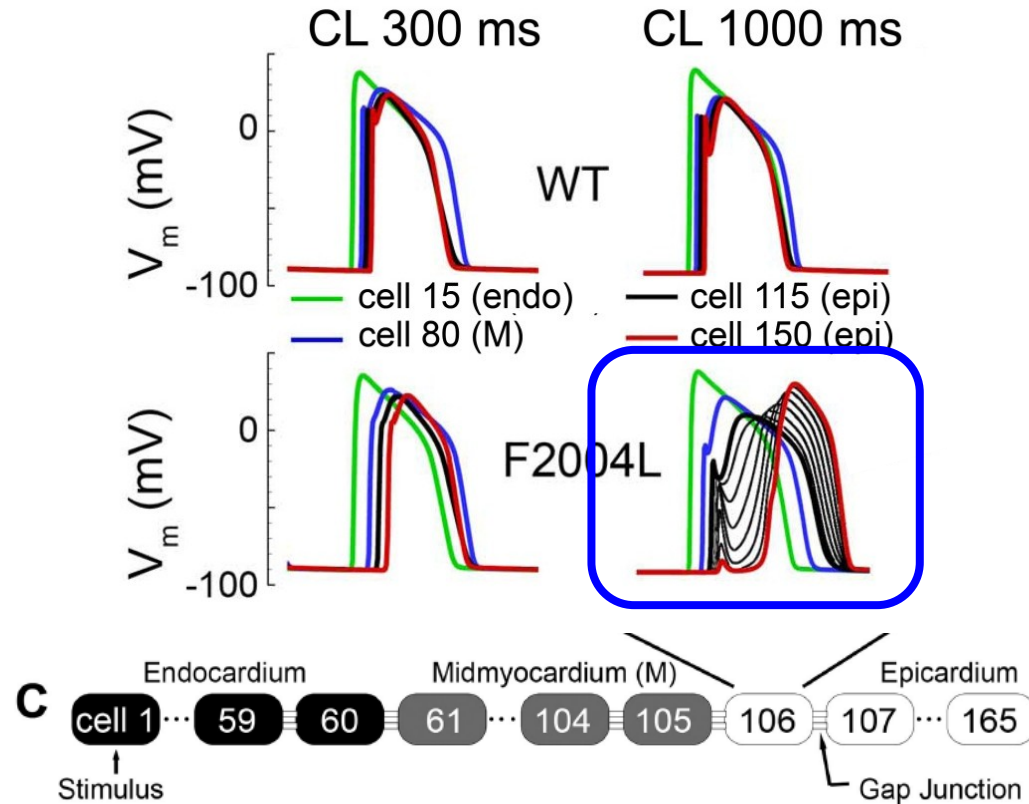
Example: Brugada syndrome

Schematic model



Example: Brugada syndrome

Schematic model





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