THE PATCH-CLAMP TECHNIQUE

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WHAT IS A PATCH- CLAMP ?

Applications:

To investigate wide range of electropysiological cell properties on single cell

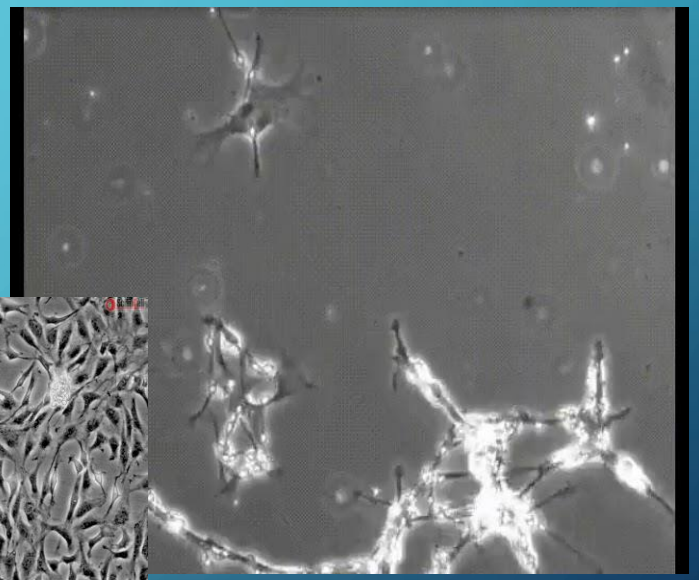
Such us:

- Current clamp, cell membrane activity and properties (Aps, Cm, Resting membrane potential…)
- Voltage clamp studies on ionic channels (activation, inactivation, voltage dependance, pharmacological blocking….)

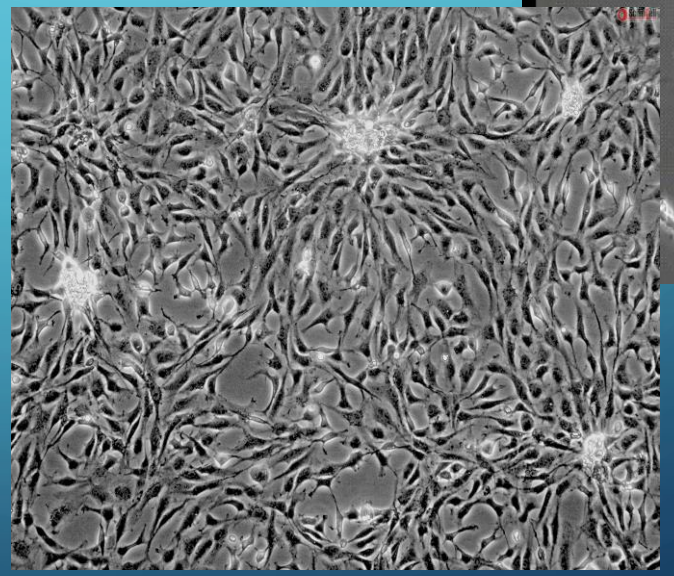
WHAT IS A PATCH- CLAMP ?

Applications:

- Neurons
- Cardiomyocytes
- Muscle fibres
- Pancreatic betta cells
- Kindey cells



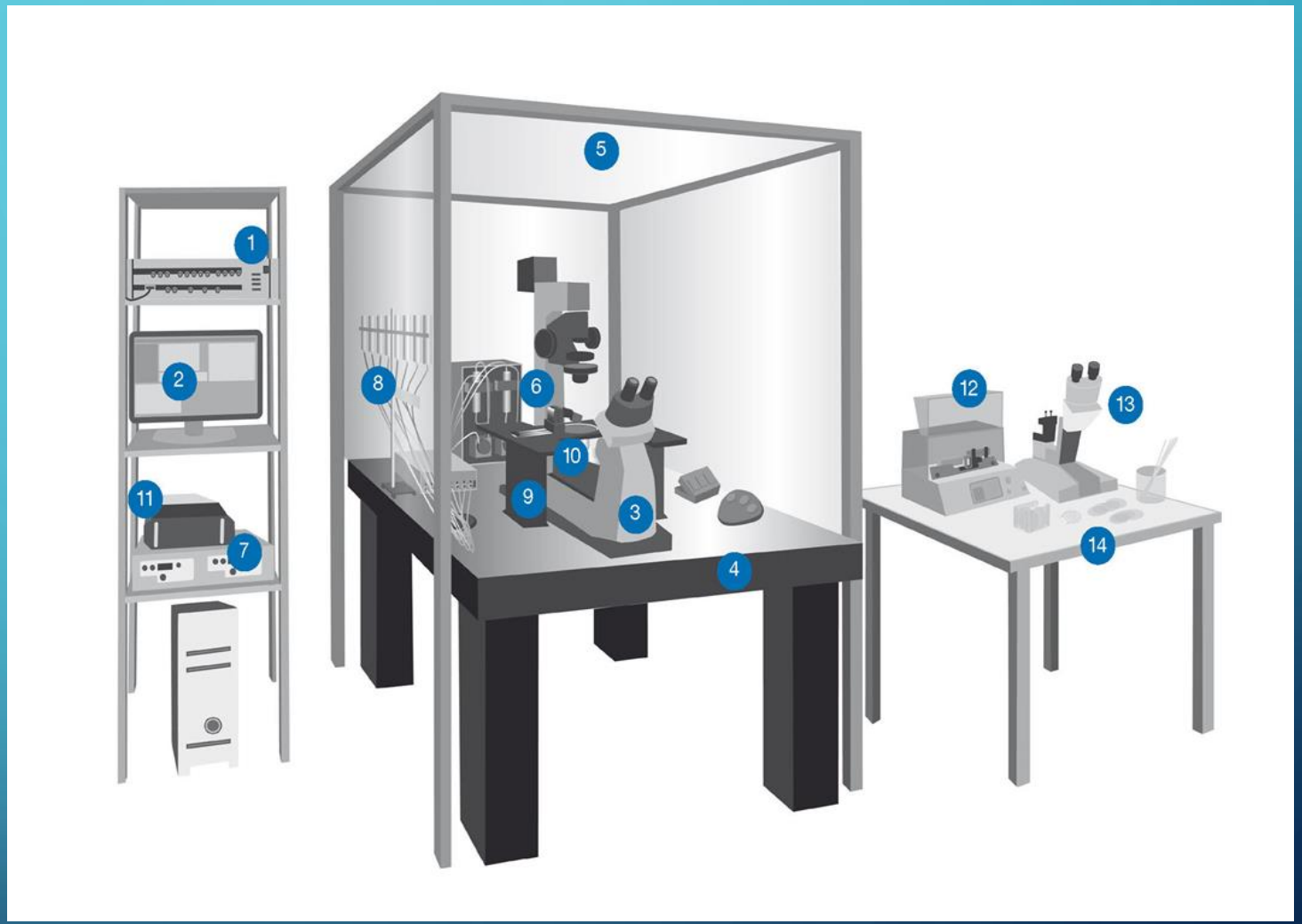
Human Neuron cells



Human Cardiac Myocytes

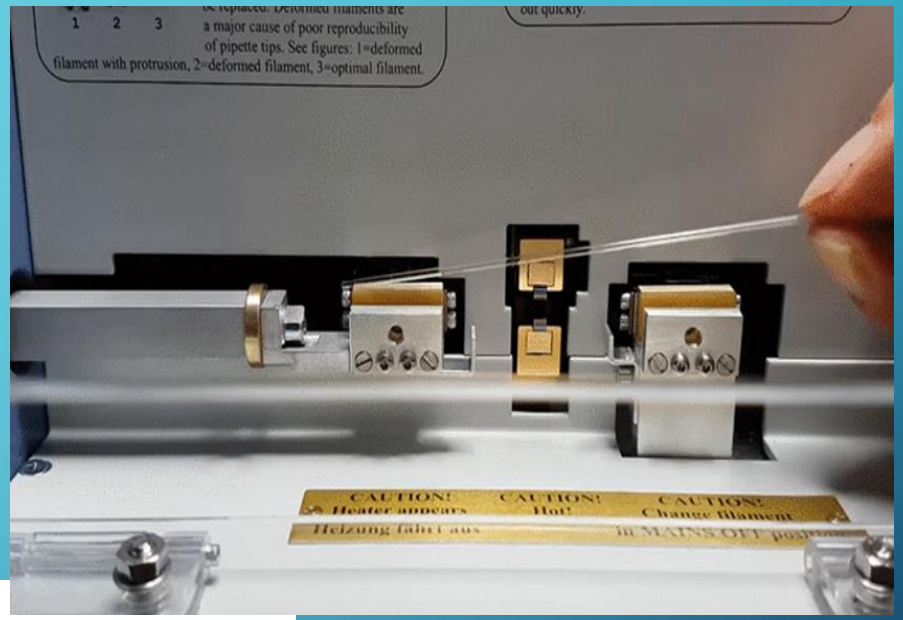
SET-UP

1. Amplifier
2. Data Acquisition Software
3. Microscope
4. Table
5. Faraday cage
6. Imaging/Recording chamber
7. Temperature control
8. Perfusion and Fluid control
9. Stage
10. Micromanipulator
11. Stimulus generator
12. Puller
13. Microforge-grinding
14. Capillary



The Puller – How to make your perfect tip ?

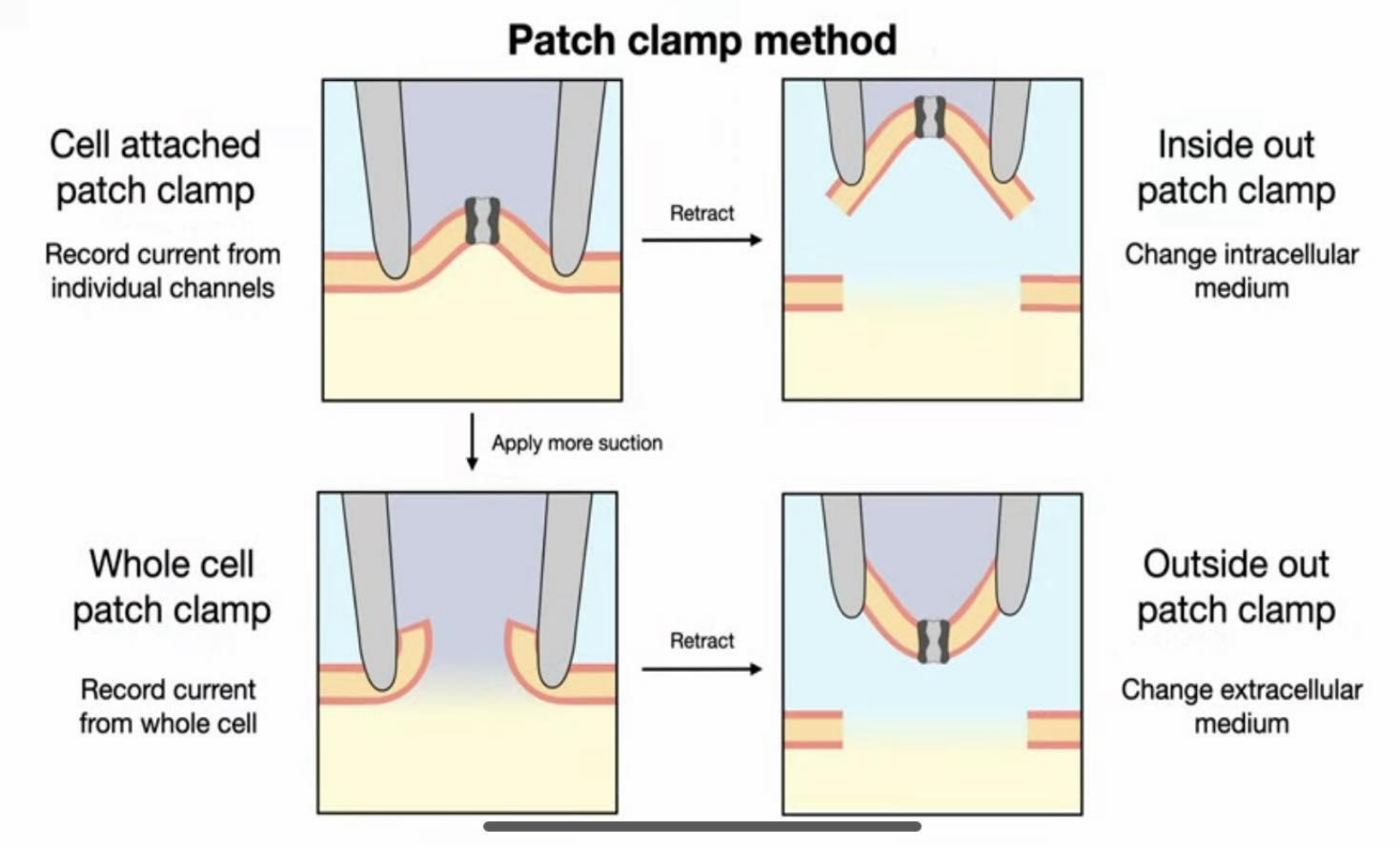
Allows to create the right pipette which is essential for successful recordings and reliable data

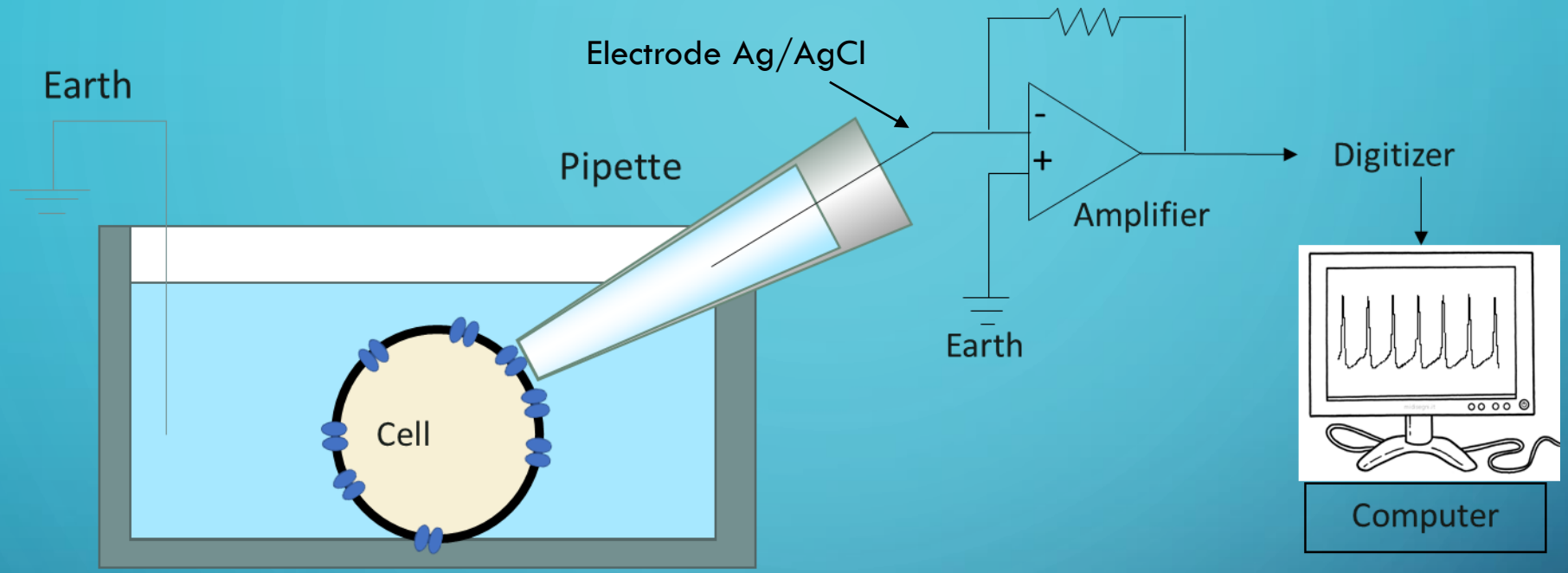


Parameters:

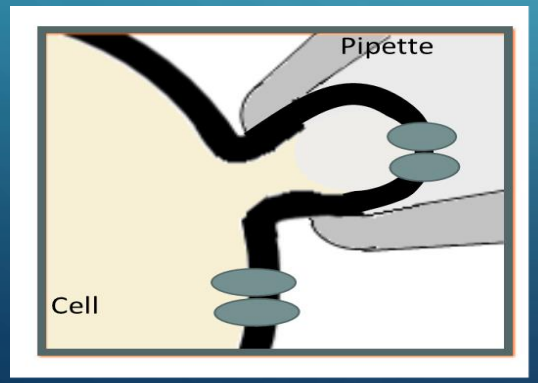
- Temperature
- Velocity
- Applied force
- Filament size

PATCH-CLAMP CONFIGURATIONS





Giga seal

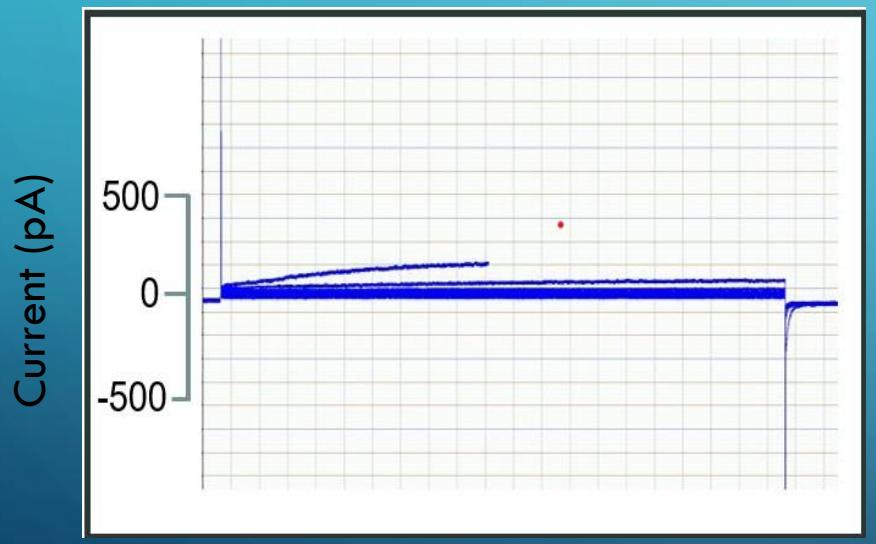


Cell-attached

WHAT CAN WE REGISTER ?

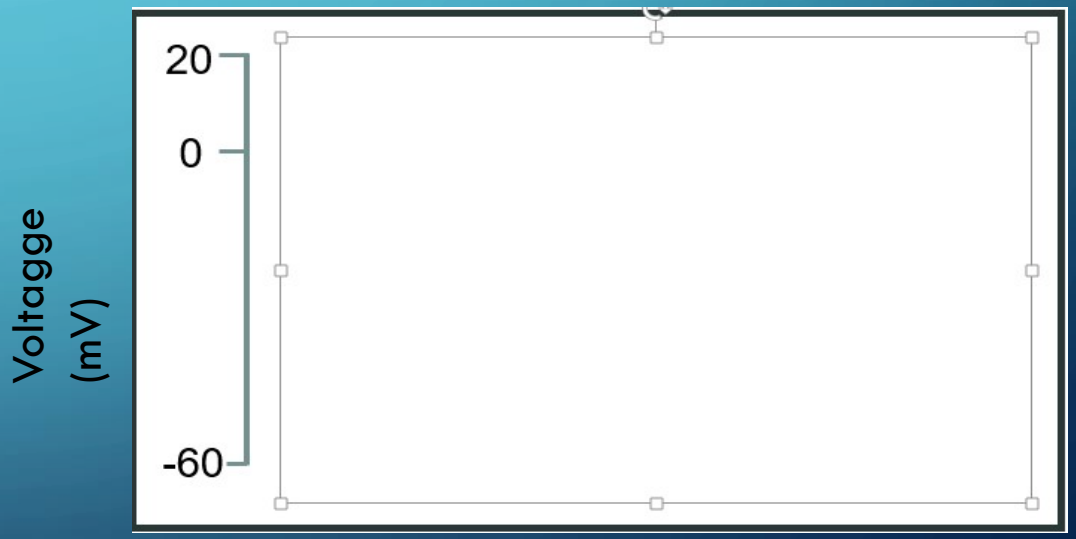
VOLTAGE CLAMP

- impose a membrane voltage
- register the evoked current



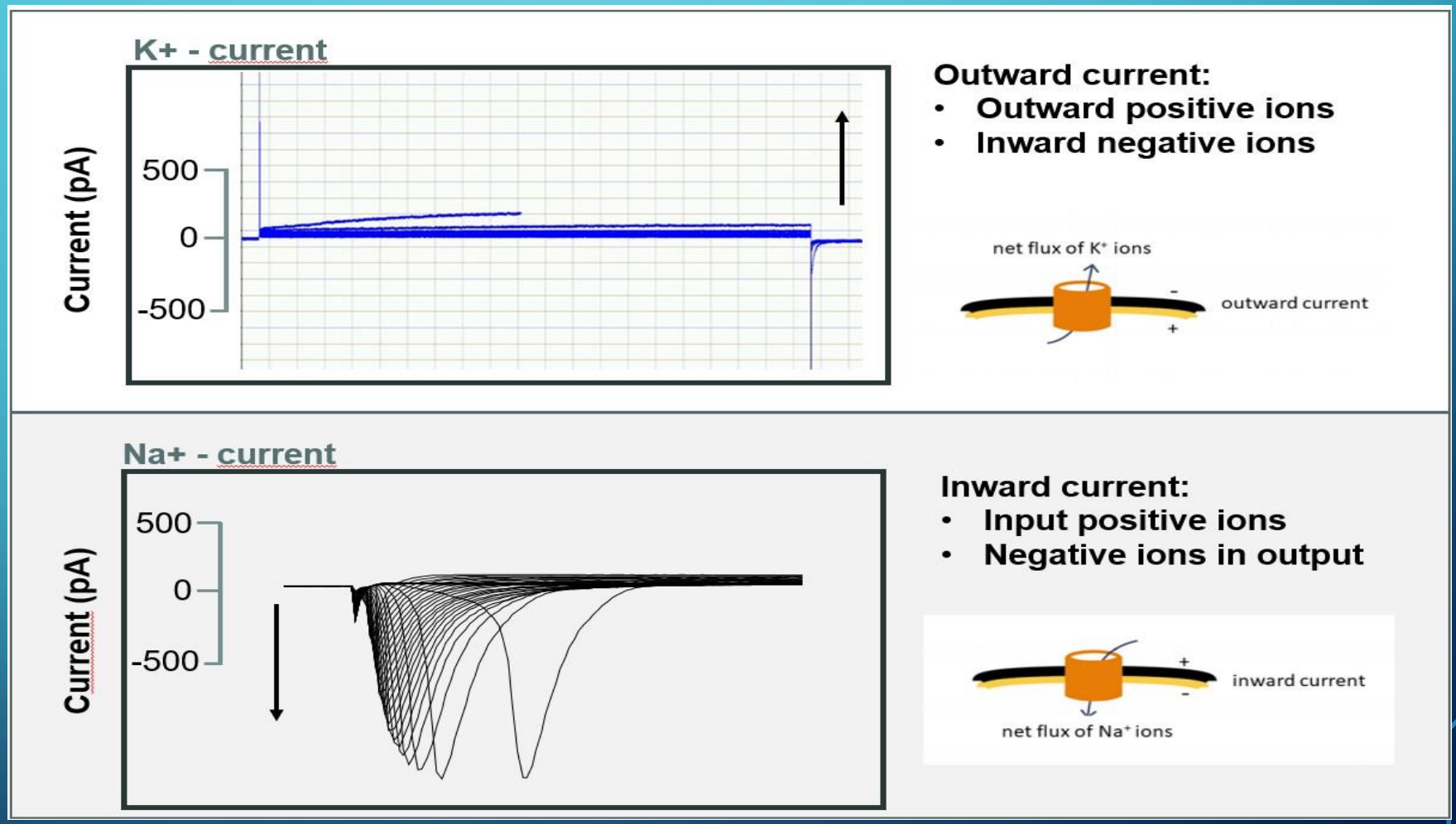
CURRENT CLAMP

- impose a current
- register the membrane potential



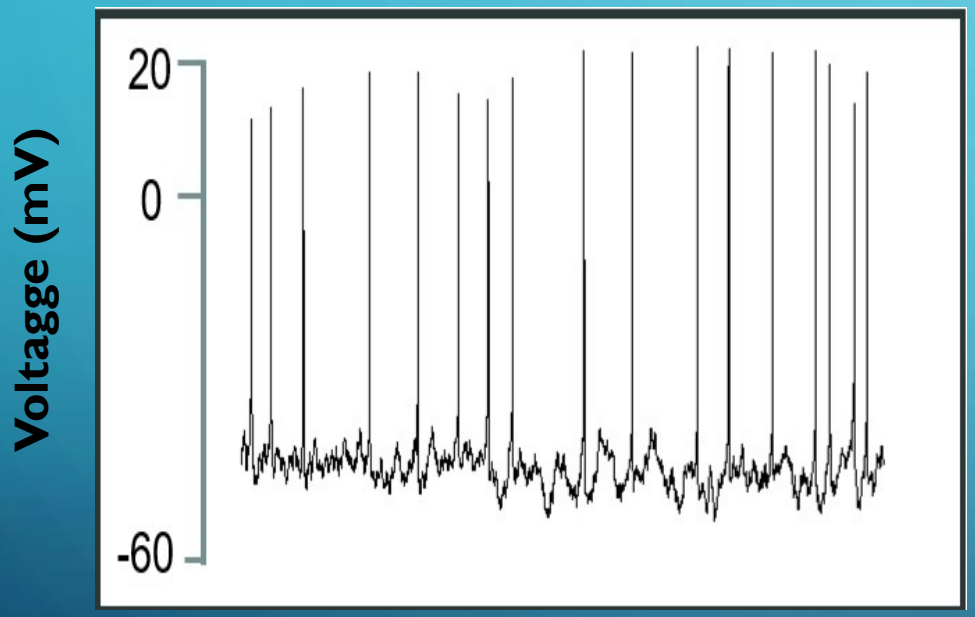
VOLTAGE CLAMP - WHAT CURRENT ARE WE RECORDING?

- Direction (inward-outward)
- Kinetics
- Voltage dependence

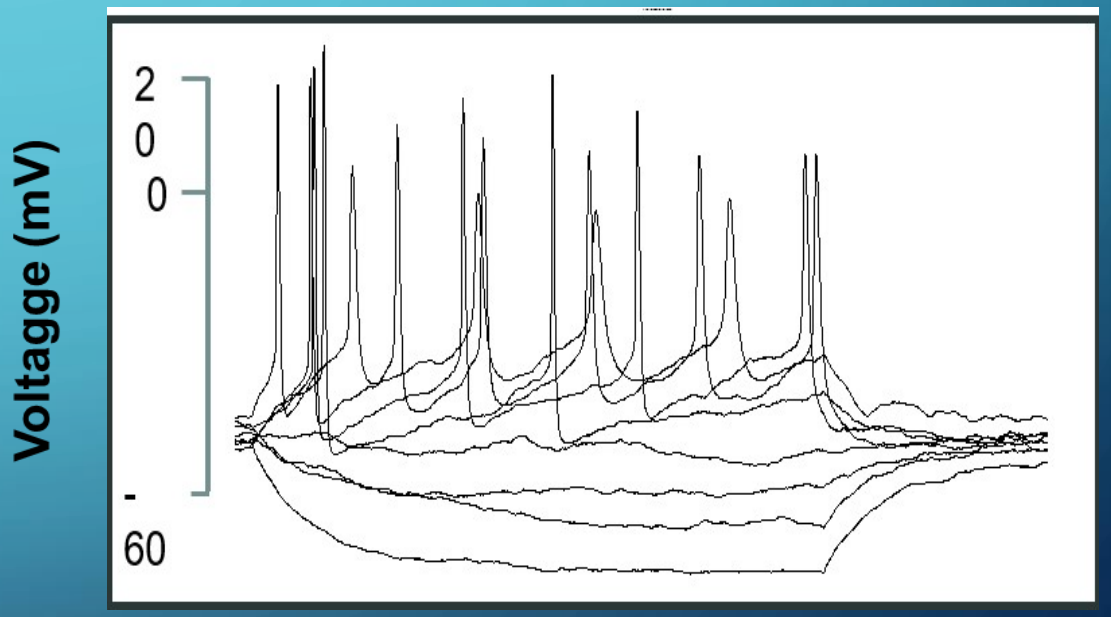


CURRENT CLAMP

- Impose a current
- Register the membrane potential



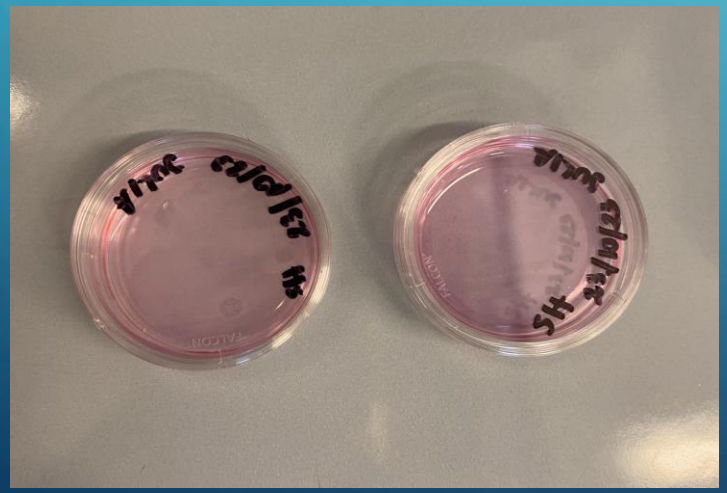
Sponetaneous action potentials

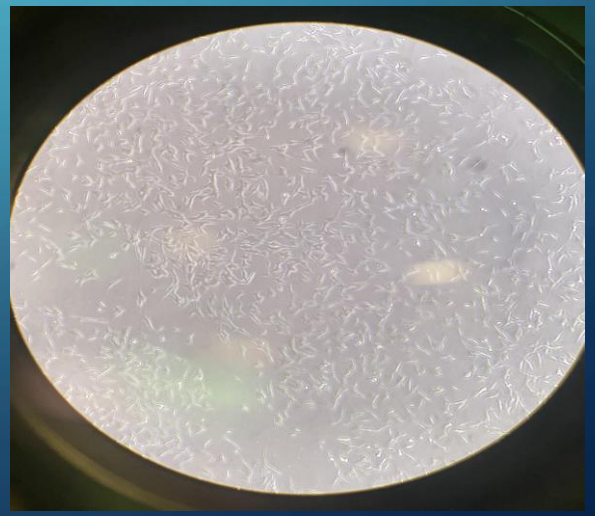


Evoked action potentials

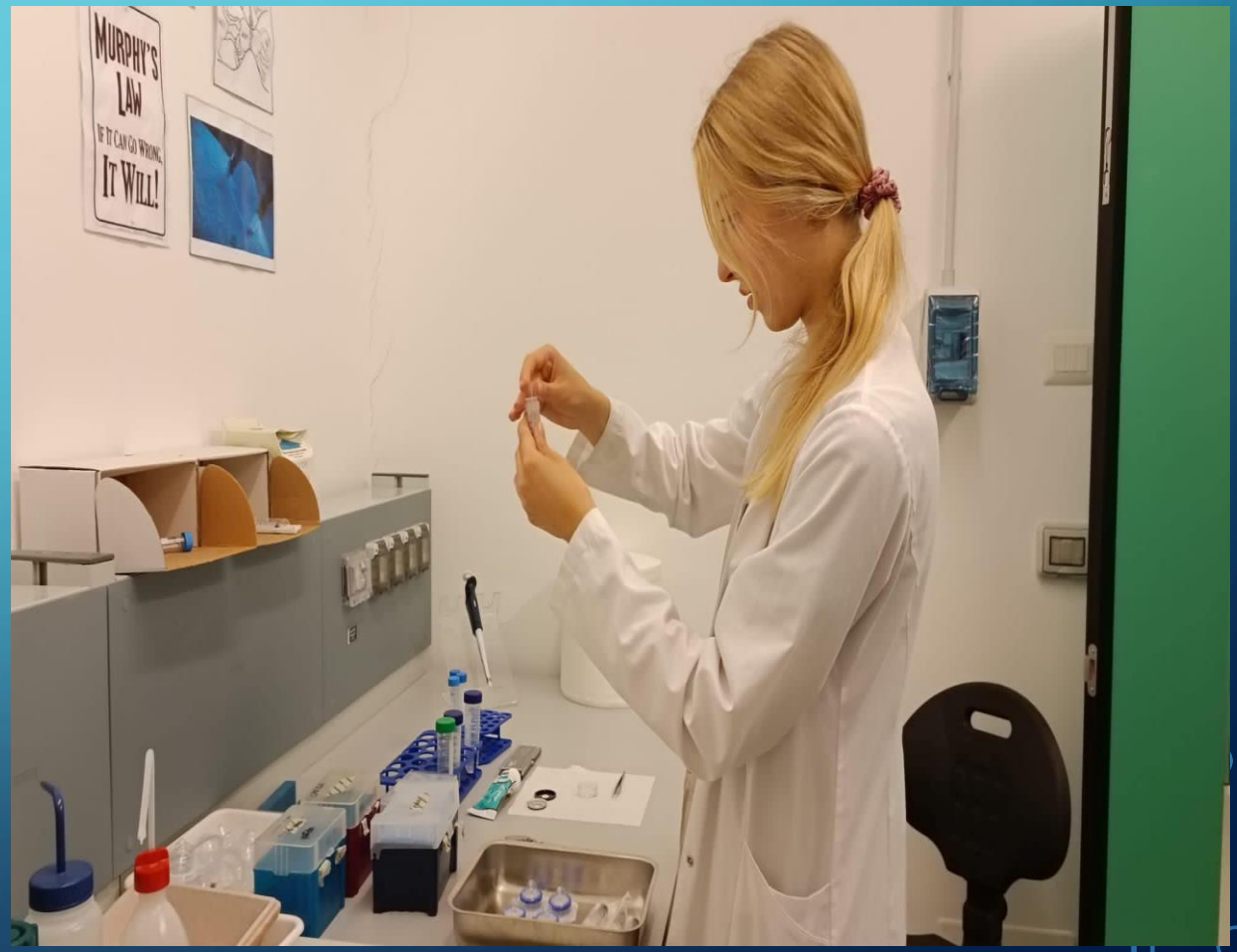
PROCEDURE

- Collection of the neuron cells from the T75 flask
- Preparation of the two dishes with 35 mm diameter
- Treatment of the dishes with the Cultrex, incubation for 1 hour
- Removal of Cultrex
- Wash with the medium
- Seeding 125000 cells in each dish with the final volume of the 2ml and adding the medium which contains all the essential components (FBS, Glut, STREEP)
- Conducting the treatment for 7 days
- Obtaining final result of differentiated neurons



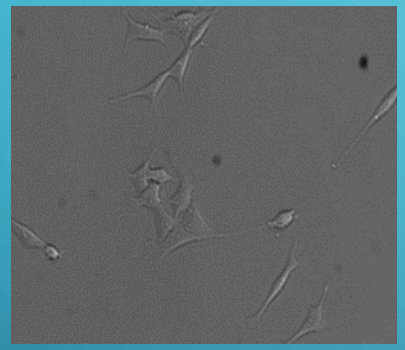


SH cells 3rd day of treatment


MURPHY'S
LAW
IF IT CAN GO WRONG,
IT WILL!

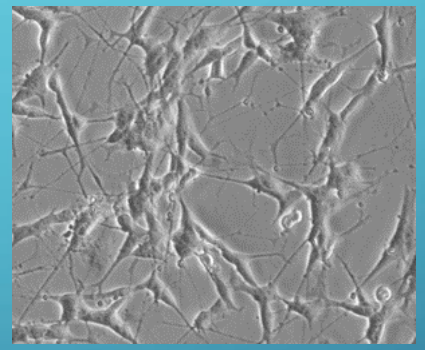
THE CONDUCTED EXPERIMENT

- TYPE OF CELLS: Undifferentiated SH-SY5Y neuron cells
- CHARACTERISTICS: SH-SY5Y cells revealed small or even no inward current, but the undifferentiated cells had the highest amplitude of outward currents



Day 1

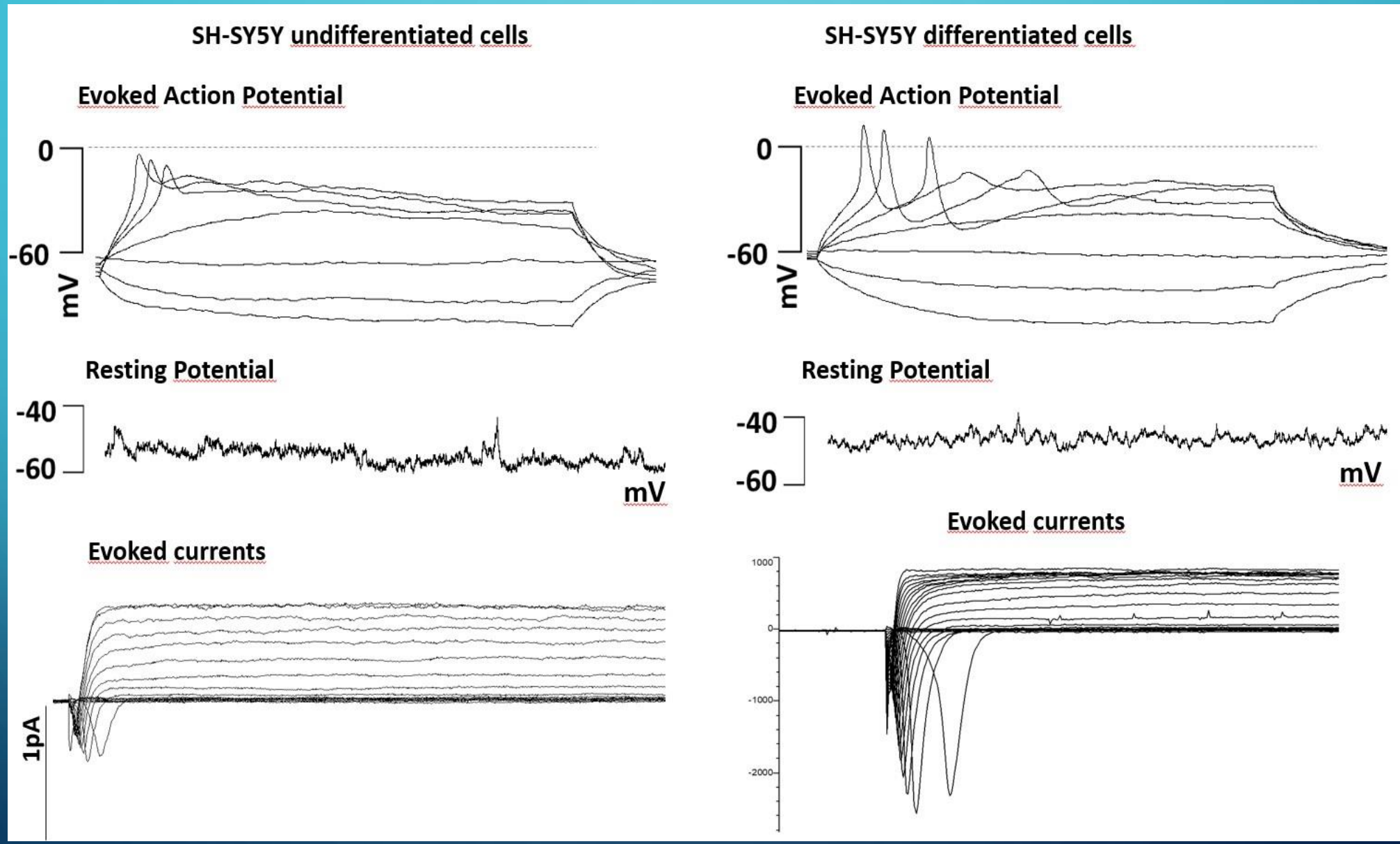
Undifferentiated neurons



Day 7

Differentiated cells

Medium contained 1 μM Retinoic Acid - 1% FBS


SH-SY5Y undifferentiated cells
Evoked Action Potential
0
-60
mV
Resting Potential
-40
-60
mV
Evoked currents
1pA
SH-SY5Y differentiated cells
Evoked Action Potential
0
-60
mV
Resting Potential
-40
-60
mV
Evoked currents
1000
0
-1000
-2000

GRAZIE PER LA VOSTRA ATTENZIONE ☺