Advanced Electrophysiology

Lab 1

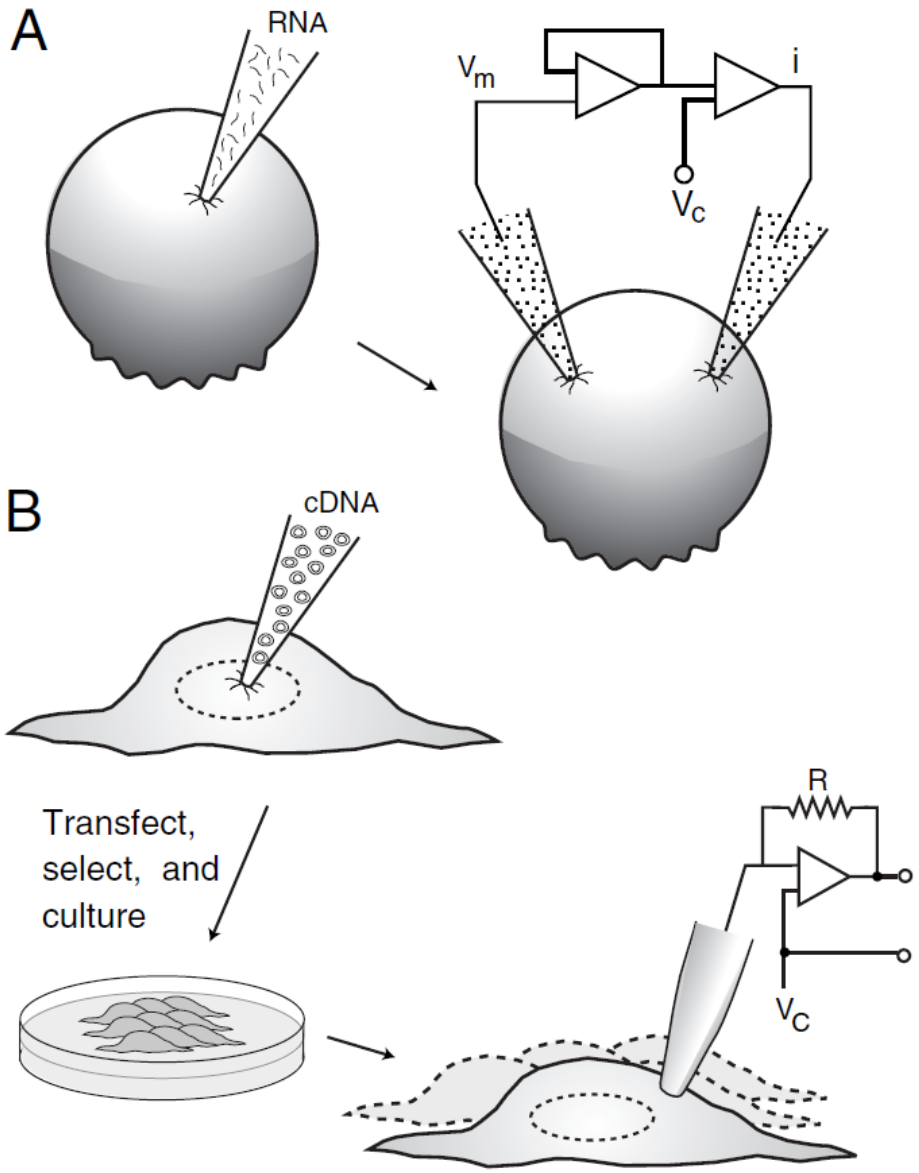
27 March 2025

Acute brain slice preparation

Preparations for single cell electrophysiology

1) **Heterologous expression systems**
   - A. **Xenopus oocytes**
   - B. **Cell lines**

→ **Investigate the function of ion channels in isolation**



Preparations for single cell electrophysiology

2) Primary neuronal cultures

Advantages:

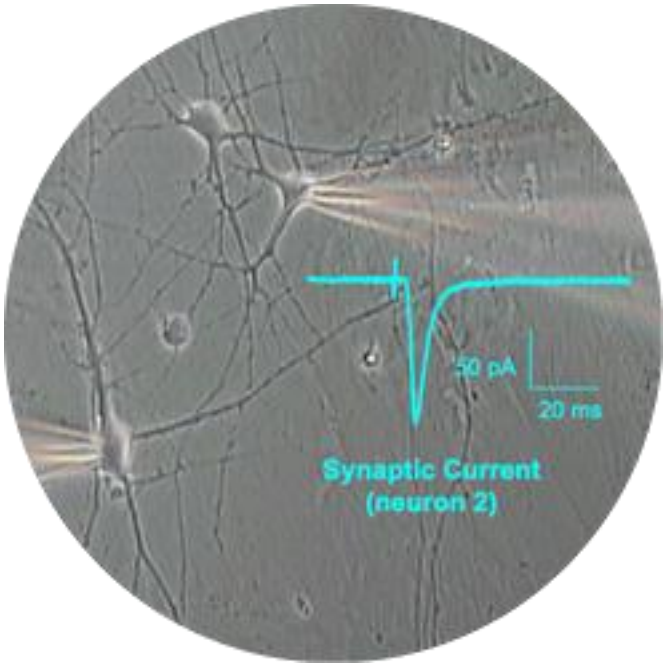
a. **Ideal optical accessibility**

b. **Simplified patching procedures**

Disadvantages:

a. **Loss of native connectivity (at least most of it)**

b. **Challenges in maintaining long stable recording**

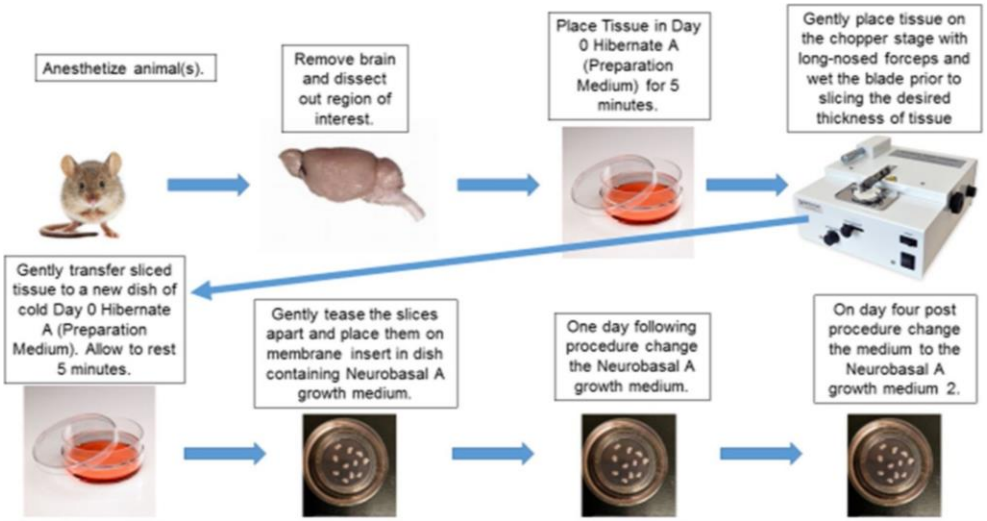


Preparations for single cell electrophysiology

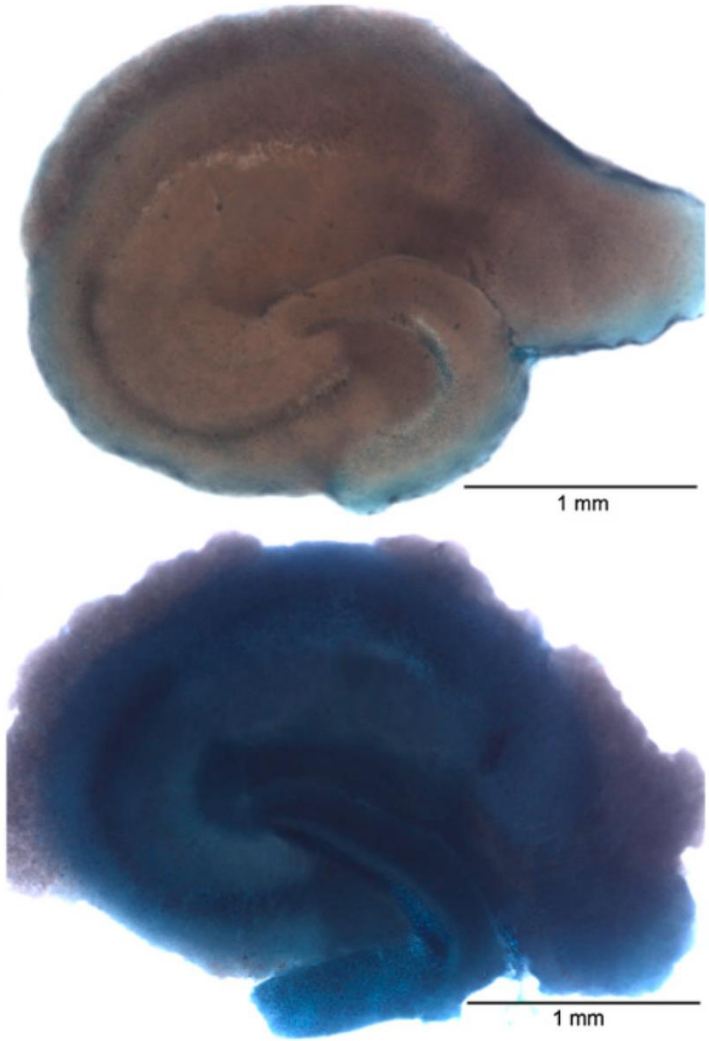
3) Brain slices

A. Organotypic brain slices

B. Acute brain slices



Example of hippocampal organotypic slice

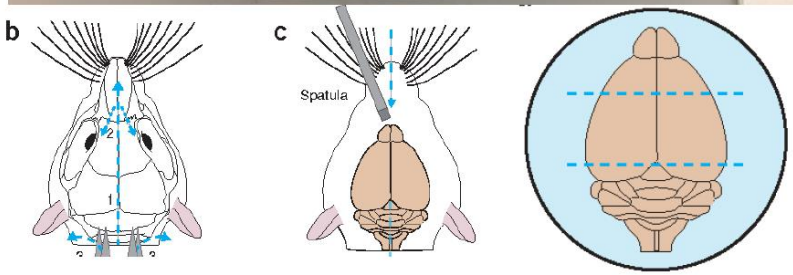


Preparations for single cell electrophysiology

3) Brain slices

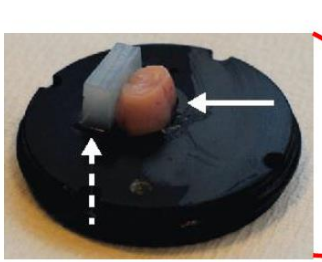
A. Organotypic brain slices

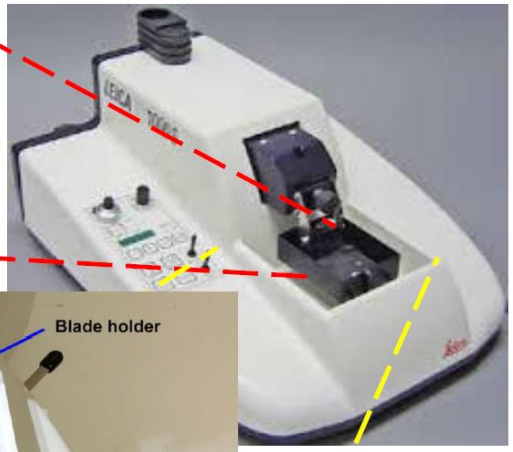
B. Acute brain slices

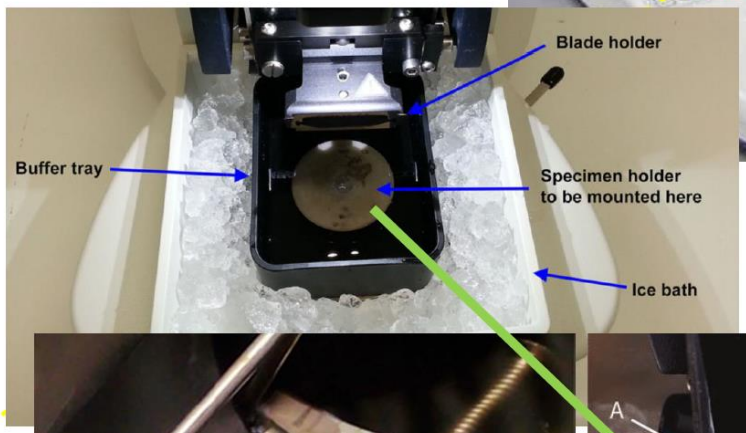


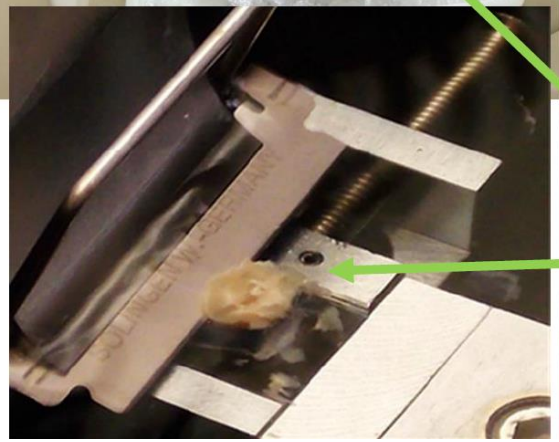
Wang & Baudry, 2019 Bio-Protocol

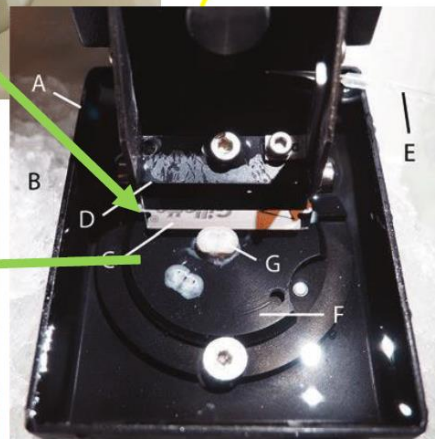
Mishra et al, 2014 Nat Prot



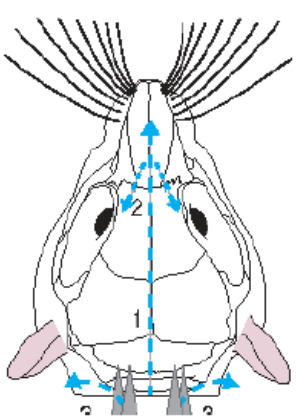


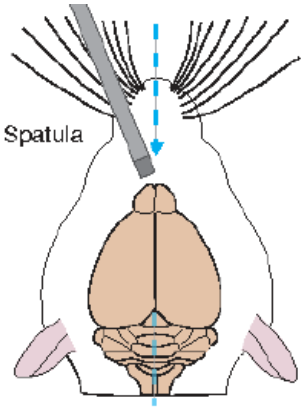


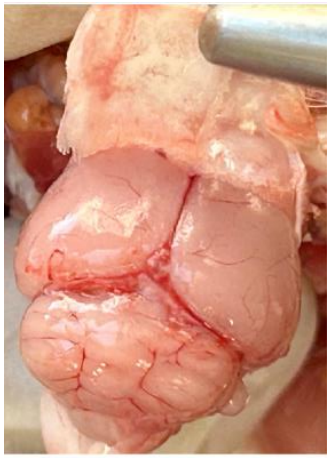


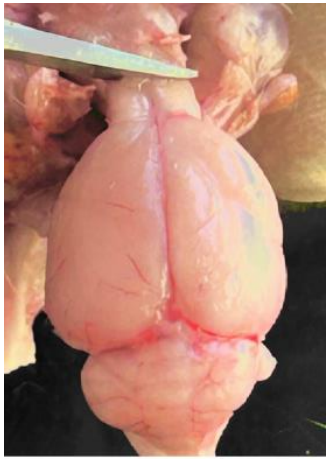


Brain dissection









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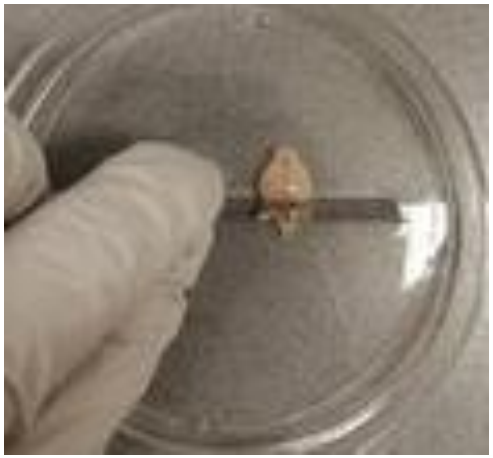
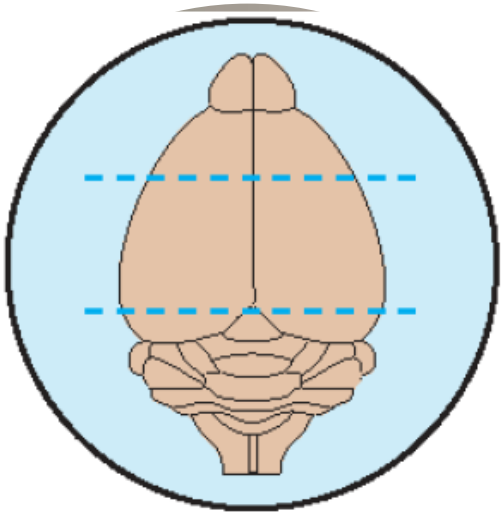
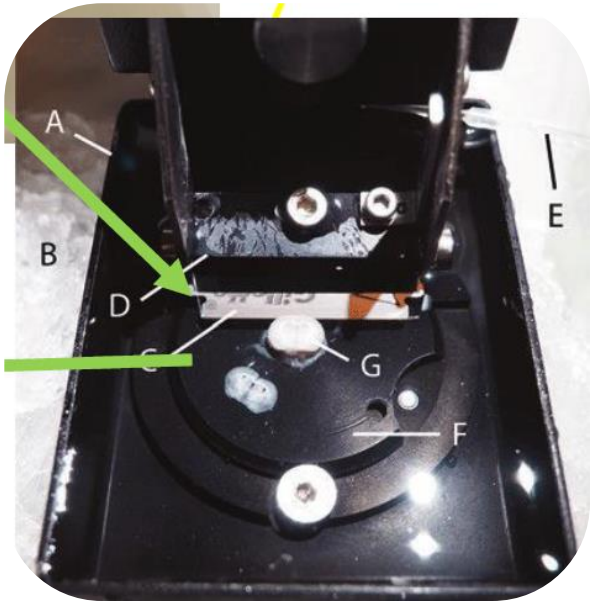
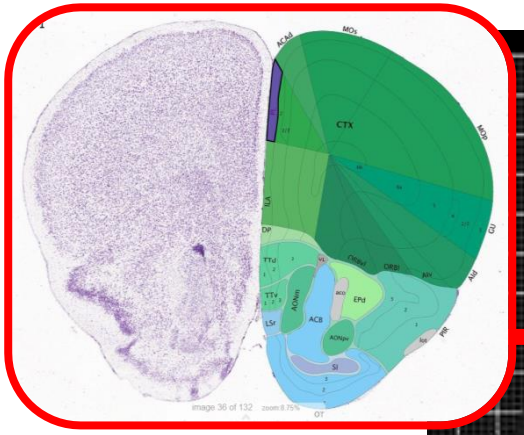
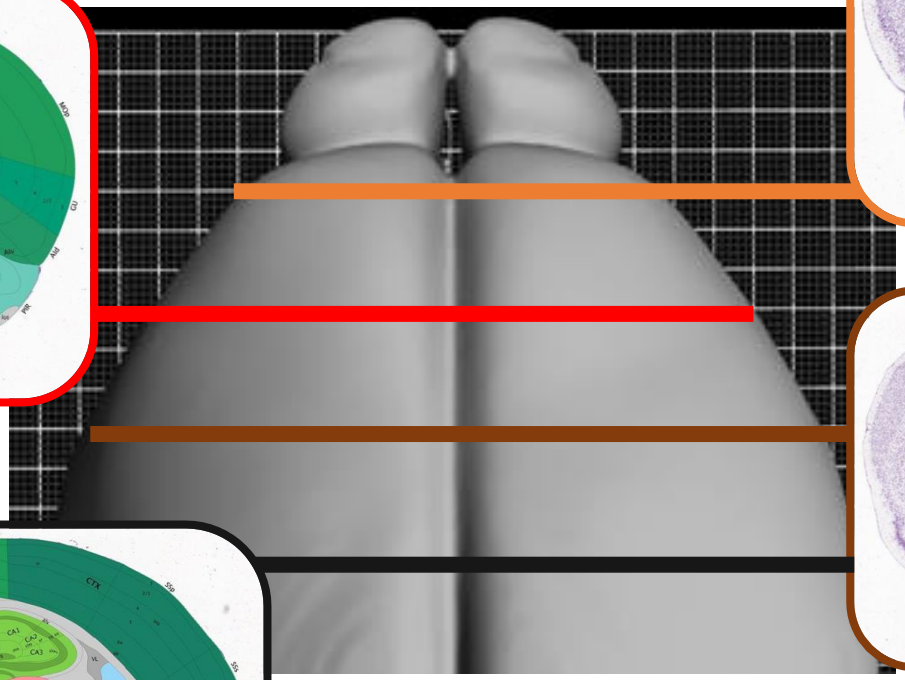


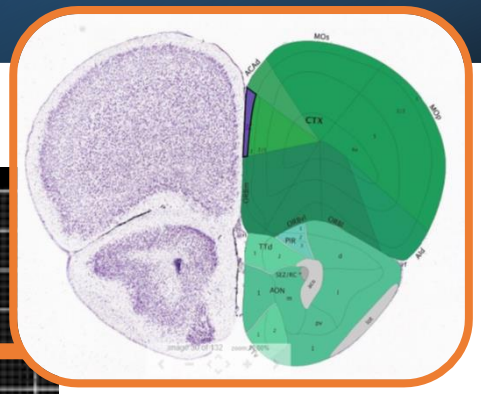


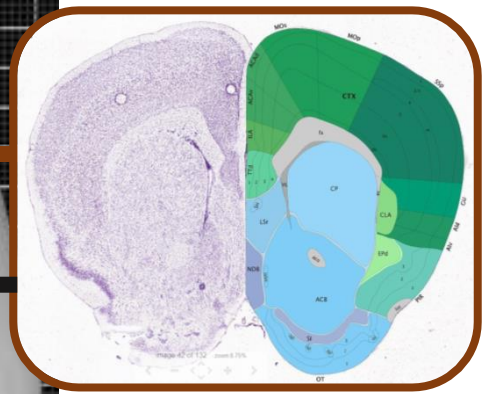


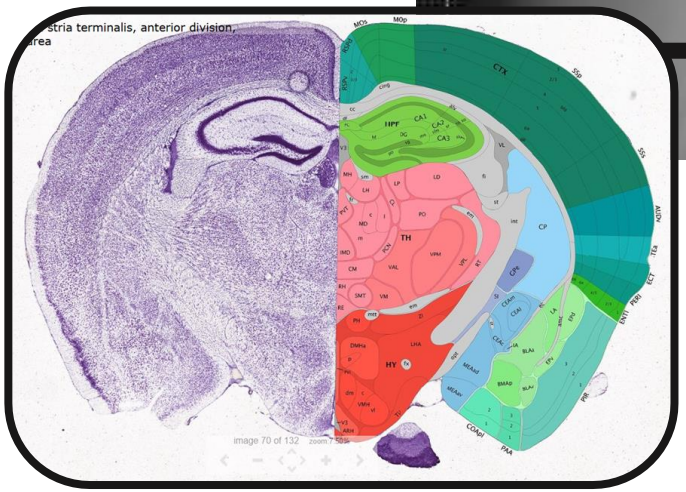
Brain slicing

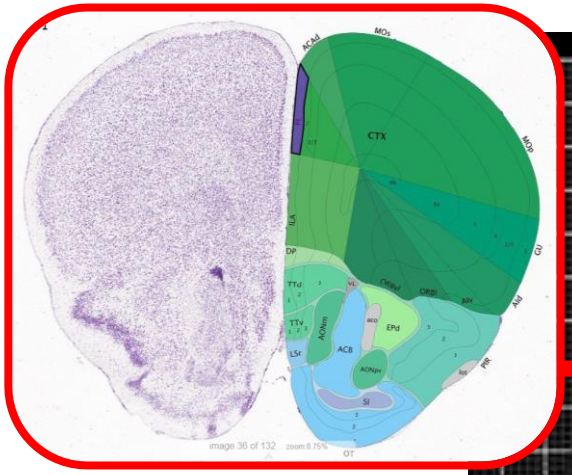








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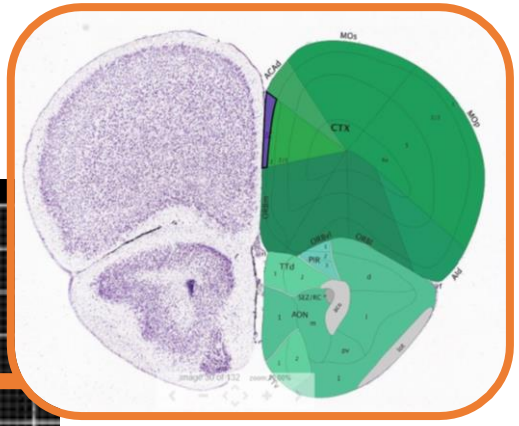
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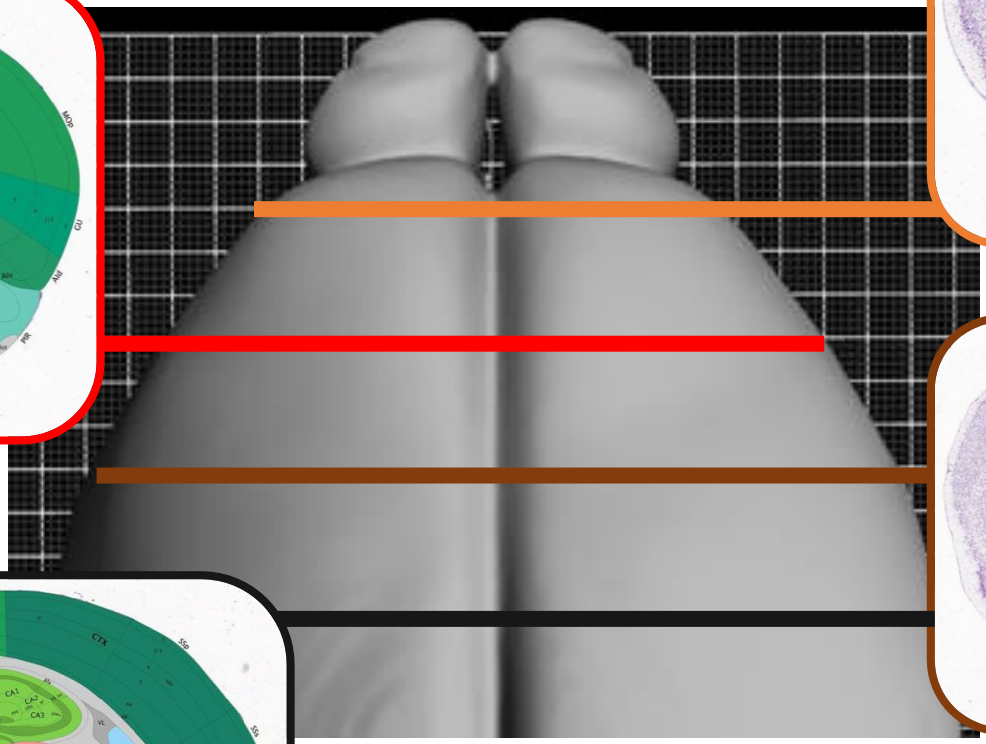

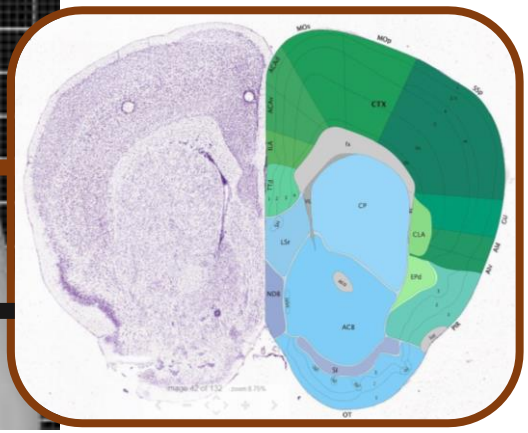
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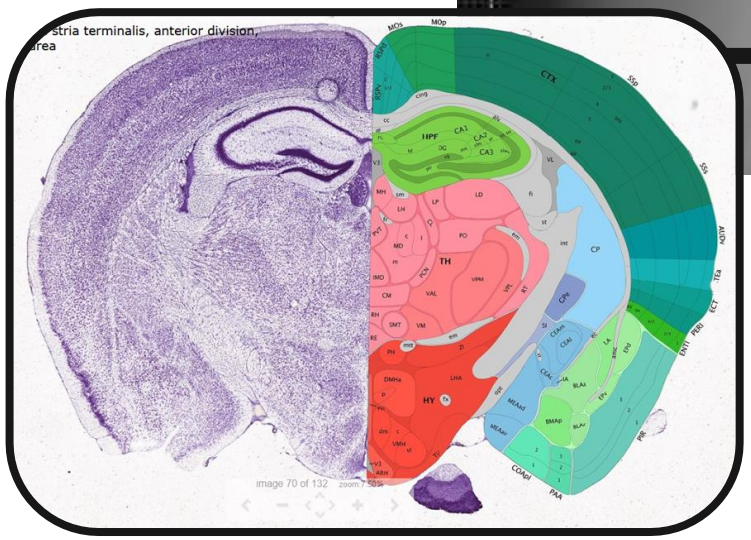
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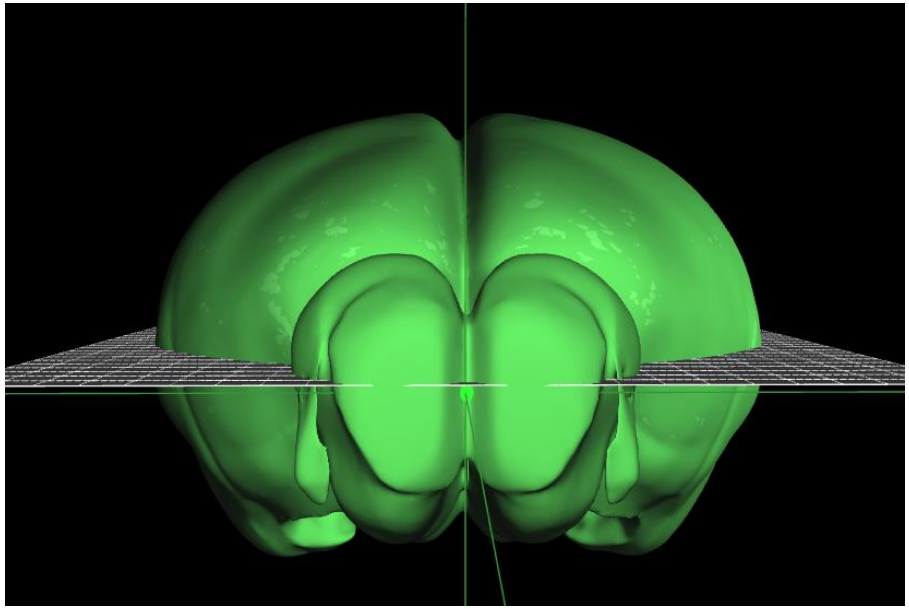
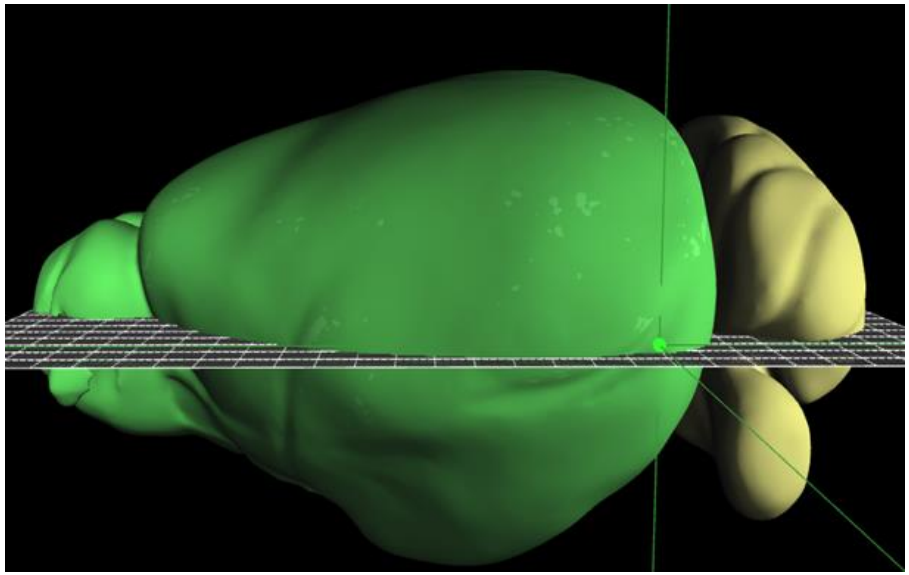
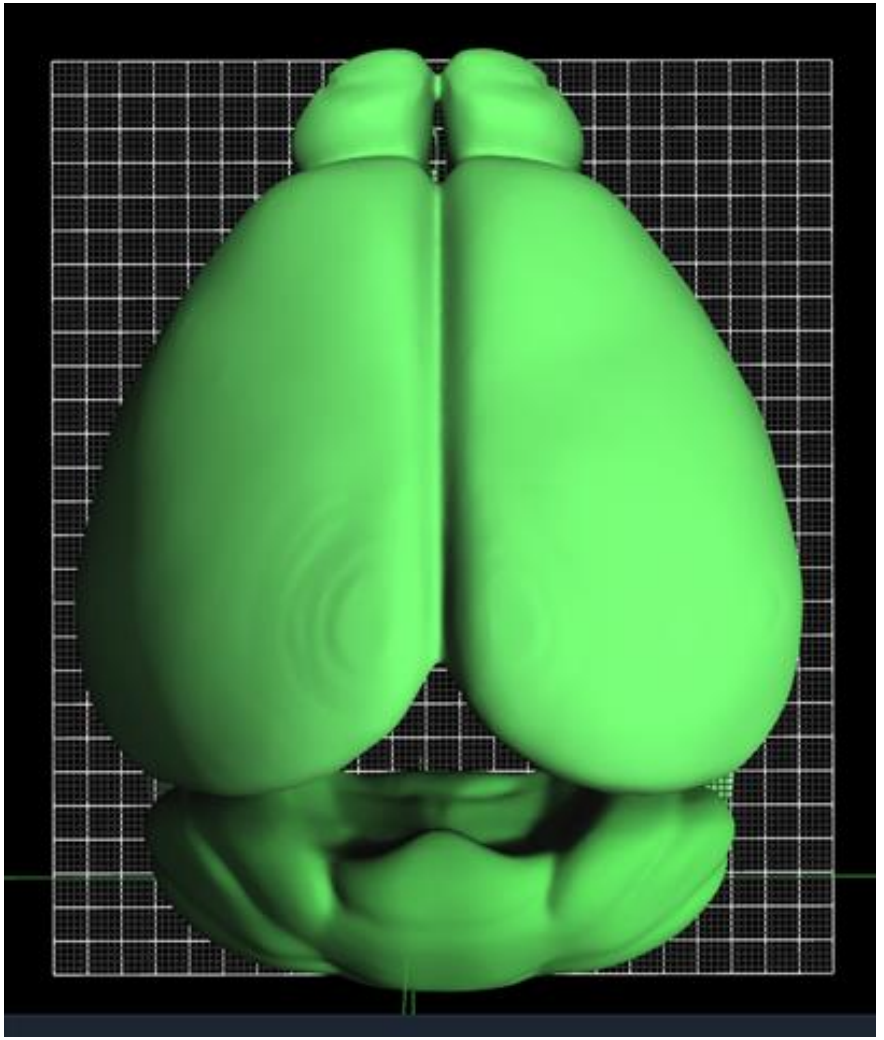
zoom 7.50%

[3d Viewer :: Allen Brain Atlas: Mouse Connectivity]

[Interactive Atlas Viewer :: Atlas Viewer]

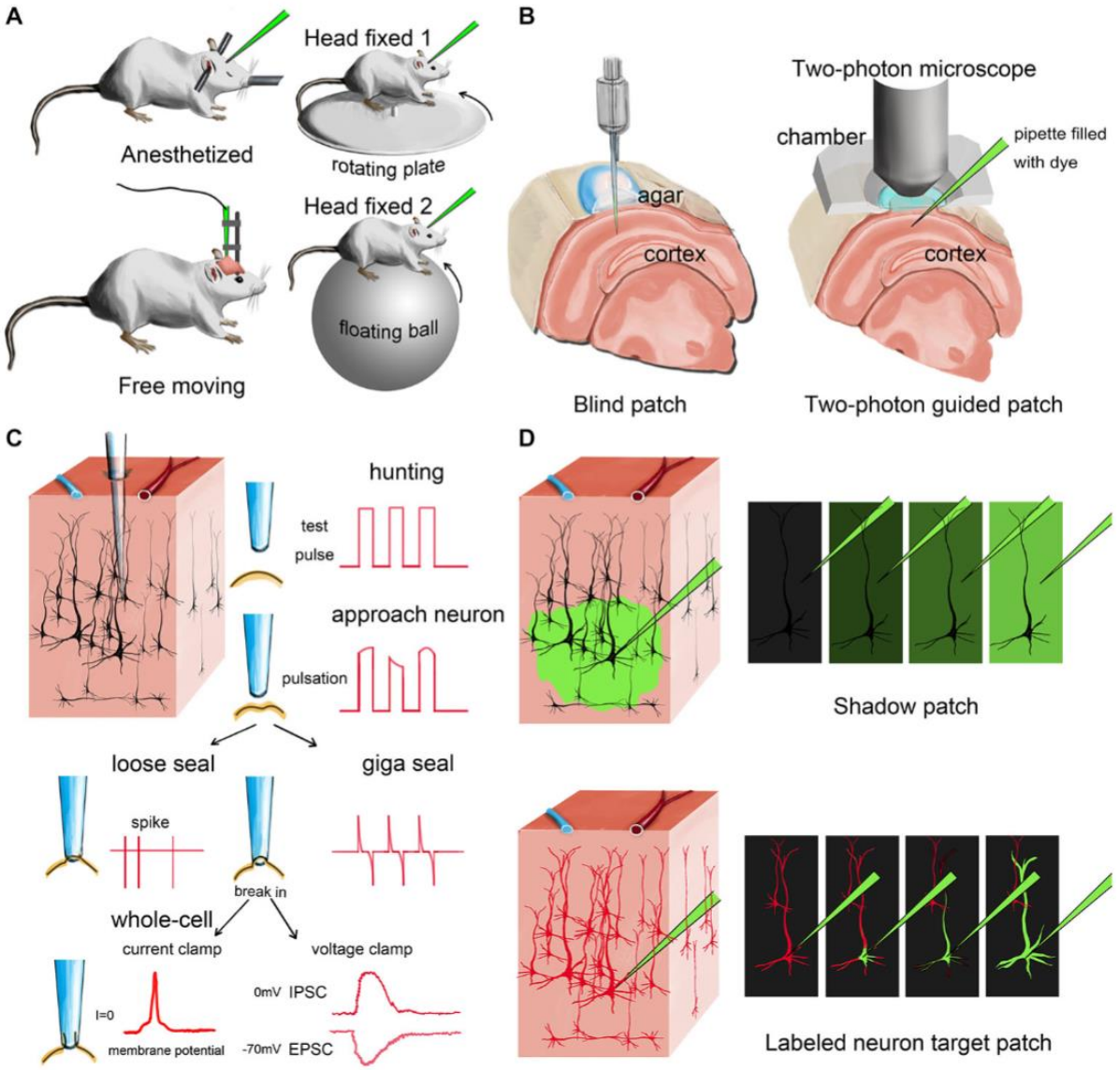






Preparations for single cell electrophysiology

4) In vivo single cell recordings



External solution: artificial cerebrospinal fluid (aCSF)

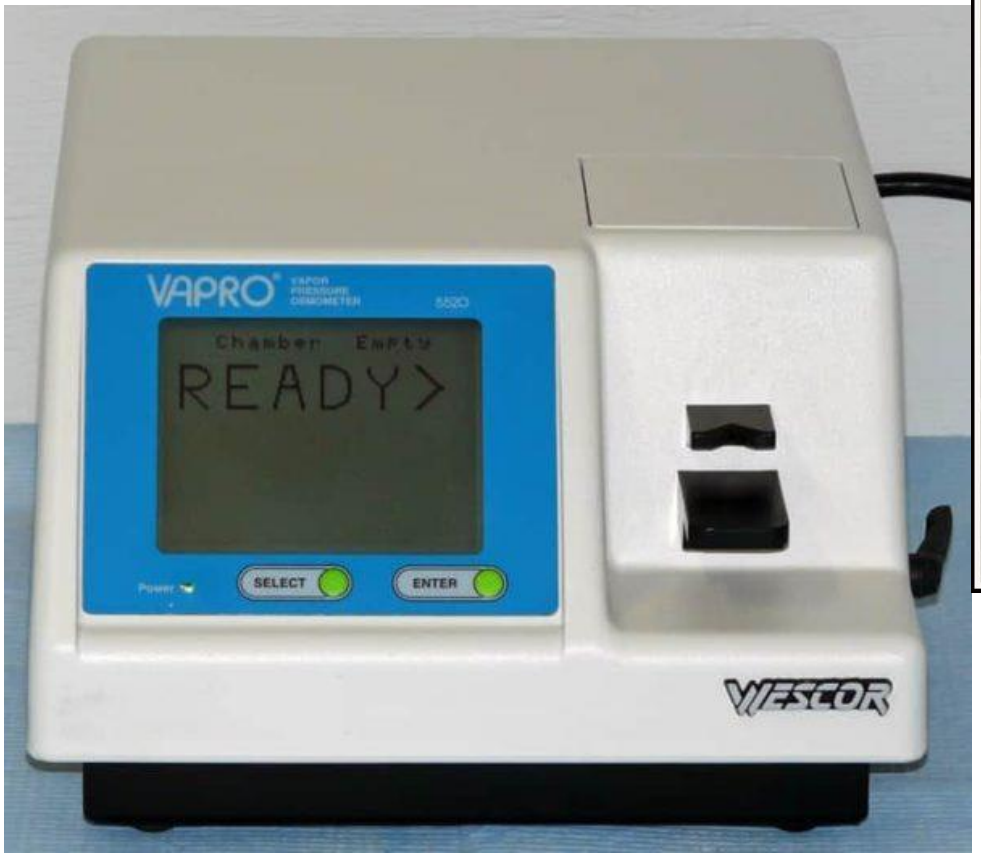
Salts	Cf (mM)
NaCl	124
KCl	5
NaH_2PO_4	1.25
$NaHCO_3$	26
$CaCl_2$	2
$MgSO_4$	2

Ion	Cf (mM)	
Na^+	151	← High Na^+
K^+	0	← Low / no K^+
Cl^-	133	← High Cl^-
Ca^{2+}	2	
Mg^{2+}	2	
HCO_3^-	26	(pH buffers)
SO_4^{2-}	2	
$H_2PO_4^-$	1.25	

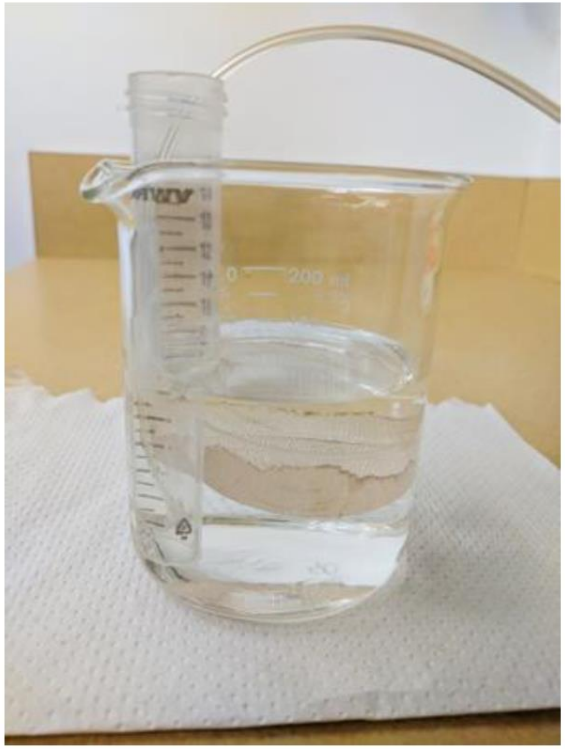
pH = ~7.4

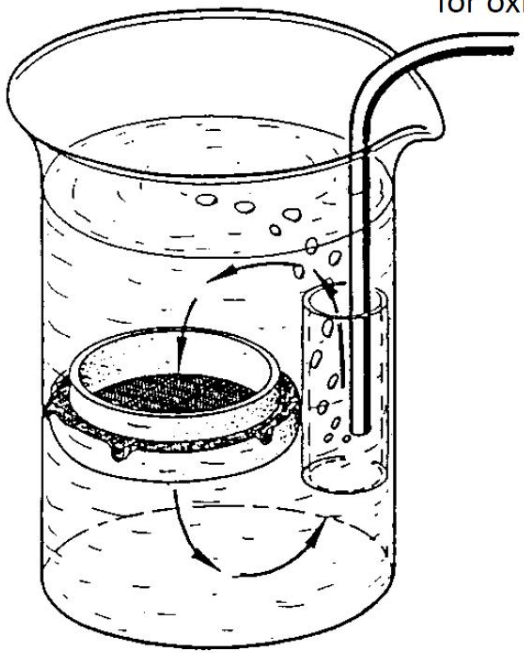
osmolarity = ~300 mOsm

How to measure the osmolarity

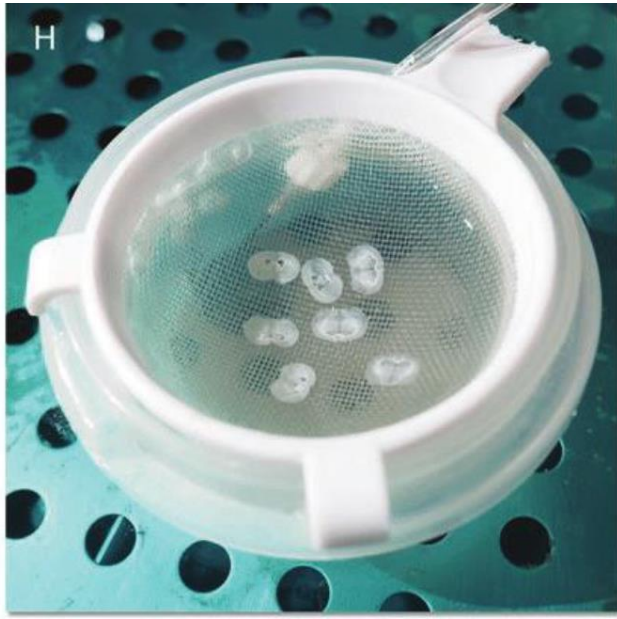


How to maintain acute brain slices

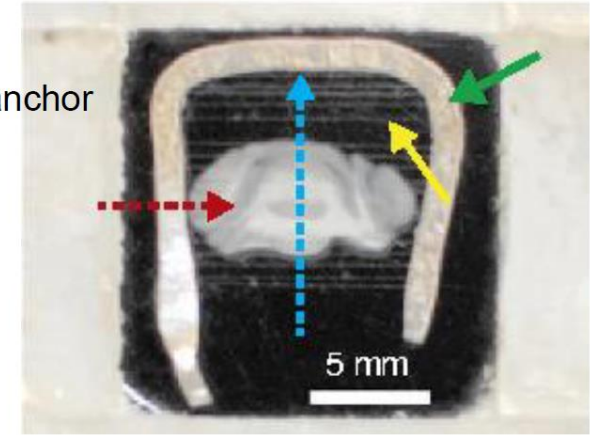




CO_2:O_2 (5:95%):
for oxigen + pH



Harp / anchor

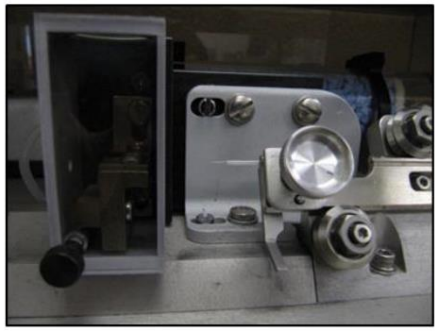


Bioprotocol

Gibb, A. J., & Edwards, F. A. (1994) Microelectrode Tech, 255-274.

How to pull a patch pipette

Horizontal puller



~ 1-3 µm tip

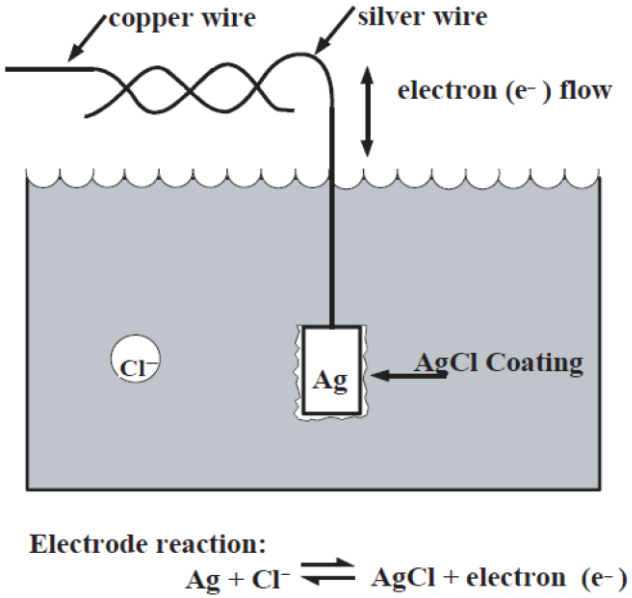
Vertical puller

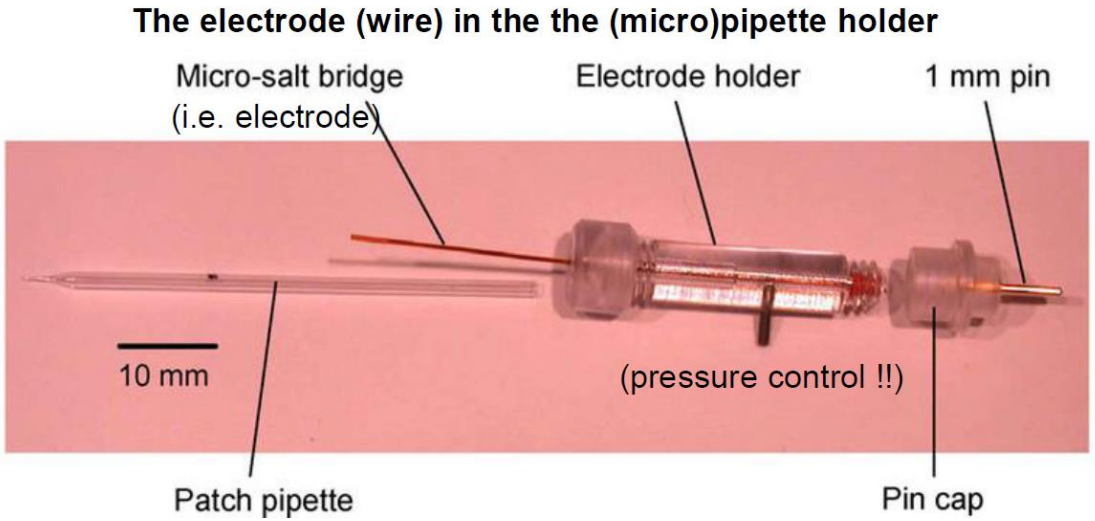
How to pull a patch pipette

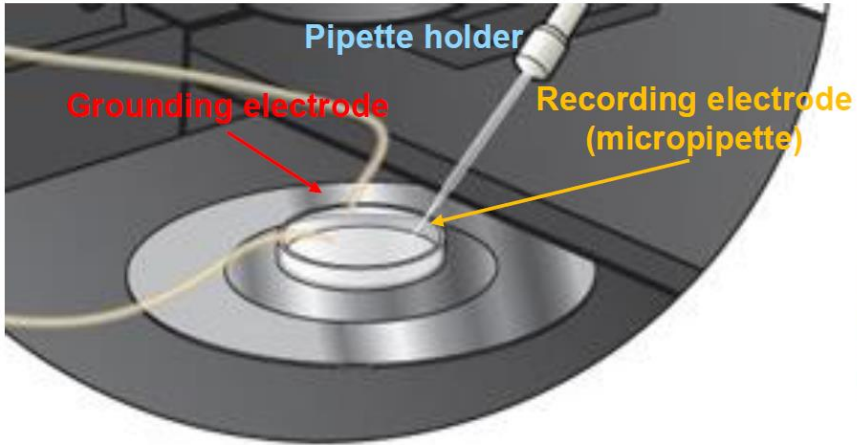
- Regulate T1 for the shape of the pipette
- Regulate T2 for the pipette resistance

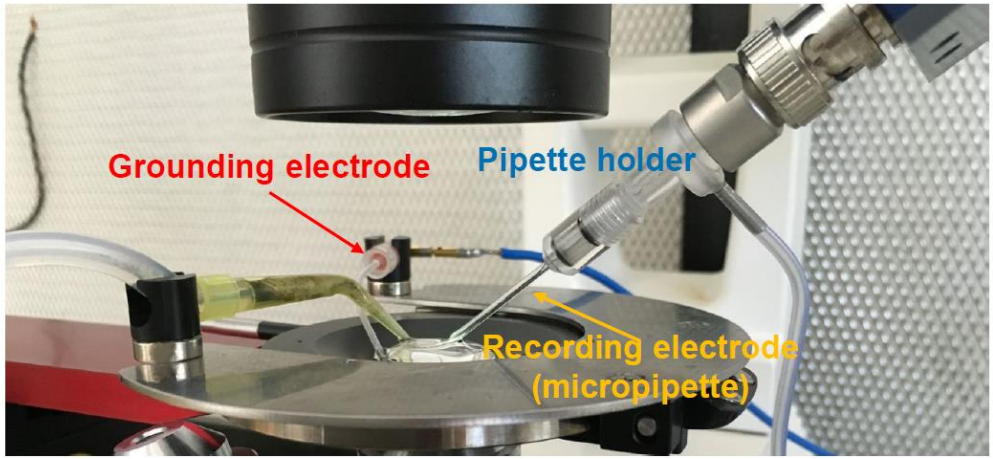
Practical Example:
You adjust T1 based on the shape you want and regulate T2 to reach the proper tip resistance (5-6 MΩ for us)

The patch-clamp pipette





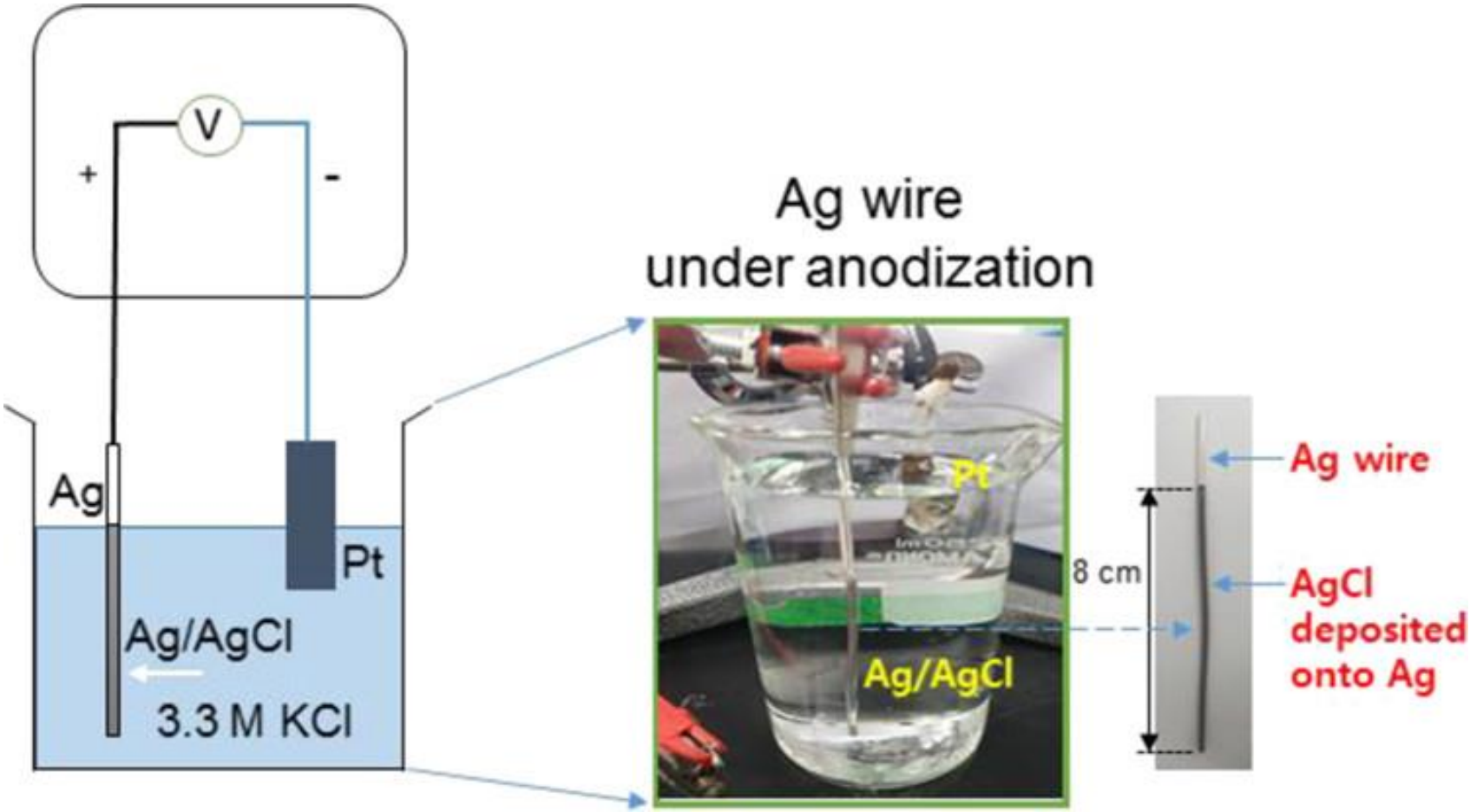




How to prepare patch-clamp electrodes:

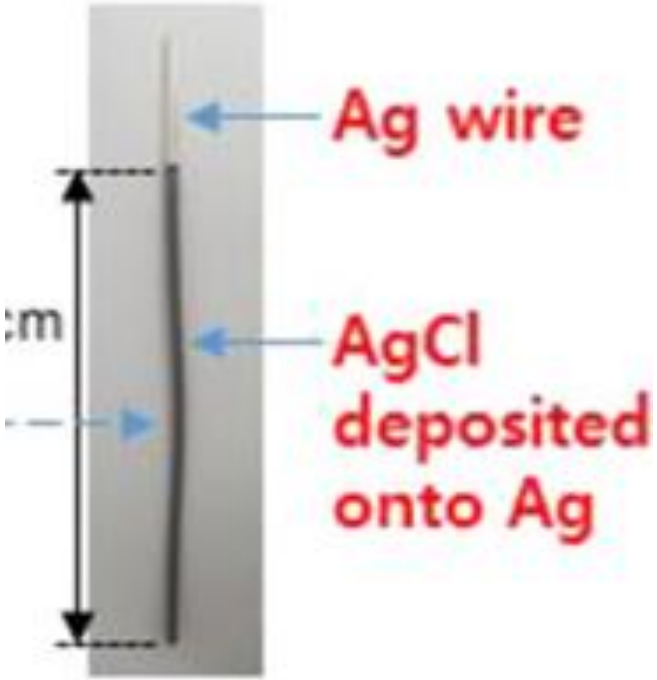
yes, you also need to be able to solder

How to chloride an electrode using a battery



How to chloride an electrode:

the bleach method



Silver reacts with chlorine in the bleach (sodium hypochlorite, NaOCl) solution.

This process forms a layer of silver chloride (AgCl) on the surface of the silver electrode.

Internal (intracellular) solution

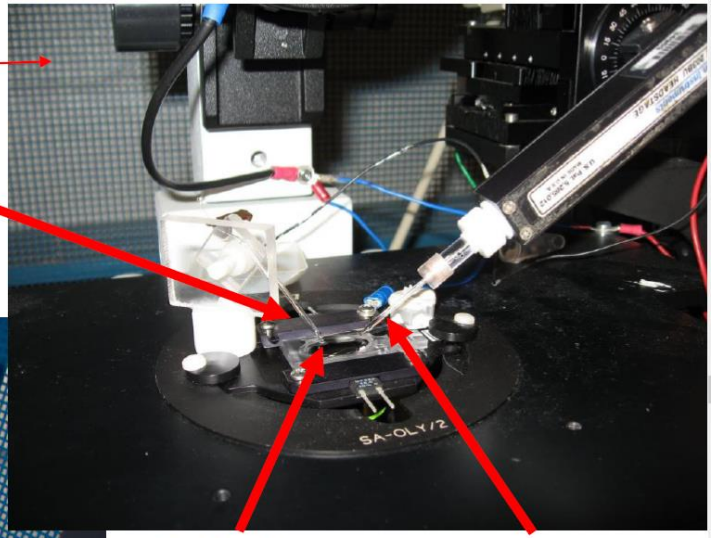
Salts	Cf (mM)
KCl	9
KOH	10
$MgCl_2$	3.48
NaCl	4
K-gluconate	120
HEPES	10
Sucrose	17.5
Na_2ATP	4
Na_3GTP	0.4

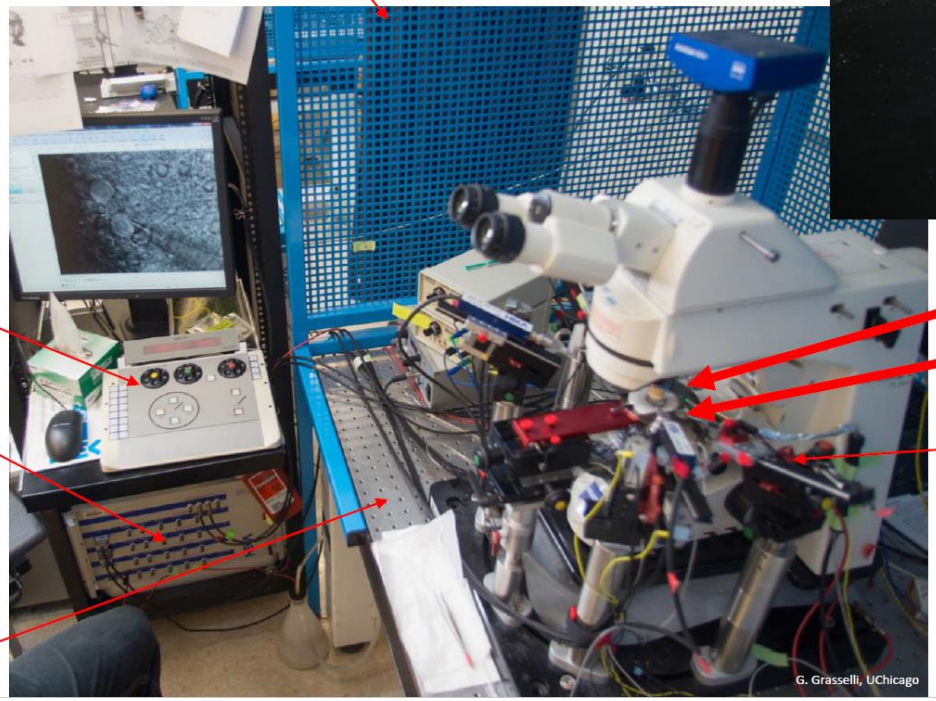
Ion	Cf (mM)	
Na^+	13.2	← Low Na^+
K^+	139	← High K^+ (or Cs^+)
Cl^-	19.96	← Low Cl^-
Ca^{2+}	0	← Low / no Ca^{2+}
Mg^{2+}	3.48	
$Gluconate^-$	120	← Anions
HEPES	10	(pH buffers)
HCO_{3-}	0	
SO_4^{2-}	0	
$H_2PO_4^-$	0	
ATP^{2-}	4	← energy
GTP^{3-}	0.4	
Sucrose	17.5	← osmolarity

pH = 7.25-7.35

osmolarity = 295-305 mOsm
(10-20 mOsm lower than aCSF)

Patch-clamp electrophysiology set-up





Faraday cage

reference electrode

Brain slice

Recording electrode

Micromanipulator controller

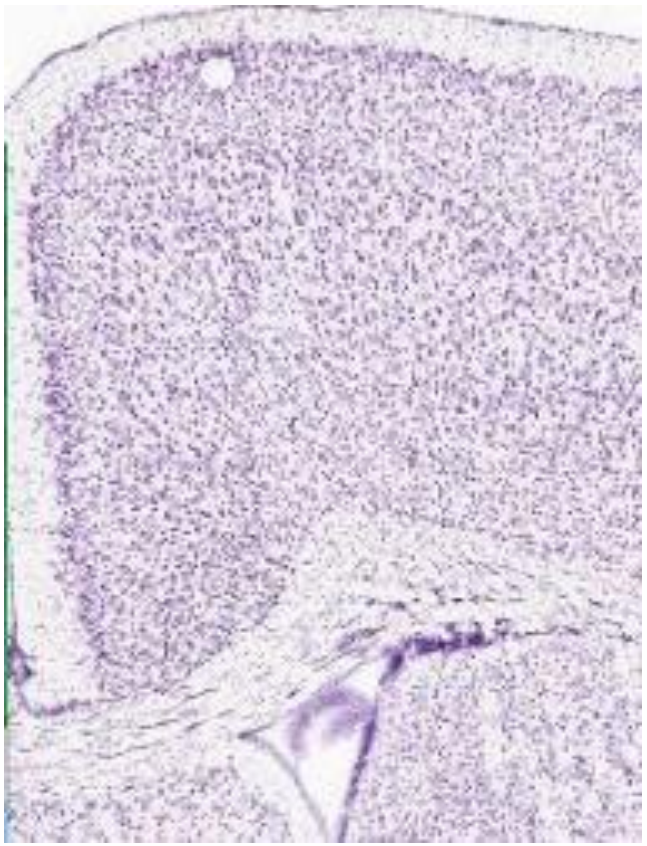
Stimulating electrode

Amplifier

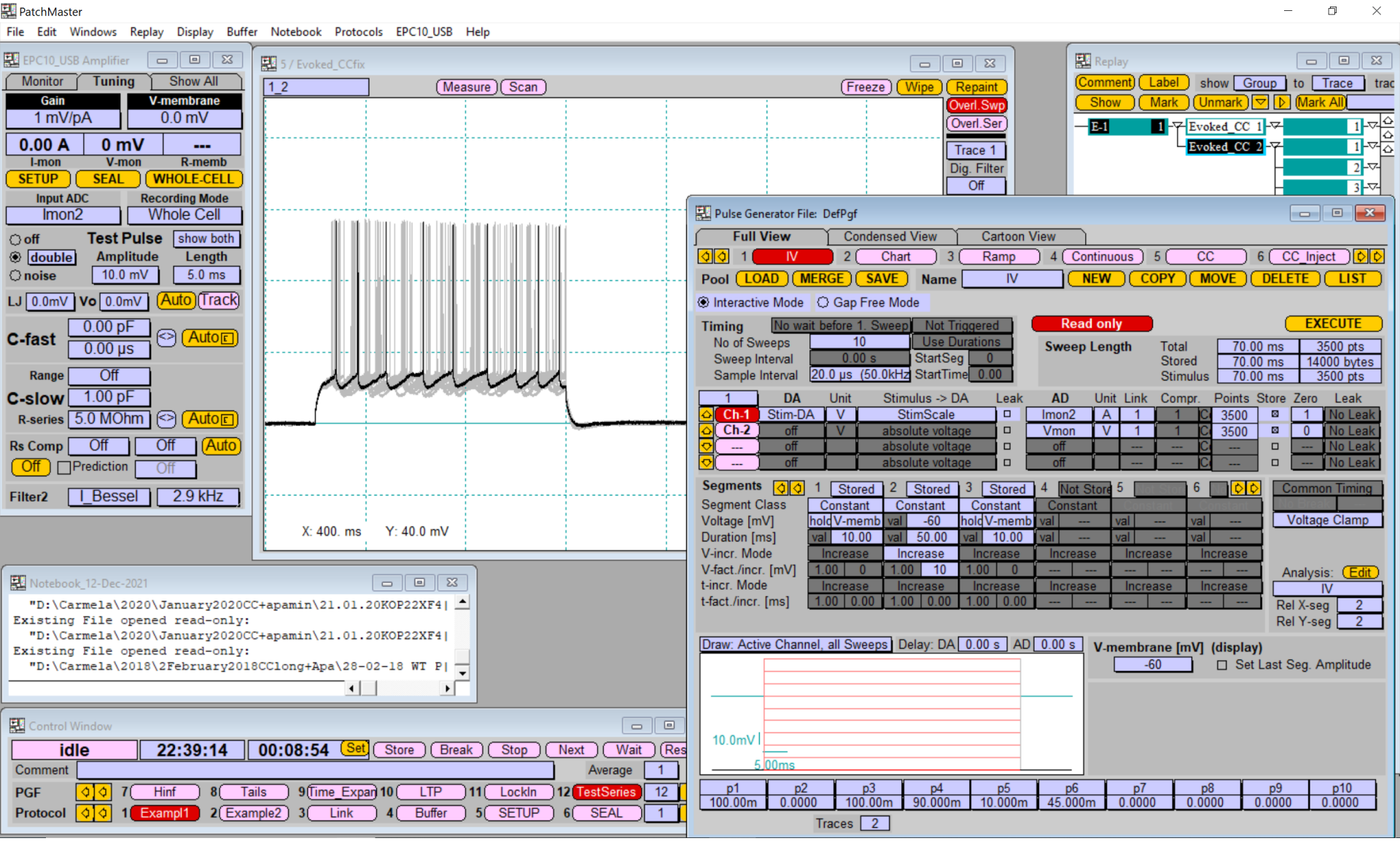
Peristaltic pump

Air table

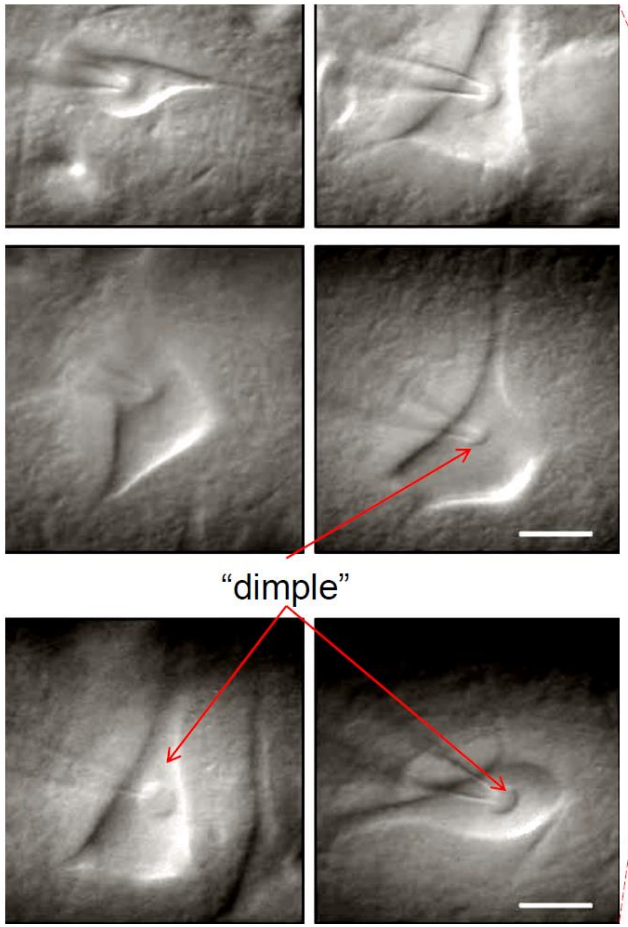
Observing brain slice at the microscope and identifying the cortical layers

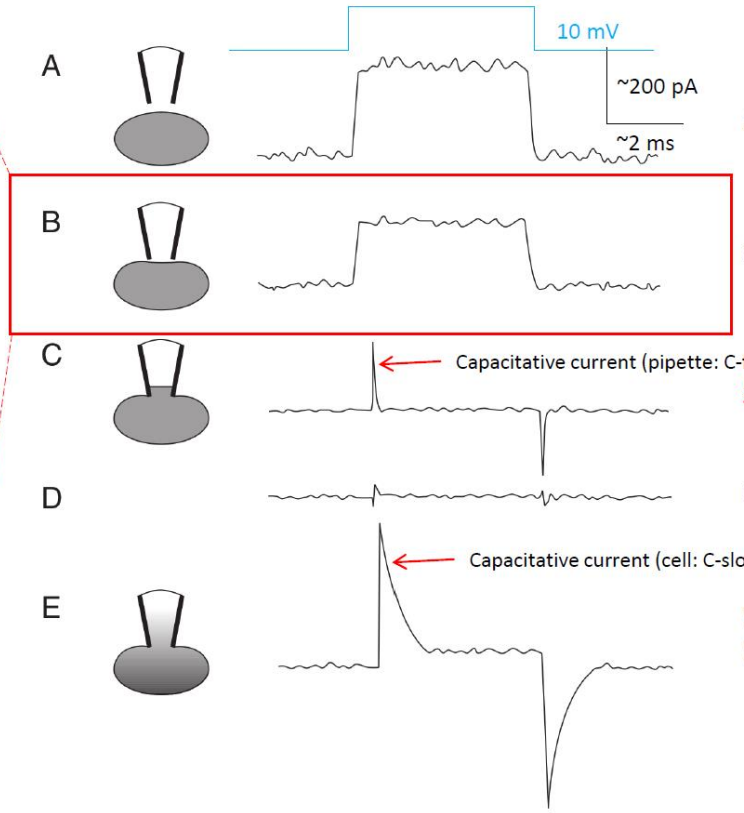


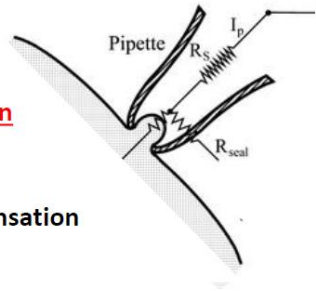
Patch-clamp electrophysiology acquisition program



The formation of a giga-seal







Learning objectives

1. **To pull patch-clamp electrodes**
2. **To coat patch-camp electrodes**
3. **To solder patch clamp electrodes**
4. **To measure the osmolarity of a solution**
5. **To slice the brain at a vibratome**
6. **To get accustomed to a patch-clamp setup and be able to identify its basic components**
7. **To be able to operate a patch-clamp micromanipulator to position a patch-clamp pipette**
8. **To observe a cortical brain slice at the microscope and be able to identify the cortical layers**
9. **To get accustomed to an electrophysiology acquisition program and its potentialities**