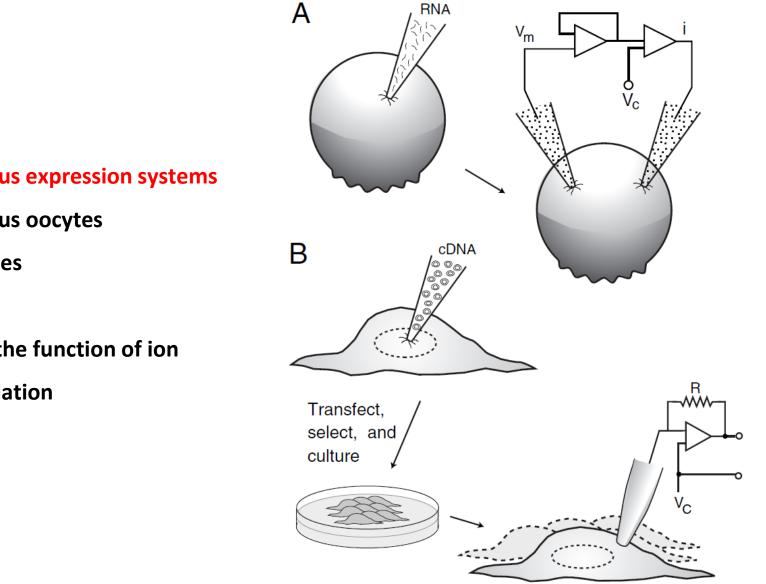
Advanced

Electrophysiology

Lab 1

27 March 2025

Acute brain slice preparation



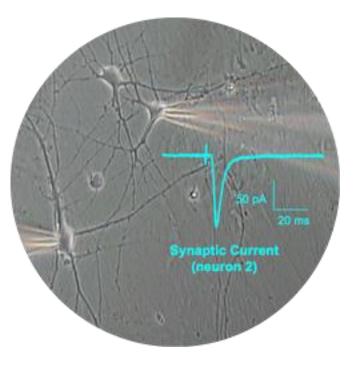
- **Heterologous expression systems** 1)
 - A. Xenopus oocytes
 - **B.** Cell lines
- \rightarrow Investigate the function of ion channels in isolation

2) Primary neuronal cultures Advantages:

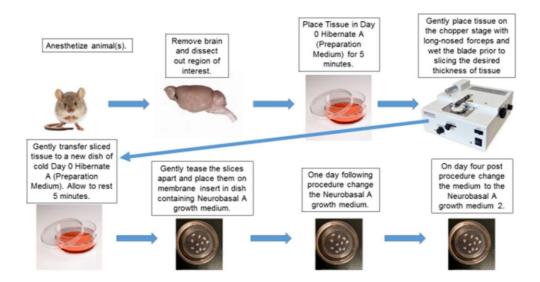
- a. Ideal optical accessibility
- b. Simplified patching procedures

Disadvantages:

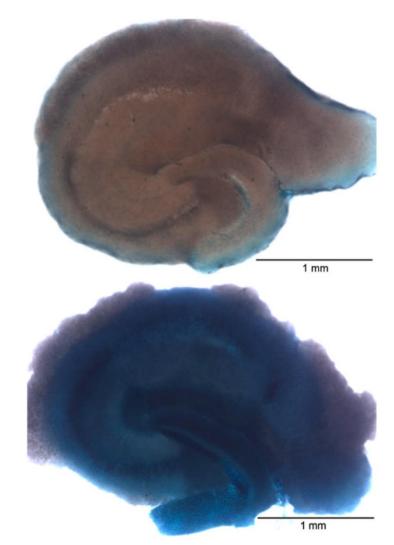
- a. Loss of native connectivity (at least most of it)
- b. Challenges in maintaining long stable recording



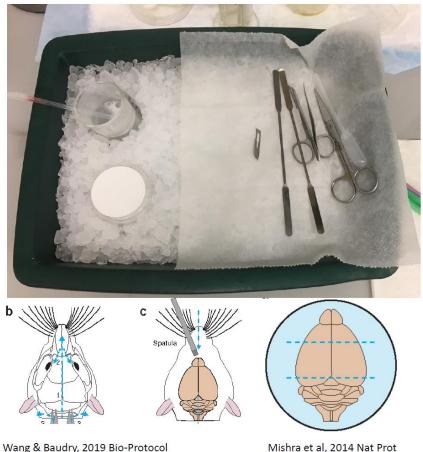
- 3) Brain slices
 - A. Organotypic brain slices
 - **B.** Acute brain slices

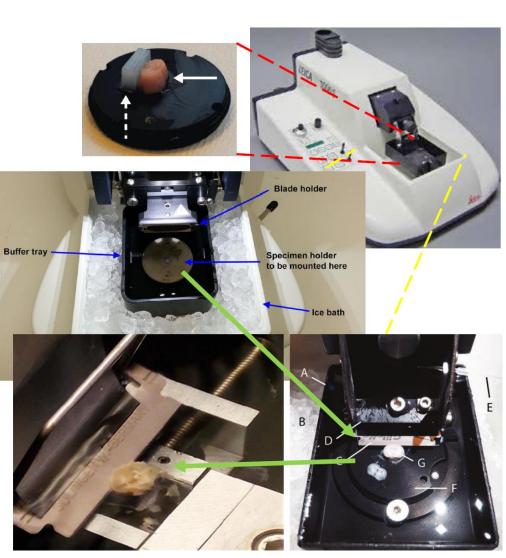


Example of hippocampal organotypic slice

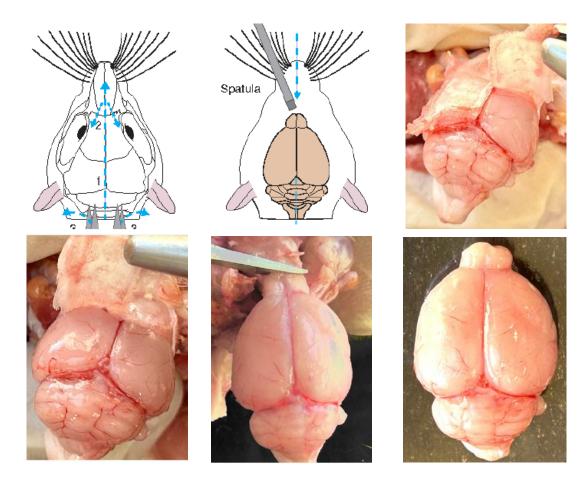


- 3) **Brain slices**
 - A. Organotypic brain slices
 - **B.** Acute brain slices



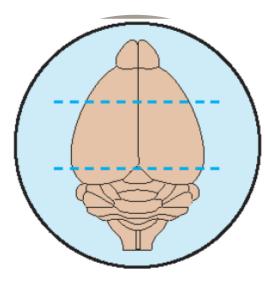


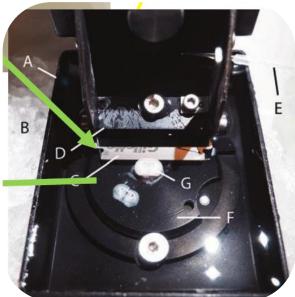
Brain dissection



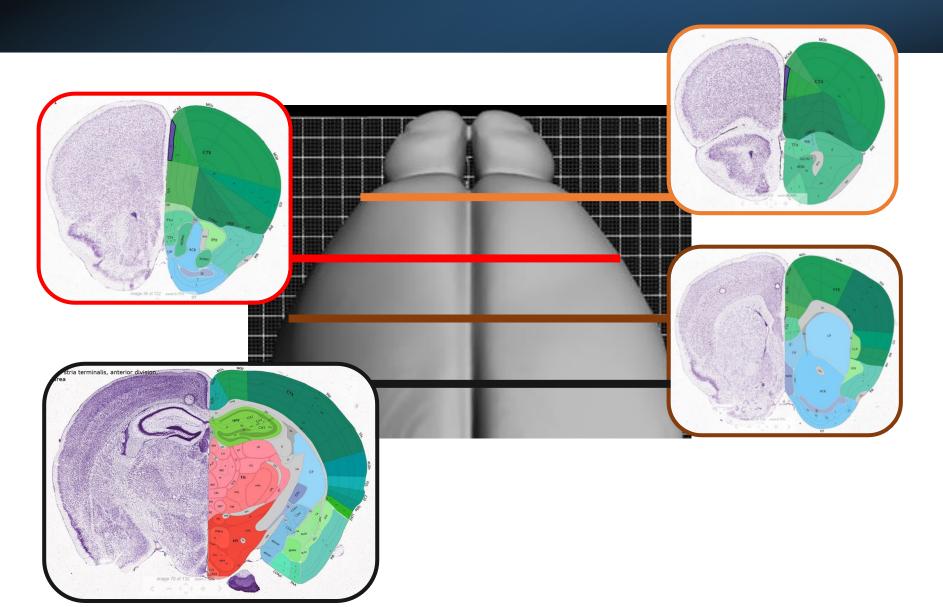
Brain dissection

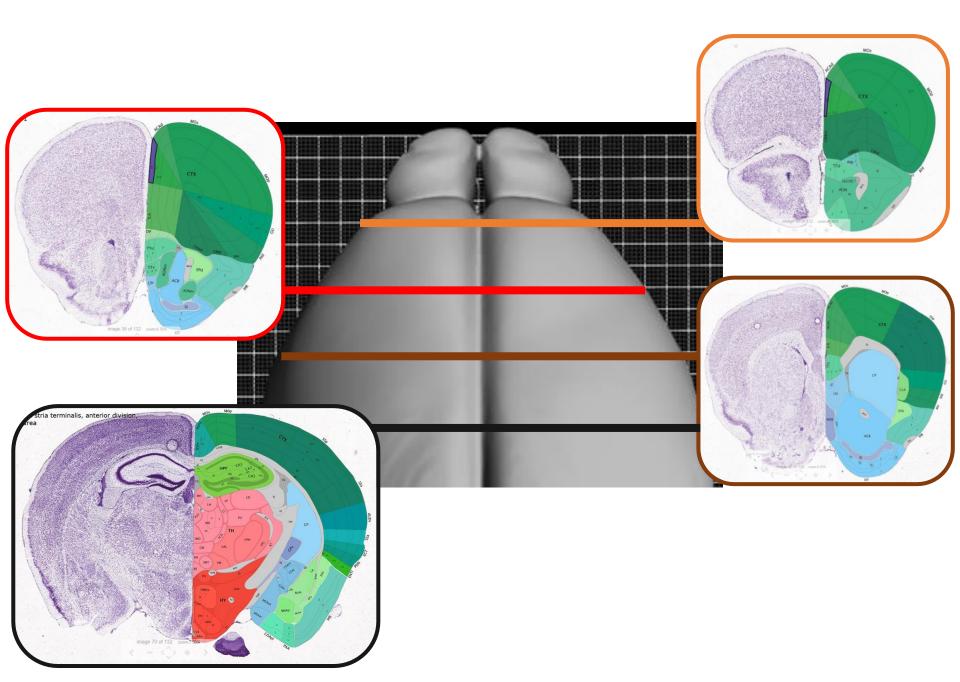






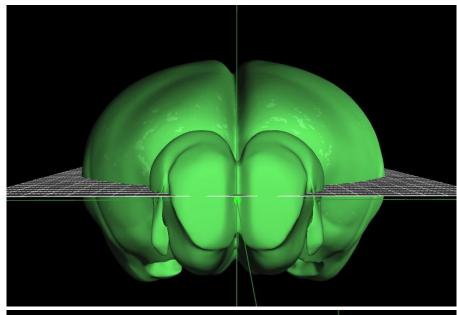
Brain slicing

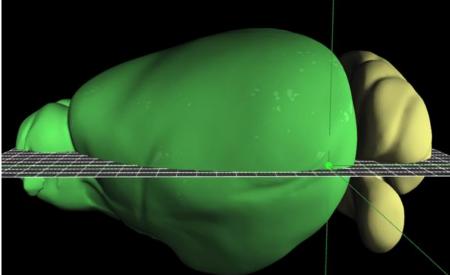


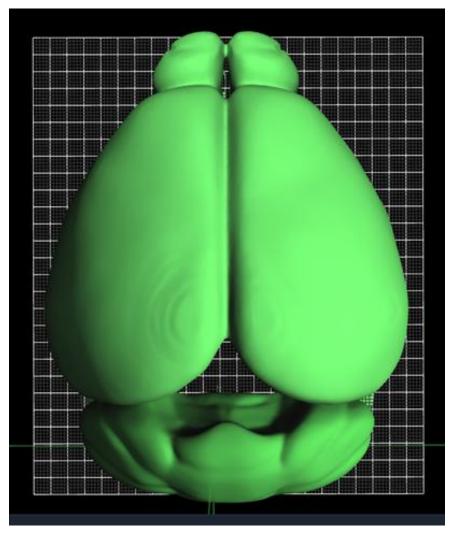


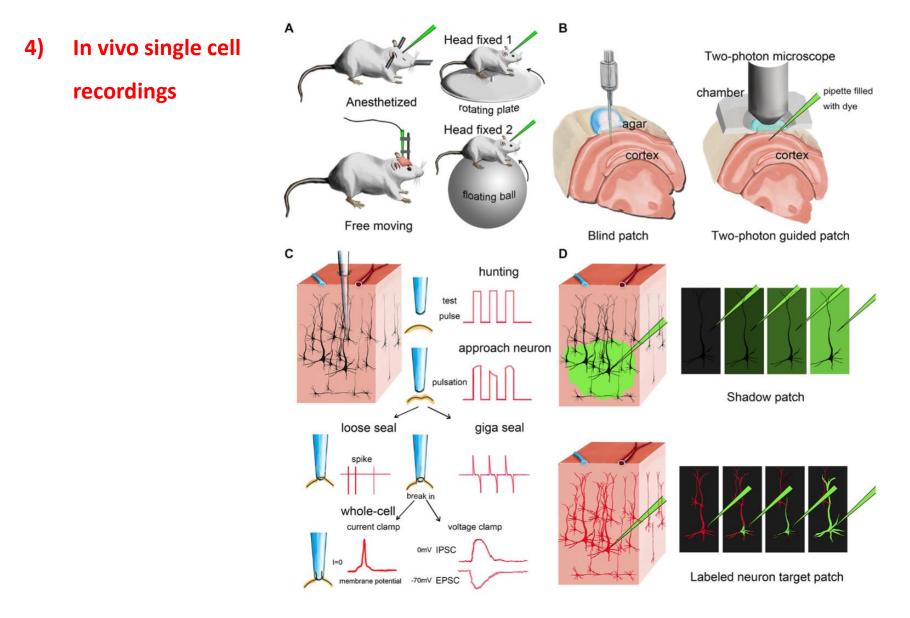
3d Viewer :: Allen Brain Atlas: Mouse Connectivity

Interactive Atlas Viewer :: Atlas Viewer









External solution: artificial cerebrospinal fluid (aCSF)

Salts	Cf (mM)
NaCl	124
KCI	5
NaH_2PO_4	1.25
NaHCO ₃	26
CaCl ₂	2
MgSO ₄	2

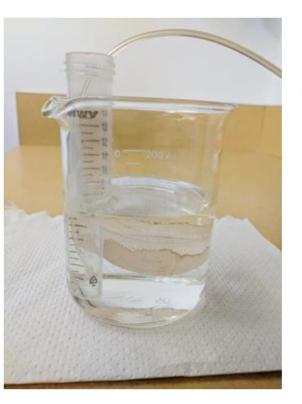
lon	Cf (mM)	
Na ⁺	151	← High Na ⁺
K+	0	← Low / no K ⁺
Cl-	133	← High Cl ⁻
Ca ²⁺	2	
Mg ²⁺	2	
HCO ₃ ⁻	26	(pH buffers)
SO ₄ ²⁻	2	
H ₂ PO ₄ ⁻	1.25	

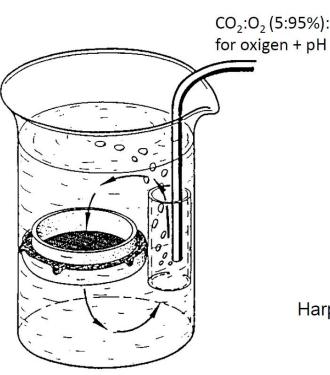
pH = ~7.4 osmolarity = ~300 mOsm

How to measure the osmolarity

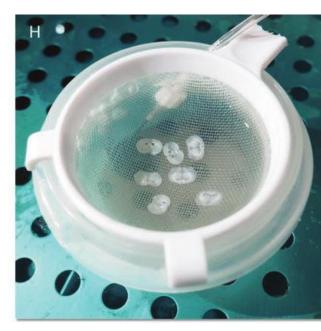


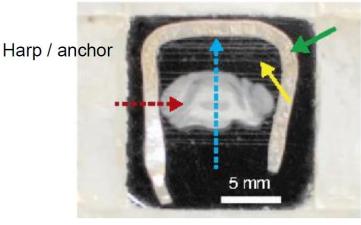
How to maintain acute brain slices





CO₂:O₂ (5:95%):





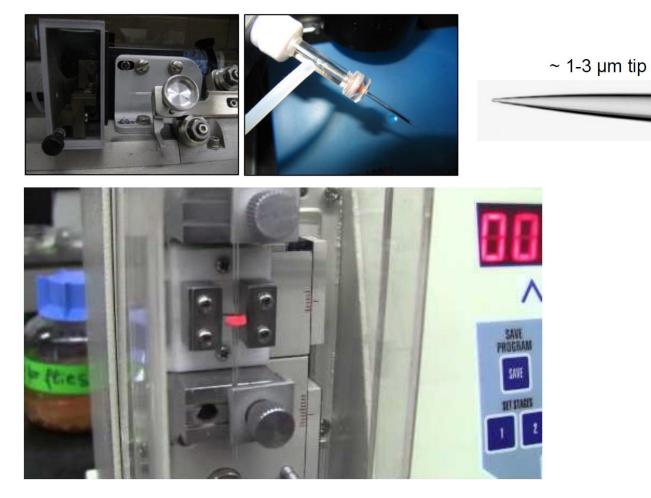
How to pull a patch pipette

Horizontal puller



Vertical puller





How to pull a patch pipette

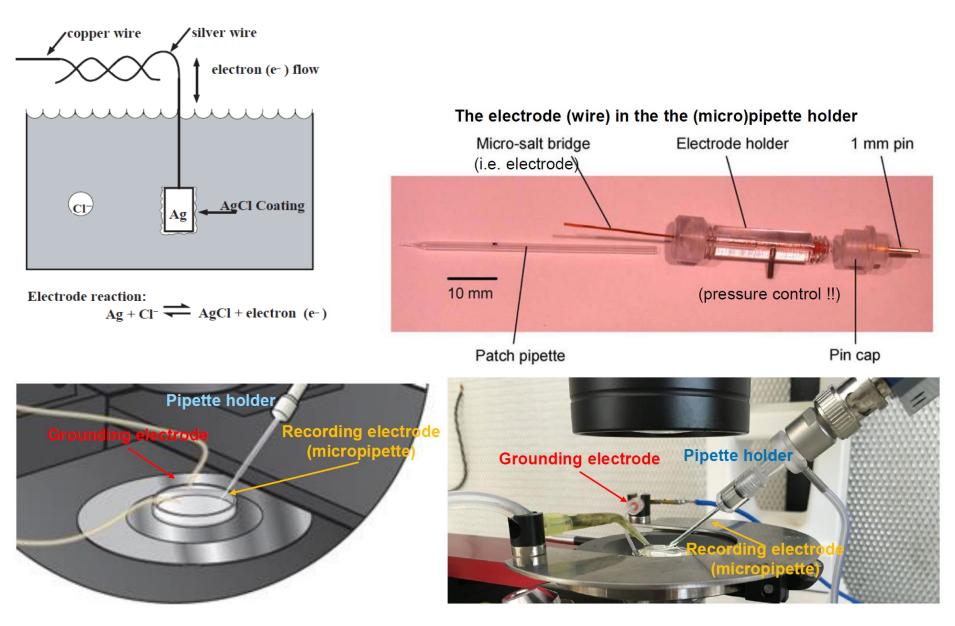


- Regulate T1 for the shape of the pipette
- Regulate T2 for the pipette resistance

Practical Example:

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

The patch-clamp pipette



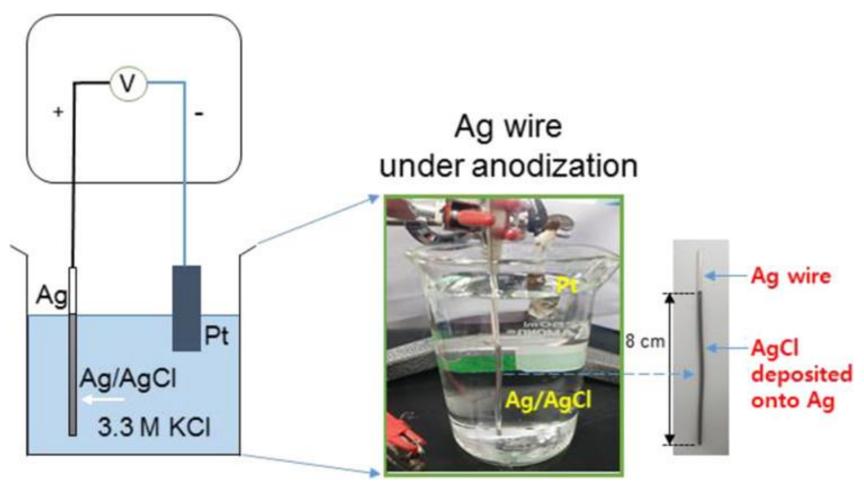
How to prepare patch-clamp electrodes:

yes, you also need to be able to solder



How to chloride an electrode





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

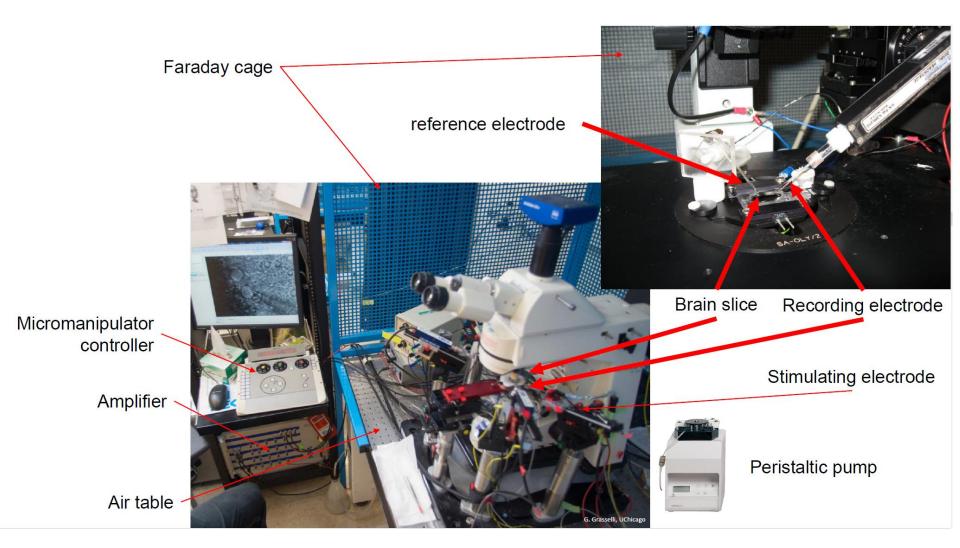
Salts	Cf (mM)
KCI	9
КОН	10
MgCl ₂	3.48
NaCl	4
K-gluconate	120
HEPES	10
Sucrose	17.5
Na ₂ ATP	4
Na ₃ GTP	0.4

pH = 7.25-7.35

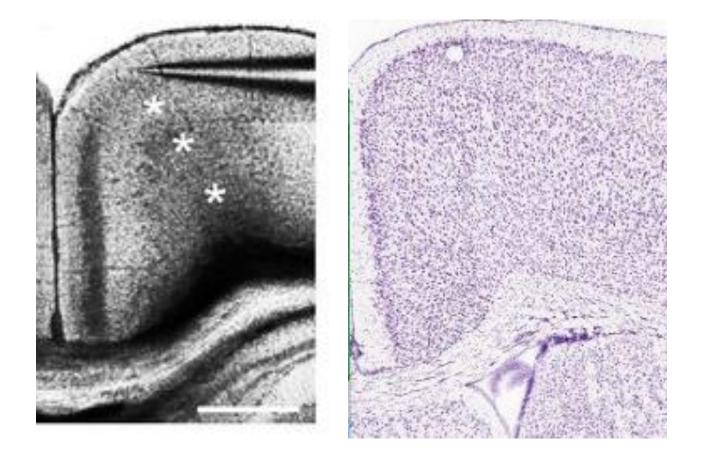
osmolarity = 295-305 mOsm (10-20 mOsm lower than aCSF)

lon	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 ₂ PO ₄ ⁻	0	
ATP ²⁻	4	energy
GTP ³⁻	0.4	energy
Sucrose	17.5	osmolarity

Patch-clamp electrophysiology set-up



Observing brain slice at the microscope and identifying the cortical layers



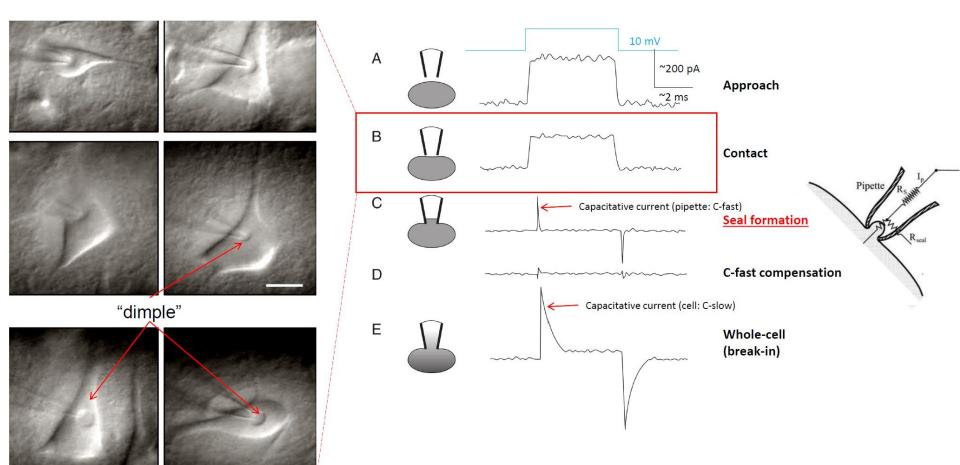
Patch-clamp electrophysiology acquisition program

🔜 PatchMaster

– 🗗 🗙

File Edit Windows Replay Display Buffer Notebook Protocols EPC10 USB Help EPC10_USB Amplifier - 0 23 Replay 🛃 5 / Evoked_CCfix 23 Monitor Tunina Show All (Comment) Label show Group to Trace trac 1 2 Measure)(Scan (Freeze) Wipe Repaint Gain V-membrane Show Mark Unmark OverI.Swp 1 mV/pA 0.0 mV 1 🗸 🏠 OverI.Ser E-1 ✓ Evoked CC 1 1-~~ 0.00 A 0 mV Evoked CC 2 Trace 1 I-mon V-mon R-memb Dig. Filter SETUP SEAL WHOLE-CELL Off Input ADC **Recording Mode** 🔜 Pulse Generator File: DefPgf Imon2 Whole Cell Full View Condensed View Cartoon View Test Pulse Ooff show both double Amplitude Length Chart Ramp 4 Continuous **6** (CC Inject 2 (3 5 10.0 mV 5.0 ms () noise Pool LOAD MERGE SAVE IV MOVE Name NEW COPY DELETE LIST Auto)(Track) LJ 0.0mV **Vo** 0.0mV Interactive Mode O Gap Free Mode Read only EXECUTE 0.00 pF Timina No wait before 1. Sv Not Triggered 🗠 🗛 🔁 C-fast No of Sweeps 10 Use Durations 0.00 us 3500 pts Total 70.00 ms Sweep Length StartSeg 0 Sweep Interval Stored 70.00 ms 14000 bytes Off 20.0 µs (50.0kHz StartTime Range Sample Interval 0.00 Stimulus 70.00 ms 3500 pts 1.00 pF C-slow DA Stimulus -> DA Unit Link Compr. Unit Leak AD Points Store Zero Leak Ch-1 Stim-DA V StimScale Imon2 Α 1 3500 \otimes 1 No Lea 5.0 MOhm 🗠 (Auto 🗈 R-series Ch-2 Vmon V 3500 \otimes 0 No Lea ute voltad 1 Of Off Auto Rs Comp off ---absolute voltag l No Lea off ---absolute voltac off -- No Les Off Prediction Segments 00 2 Stored 1 Stored 3 Stored Common Timino 2.9 kHz l Bessel Filter2 Constant Segment Class Constant Constant Constant Voltage [mV] -60 Voltage Clamp holdV-memb holdV-memb X: 400, ms Y: 40.0 mV 10.00 Duration [ms] 50.00 10.00 V-incr. Mode Increase Increase Incr V-fact./incr. [mV] 1.00 10 Analysis: (Edit Notebook 12-Dec-2021 23 t-incr. Mode IV t-fact./incr. [ms] "D:\Carmela\2020\January2020CC+apamin\21.01.20KOP22XF4| Rel X-seg 2 Existing File opened read-only: Rel Y-seg 2 "D:\Carmela\2020\January2020CC+apamin\21.01.20KOP22XF4| Draw: Active Channel, all Sweeps Delay: DA 0.00 s AD 0.00 s V-membrane [mV] (display) Existing File opened read-only: -60 Set Last Seq. Amplitude "D:\Carmela\2018\2February2018CClong+Apa\28-02-18 WT P| • • Control Window - 0 10.0mV idle 22:39:14 00:08:54 Store (Break Stop Next Wait (Res 5.00ms 1 Comment Average p1 p2 p3 p4 p5 p6 p7 p8 p9 p10 Hinf 9(Time Expan 10) LTP LockIn 12 PGF 8 Tails 111 12 TestSeries 100.00m 0.0000 100.00m 90.000m 10.000m 0.0000 0.0000 0.0000 0.0000 45.000m Protocol 2 Example2 3 Link Buffer 5 SETUP SEAL 1 =xamnl1 Traces 2

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