

Advanced Electrophysiology

Lab 1

27 February 2026

Acute brain slice preparation

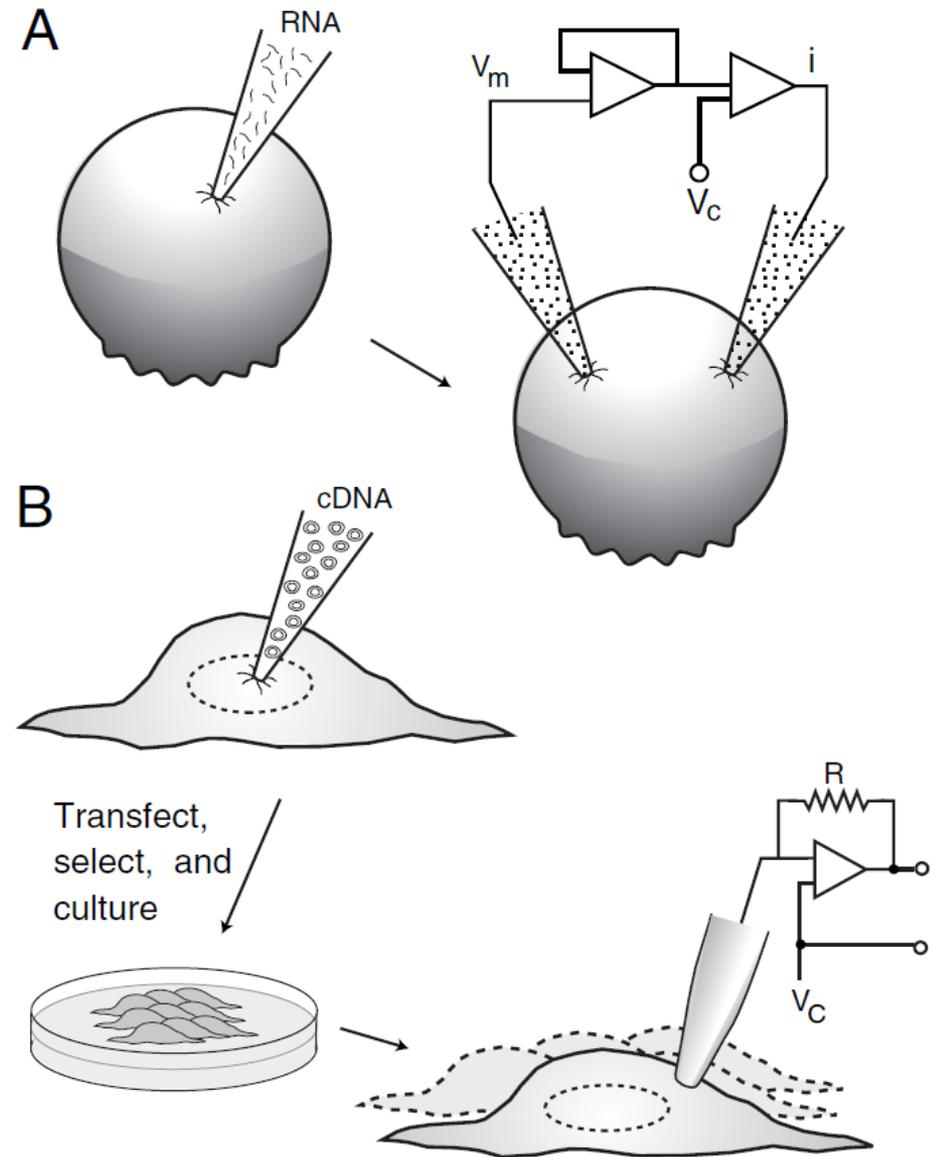
Preparations for single cell electrophysiology

1) Heterologous expression systems

A. *Xenopus* oocytes

B. Cell lines

→ Investigate the function of ion channels in isolation



Preparations for single cell electrophysiology

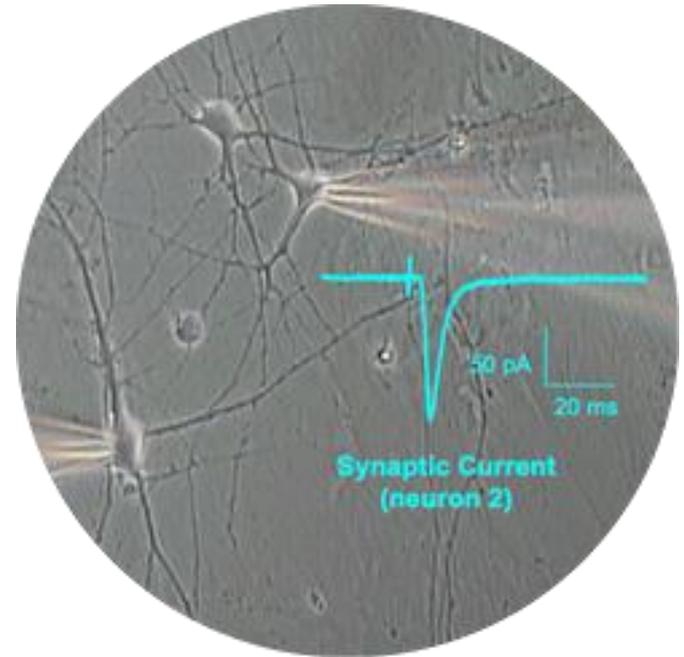
2) Primary neuronal cultures

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



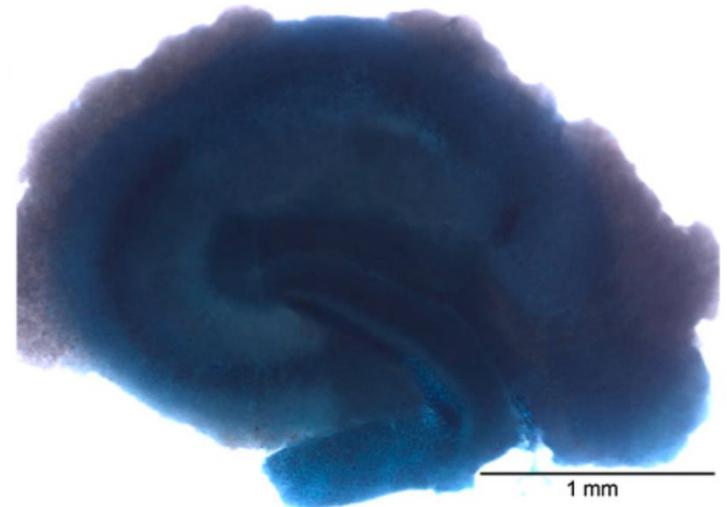
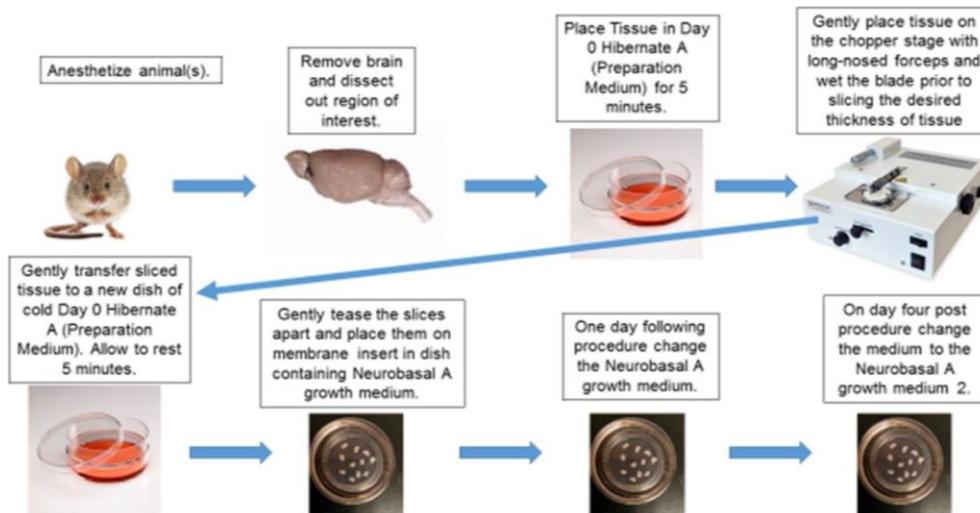
Preparations for single cell electrophysiology

3) Brain slices

A. Organotypic brain slices

B. Acute brain slices

Example of hippocampal organotypic slice

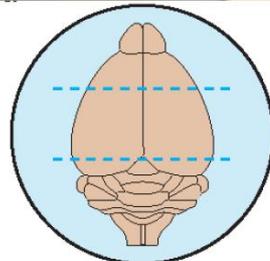
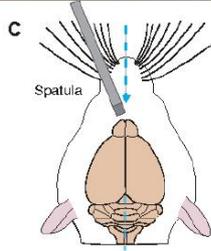
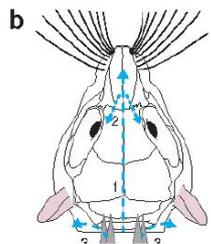


Preparations for single cell electrophysiology

3) Brain slices

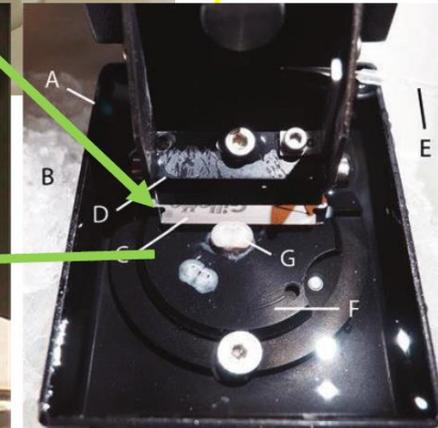
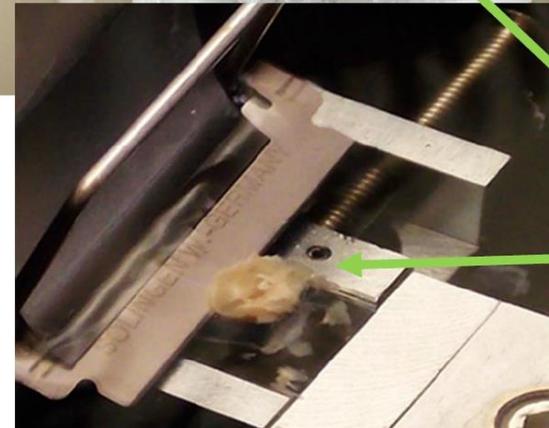
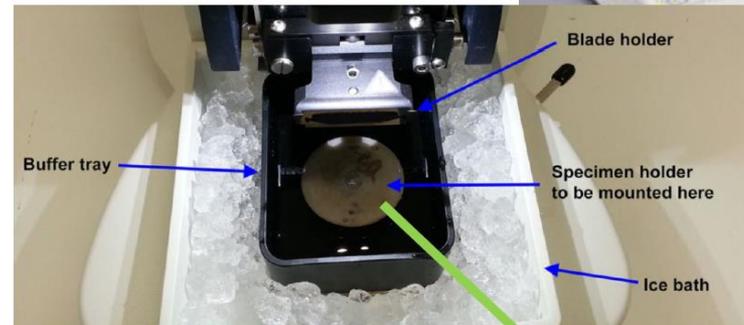
A. Organotypic brain slices

B. Acute brain slices

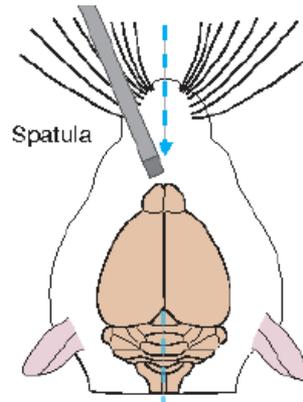
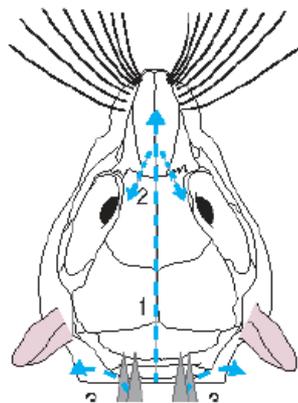


Wang & Baudry, 2019 Bio-Protocol

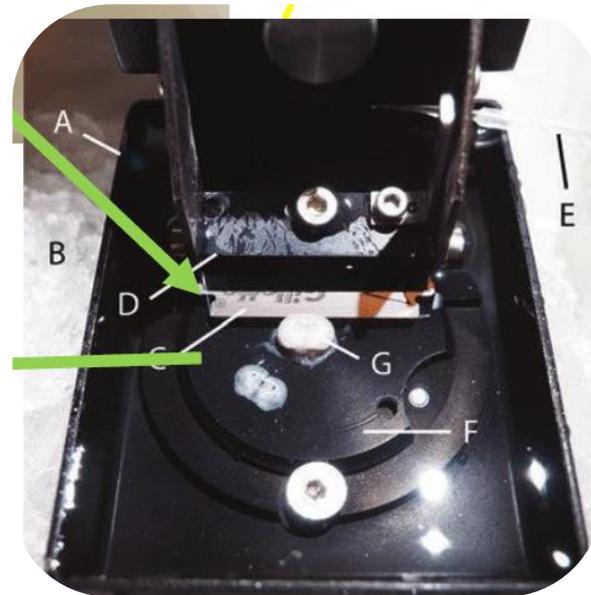
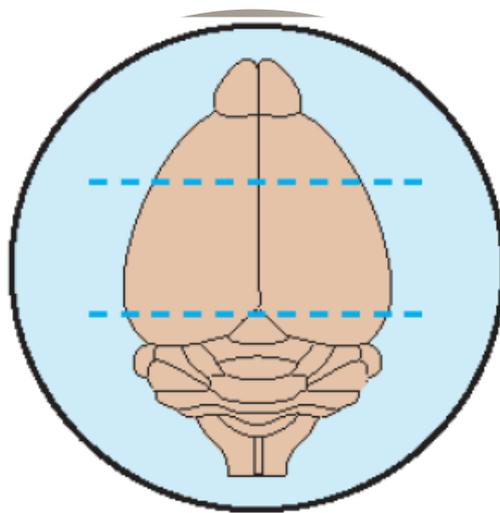
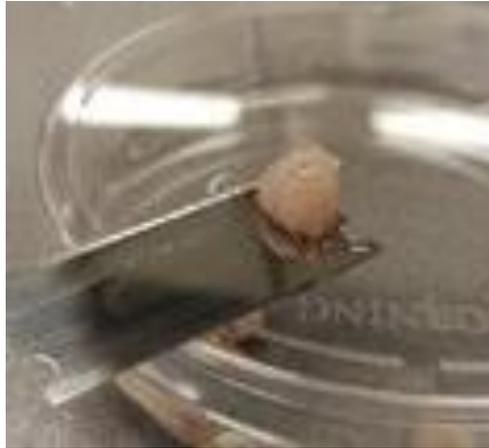
Mishra et al, 2014 Nat Prot



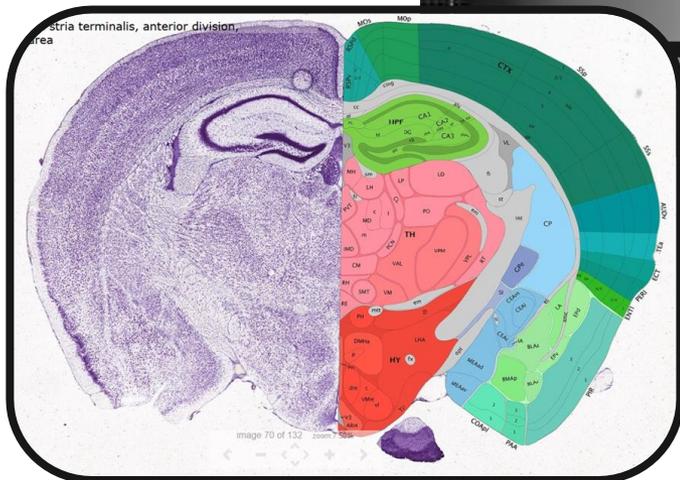
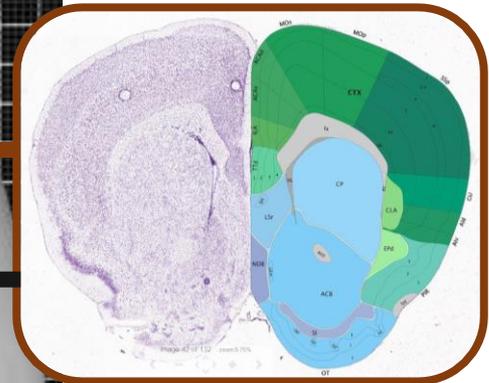
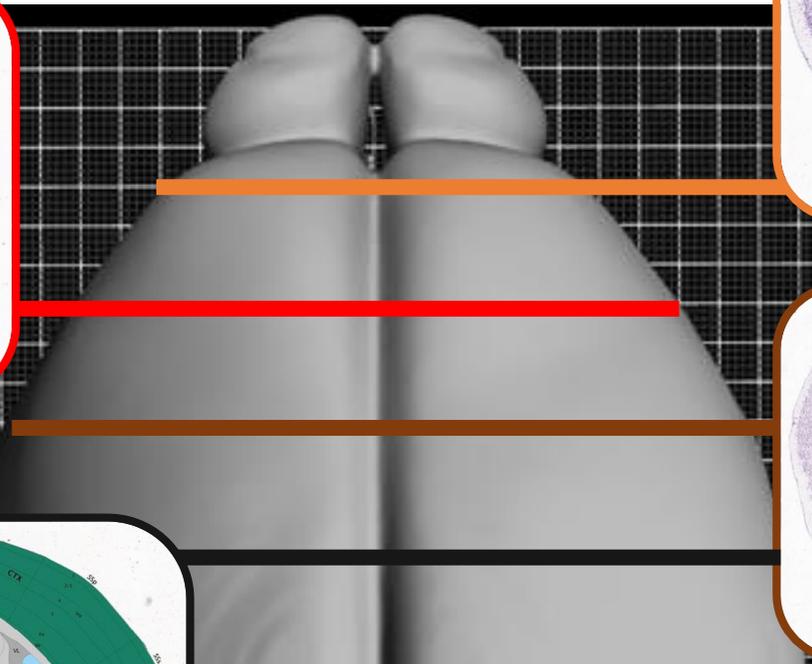
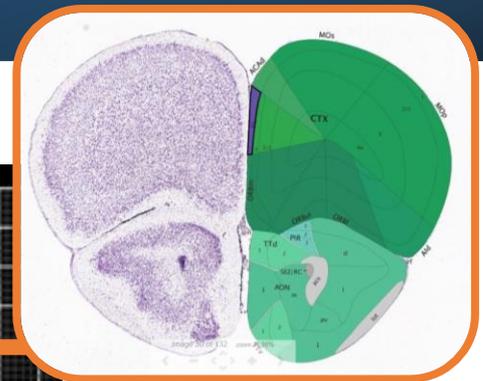
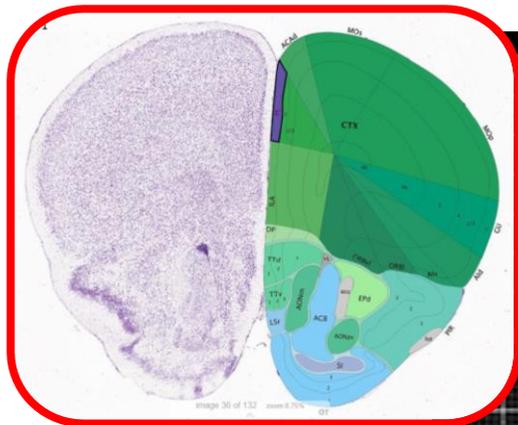
Brain dissection



Brain dissection

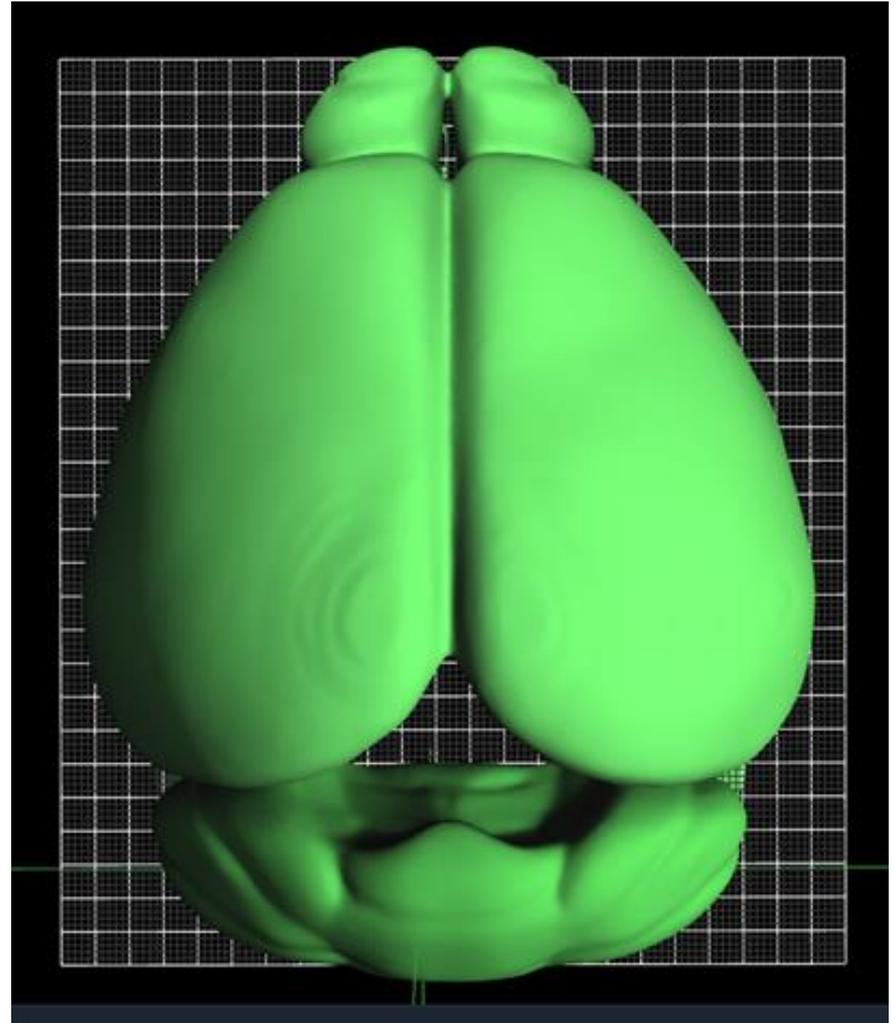
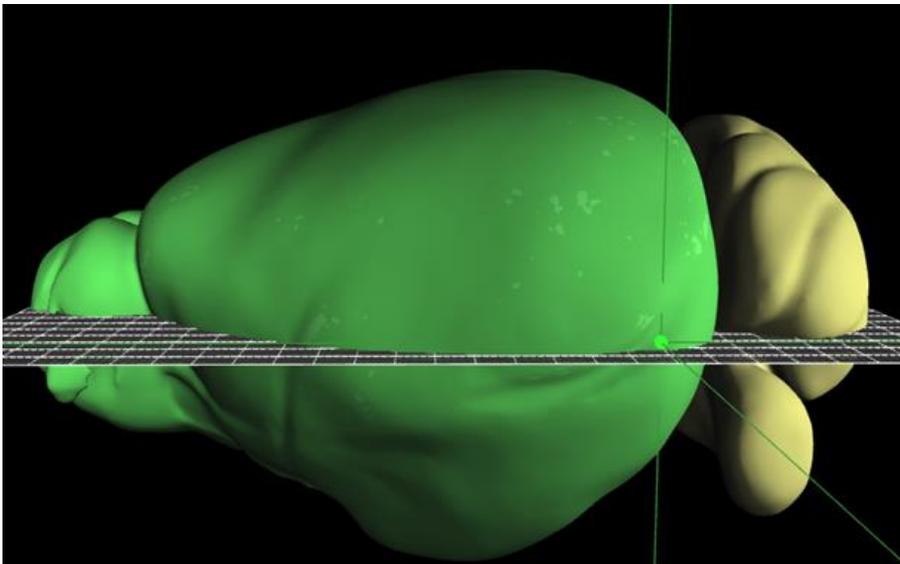
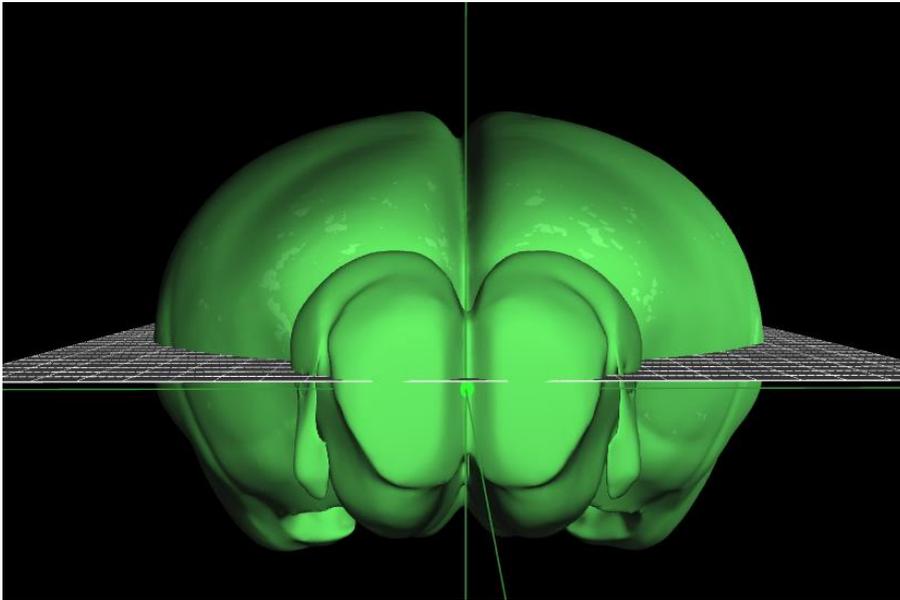


Brain slicing



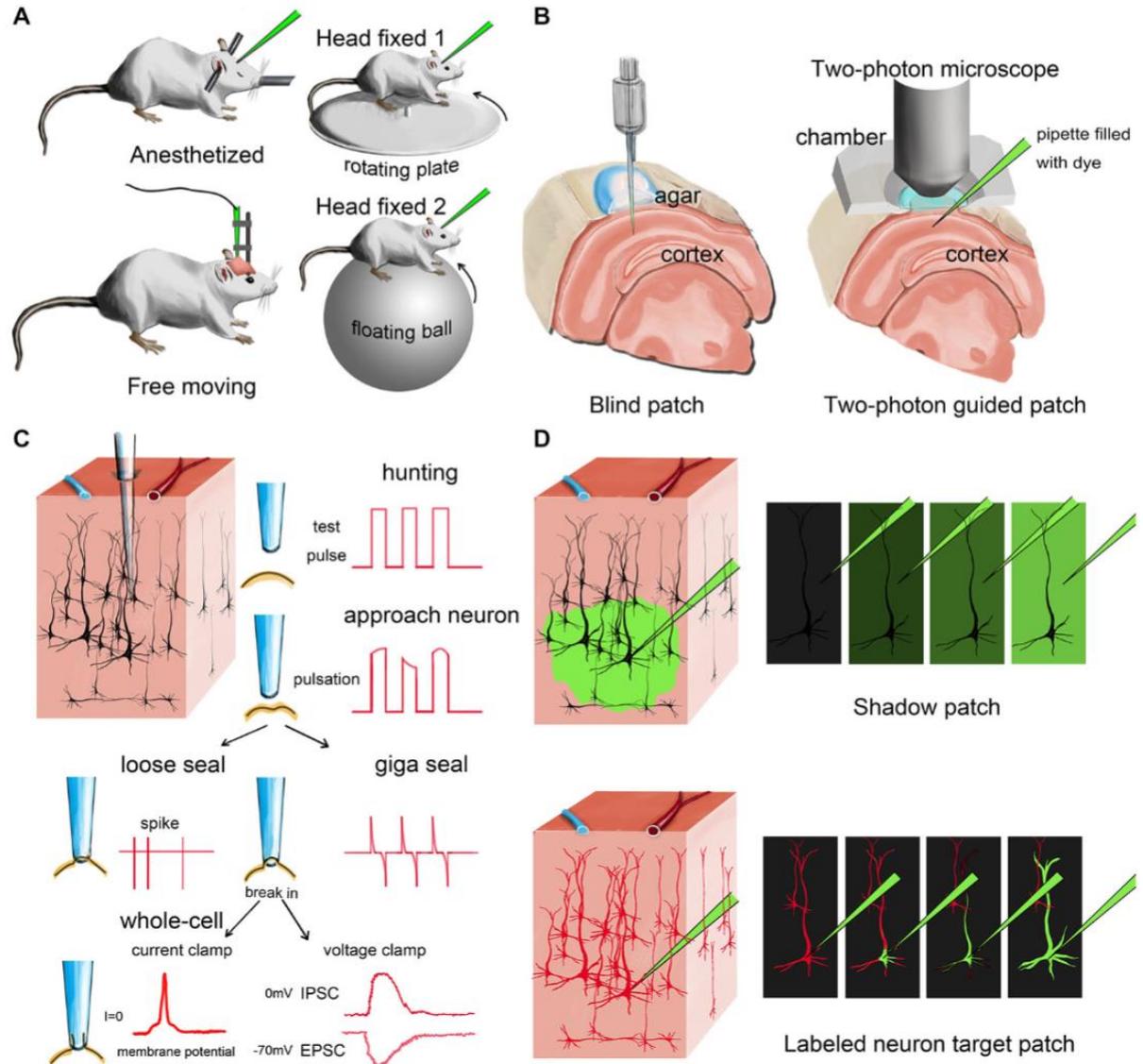
[3d Viewer :: Allen Brain Atlas: Mouse Connectivity](#)

[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 ₂ PO ₄	1.25
NaHCO ₃	26
CaCl ₂	2
MgSO ₄	2

Ion	Cf (mM)
Na ⁺	151
K ⁺	0
Cl ⁻	133
Ca ²⁺	2
Mg ²⁺	2
HCO ₃ ⁻	26
SO ₄ ²⁻	2
H ₂ PO ₄ ⁻	1.25

← High Na⁺

← Low / no K⁺

← High Cl⁻

(pH buffers)

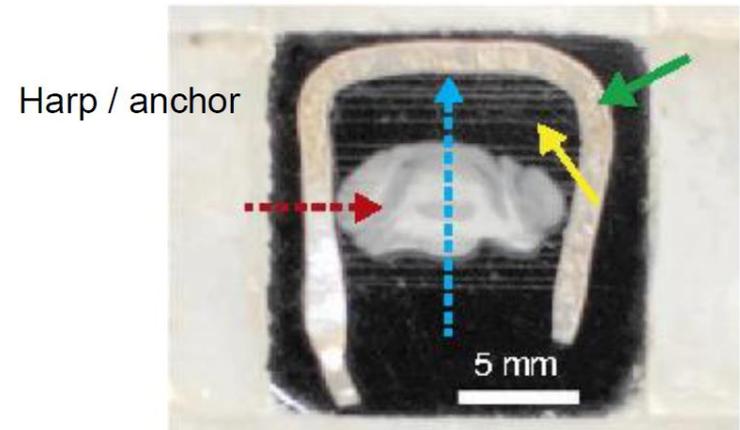
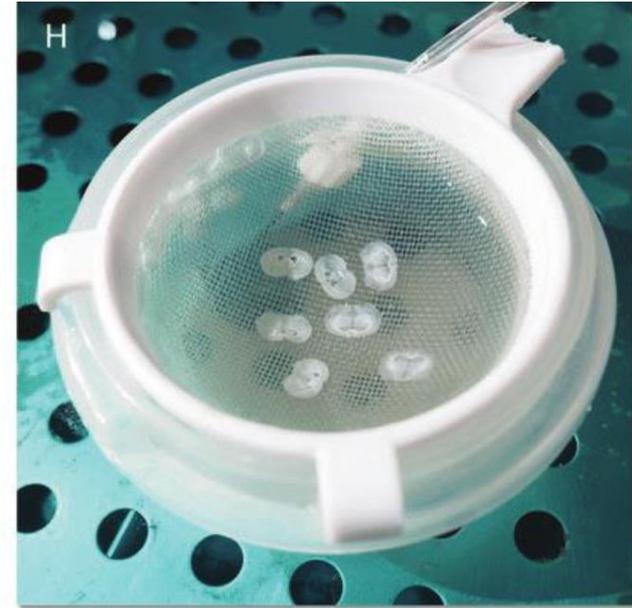
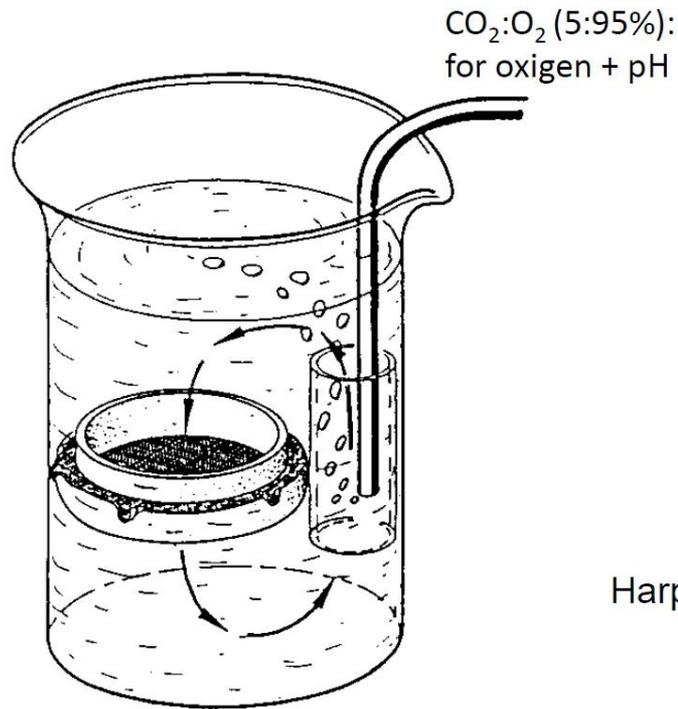
pH = ~7.4

osmolarity = ~300 mOsm

How to measure the osmolarity

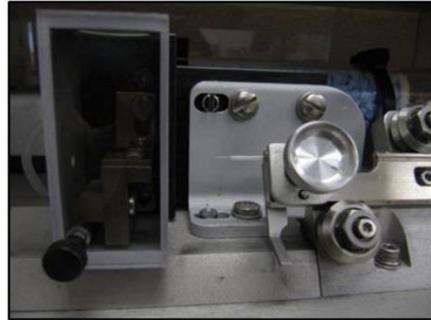


How to maintain acute brain slices



How to pull a patch pipette

Horizontal puller



~ 1-3 μm tip



Vertical puller



How to pull a patch pipette

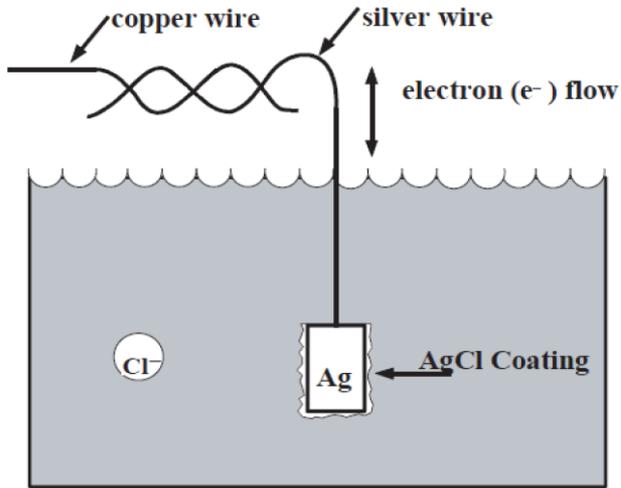


- Regulate T1 for the shape of the pipette
- Regulate T2 for the pipette resistance

Practical Example:

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

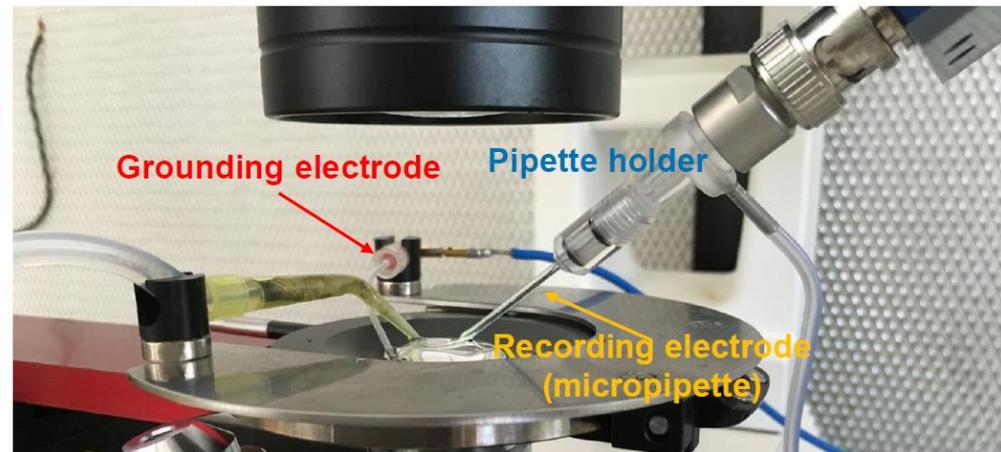
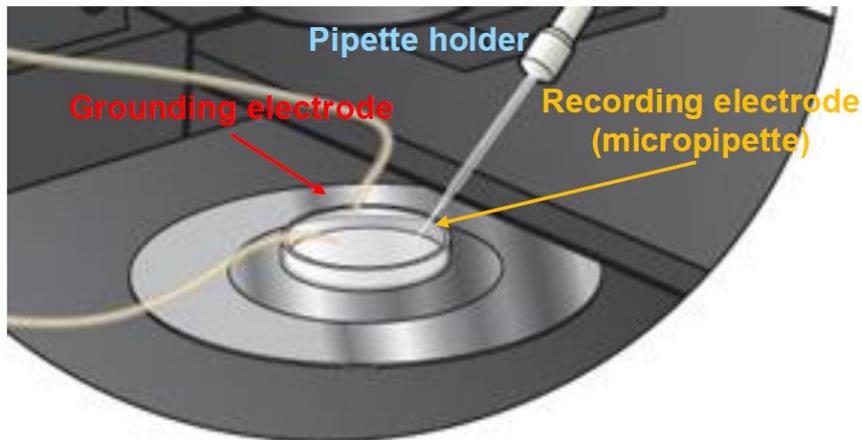
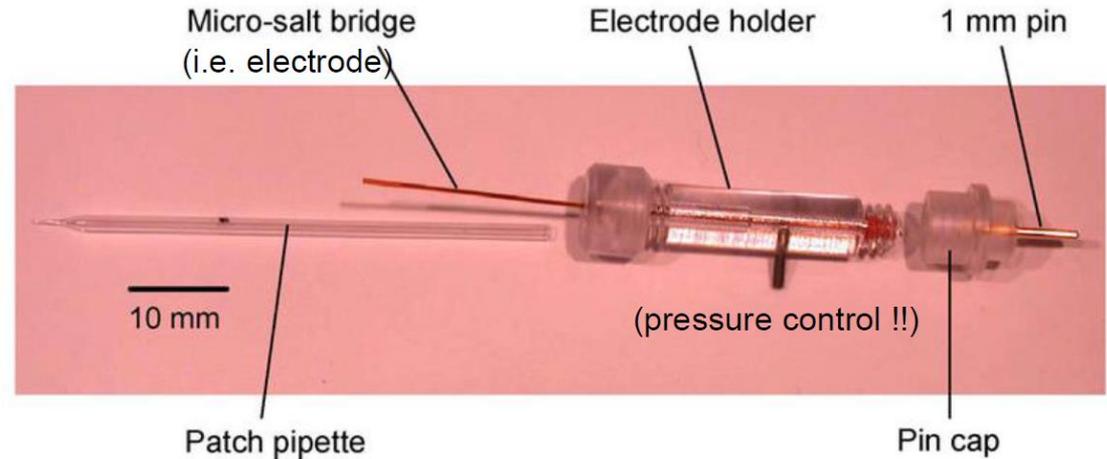
The patch-clamp pipette



Electrode reaction:



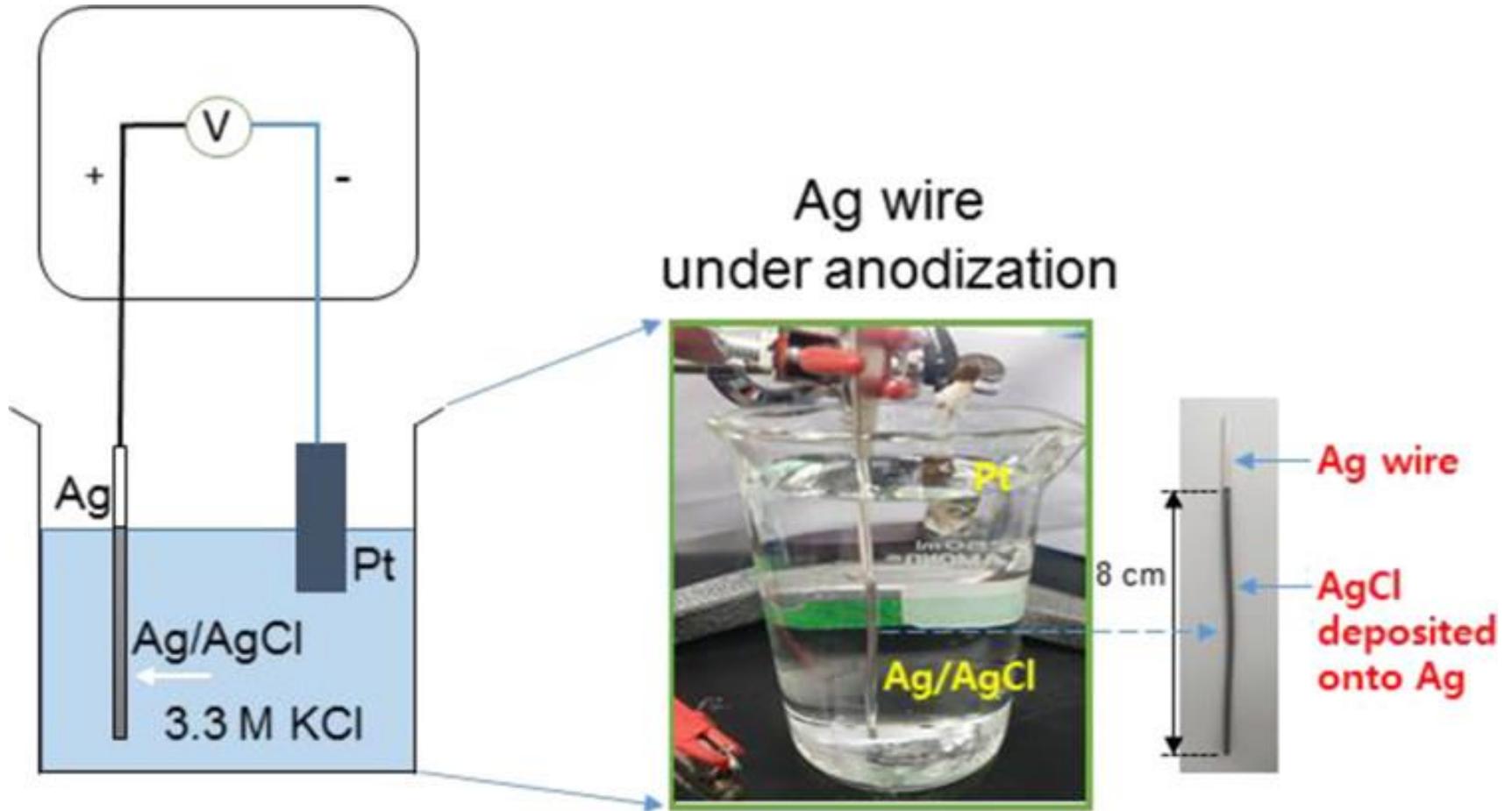
The electrode (wire) in the the (micro)pipette holder



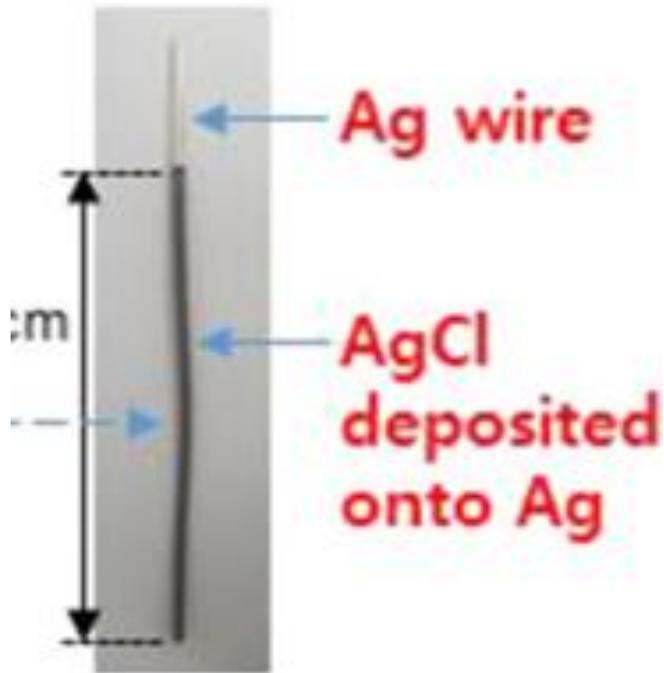
How to prepare patch-clamp electrodes: yes, you also need to be able to solder



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

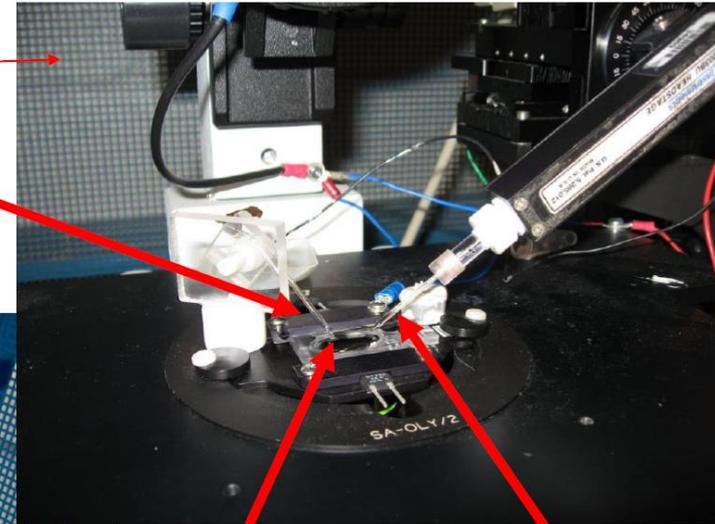
pH = 7.25-7.35

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

Patch-clamp electrophysiology set-up

Faraday cage

reference electrode



Brain slice

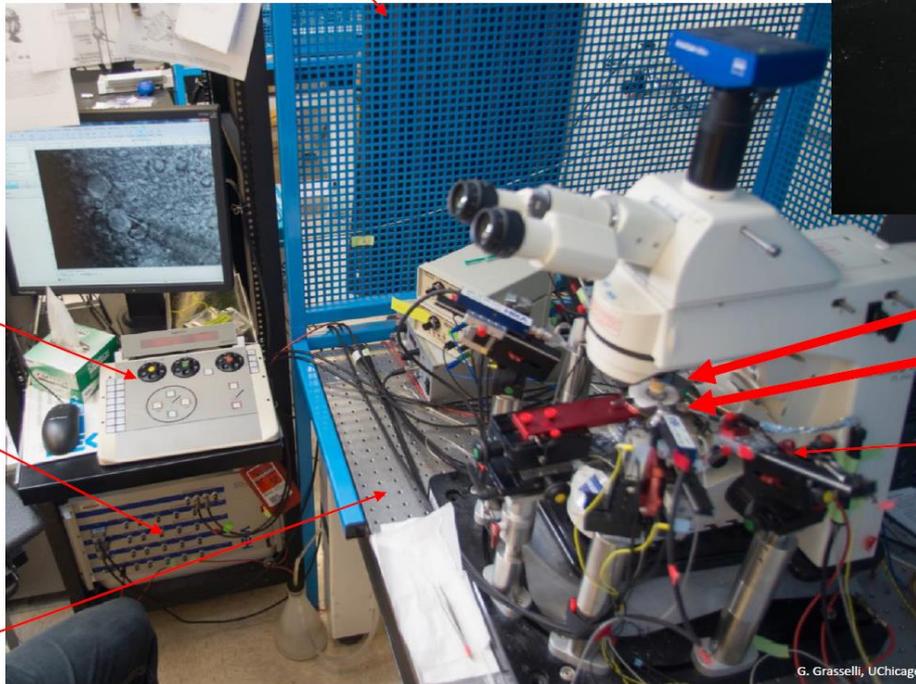
Recording electrode

Micromanipulator controller

Amplifier

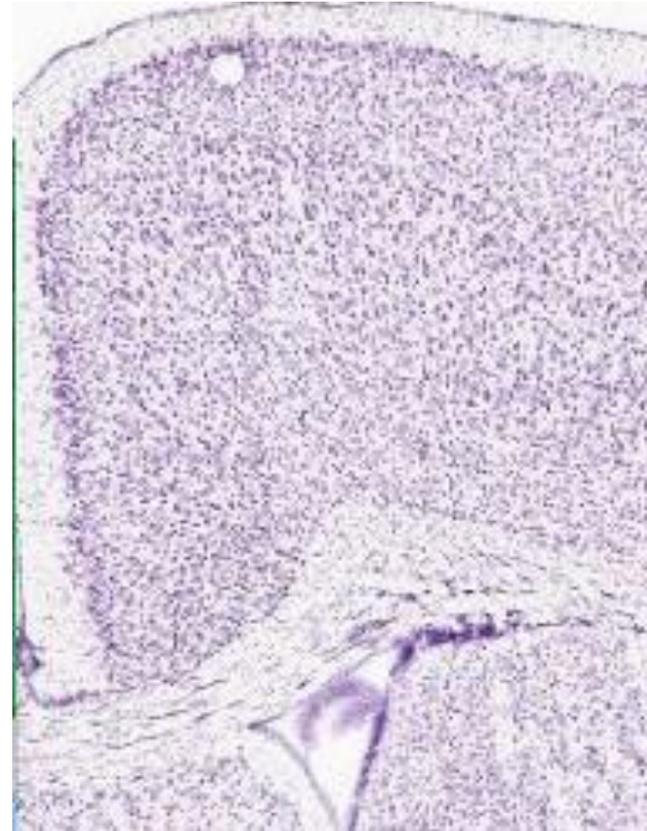
Stimulating electrode

Air table



Peristaltic pump

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

Monitor Tuning Show All

Gain 1 mV/pA V-membrane 0.0 mV

0.00 A 0 mV ---

I-mon V-mon R-memb

SETUP SEAL WHOLE-CELL

Input ADC Recording Mode

Imon2 Whole Cell

Test Pulse show both

double Amplitude Length

10.0 mV 5.0 ms

LJ 0.0mV Vo 0.0mV Auto Track

C-fast 0.00 pF AutoE

0.00 μ s

Range Off

C-slow 1.00 pF

R-series 5.0 MOhm AutoE

Rs Comp Off Off Auto

Off Prediction Off

Filter2 I_Bessel 2.9 kHz

5 / Evoked_CCfix

Measure Scan Freeze Wipe Repaint

Overl.Swp Overl.Ser

Trace 1 Dig. Filter Off

Replay

Comment Label show Group to Trace trace

Show Mark Unmark Mark All

E-1 1 Evoked CC 1 1

Evoked CC 2 1

2 2

3 3

Pulse Generator File: DefPgf

Full View Condensed View Cartoon View

1 IV 2 Chart 3 Ramp 4 Continuous 5 CC 6 CC Inject

Pool LOAD MERGE SAVE Name IV NEW COPY MOVE DELETE LIST

Interactive Mode Gap Free Mode

Timing No wait before 1. Sweep Not Triggered

No of Sweeps 10 Use Durations

Sweep Interval 0.00 s StartSeg 0

Sample Interval 20.0 μ s (50.0kHz) StartTime 0.00

Read only EXECUTE

Sweep Length Total Stored Stimulus

70.00 ms 3500 pts

70.00 ms 14000 bytes

70.00 ms 3500 pts

1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points	Store	Zero	Leak
Ch-1	Stim-DA	V	StimScale	<input type="checkbox"/>	Imon2	A	1	1	3500	<input type="checkbox"/>	1	No Leak
Ch-2	off	V	absolute voltage	<input type="checkbox"/>	Vmon	V	1	1	3500	<input type="checkbox"/>	0	No Leak
---	off		absolute voltage	<input type="checkbox"/>	off	---	---	---	---	<input type="checkbox"/>	---	No Leak
---	off		absolute voltage	<input type="checkbox"/>	off	---	---	---	---	<input type="checkbox"/>	---	No Leak

Segments

1	Stored	2	Stored	3	Stored	4	Not Store	5	6	Common Timing
Segment Class	Constant	Constant	Constant	Constant	Constant	Constant	Constant	Constant	Constant	
Voltage [mV]	hold V-memb	val -60	hold V-memb	val ---	val ---	val ---	val ---	val ---	val ---	Voltage Clamp
Duration [ms]	val 10.00	val 50.00	val 10.00	val ---	val ---	val ---	val ---	val ---	val ---	
V-incr. Mode	Increase	Increase	Increase	Increase	Increase	Increase	Increase	Increase	Increase	
V-fact./incr. [mV]	1.00	0	1.00	10	1.00	0	---	---	---	
t-incr. Mode	Increase	Increase	Increase	Increase	Increase	Increase	Increase	Increase	Increase	
t-fact./incr. [ms]	1.00	0.00	1.00	0.00	1.00	0.00	---	---	---	

Analysis: Edit

Rel X-seg 2

Rel Y-seg 2

Draw: Active Channel, all Sweeps Delay: DA 0.00 s AD 0.00 s

V-membrane [mV] (display)

-60 Set Last Seg. Amplitude

10.0mV

5.00ms

p1	p2	p3	p4	p5	p6	p7	p8	p9	p10
100.00m	0.0000	100.00m	90.000m	10.000m	45.000m	0.0000	0.0000	0.0000	0.0000

Traces 2

Notebook_12-Dec-2021

"D:\Carmela\2020\January2020CC+apamin\21.01.20KOP22XF4 |

Existing File opened read-only:

"D:\Carmela\2020\January2020CC+apamin\21.01.20KOP22XF4 |

Existing File opened read-only:

"D:\Carmela\2018\2February2018CClong+Apa\28-02-18 WT P |

Control Window

idle 22:39:14 00:08:54 Set Store Break Stop Next Wait Res

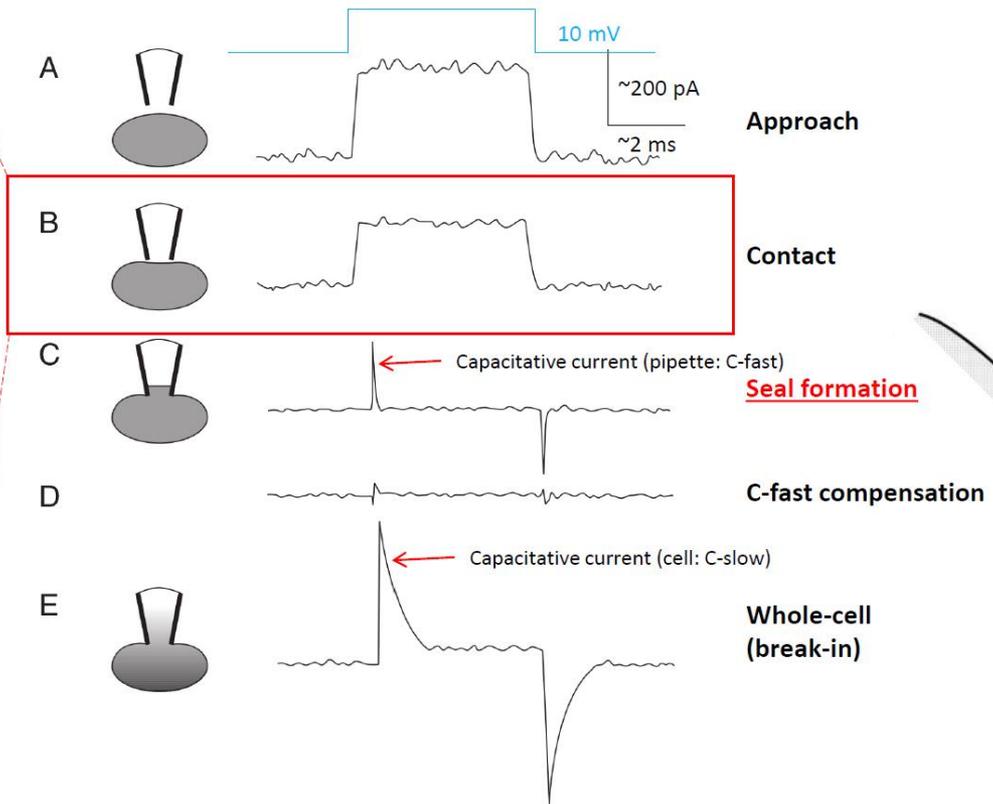
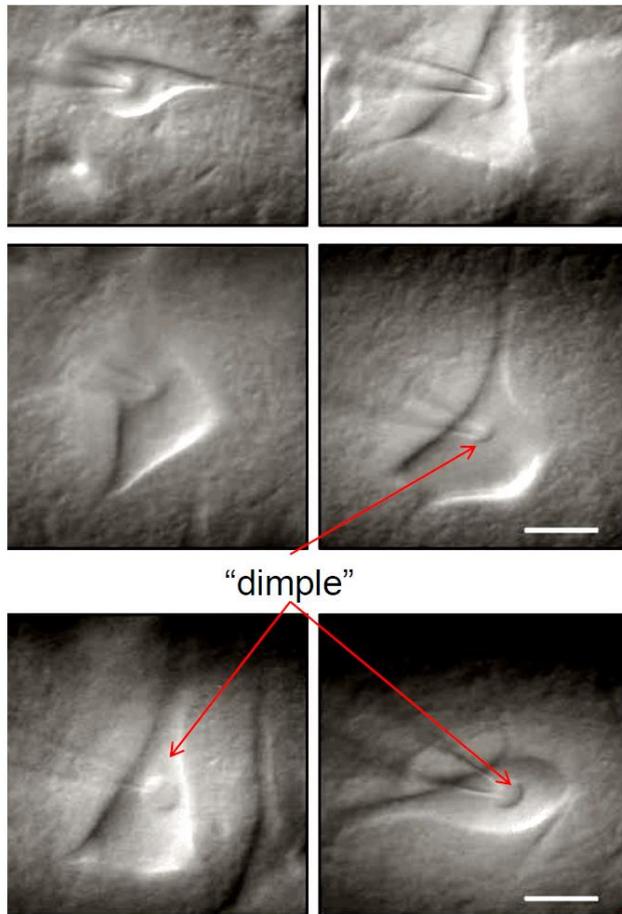
Comment

Average 1

PGF 7 Hinf 8 Tails 9 Time Expar 10 LTP 11 LockIn 12 TestSeries 12

Protocol 1 Examp1 2 Example2 3 Link 4 Buffer 5 SETUP 6 SEAL 1

The formation of a giga-seal



Learning objectives

1. To pull patch-clamp electrodes
2. To coat patch-clamp 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