UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Department of Neurobiology and Developmental Sciences
Center for Translational Neuroscience
College of Medicine

Neuronal Signals - NBDS 5161
Session 3: Single Cell Recordings

Abdallah HAYAR

Lectures can be downloaded from
http://hayar.net/NBDS5161

Updated Tentative Schedule for Neuronal Signals (NBDS 5161)
One Credit–Hour, Summer 2010
Location: Biomedical Research Building II, 6th floor, conference room,
Time: 9:00 -10:20 am

Session	Day	Date	Topic	Instructor
1	Tue	6/1	Design of an electrophysiology setup	Hayar
2	Thu	6/3	Neural population recordings	Hayar
3	Thu	6/10	Single cell recordings	Hayar
4	Fri	6/11	Analyzing synaptic activity	Hayar
5	Mon	6/14	Data acquisition and analysis	Hayar
6	Wed	6/16	Analyzing and plotting data using OriginLab	Hayar
7	Fri	6/18	Detecting electrophysiological events	Hayar
8	Mon	6/21	Writing algorithms in OriginLab®	Hayar
9	Wed	6/23	Imaging neuronal activity	Hayar
10	Fri	6/25	Laboratory demonstration of an electrophysiology and imaging experiment	Hayar
11	Fri	7/9	Article presentation I: Electrophysiology	Hayar
12	Mon	7/12	Article presentation II: Imaging	Hayar
13	Wed	7/14	Exam and students' survey about the course	Hayar

Student List

	Name	E-mail	Regular/Auditor	Department	Position
1	Simon, Christen	CSimon@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
2	Kezunovic, Nebojsa	NKezunovic@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
3	Hyde, James R	JRHyde@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
4	Yadlapalli, Krishnapraveen	KYadlapalli@uams.edu	Regular (form signed)	Pediatrics	Research Technologist – Mentor: Dr. Alchaer
5	Pathan, Asif	APATHAN@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – Mentor: Dr. Rusch
6	Kharade, Sujay	SKHARADE@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – 4th year - Mentor: Dr. Rusch
7	Howell, Matthew	MHOWELL2@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Interdisciplinary Toxicology - 3rd year - Mentor: Dr. Gottschall
8	Beck, Paige B	PBBeck@uams.edu	Regular (form signed)	College of Medicine	Medical Student – 2nd Year - Mentor: Dr. Garcia-Rill
9	Atcherson, Samuel R	SRAtcherson@uams.edu	Auditor (form signed)	Audiology & Speech Pathology	Assistant Professor
10	Detweiler, Neil D	NDDETWEILER@uams.edu	Auditor (form not signed)	Pharmacology & Toxicology	Graduate Pharmacology – 1st year
11	Thakali, Keshari M	KMThakali@uams.edu	Unofficial auditor	Pharmacology & Toxicology	Postdoctoral Fellow – Mentor: Dr. Rusch
12	Boursoulian, Feras	FBoursoulian@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Postdoctoral Fellow – Mentor: Dr. Hayar
13	Steele, James S	JSSTEELE@uams.edu	Unofficial auditor	College of Medicine	Medical Student – 1st Year – Mentor: Dr. Hayar
14	Smith, Kristen M	KMSmith2@uams.edu	Unofficial auditor	Neurobiology & Developmental Sciences	Research Technologist – Mentor: Dr. Garcia-Rill
15	Gruenwald, Konstantin	kjoachimg@gmail.com	Unofficial auditor	Neurobiology & Developmental Sciences	High school Student – Mentor: Dr. Hayar
	Yang, Dong	YangDong@uams.edu	Unable to attend	Pediatrics Pulmonary	Research Assistant – Accepted in Neuroscience

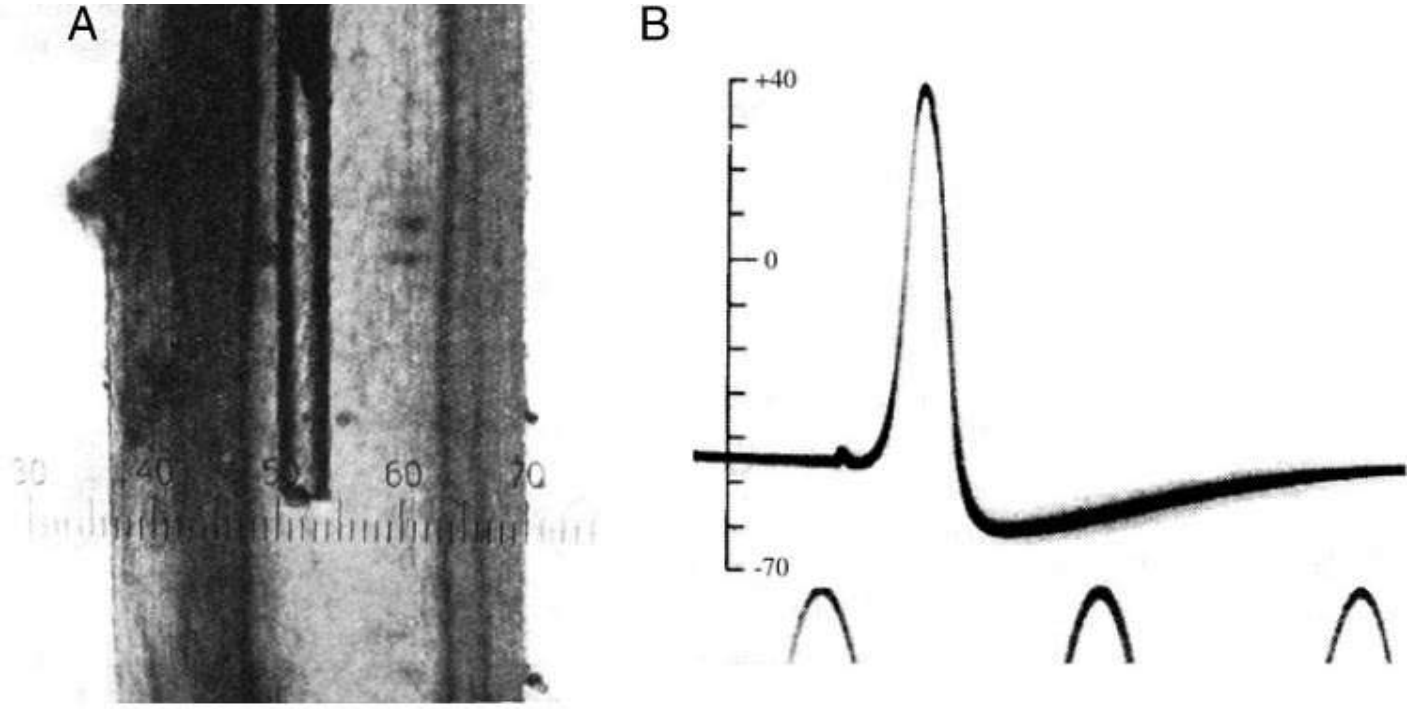
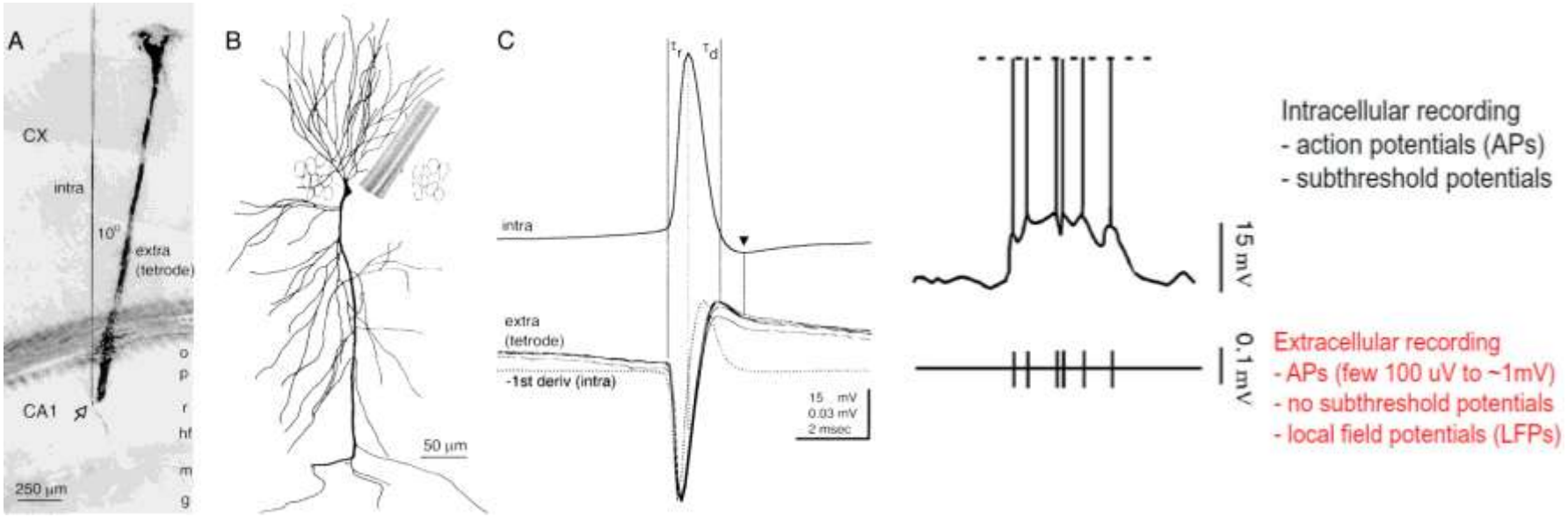


FIGURE 1 Intracellular recording of the membrane potential and action potential generation in the squid giant axon. (A) A glass micropipette, about 100 µm in diameter, was filled with seawater and lowered into the giant axon of the squid after it had been dissected free. The axon is about 1 mm in diameter and is transilluminated from behind. (B) One action potential recorded between the inside and the outside of the axon. Peaks of a sine wave at the bottom provided a scale for timing, with 2 ms between peaks. From Hodgkin and Huxley (1939).

Intracellular vs. Extracellular recordings

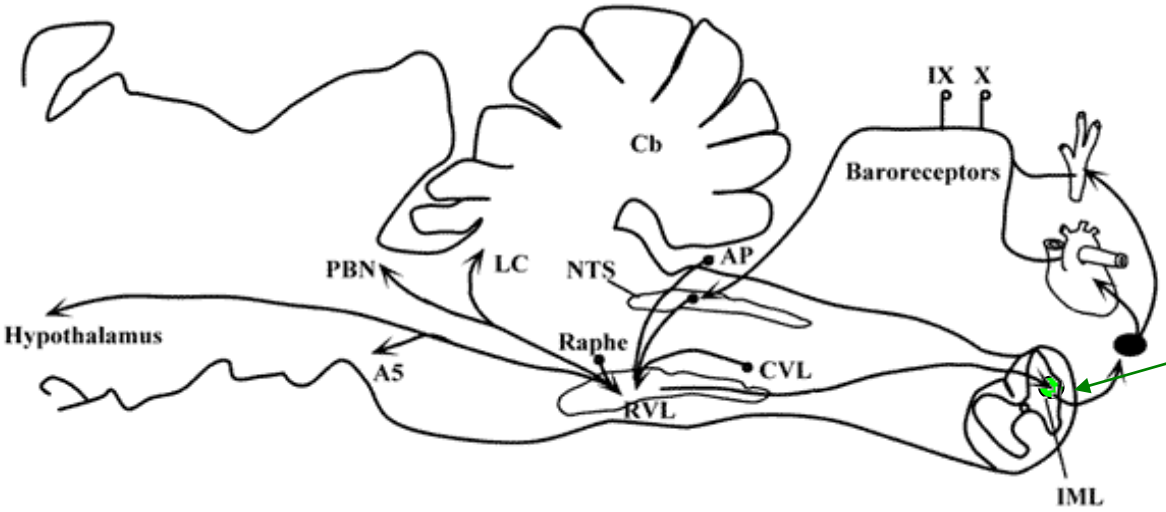


Extracellular waveform is *almost* derivative of intracellular

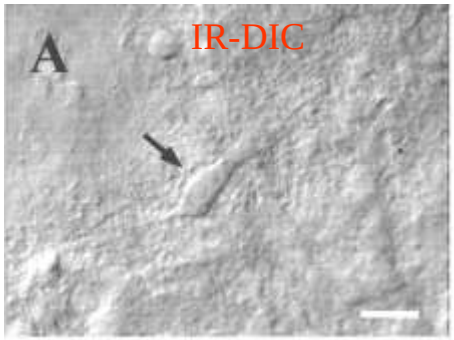
Intracellular recordings: Sharp vs. Patch clamp recording

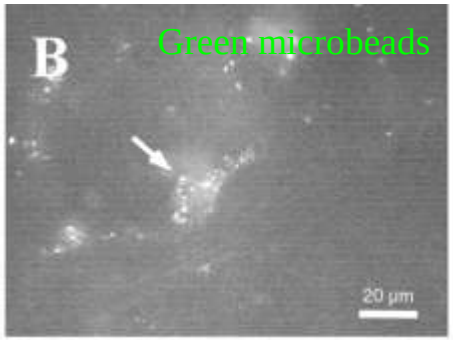
	Sharp 	Patch clamp 
Pipette solution	3000 mM KCl or KAc	Mainly 124 mM Kgluconate or CsMeSO4 +ATP +GTP
Tip Diameter	0.1 µm, sharp enough to impale the cell	1-2 µm, seal with the plasma membrane
Seal with membrane	Leaky seal	GigaOhm tight seal
Stability of recording	Stable with no significant rundown of membrane properties	Rundown of the properties of membrane channel
Dialysis	Excess chloride or acetate leaks into the cell	Intracellular messengers and soluble enzymes will diffuse out of the cell
Membrane resistance	Will appear lower because of leak: ex 30 MOhm	Accurate measurement ex 300 MOhm
Electrode resistance	60-120 MOhm	3-8 MOhm
Space clamp	Cannot do adequate voltage clamping, better for current clamp	Much better access to cell, can record in voltage clamp
Pharmacology	Drugs or fluorescent dyes diffuse very slowly into cell	Can block potassium channel by intracellular cesium and sodium channel by QX-314

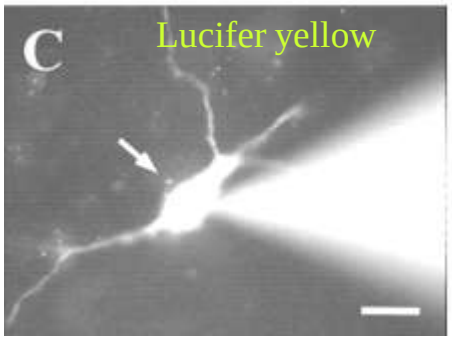
Combining anatomy and immunocytochemistry with patch-clamping

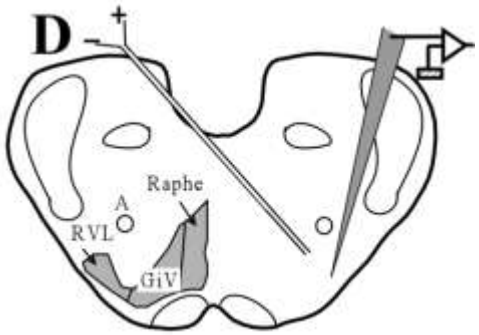


Micro-beads injection



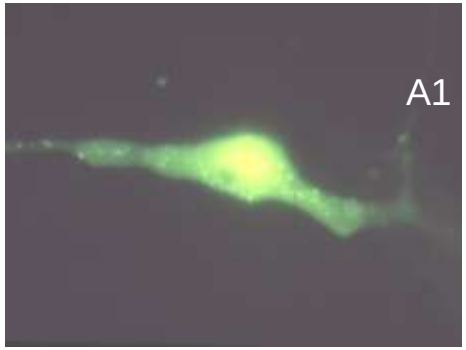


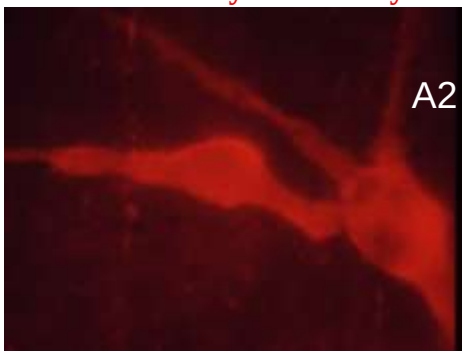


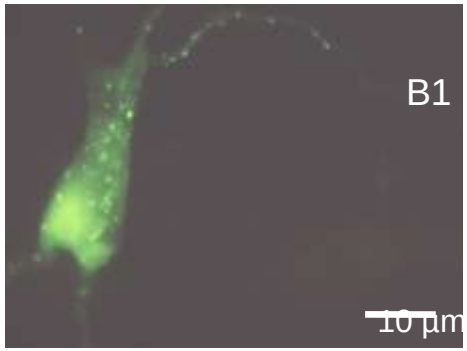


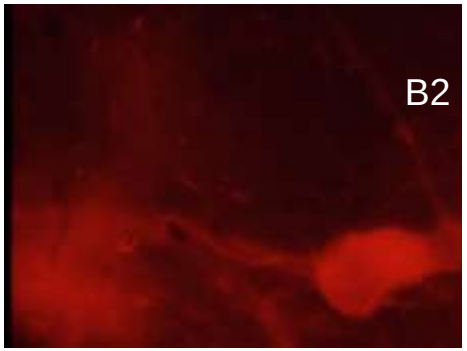
Microbeads and Lucifer Yellow

Tyrosine hydroxylase-immunocytochemistry







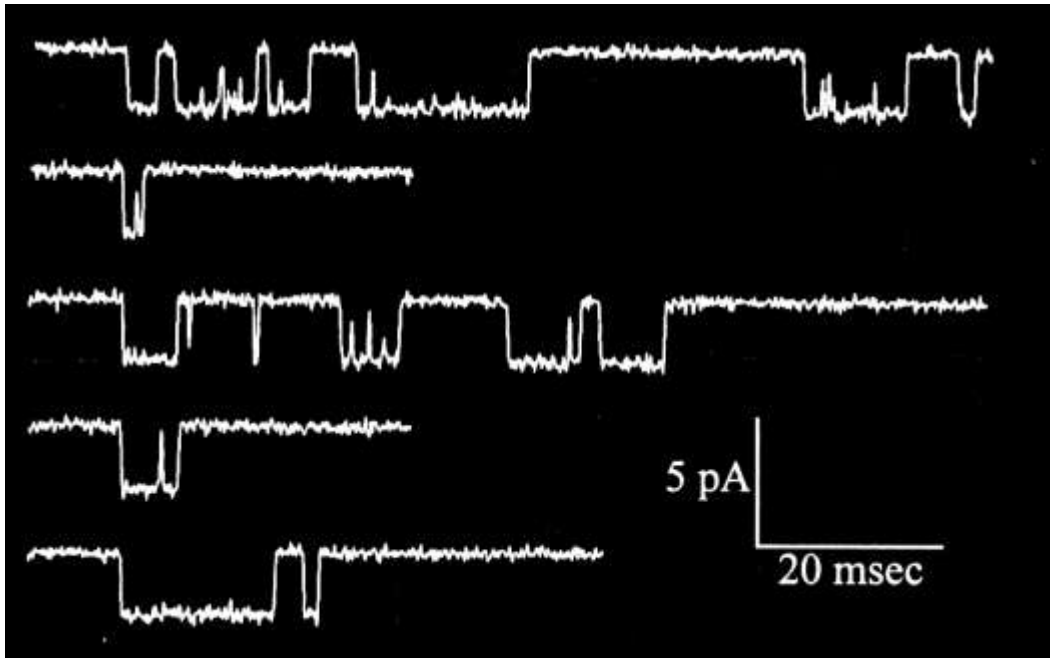


Historical Background

Neher and Sakmann

Ion channels

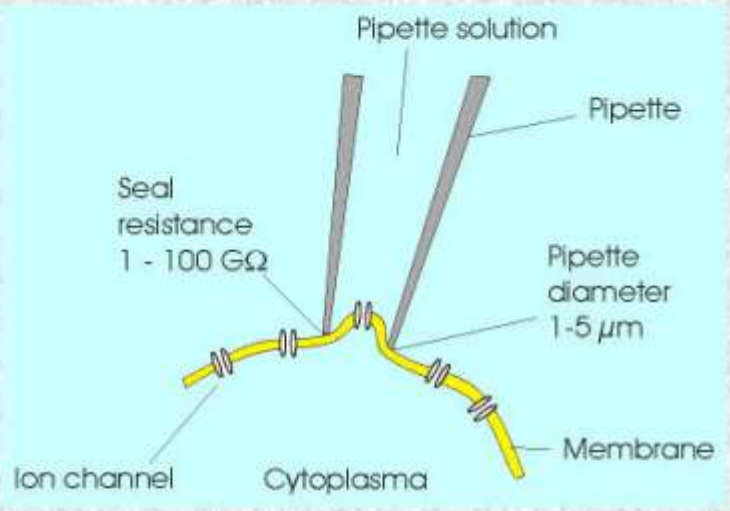
Following Hodgkin & Huxley's results in the 1950's two classes of transport mechanisms competed to explain their results: *carrier molecules* and *pores* - and there was no direct evidence for either.
It was not until the 1970's that the nicotinic ACh receptor and the Na^+ channel were chemically isolated, purified, and identified as proteins.

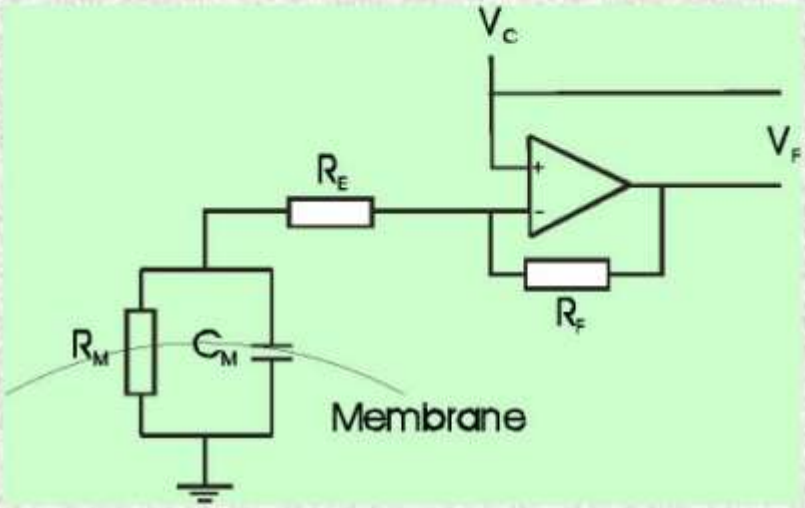


Patch-clamp recording from a single ACh-activated channel on a cultured muscle cell with the patch clamped to -80mV. Openings of the channel (downward events) caused a unitary 3 pA current to flow, often interrupted by a brief closing. Notice the random openings and closing, characteristic of all ion channels. Reproduced from Sigworth FJ (1983) **An example of analysis** in *Single Channel Recording*, eds. Sakmann B, Neher E. Pp 301-321. Plenum Press.

The technical breakthrough of the *patch-clamp* techniques developed by Neher and Sakmann (1976) allowed them to report the first direct measurement of electrical current flowing through a single channel for which they received the 1991 Nobel prize.

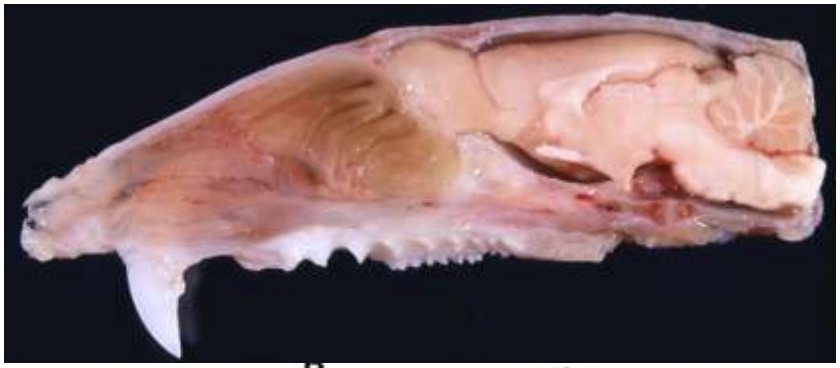
The Patch-Clamp Technique

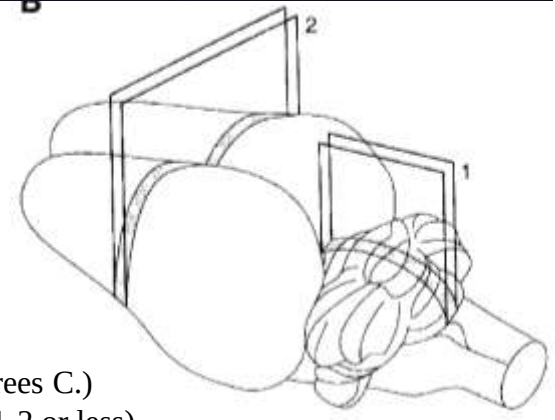




- Has been developed in the late 70ies by Neher and Sakmann (Nobel prize 1991)
- Patch-Clamp (PC) is a "voltage-clamp" method
- PC deals with picoampere (10^{-12} A) currents
- Patch electrodes do not penetrate the cell membrane
- membrane is "sealed" to the electrode opening
- suitable for "small cells" but not only

Dissecting the Brain





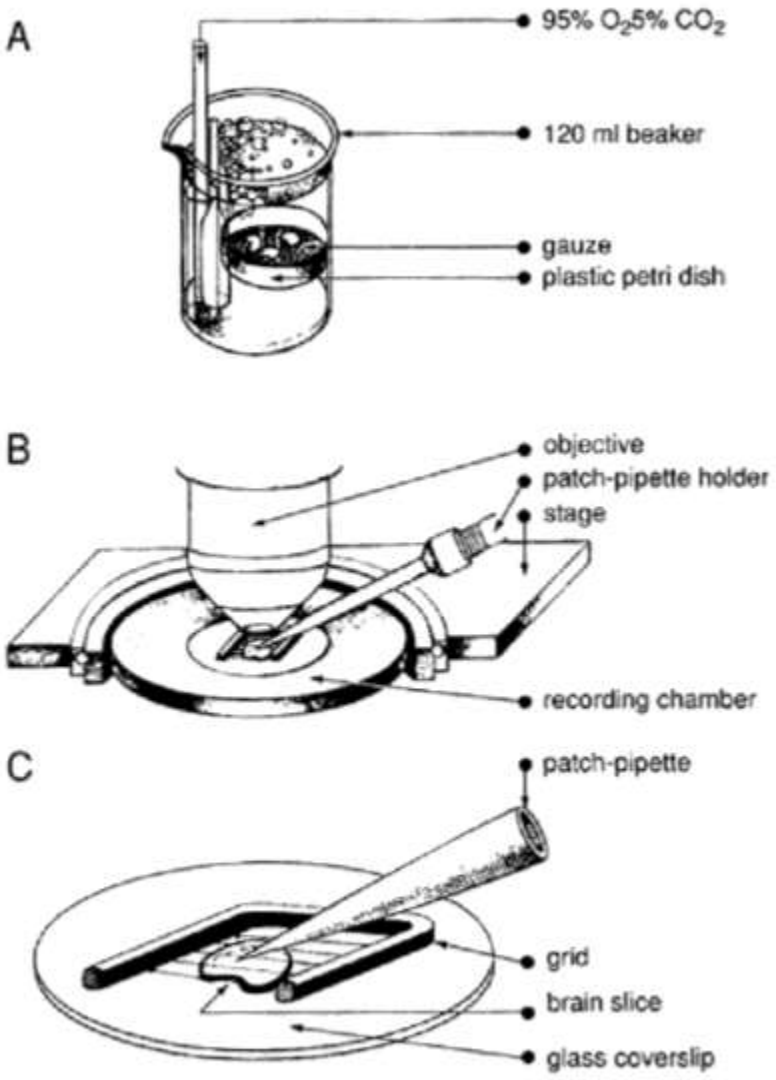
The principle of the VIBRATOME is a Vibrating blade intersecting the specimen underneath the surface of a liquid bath, which lubricates the cut. Next there are 5 parameters under the control of the operator:
1. Nature of the bath (usually a physiologically compatible buffer).
2. Temperature of the bath (the softer the specimen, the colder the bath should be).
3. Speed (the softer the specimen, the slower the speed).
4. Amplitude (the softer the specimen, the higher the amplitude).
5. Blade Angle (the softer the specimen, the steeper angle of attack required).

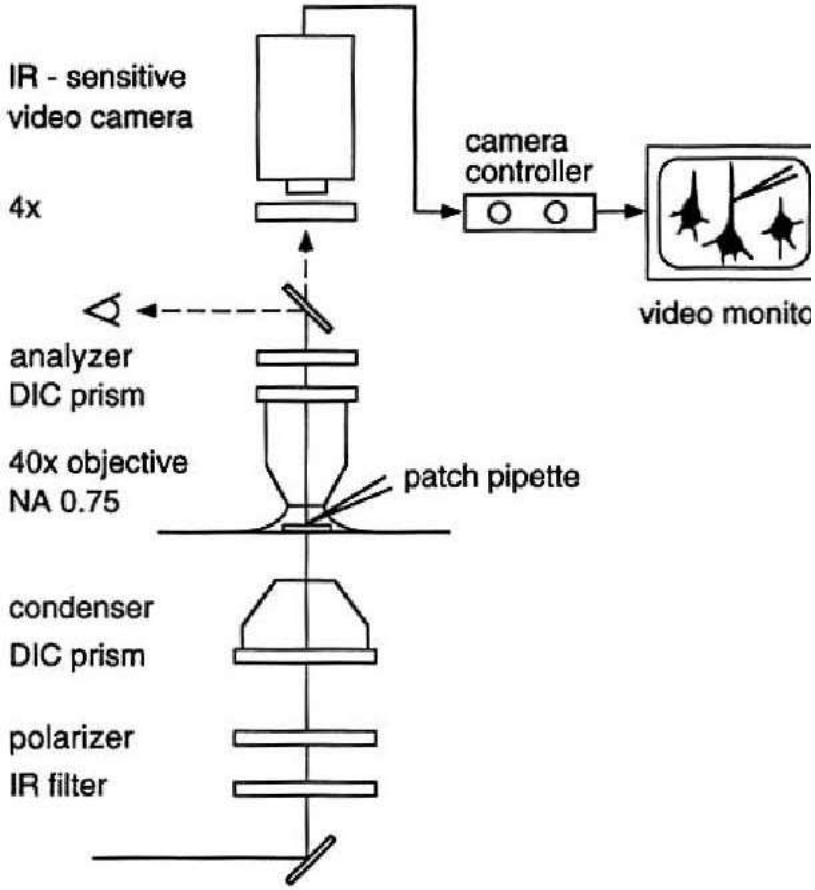
General rules for brain
Bath – cold (2 – 6 degrees C.)
Speed – slow (setting 1-2 or less)
Amplitude – High (8 +/-)
Blade Angle – Steep (25 degrees +/-)

Embedding when necessary (i.e. Fresh brain at 20 microns). Do not use agar. Use 2-5% neutral agarose. Trial and error on the exact concentration. The trick is to match the density of the agarose with that of your specimen.

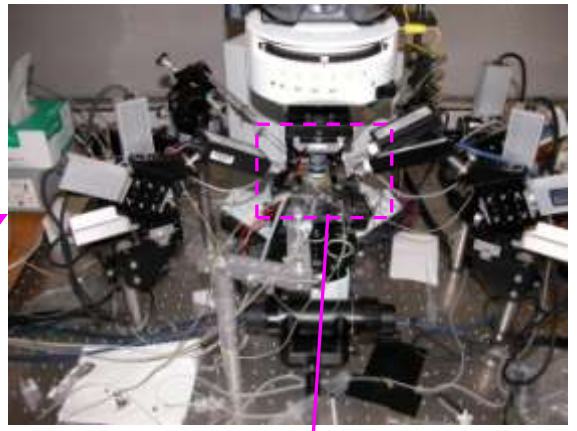
Vibratome makes no claim for less than 20-micron sections using a standard razor blade. However, many people have successfully cut firmer specimens at 10 microns using a glass or sapphire knife.

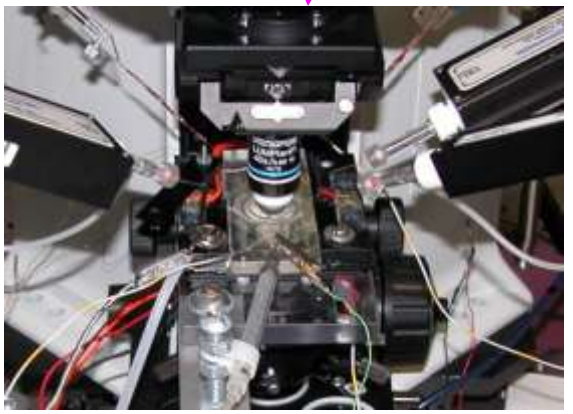
Set-up for slice patch recording

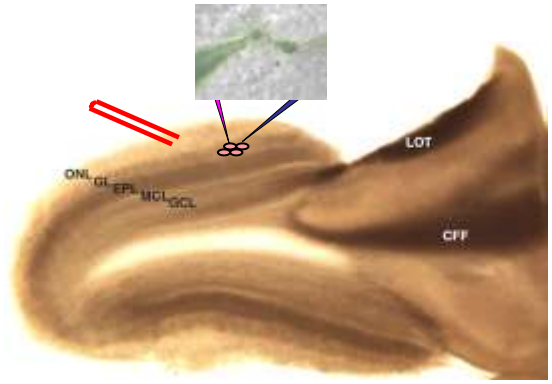




Electrophysiological and imaging setup

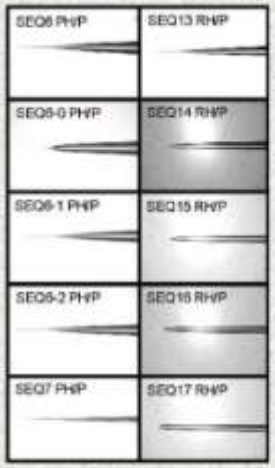






- Olfactory bulb slices (400 µm thick) were cut from young rats (21- 30 days).
- Single and paired whole-cell current and voltage clamp recordings were made at 30°C; drugs applied by perfusion.
- Recorded neurons filled with Lucifer Yellow and biocytin and their morphology was reconstructed.
- Olfactory nerve (ON) electrical stimulation.

The Patch Pipette



* Patch pipettes are microelectrodes made of glass capillaries
* Tip diameter 1 - 50 µm, heatpolished tip
* filled with "physiological" solution
* electrical contact by Ag/AgCl-electrode (chlorided silver wire)
* used only once

P-1000 & P-97 PIPETTE COOKBOOK 2009

http://www.sutter.com/contact/faqs/pipette_cookbook.pdf

Standard or Thick Walled Glass 1.0mm x 0.5mm glass, 0.06µm Tip, 12mm taper **(400x mag.)**

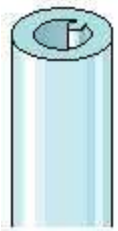
Thick Walled Glass (400X mag) 1.5mm x 0.86mm glass, ~2µm Tip, 3-4 mm taper General Look Up Tables - Prog #51, four to five loops

Micropipette Accessories

http://www.siskiyou.com/stable-tip-electrode-holder.shtml

The new ST50 Stable-tip electrode holders eliminate the final instability link in the electrophysiology experimental setup. **We've taken a thermally stable base material and coated it with alumina oxide. This coating has two benefits: first, it is non-conductive so the holder does not act as an electrical antenna; second, it is very resistant to corrosion.**

http://www.wpiinc.com/



Standard Glass Capillaries
WPI Cat# 1B150F-3
3 in. (76 mm)
1.5 / 0.84 OD/ID (mm)
Filament

•MicroFil 28g, 28 gauge
•250 um ID
•350 um OD
•67 mm long
WPI Cat# MF28G67-5

Silver Wire:
30 AWG, 0.25 mm,
diameter ,10 ft
WPI Cat# AGW1010

24 AWG, 0.5 mm,
diameter, 30 ft
WPI Cat# AGW2030

Micropipette Storage Jar for 1.5 mm OD micropipettes, WPI Cat# E215

Fabrication of Patch Pipettes

Glass	Loss Factor	Log_{10} Volume Resistivity	Dielectric Constant	Softening Temp. °C	Description
7940	.0038	11.8	3.8	1580	Quartz (fused silica)
1724	.0066	13.8	6.6	926	Aluminosilicate
7070	.25	11.2	4.1	----	Low loss borosilicate
8161	.50	12.0	8.3	604	High lead
Sylgard	.58	13.0	2.9	----	#184 Coating compound
7059	.584	13.1	5.8	844	Barium-borosilicate
7760	.79	9.4	4.5	780	Borosilicate
EG-6	.80	9.6	7.0	625	High lead
0120	.80	10.1	6.7	630	High lead
EG-16	.90	11.3	9.6	580	High lead
7040	1.00	9.6	4.8	700	Kovar seal borosilicate
KG-12	1.00	9.9	6.7	632	High lead
1723	1.00	13.5	6.3	910	Aluminosilicate
0010	1.07	8.9	6.7	625	High lead
7052	1.30	9.2	4.9	710	Kovar seal borosilicate
EN-1	1.30	9.0	5.1	716	Kovar seal borosilicate
7720	1.30	8.8	4.7	755	Tungsten seal borosilicate
7056	1.50	10.2	5.7	720	Kovar seal borosilicate
3320	1.50	8.6	4.9	780	Tungsten seal borosilicate
7050	1.60	8.8	4.9	705	Series seal borosilicate
KG-33	2.20	7.9	4.6	827	Kimax borosilicate
7740	2.60	8.1	5.1	820	Pyrex borosilicate
1720	2.70	11.4	7.2	915	Aluminosilicate
N-51A	3.70	7.2	5.9	785	Borosilicate
R-6	5.10	6.6	7.3	700	Soda lime
0080	6.50	6.4	7.2	695	Soda lime

	Chemical Constituent												
Glass	SiO_2	B_2O_3	Al_2O_3	Fe_2O_3	PbO	BaO	CaO	MgO	Na_2O	K_2O	Li_2O	As_2O_3	Sb_2O_3	SO_3
1724	*Not available*													
7070	70.7	24.6	1.9	___	___	.2	.8	.8	___	___	.56	___	___	___
8161	38.7	___	0.2	___	51.4	2.0	0.3	.04	.2	6.6	___	.04	.38	___
7059	50.3	13.9	10.4	___	___	25	___	___	.08	___	___	___	___	___
7760	78.4	14.5	1.7	___	___	___	.1	.1	2.7	1.5	___	.18	___	___
EG-6	54.1	___	1.0	3.9	27.1	___	.1	.1	3.4	9.2	___	.2	___	___
0120	55.8	___	___	.03	29.5	___	.25	___	3.6	8.9	___	.4	___	___
EG-16	34.8	___	0.3	___	58.8	___	0.5	0.5	0.1	5.5	___	.2	.3	___
7040	66.1	23.8	2.9	___	___	___	.1	.1	4.1	2.7	___	.1	___	___
KG-12	56.5	___	1.5	___	28.95	___	.1	.1	3.7	8.6	___	.4	.25	___
1723	57.0	4.0	16.0	___	___	6.0	10.0	7.0	___	___	___	___	___	___
0010	61.1	___	___	___	22.5	___	.3	.1	7.2	7.3	___	___	___	___
7052	65.0	18.3	7.4	___	___	2.7	.2	.1	2.4	2.9	.6	___	___	___
EN-1	65.0	18.0	7.6	___	.01	2.7	.1	.1	2.3	3.2	.6	___	___	___
7720	71.4	15.2	2.0	___	6.1	.3	.2	.1	3.7	.3	___	___	.5	___
7056	69.0	17.3	3.9	___	___	___	.12		.91	7.5	.68	.48	___	___
3320	75.3	14.3	___	___	___	___	.1	.1	4.0	___	___	___	.8	___
7050	67.6	23.0	3.2	___	___	.1	.1	.1	5.1	.2	___	___	___	___
KG-33	80.4	12.9	2.6	___	.005	___	.05		4.0	.05	___	___	___	___
7740	80.4	13.0	2.1	___	___	___	.1	.1	4.1	___	___	___	___	___
1720	62.0	5.3	17.0	___	___	___	8.0	7.0	1.0	___	___	___	___	___
N51-A	72.3	9.9	7.3	___	.02	___	.9	.05	6.5	.7	___	.02	___	___
R-6	67.7	1.5	2.8	___	___	2.0	5.7	3.9	15.6	.6	___	___	___	.2
0080	73.0	.04	___	___	___	.1	4.8	3.2	16.8	.4	___	___	___	.22

-A thick wall (0.2 - 0.3 mm) generates lower electrical noise and increases bluntness at the tip, thereby preventing penetration into the cell during seal formation.
-To obtain blunter tip, better use multistage electrode pullers.
-To lower the noise, coat the pipette up to within 100 µm from its tip with Sylgard#184, a hydrophobic material
-To promote gigaohm seals and to reduce the possibility of tip penetration into the cell during seal formation, pipette tips should be fire-polished.
- Glasses with the lowest loss factor (describes dielectric properties) exhibit the lowest noise.

The Silver/Silver Chloride Electrode

-The flowing electric charge is typically carried by moving electrons in a conductor such as a wire; whereas in an electrolyte, it is instead carried by ions.

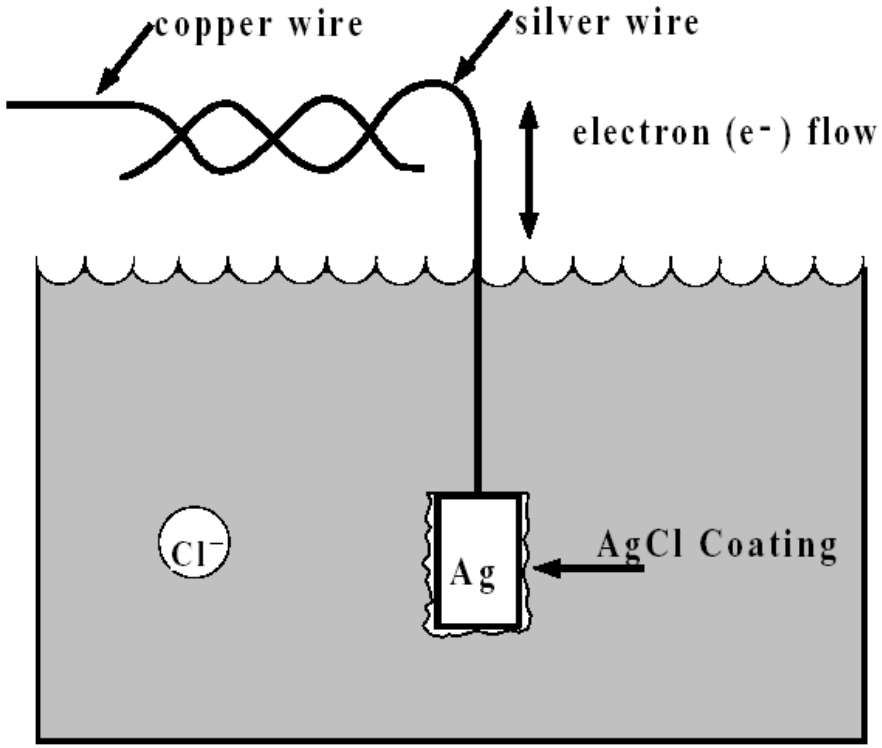
-The Ag/AgCl electrode is reversible (current can flow in both directions)

-If electrons flow from the copper wire through the silver wire to the electrode AgCl pellet, they convert the AgCl to Ag atoms and the Cl- ions become hydrated and enter the solution.

- If electrons flow in the reverse direction, Ag atoms in the silver wire that is coated with AgCl give up their electrons (one electron per atom) and combine with Cl ions that are in the solution to make insoluble AgCl.

-This electrode perform well only in solutions containing chloride ions.

-If the 2 electrodes face different Cl- concentrations (for instance, 3 M KCl inside a micropipette* and 120 mM NaCl in a bathing solution surrounding the cell), there will be steady potential difference, termed *liquid junction potential, which can be subtracted electronically*



Electrode reaction:

$$Ag + Cl^- \rightleftharpoons AgCl + electron\ (e^-)$$

This reaction can also be presented by:

$$AgCl \rightleftharpoons Ag^+ + Cl^-$$

$$Ag^+ \overset{+e^-}{\underset{-e^-}{\rightleftharpoons}} Ag$$

4.0 mm dia. x 1 mm Ag/AgCl Electrode

Correction for liquid junction potentials in patch clamp experiments

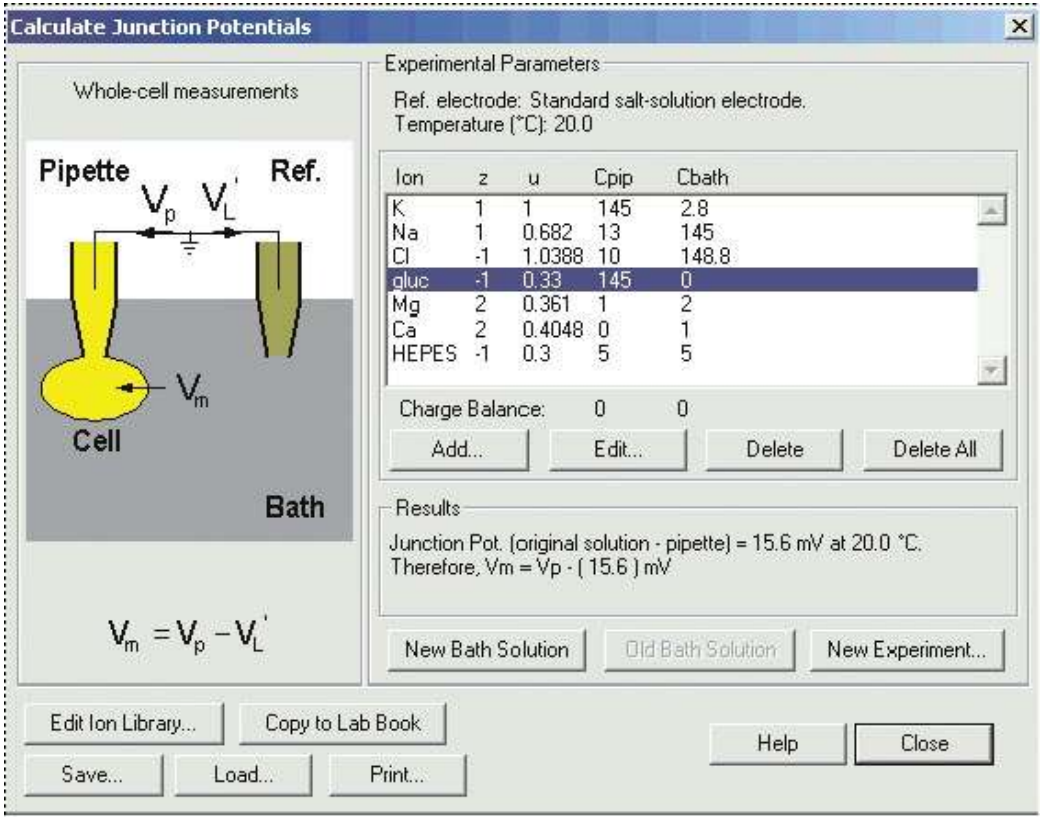
Experimentally:

-Fill an electrode pipette with intracellular solution
-Fill the bath with the same intracellular solution and insert electrode into bath
-Use Current clamp mode and adjust pipette offset potentiometer to zero the voltage read on the meter
-Change the bath solution to aCSF or extracellular solution
-Read the voltage, it should be around -10 mV.
-The Junction potential should be -(-10)=+10 mV
-Your Real resting potential should be Vmeasured - Junction Potential
-Note: It is recommended that the reference electrode is a 2-3 M KCl microelectrode or a 2-3 M KCl agar bridge

Calculation:

The second option is to calculate the LJP using a generalized version of the Henderson equation (Barry and Lynch, 1991; Barry, 1994). JPCalc software, developed by Peter
Barry, is one such calculator.
Although JPCalc was designed to be stand-alone software, it is incorporated into Clampex.

Clampex - Tools – Junction Potential



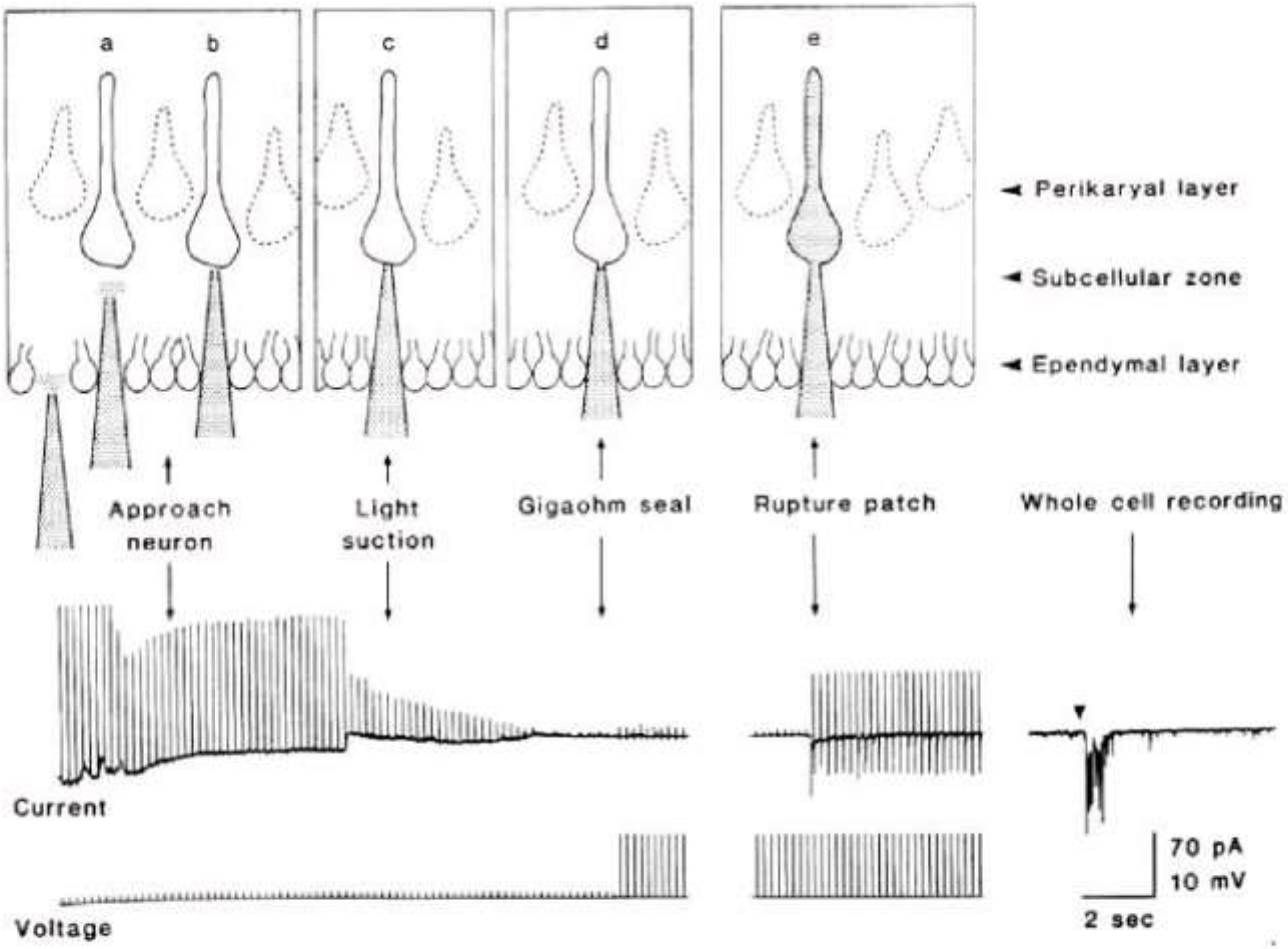
$$V^S - V^P = (RT/F) \quad S_F \quad \ln\{\sum_{i=1}^{N} z_i^2 u_i a_i^P / \sum_{i=1}^{N} z_i^2 u_i a_i^S\} \qquad (1)$$

where

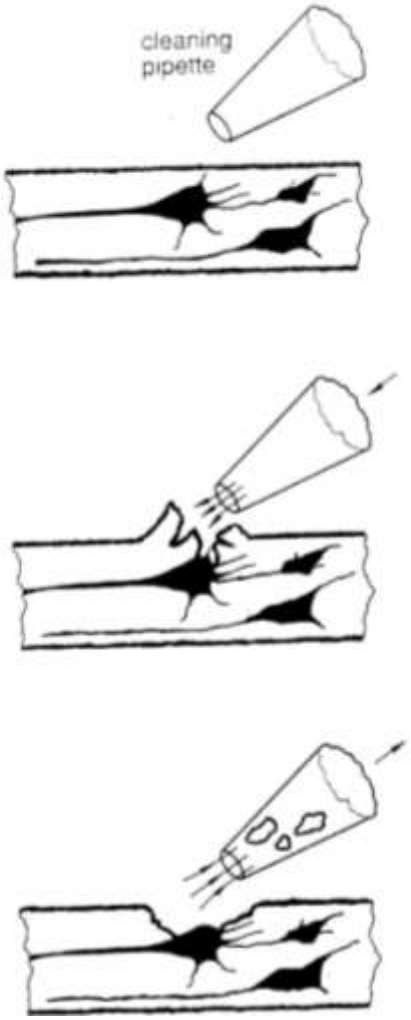
$$S_F = \sum_{i=1}^{N}[(z_i u_i)(a_i^S - a_i^P)] \quad / \quad \sum_{i=1}^{N}[z_i^2 u_i \quad (a_i^S - a_i^P)]$$

where $V^s - V^p$ represents the potential of the solution (S) with respect to the pipette (P) or electrode and u, a and z represent the mobility, activity and valency (including sign) of each ion species (i); R is the gas constant, T is the temperature in K and F is the Faraday, so that RT/F ln = 58.2 log10 in mV at a temperature of 20°C.

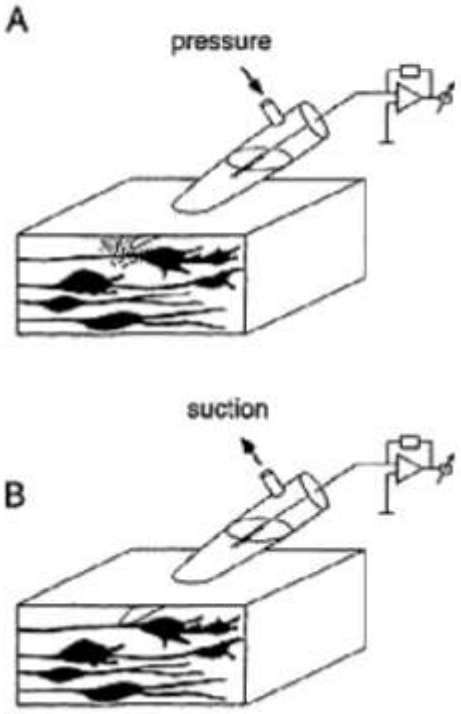
Blind patching technique

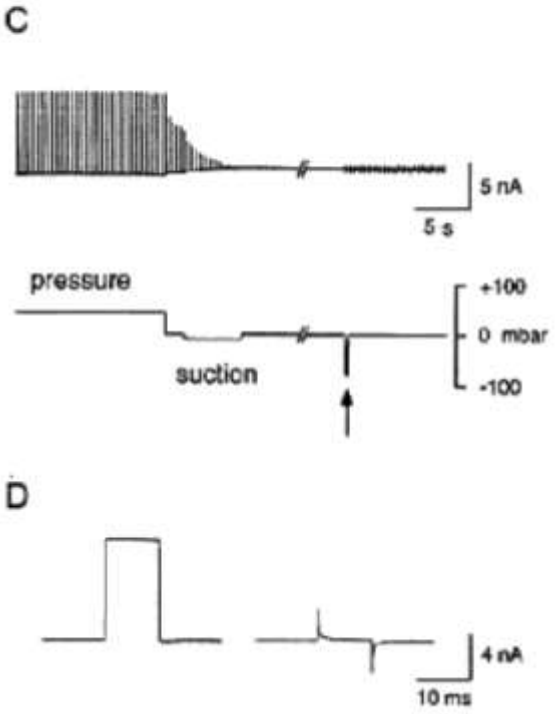


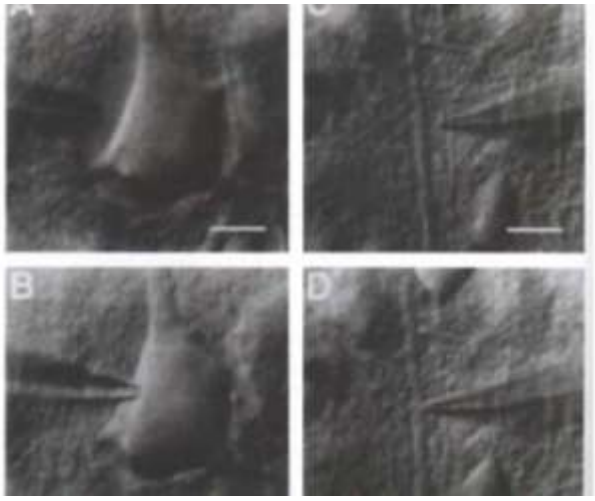
Slice "cleaning" technique



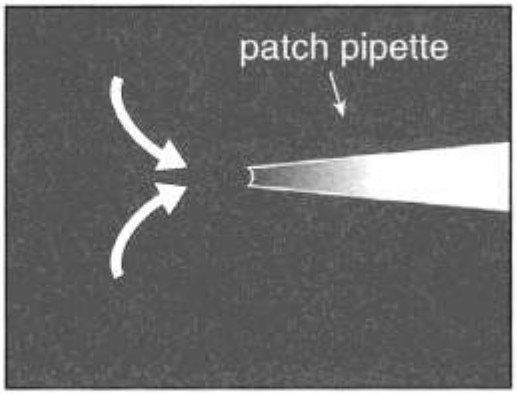
"Blow and seal" technique



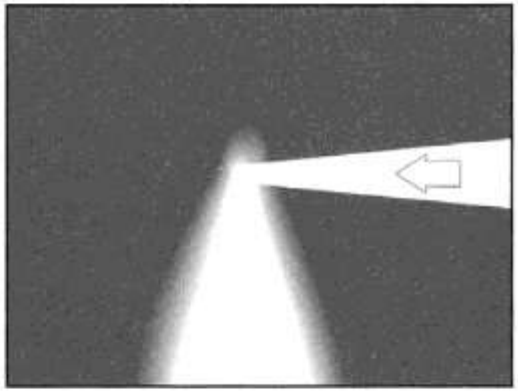




a) no pressure



b) too much pressure



c) correct pressure

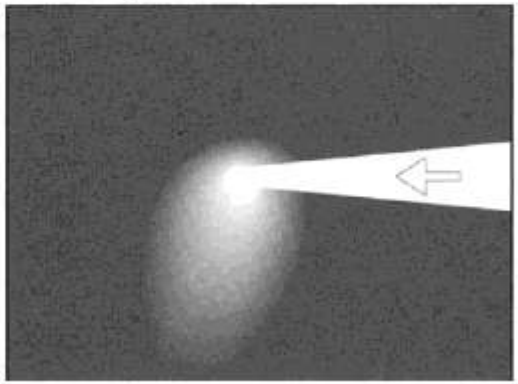
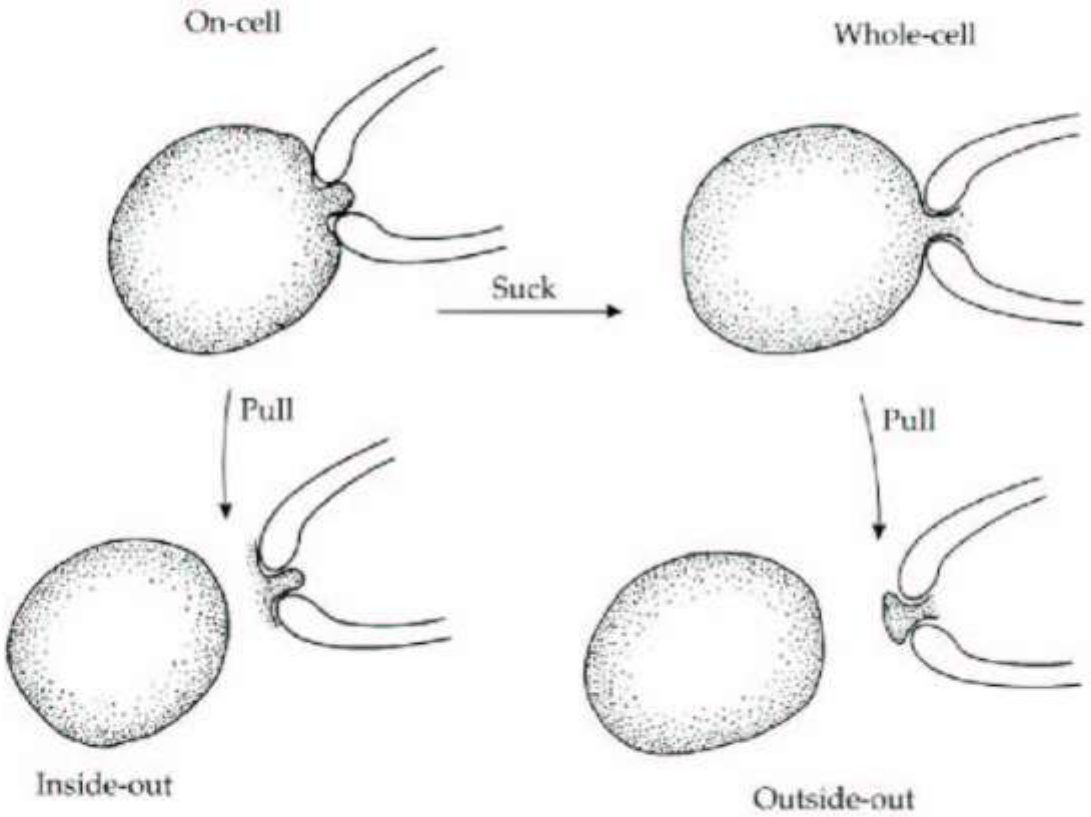


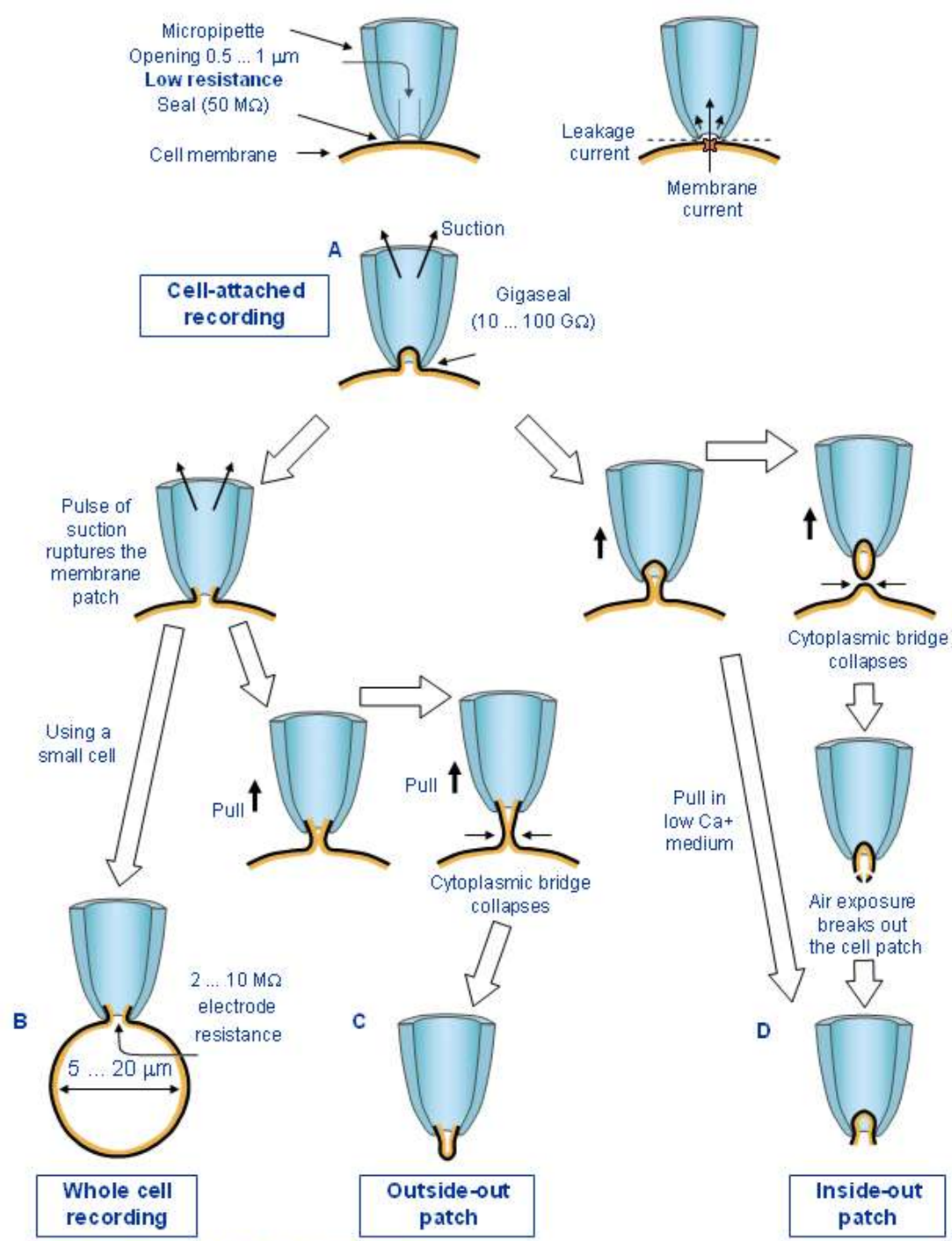
Figure 20 – Proper application of positive pressure is critical when using dye-filled patch pipettes in brain slices.

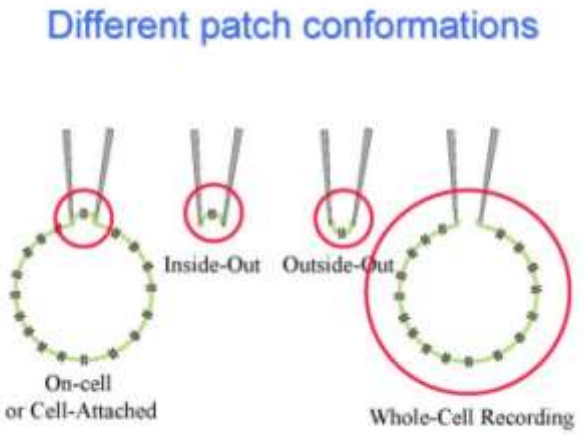
Patch-clamp configurations



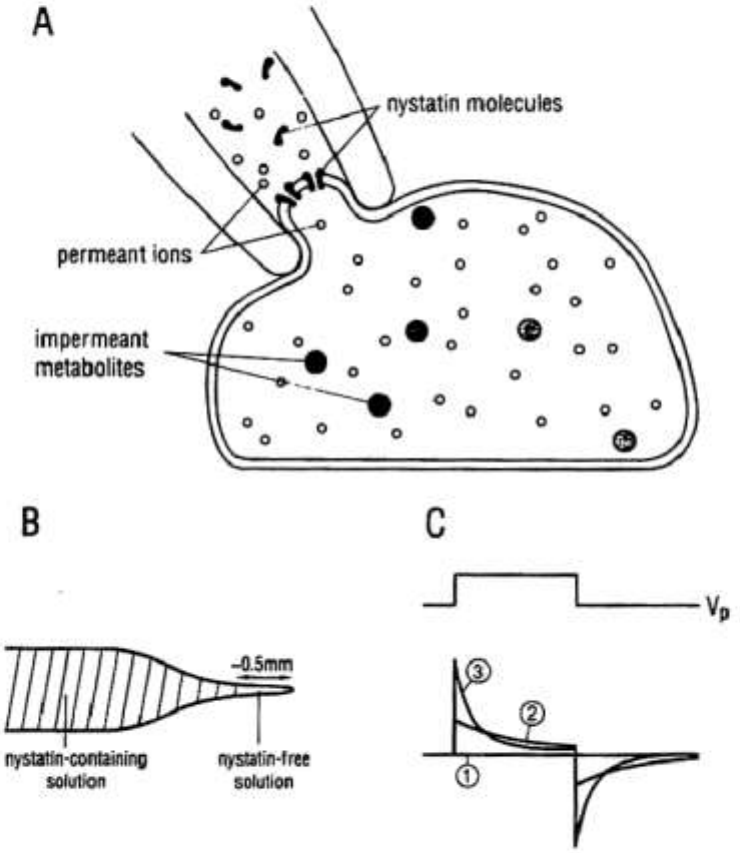
Four Gigaseal Recording Methods

Patch Clamp Applications

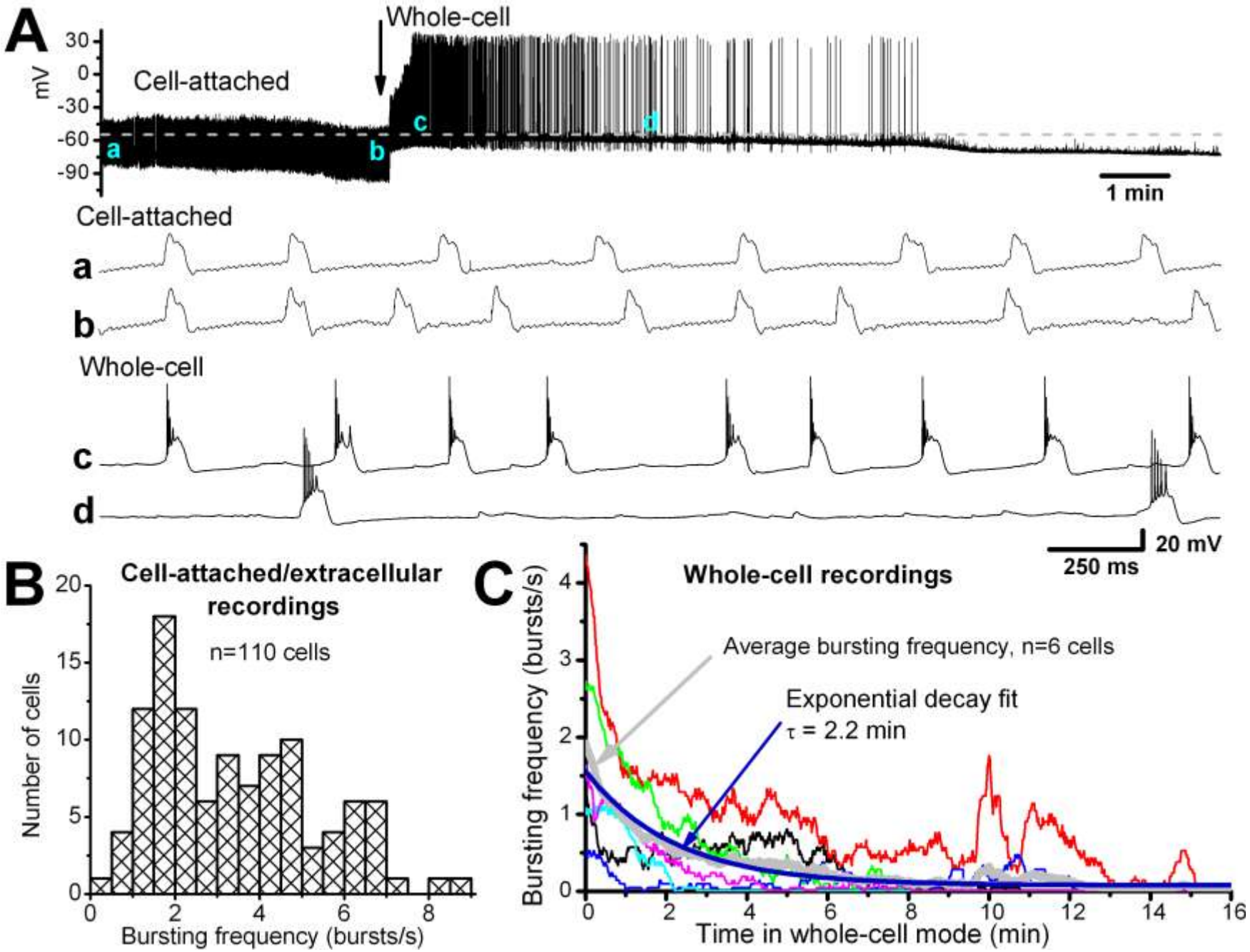




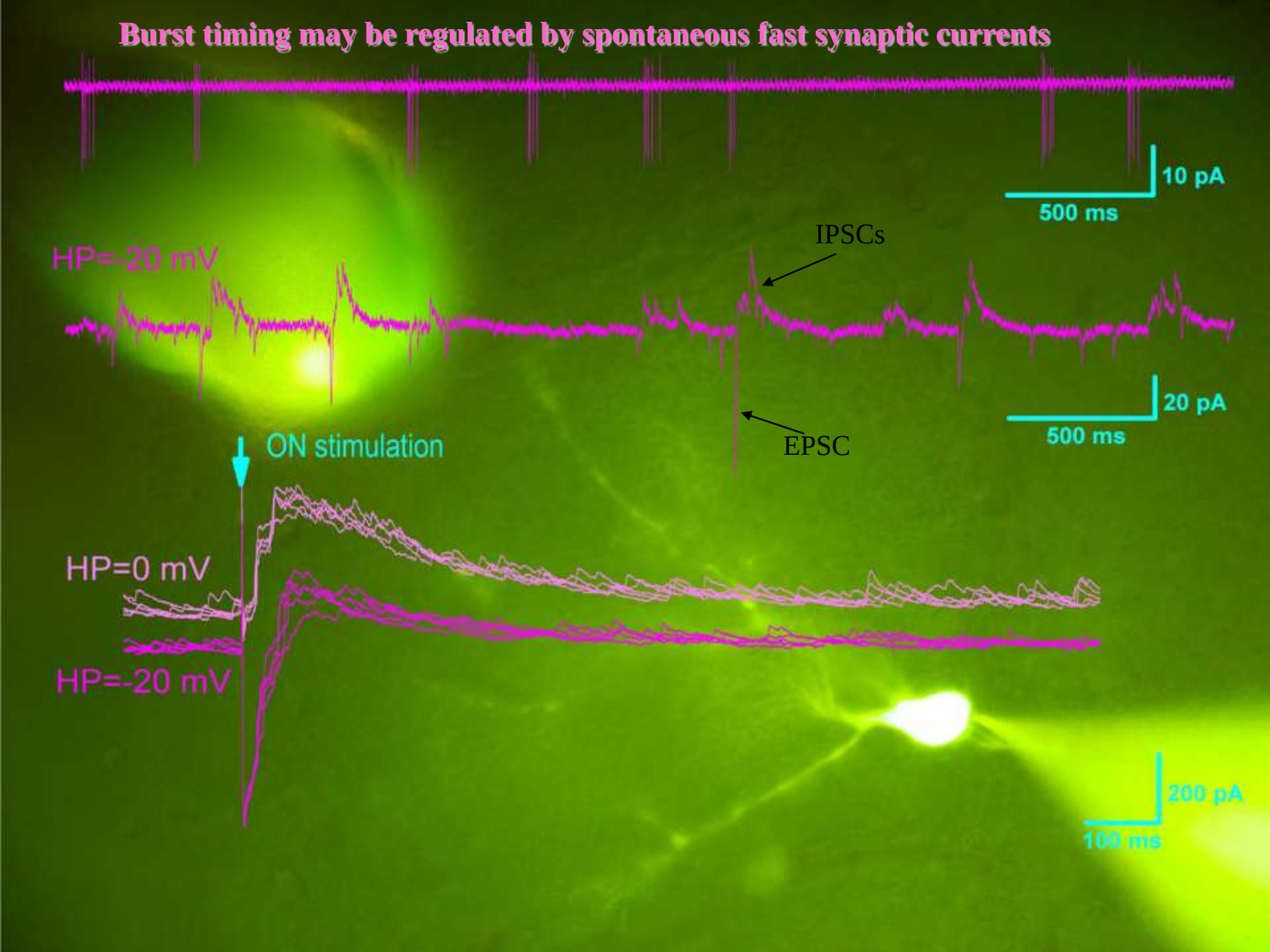
Perforated patch whole-cell recording



Cell-Attached vs. Whole Cell: Bursting exhibit rundown in whole-cell recording



Burst timing may be regulated by spontaneous fast synaptic currents



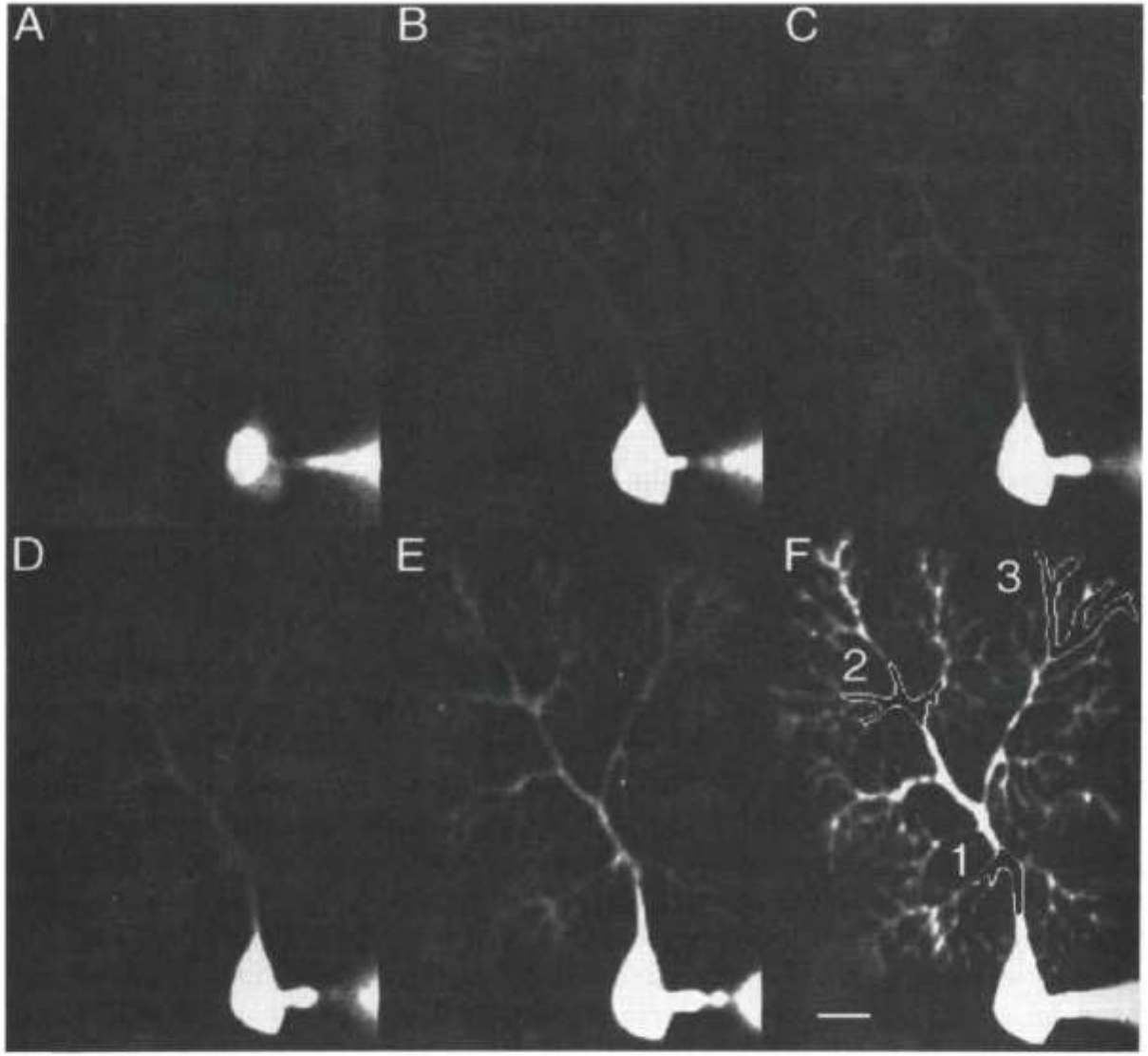
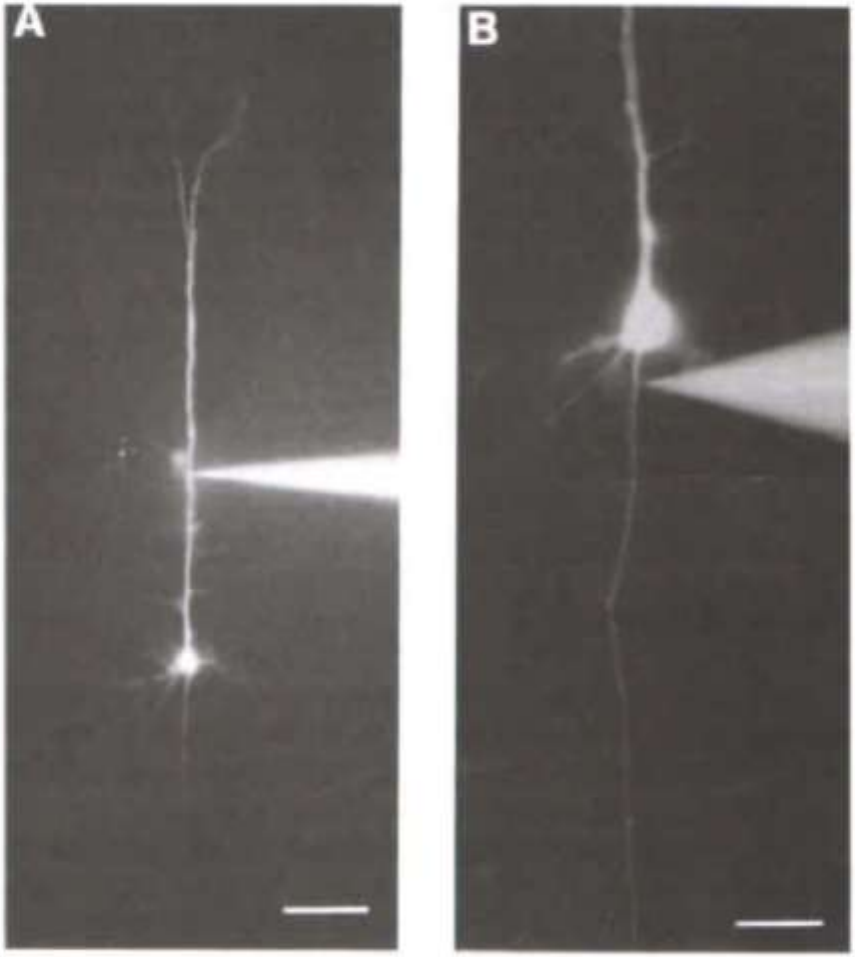


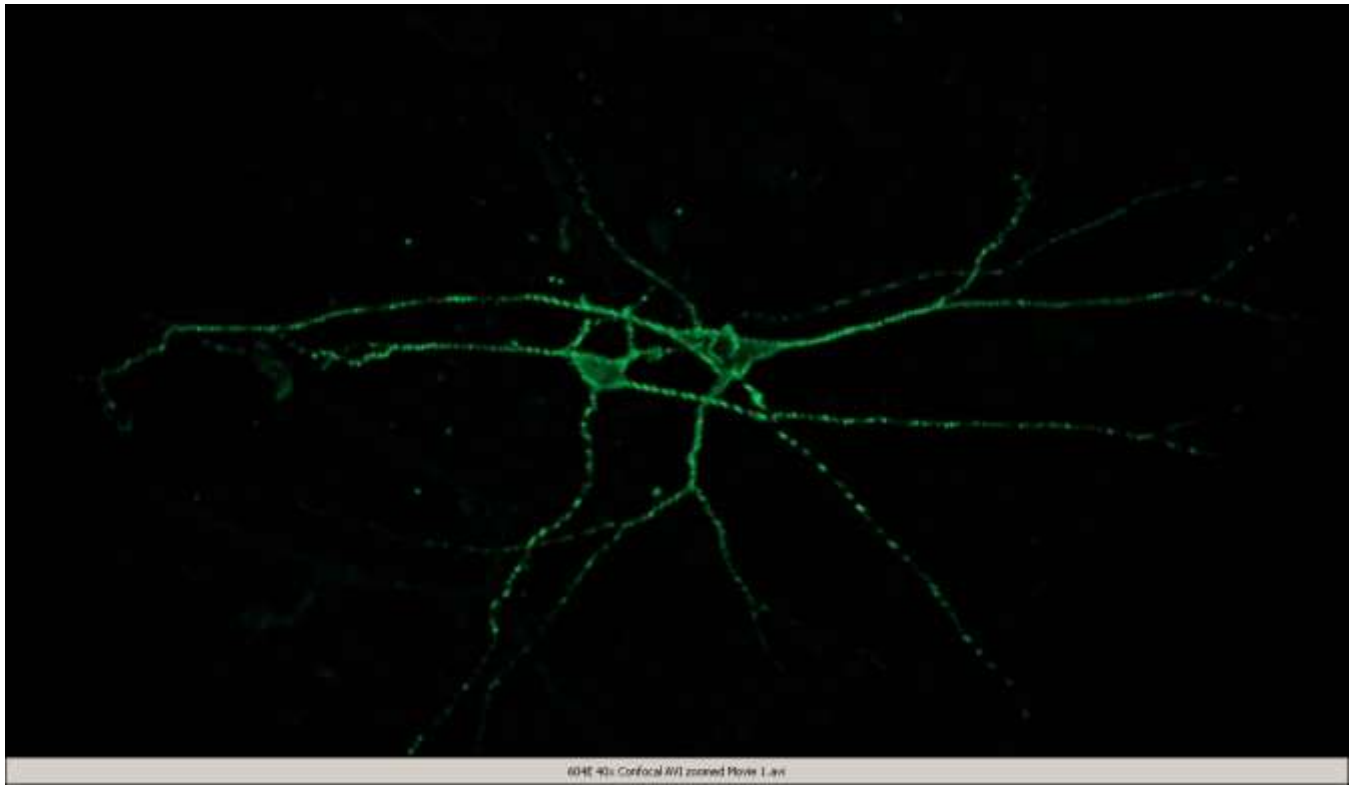
Figure 21 – Time-dependent diffusion of dye solution from a patch pipette to the dendrites of a cerebellar Purkinje neuron.

Dialysis during whole-cell recordings

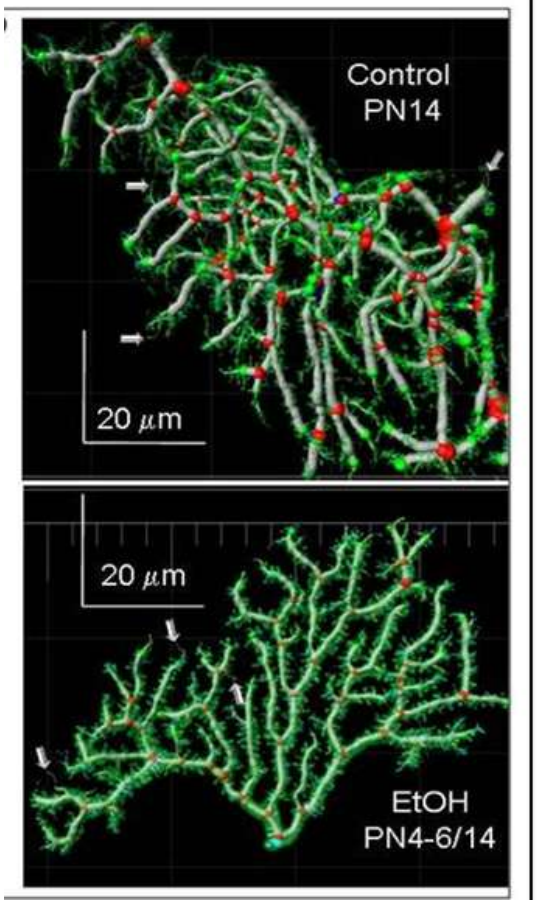


Confocal imaging of neurons filled with fluorescent dyes during patch clamping

Pedonculopontine neurons



Purkinje cells



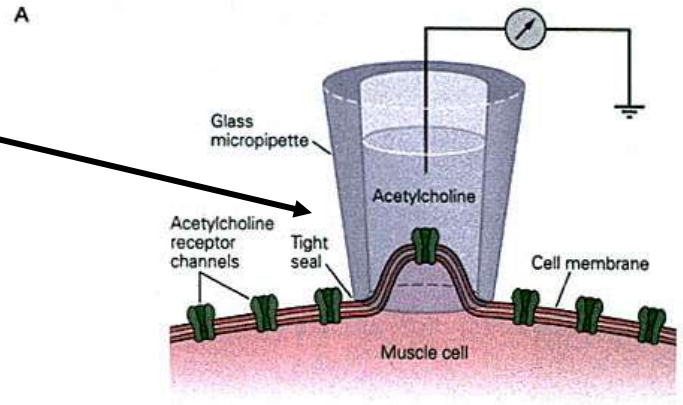
Patch clamp

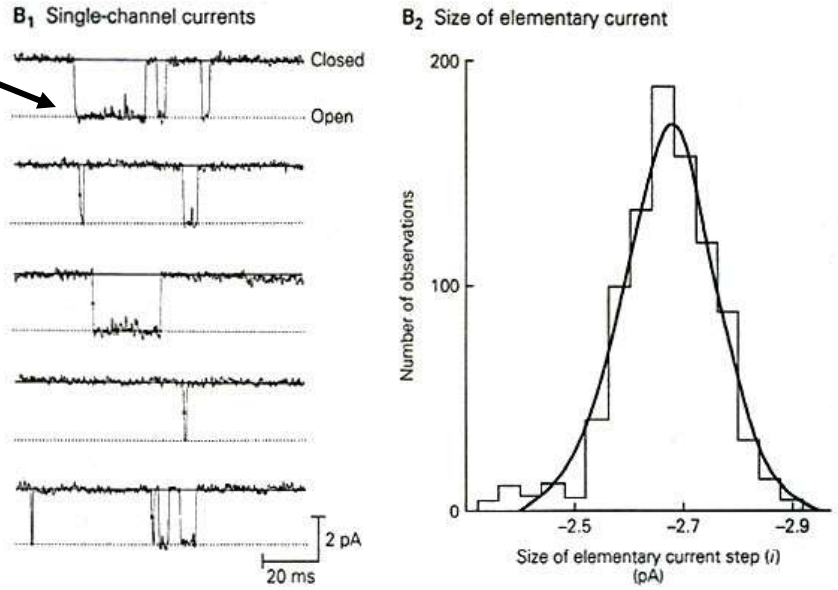
Measure opening and closing of single ACh receptor channels

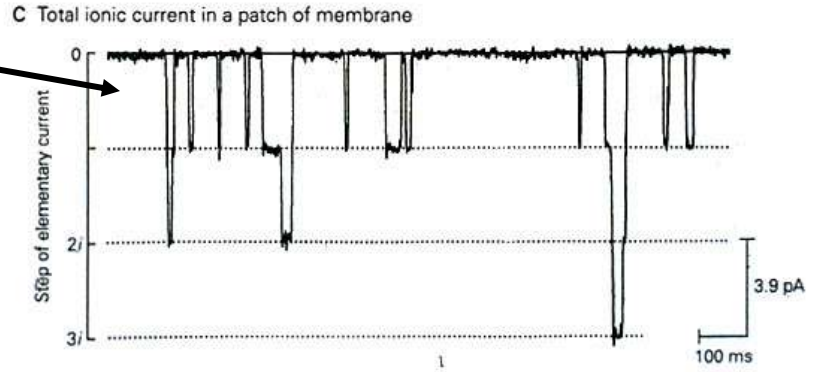
Opening & closing rates are almost instantaneous.

Small depolarizations that occur last ~2 msec and are ~0.25 microvolts in amplitude.

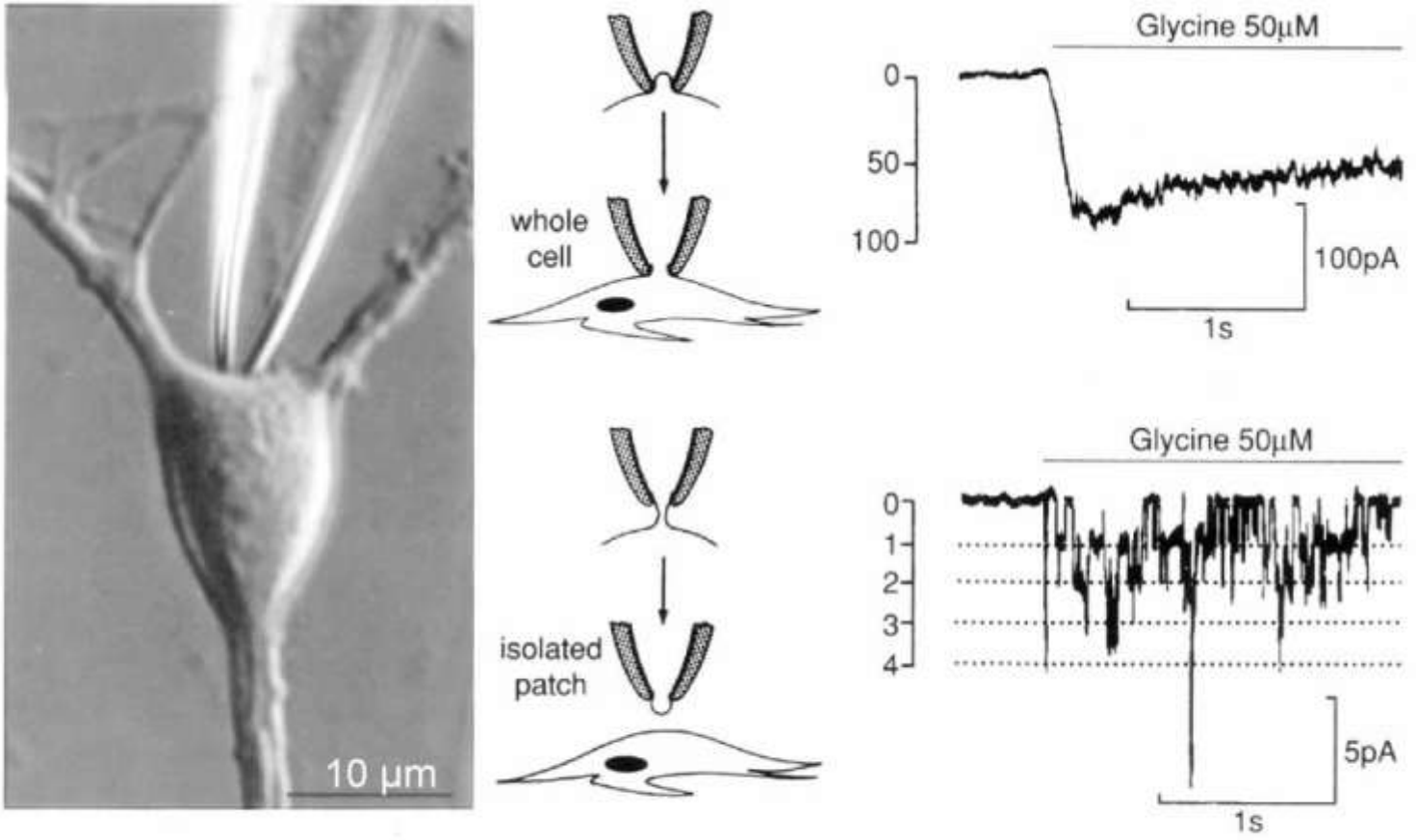
The mean open time = $t_{1/2}$ of decay of the MEPP







Whole-Cell and Outside-Out Patch



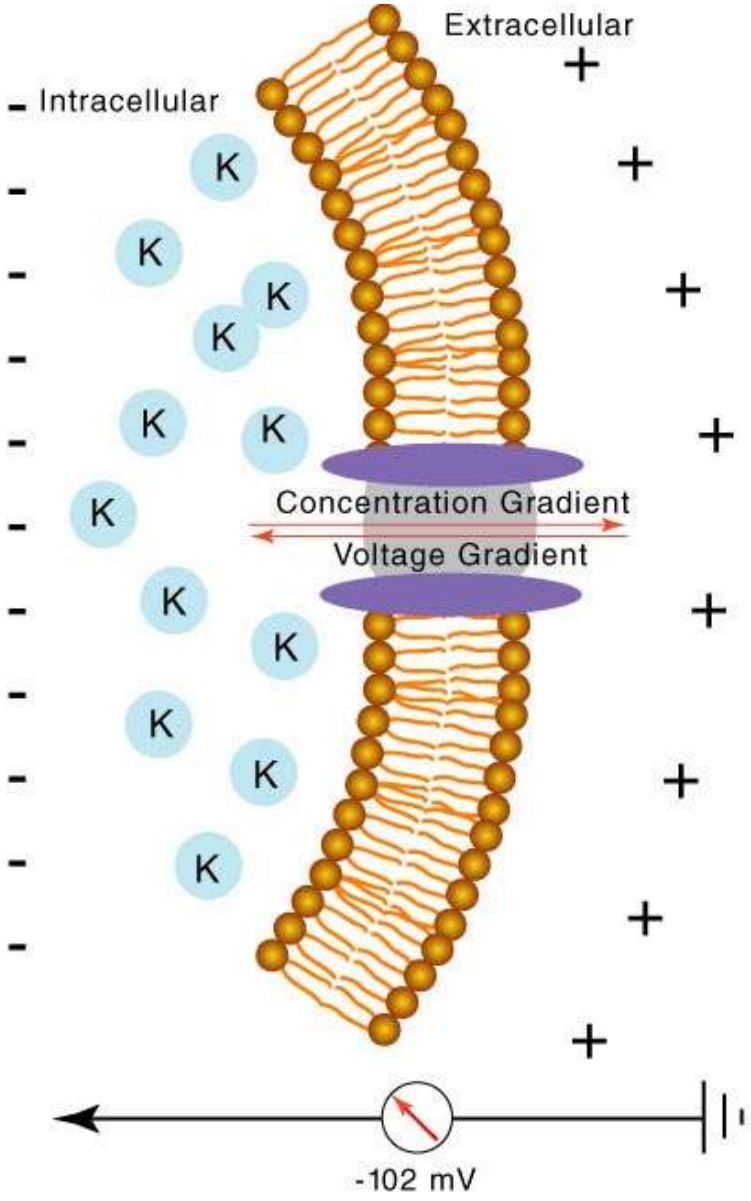


FIGURE 3 The equilibrium potential is influenced by the concentration gradient and the voltage difference across the membrane. Neurons actively concentrate K+ inside the cell. These K+ ions tend to flow down their concentration gradient from inside to outside the cell. However, the negative membrane potential inside the cell provides an attraction for K+ ions to enter or remain within the cell. These two factors balance one another at the equilibrium potential, which in a typical mammalian neuron is -102 mV for K+.

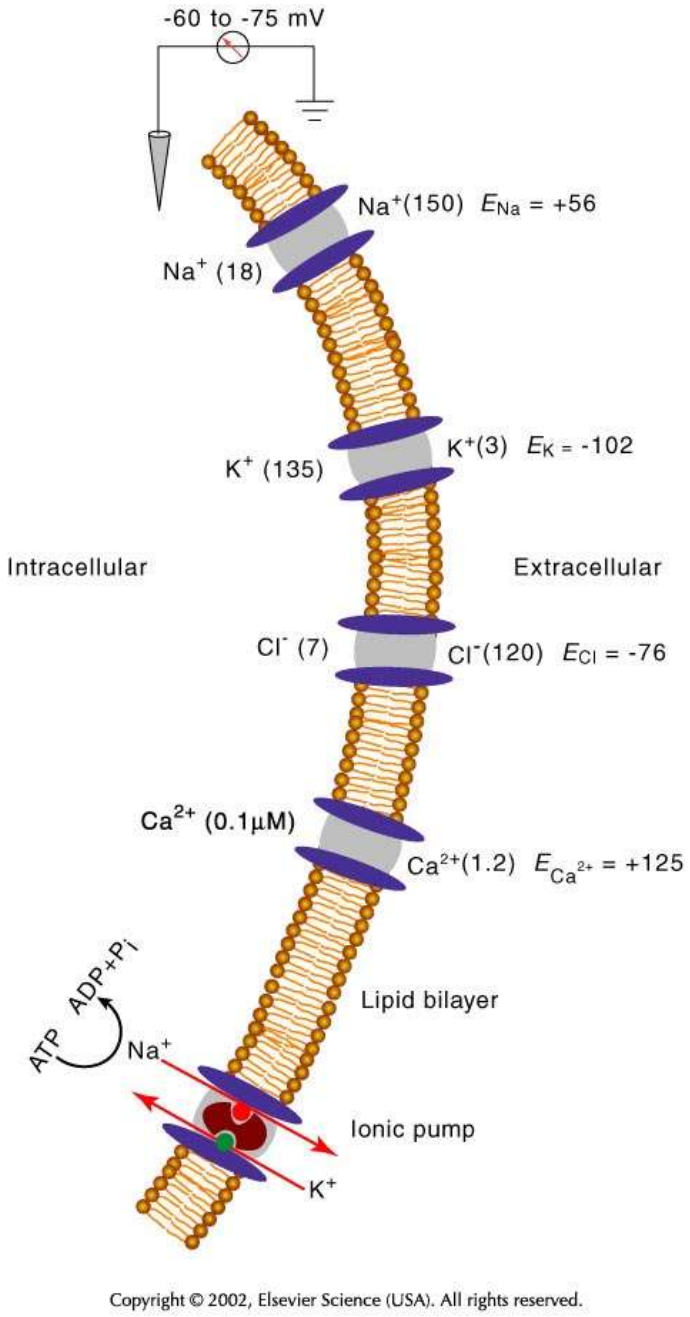
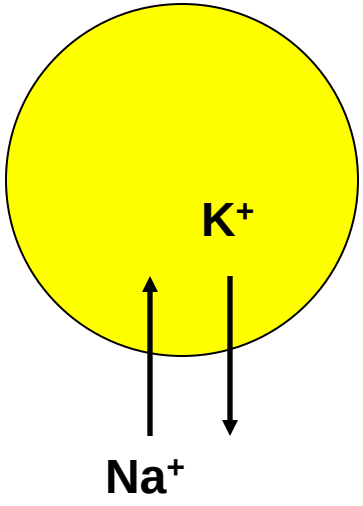


FIGURE 2 Differential distribution of ions inside and outside plasma membrane of neurons and neuronal processes, showing ionic channels for Na+, K+, Cl-, and Ca2+, as well as an electrogenic Na+–K+ ionic pump (also known as Na+, K+–ATPase). Concentrations (in millimoles except that for intracellular Ca2+) of the ions are given in parentheses; their equilibrium potentials (*E*) for a typical mammalian neuron are indicated.

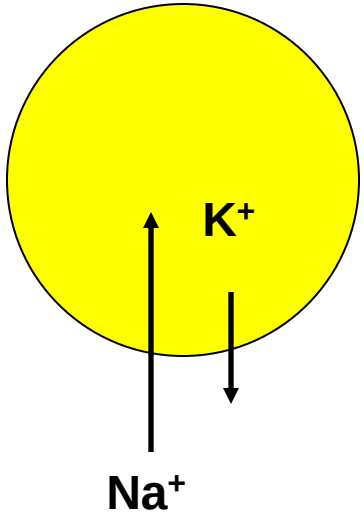
A Net Current Alters the Membrane Potential

Resting Potential



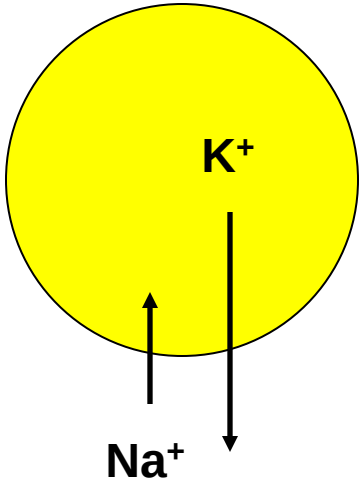
$I_{Na} = I_K$

Depolarization



$I_{Na} > I_K$

Hyperpolarization



$I_{Na} < I_K$

The **Nernst equation** has a physiological application when used to calculate the potential of an ion of charge *z* across a membrane. This potential is determined using the concentration of the ion both inside and outside the cell:

$$E = \frac{RT}{zF} \ln \frac{[\text{ion outside cell}]}{[\text{ion inside cell}]}.$$

R is the universal gas constant

T is the absolute temperature

z is the number of electrons

F is the Faraday constant, the number of coulombs per mole of electrons

$$E_{eq,K^+} = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i},$$

$$E_{eq,K^+} = 61.54mV \log \frac{[K^+]_o}{[K^+]_i},$$

The **Goldman-Hodgkin-Katz voltage equation**, more commonly known as the **Goldman equation** is used in cell membrane physiology to determine the potential across a cell's membrane taking into account all of the ions that are permeant through that membrane.

$$E_{m,K_xNa_{1-x}Cl} = \frac{RT}{F} \ln \left(\frac{P_{Na^+}[Na^+]_{\text{out}} + P_{K^+}[K^+]_{\text{out}} + P_{Cl^-}[Cl^-]_{\text{in}}}{P_{Na^+}[Na^+]_{\text{in}} + P_{K^+}[K^+]_{\text{in}} + P_{Cl^-}[Cl^-]_{\text{out}}} \right)$$

How to Measure Current

A voltage clamp can control the voltage across the membrane and record the current that passes through the membrane.

Ohm's Law says: **Voltage = Current * Resistance**

Circuitry in the voltage clamp controls **Voltage**
When channels open, **Resistance** will change and the device will measure **Current**.

$$V = IR \quad \text{or} \quad I = \frac{V}{R} \quad \text{or} \quad R = \frac{V}{I}$$

Voltage drop across the resistor "V" is in volts, abbreviated "V". The abbreviation "V" for "volts" is not to be confused with the voltage "V" in Ohm's law.

$$G = \frac{1}{R} = \frac{I}{V}$$

Current "I" is in amperes, often shortened to "amps", abbreviated "A".

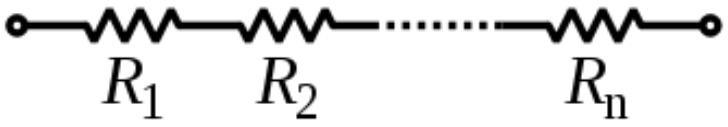
Resistance "R" is in ohms, often represented by the Greek symbol capital omega (Ω). "K" or "k" signifies a multiplier of a "thousand" ohms, 1,000 Ω = 1 K Ω
"M" or "MEG" signifies multiplier of a "million" ohms. 1,000,000 Ω = 1 M Ω
Giga ohm = 1,000,000,000 Ω = 1 G Ω

Resistors

Resistors are elements of electrical networks and electronic circuits and are ubiquitous in most electronic equipment.

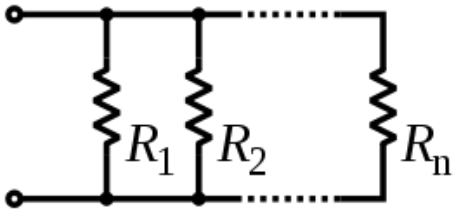
The electrical resistance of an object is a measure of its opposition to the passage of a steady electric current. Discovered by Georg Ohm in the late 1820s, electrical resistance shares some conceptual parallels with the mechanical notion of friction.
The SI unit of electrical resistance is the ohm, symbol Ω.
Resistance's reciprocal quantity is electrical conductance measured in siemens, symbol S.

For resistors in series



$$R_{eq} = R_1 + R_2 + \cdots + R_n$$

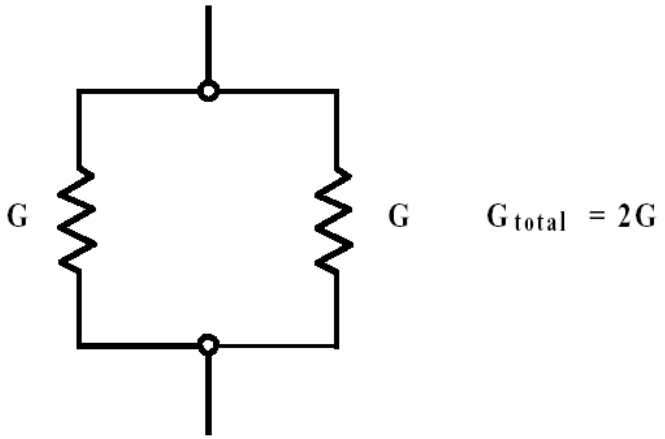
For resistors in parallel

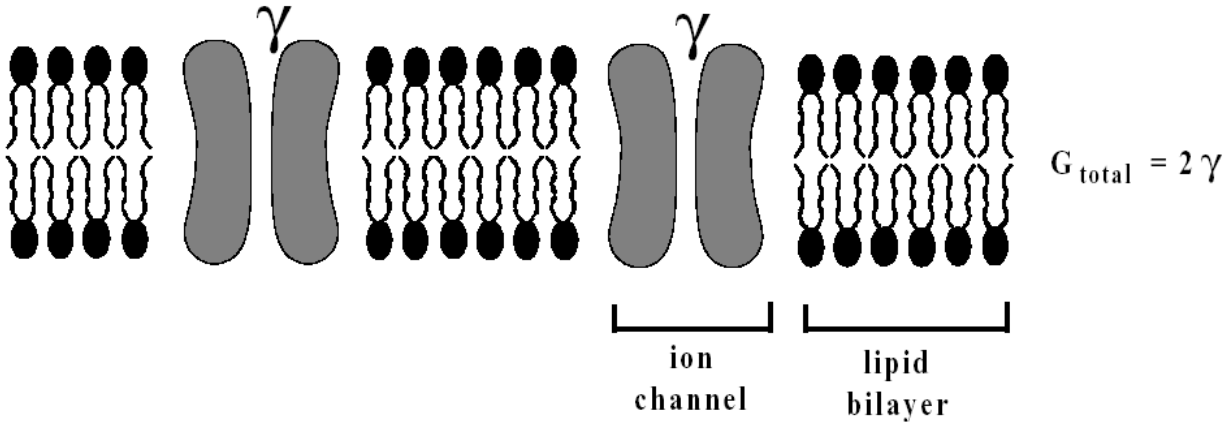


$$\frac{1}{R_{eq}} = \frac{1}{R_1} + \frac{1}{R_2} + \cdots + \frac{1}{R_n}$$

Summation of Conductance

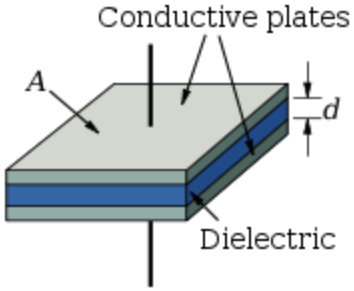
In electrophysiology, it is convenient to discuss currents in terms of conductance because side-by-side ("parallel“) conductances simply summate. The most important application of the parallel conductances involves ion channels. When several ion channels in a membrane are open simultaneously, the total conductance is simply the sum of the conductances of the individual open channels.





Conductances in parallel summate together, whether they are resistors or channels.

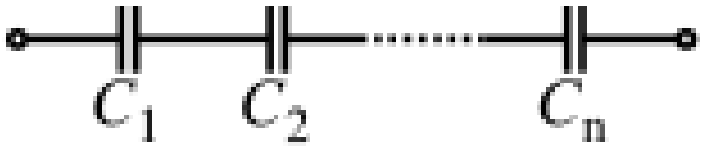
A capacitor or condenser is a passive electronic component consisting of a pair of conductors separated by a dielectric. When a voltage potential difference exists between the conductors, an electric field is present in the dielectric.



$$C = \frac{\varepsilon A}{d}$$

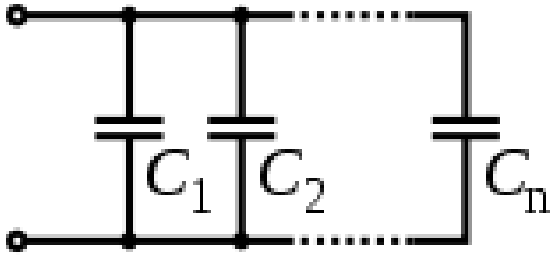
Dielectric is placed between two conducting plates, each of area *A* and with a separation of *d*.

For capacitors in series



$$\frac{1}{C_{eq}} = \frac{1}{C_1} + \frac{1}{C_2} + \cdots + \frac{1}{C_n}$$

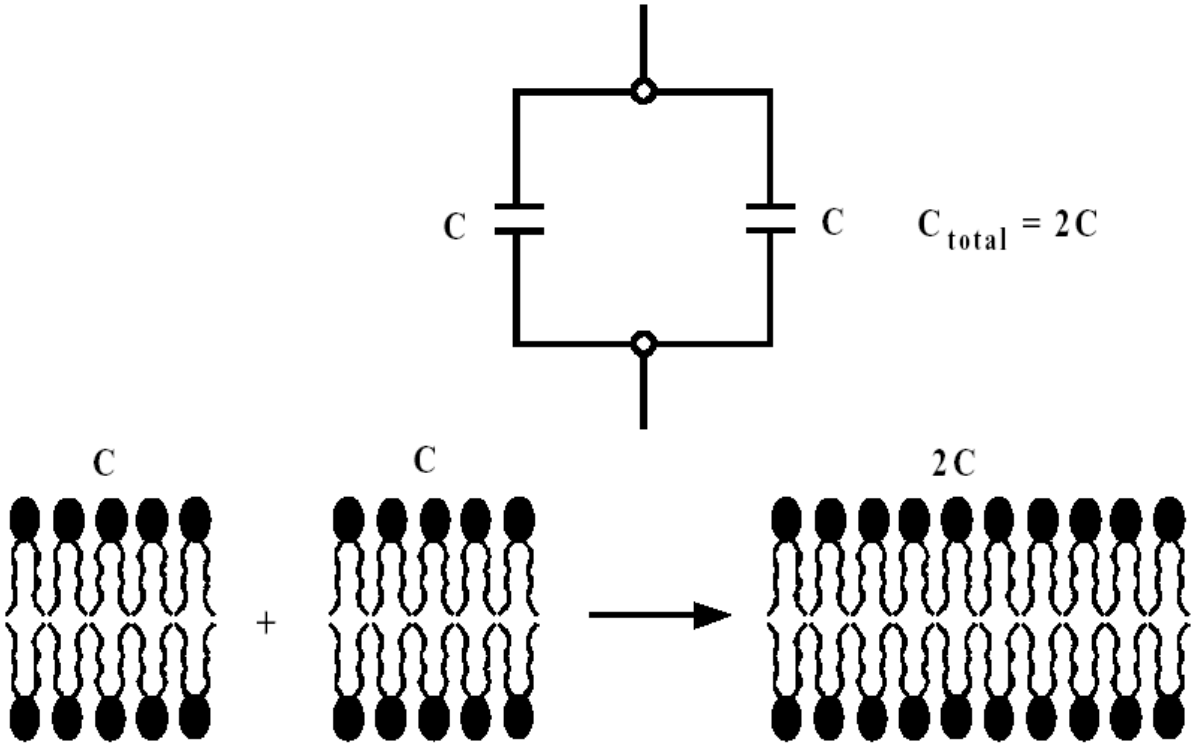
For capacitors in parallel



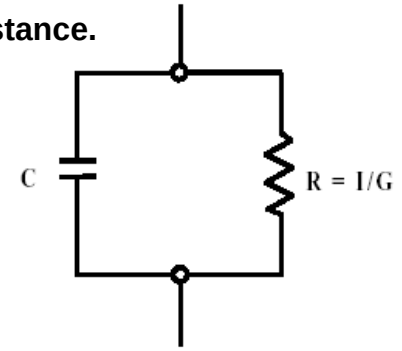
$$C_{eq} = C_1 + C_2 + \cdots + C_n$$

Capacitors in Parallel Add Their Values

When multiple capacitors are connected in parallel, this is electronically equivalent to a single large capacitor; that is, the total capacitance is the sum of their individual capacitance values. Thus, membrane capacitance increases with cell size. Membrane capacitance is usually expressed as value per unit area; nearly all lipid bilayer membranes of cells have a capacitance of 1 µF/cm2 (0.01 pF/µm2).



RC circuit: A membrane behaves electrically like a capacitance in parallel with a resistance.



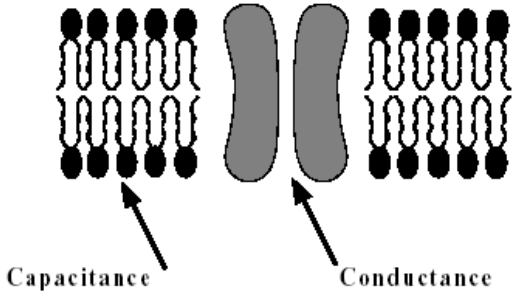
A simple resistor-capacitor (RC) circuit is a capacitor and a resistor in series.

The capacitor discharges its energy into the resistor. This voltage across the capacitor over time could be found through Kirchhoff's current law, where the current coming out of the capacitor must equal the current going through the resistor.

$$V(t) = V_0 e^{-\frac{t}{RC}} ,$$

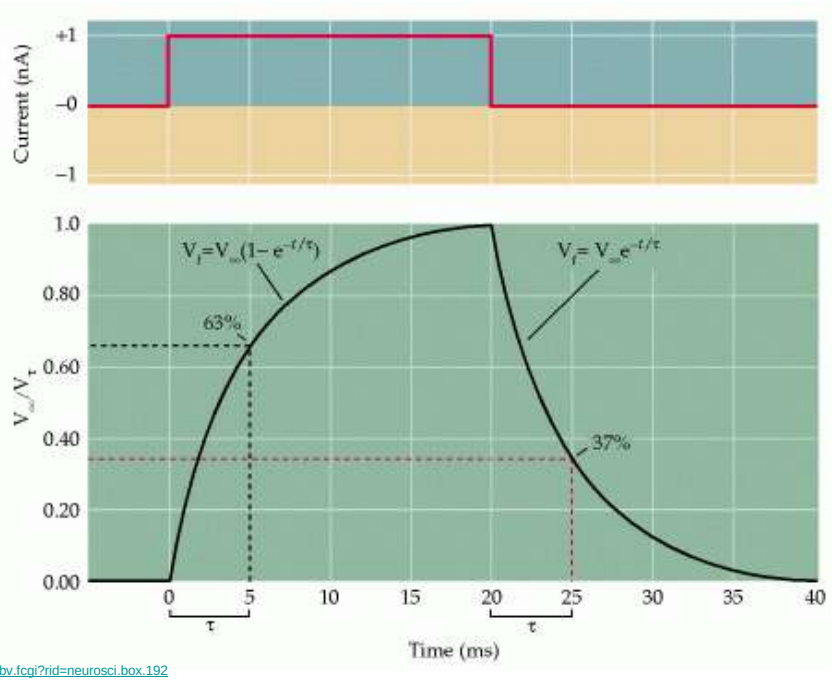
The time constant τ (in seconds) is: $\tau = RC$.

R is the resistance (in ohms) and C is the capacitance (in farads).



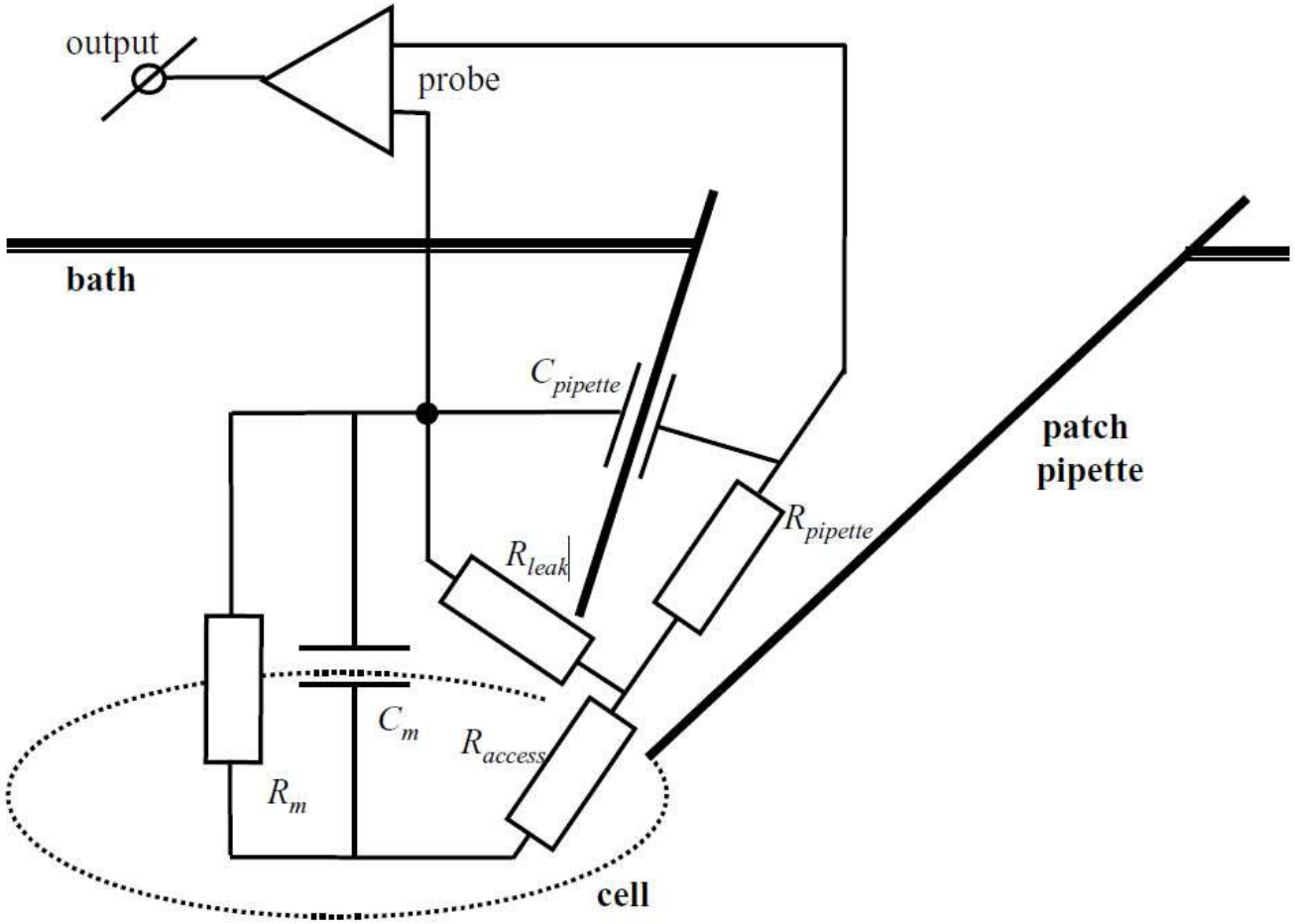
The resistance across the membrane is a function of the number of open ion channels

The capacitance is a function of the properties of the lipid bilayer.

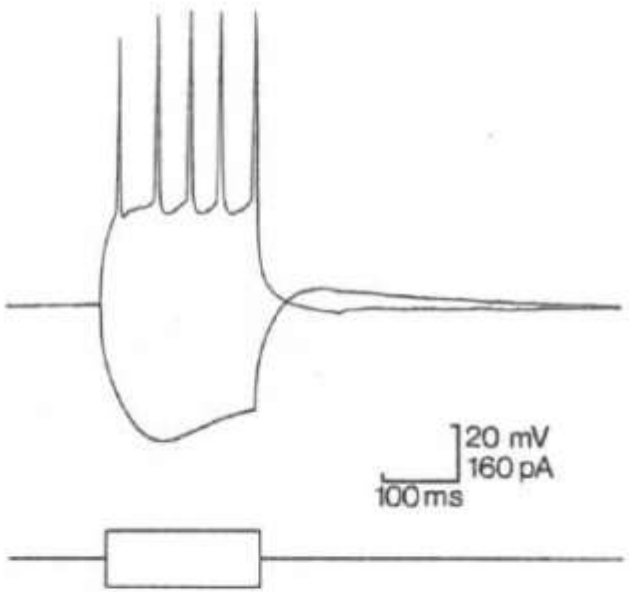


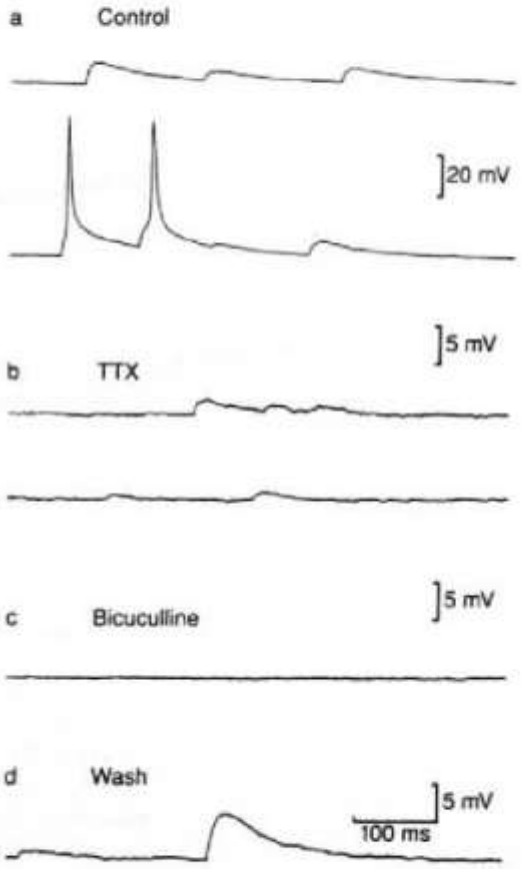
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=neurosci.box.192

Equivalent circuit for the whole-cell configuration



Current clamp recordings in slices



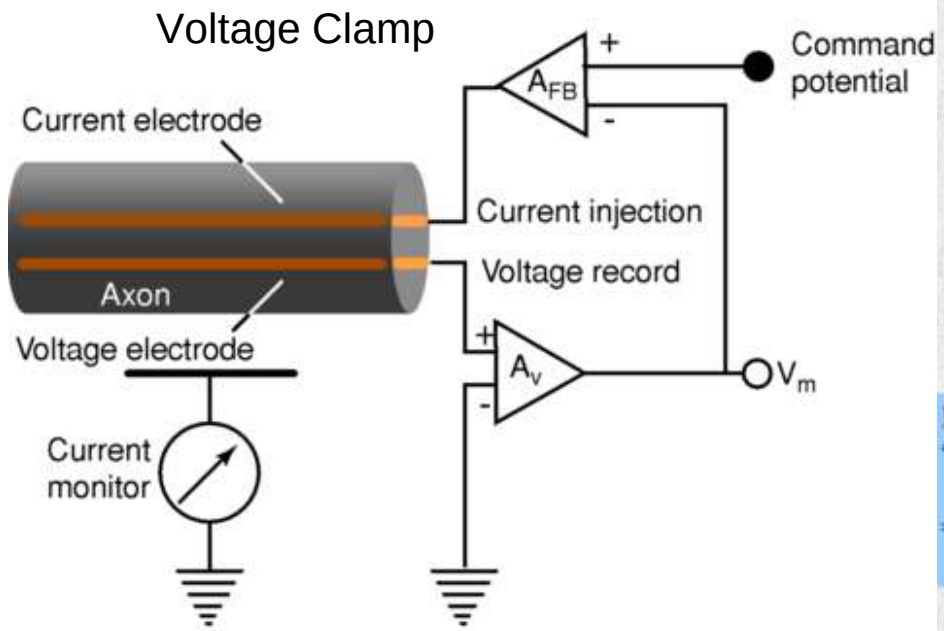


Why study voltage clamping?

- Historical: This is the method invented by Hodgkin and Huxley to discover the voltage-dependent behavior of sodium and potassium currents.

- Factual: To understand the voltage and time dependence of sodium and potassium currents underlying the action potential.

- Methodological:
  - The same method, in principle, is still used to study many other types of membrane currents (calcium currents, chloride currents, pump currents, etc.)
  - The same method is used to study the currents that go through single ion channels.



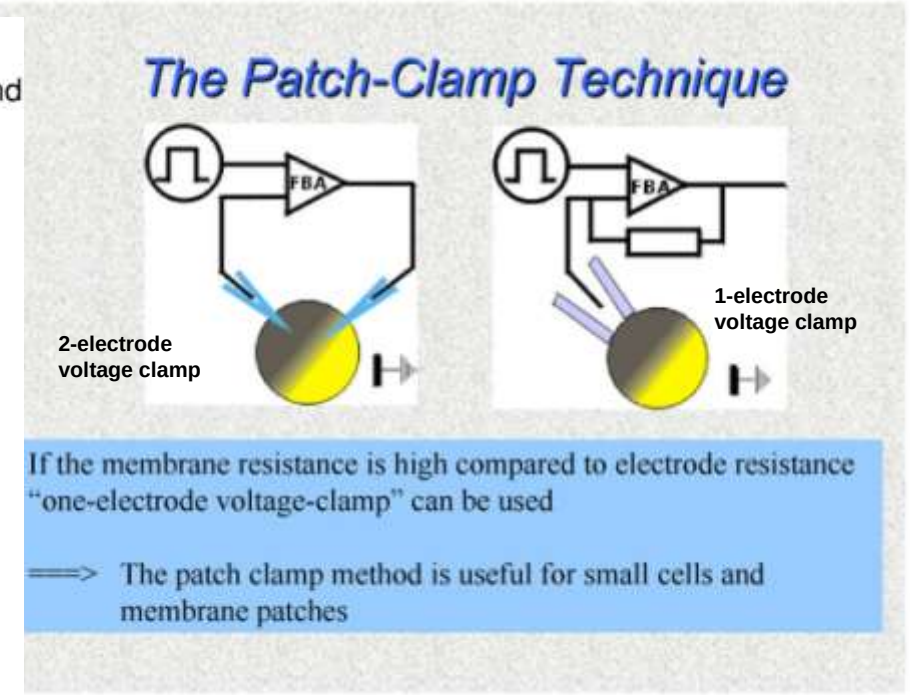


Fig. 5. The voltage-clamp technique keeps the voltage across the membrane constant so that the amplitude and time course of ionic currents can be measured. In the two-electrode voltage-clamp technique, one electrode measures the voltage across the membrane while the other injects current into the cell to keep the voltage constant. The experimenter sets a voltage to which the axon or neuron is to be stepped (the command potential). Current is then injected into the cell in proportion to the difference between the present membrane potential and the command potential. This feedback cycle occurs continuously, thereby clamping the membrane potential to the command potential. By measuring the amount of current injected, the experimenter can determine the amplitude and time course of the ionic currents flowing across the membrane.

Voltage Clamp

The voltage clamp is used by electrophysiologists to measure the ion currents across a neuronal membrane while holding the membrane voltage at a set level. Neuronal membranes contain many different kinds of ion channels, some of which are voltage gated. The voltage clamp allows the membrane voltage to be manipulated independently of the ionic currents, allowing the current-voltage relationships of membrane channels to be studied

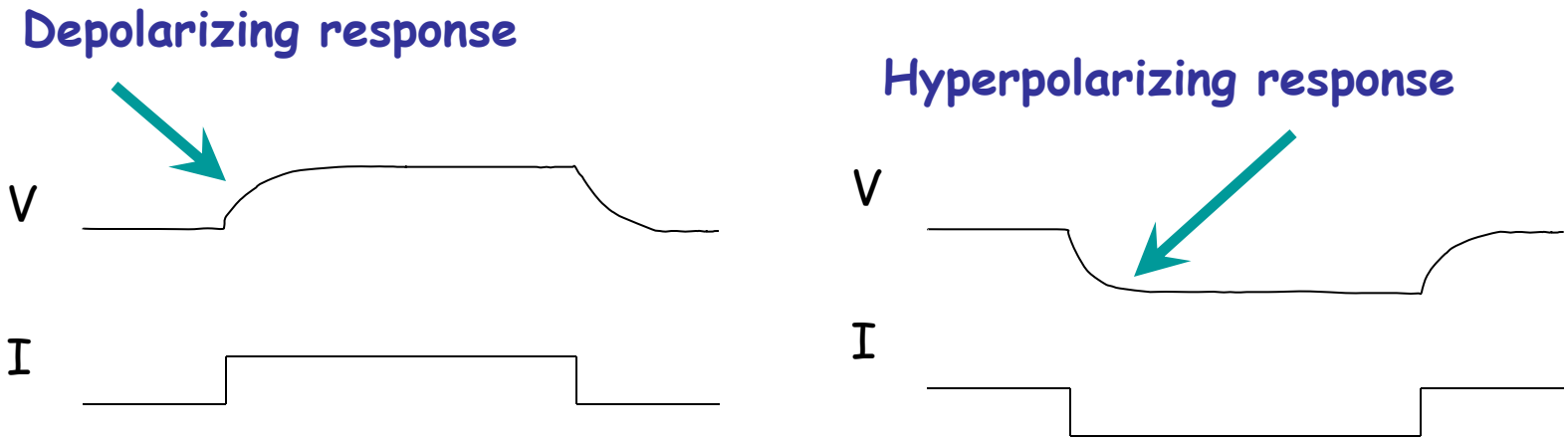
- Measure V_M = Inside - Outside
- Choose Clamp potential (V_C)
- Calculate $V_C - V_M$
- Inject current = $\gamma (V_C - V_M)$

- If $V_C > V_M$, current is positive
  - Membrane potential increases
  - $V_C - V_M$ decreases
- If $V_C < V_M$, current is negative
  - Membrane potential decreases
  - $V_C - V_M$ decreases

- Early inward current
  - Negative current injected by clamp to maintain membrane potential.
  - Negative current is compensating inward flow of positive ions
  - Transient current
- Later outward current
  - Positive current injected to compensate for outward flow of positive ions
  - Persistent current

Voltage clamping: 3 principles

(1) Injecting positive current into the cell depolarizes the cell (injecting negative current hyperpolarizes it).



(2) When current is injected into the cell, it takes some time to hyperpolarize/depolarize the cell because the cell's capacitance must be charged/discharged.

(3) When there is no net flow of ions into the cell, the membrane potential doesn't change.

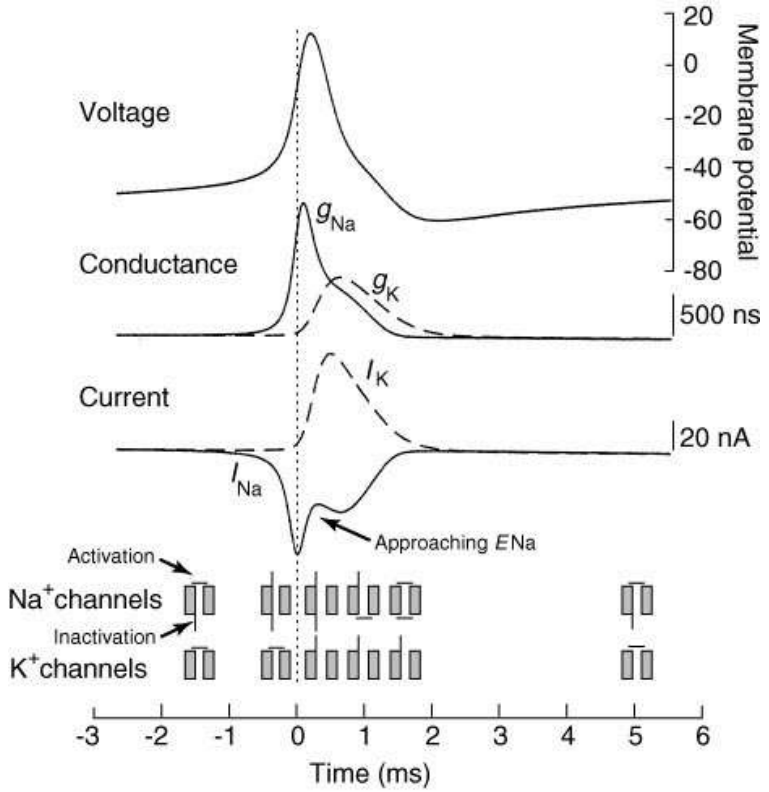
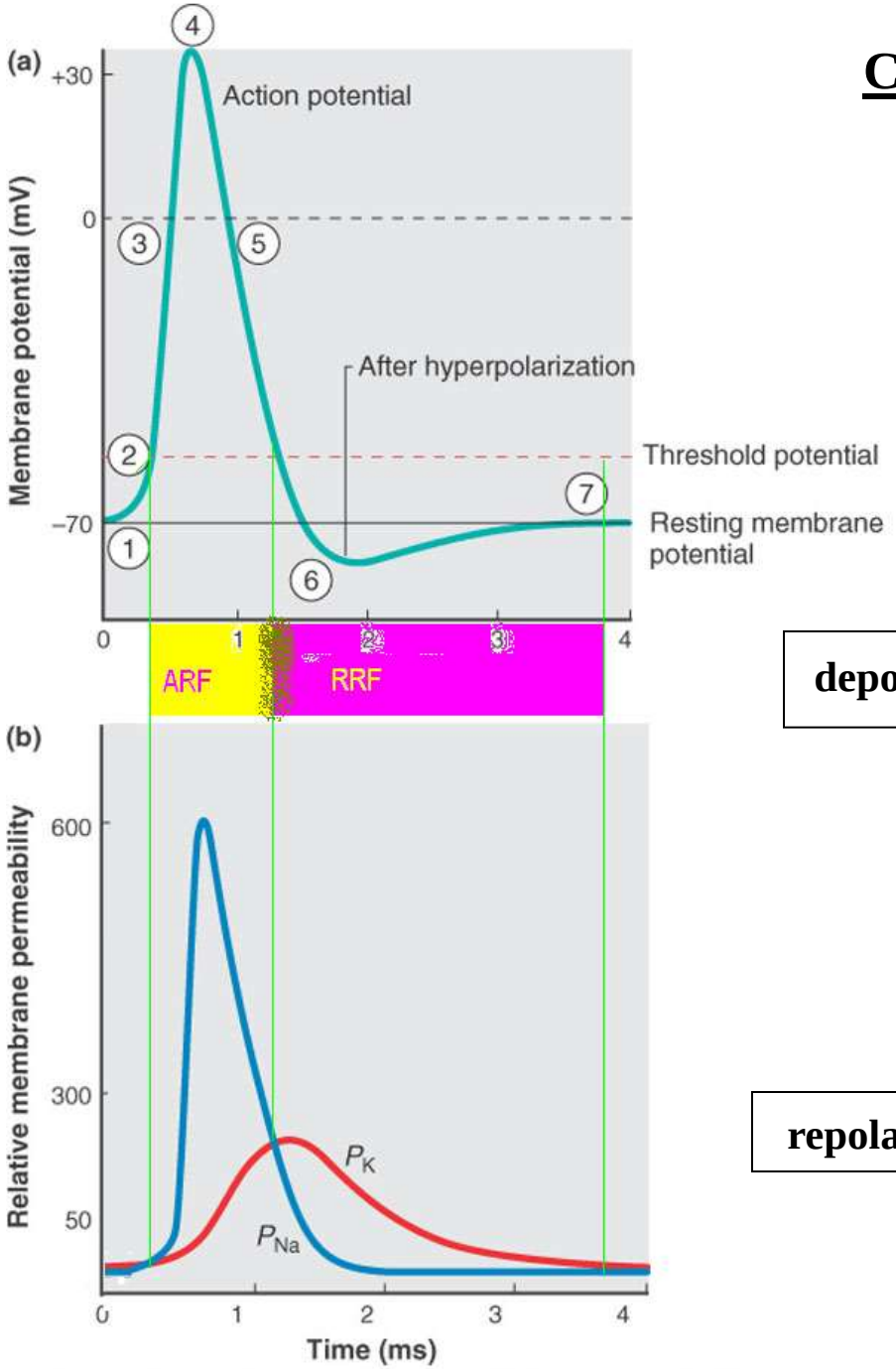


FIGURE 7 Generation of the action potential is associated with an increase in membrane Na+ conductance and Na+ current followed by an increase in K+ conductance and K+ current. Before action potential generation, Na+ channels are neither activated nor inactivated (illustrated at the bottom of the figure). Activation of Na+ channels allows Na+ ions to enter the cell, depolarizing the membrane potential. This depolarization also activates K+ channels. After activation and depolarization, the inactivation particle on the Na+ channels closes and the membrane potential repolarizes. The persistence of the activation of K+ channels (and other membrane properties) generates an afterhyperpolarization. During this period, the inactivation particle of the Na+ channel is removed and the K+ channels close. From Huguenard and McCormick.61

Comparison of Na^+ and K^+ Currents following a Depolarization



Na^+ influx

opens Na^+ channels

depolarization

opens K^+ channels

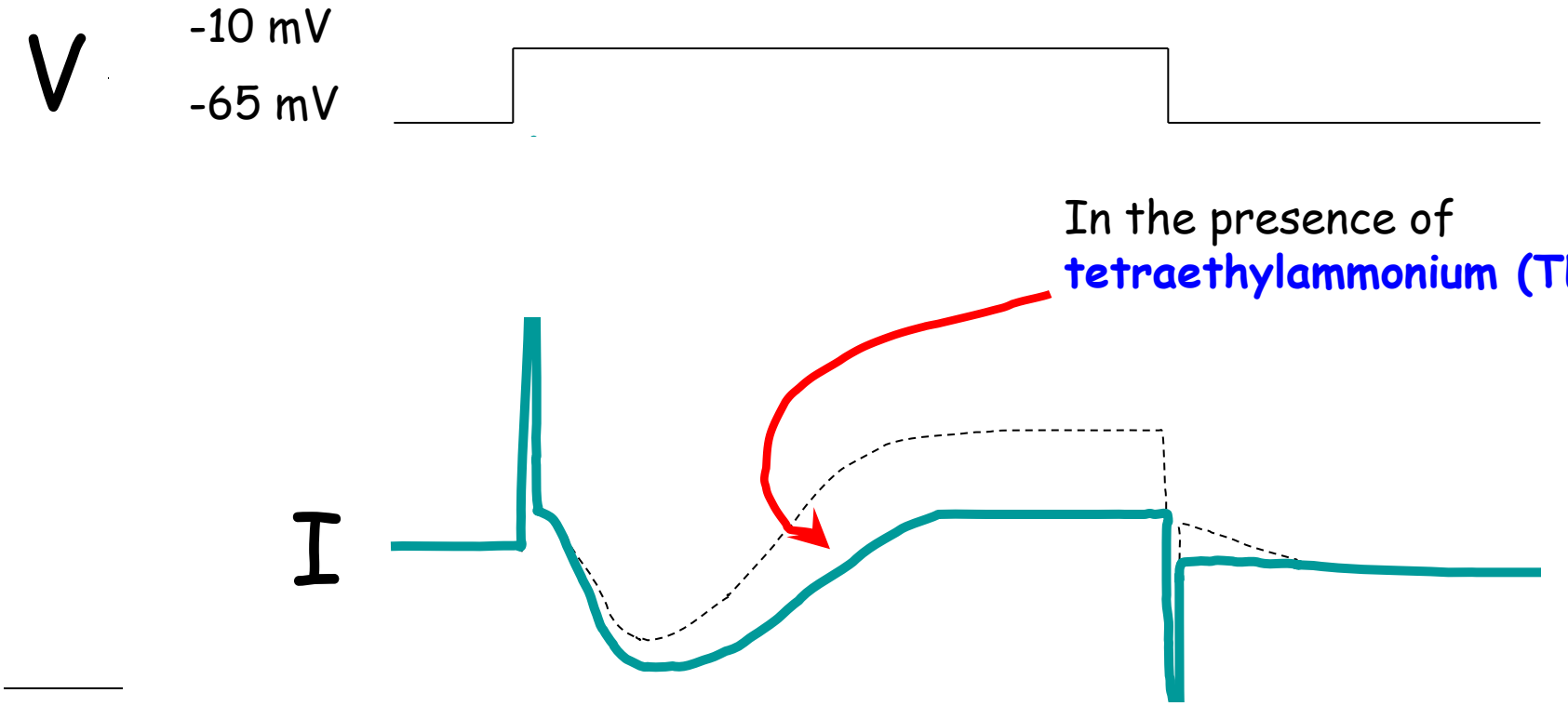
K^+ efflux

repolarization

closes K^+ channels

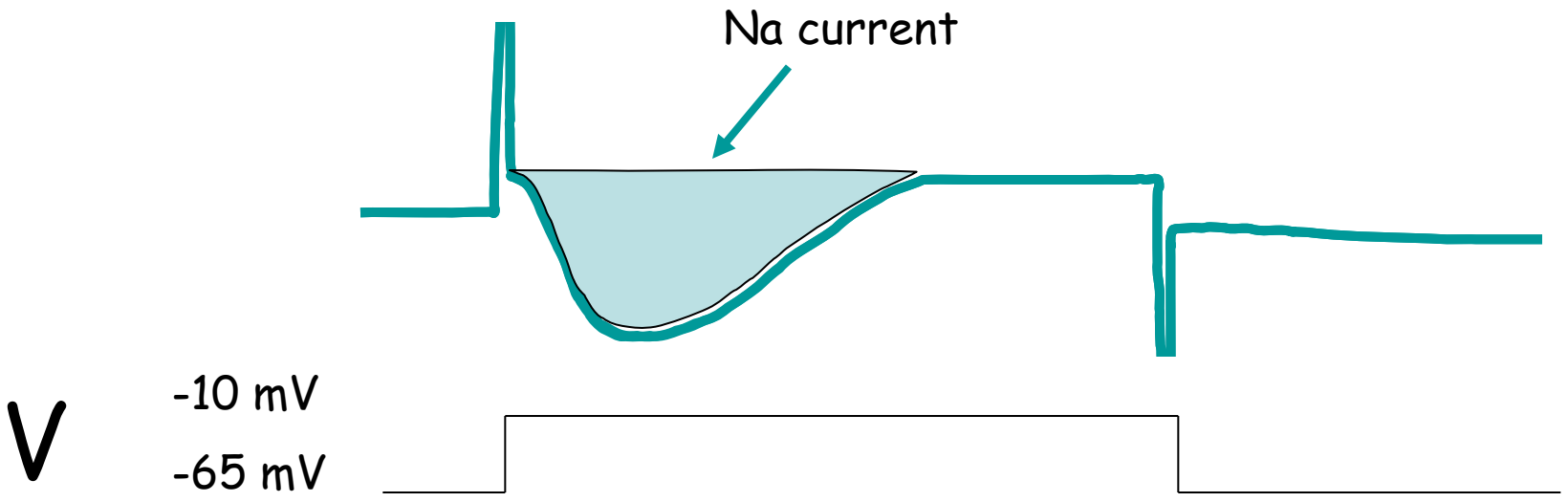
Voltage clamp: what is the behavior of voltage dependent sodium current?

The pharmacological method: Block the potassium current with a drug: **tetraethylammonium**. The voltage-dependent current that remains is the voltage-dependent sodium current.



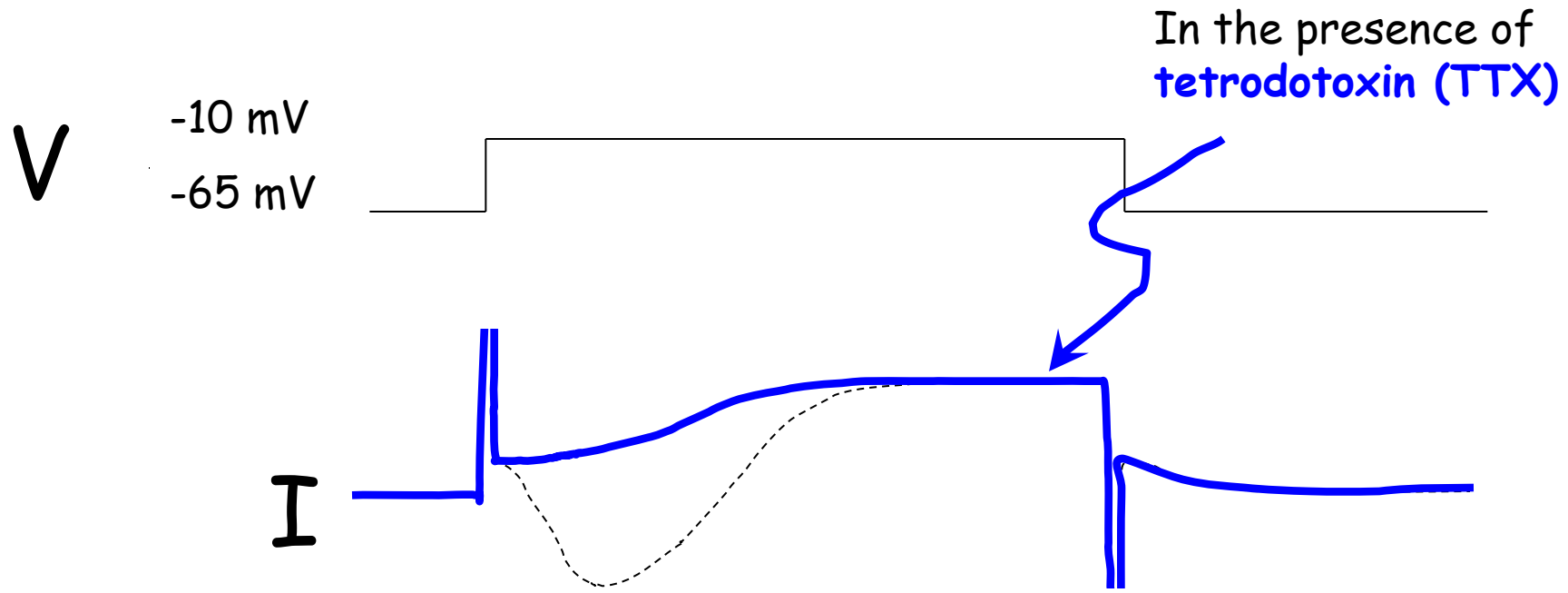
Voltage clamp: what is the behavior of voltage dependent sodium current?

The pharmacological method: Block the potassium current with a drug: **tetraethylammonium**. The voltage-dependent current that remains is the voltage-dependent sodium current.
Note: even with a constant voltage, the sodium current first increases, and then automatically, while the depolarization is maintained, the current decreases (inactivation)



Voltage clamp: what is the behavior of voltage dependent potassium current?

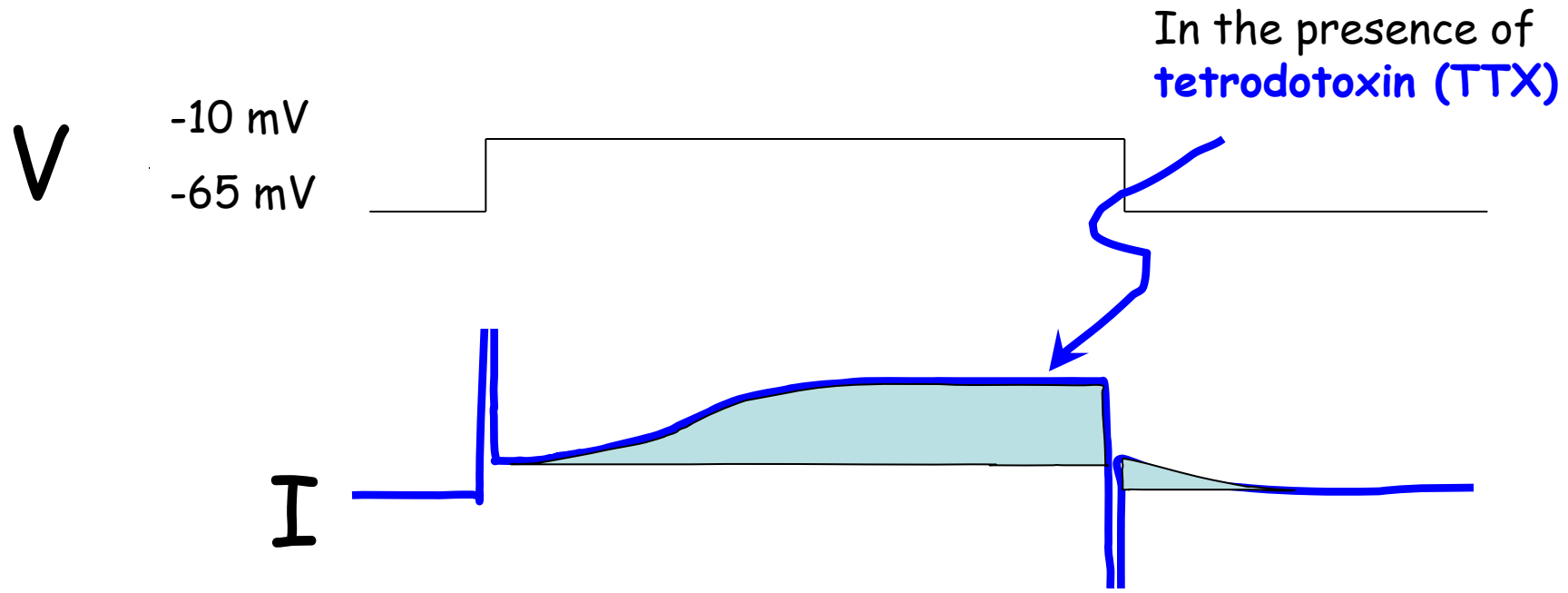
Pharmacological method: Block the voltage-dependent sodium current with **tetrodotoxin**. The current that remains is the voltage-dependent potassium current.



Note: (1) the potassium current is slower to activate than the sodium current. Therefore, sometimes called "delayed current"
(2) the potassium current is maintained for as long as the depolarization is maintained. (only closes after repolarization)

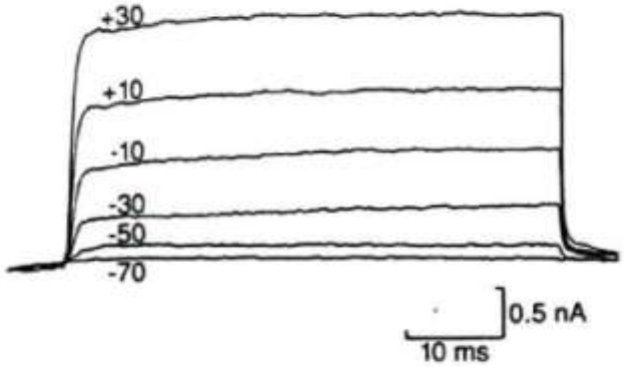
Voltage clamp: what is the behavior of voltage dependent potassium current?

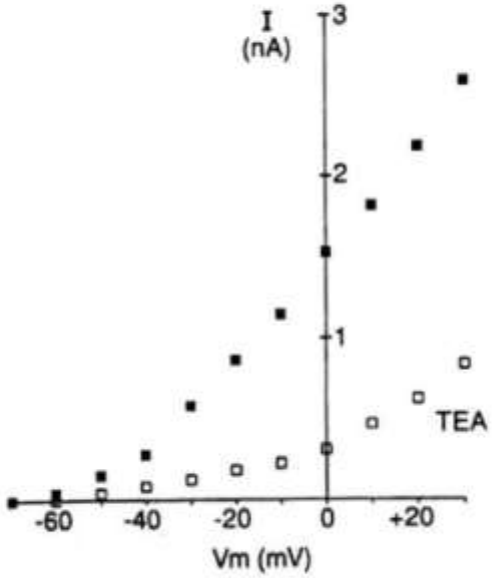
Pharmacological method: Block the voltage-dependent sodium current with tetrodotoxin. The current that remains is the voltage-dependent potassium current.



Note: (1) the potassium current is slower to activate than the sodium current. Therefore, sometimes called "delayed current"
(2) the potassium current is maintained for as long as the depolarization is maintained. (only closes after repolarization)

Whole-cell recording of voltage-gated currents





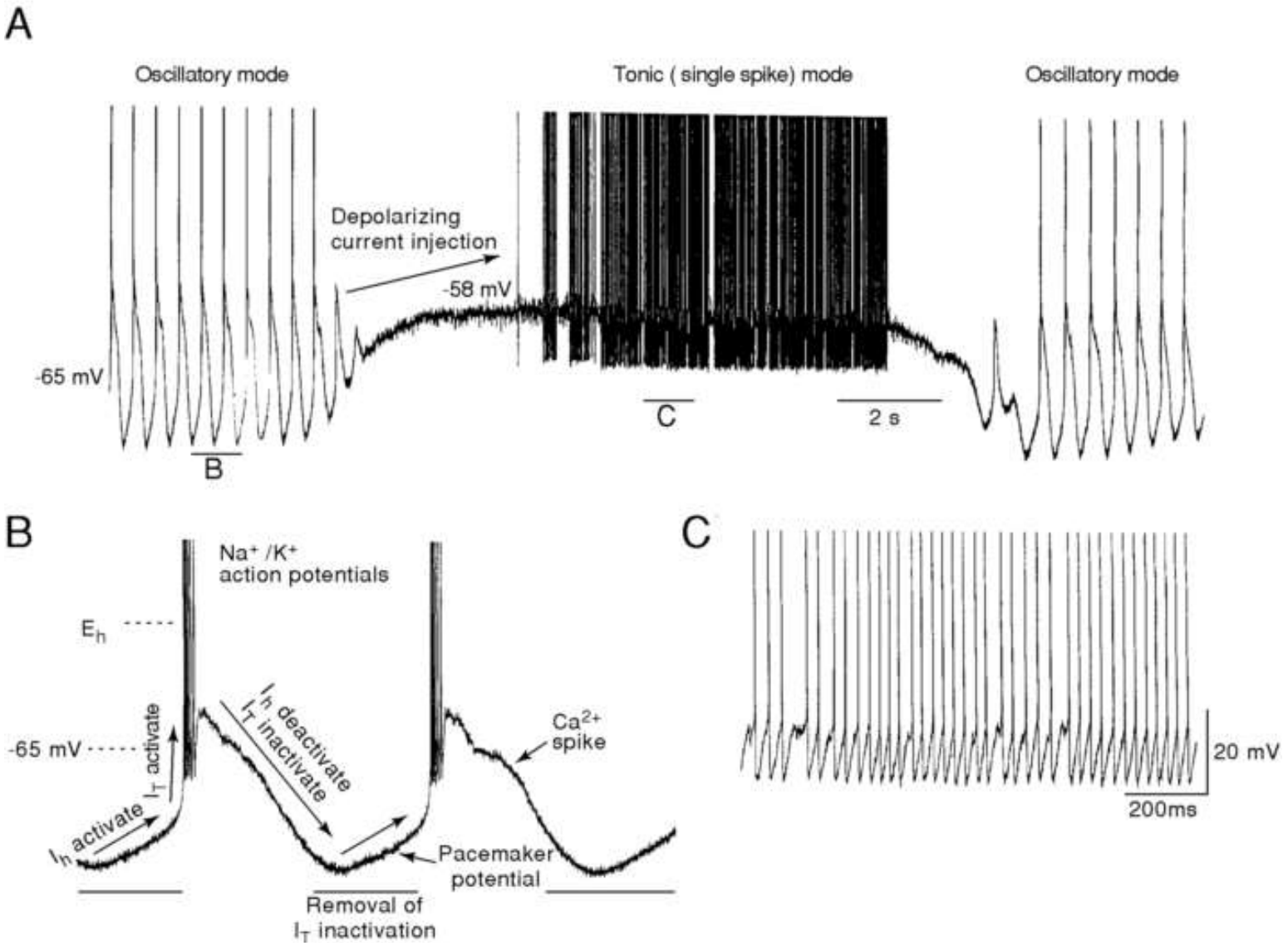


Fig 13. Two different patterns of activity generated in the same neuron, depending on membrane potential. (A) The thalamic neuron spontaneously generates rhythmic bursts of action potentials due to the interaction of the Ca2+ current *I*T and the inward "pacemaker" current *I*h. Depolarization of the neuron changes the firing mode from rhythmic burst firing to tonic action potential generation in which spikes are generated one at a time. Removal of this depolarization reinstates rhythmic burst firing. This transition from rhythmic burst firing to tonic activity is similar to that which occurs in the transition from sleep to waking. (B) Expansion of detail of rhythmic burst firing. (C) Expansion of detail of tonic firing. From McCormick and Pape (1990).

Voltage Dependent Channels

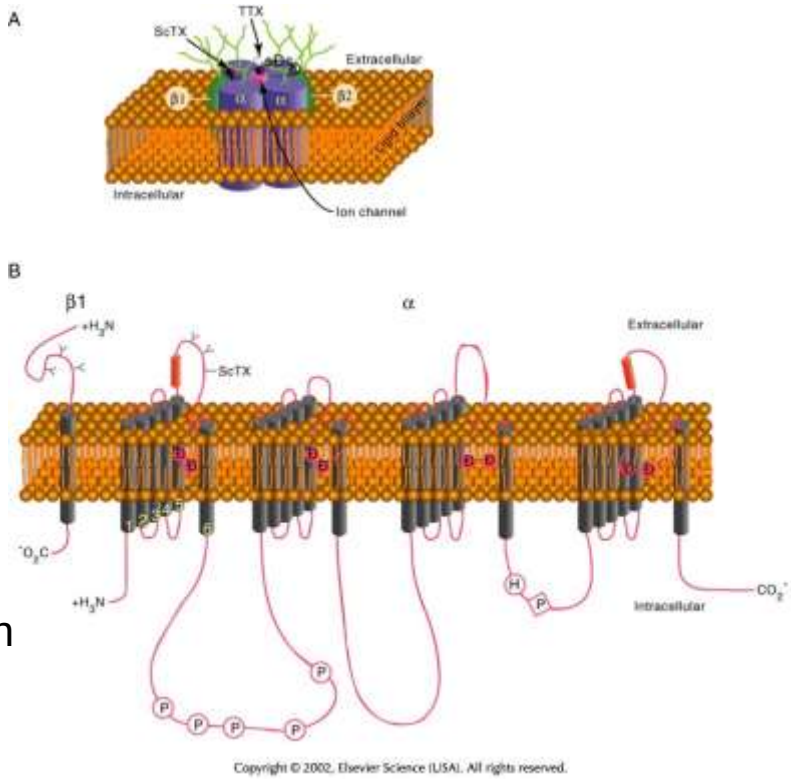
- Diversity of firing patterns produced by myriad voltage dependent channels
- Channels differ by
  - Ion selectivity (e.g. K, Na, Ca)
  - Distribution (Dendrites, soma, axon)
  - Activation and Inactivation properties
  - Sensitivity to drugs
- Sodium channel has two types of gates
  - Activation gate
    - Opens with depolarization
    - Closes with hyperpolarization
  - Inactivation gate
    - Closes with depolarization
    - Opens with hyperpolarization
- Current flows when both gates open

Terminology

- Activation
  - Turning on of current with depolarization
- De-activation
  - Turning off of current with repolarization
- Inactivation
  - Turning off of current with sustained depolarization
- De-inactivation
  - Removal of inactivation (block) by repolarization

Sodium Currents

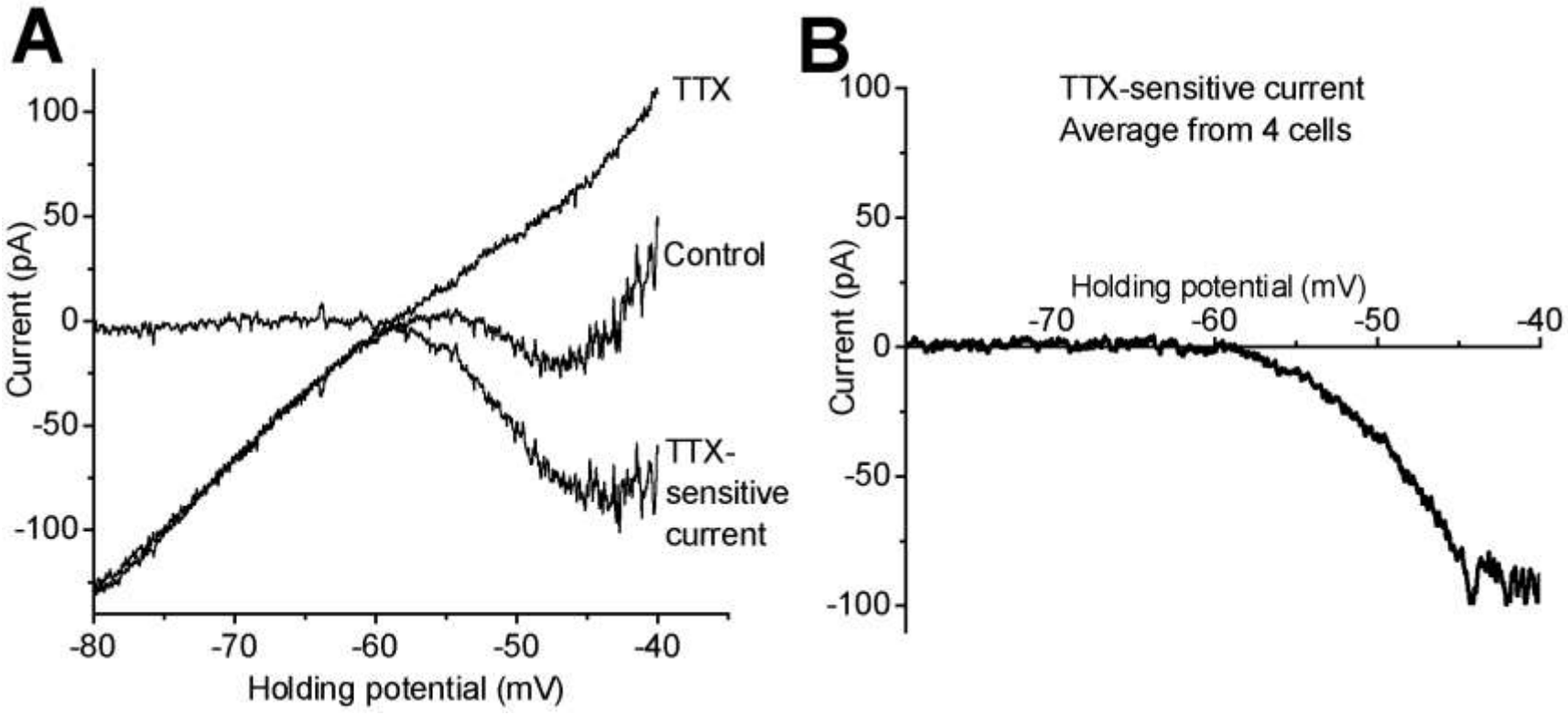
- Transient, I_{NaF}
  - Responsible for Action Potential
- Persistent, I_{NaP}
  - Threshold near resting potential
  - Origin:
    - Window current, or
    - Different gating mode of I_{NaF}, or
    - Separate channel protein



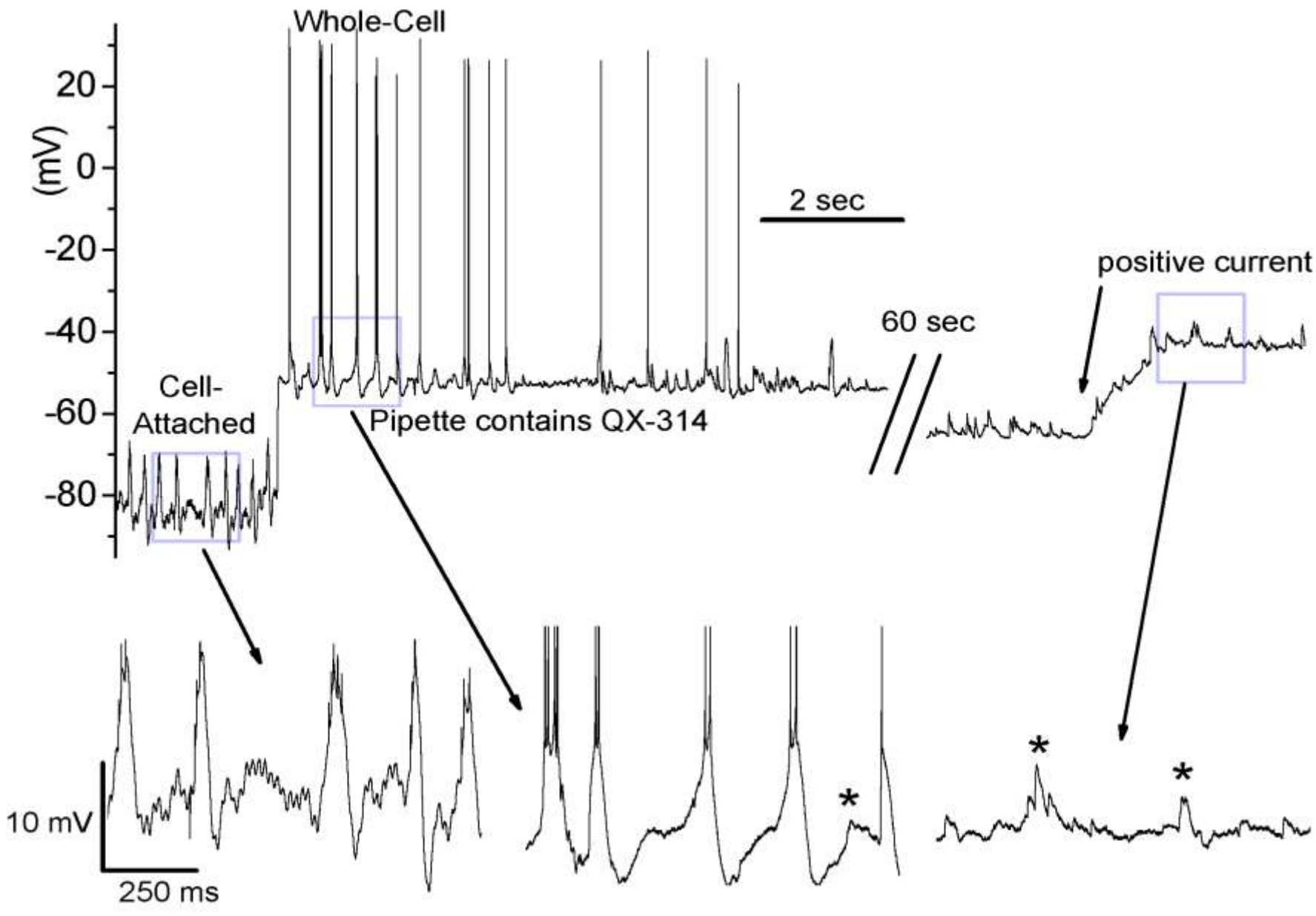
Function of Persistent current
-Enhancement of subthreshold synaptic potentials
-Depolarization activates INaP, => more depolarization
-Hyperpolarization de-activates INaP, which produces more hyperpolarization

Plateau potential
-Prolonged potential that remains after synaptic inputs or current injection is removed
-Contributes to persistent firing

Persistent sodium current is activated by a slow depolarizing voltage ramp (> -60 mV) and is blocked by Tetrodotoxin (TTX)



Bursting is mediated by sodium channel activation and is blocked by intracellular application of a sodium channel blocker QX-314



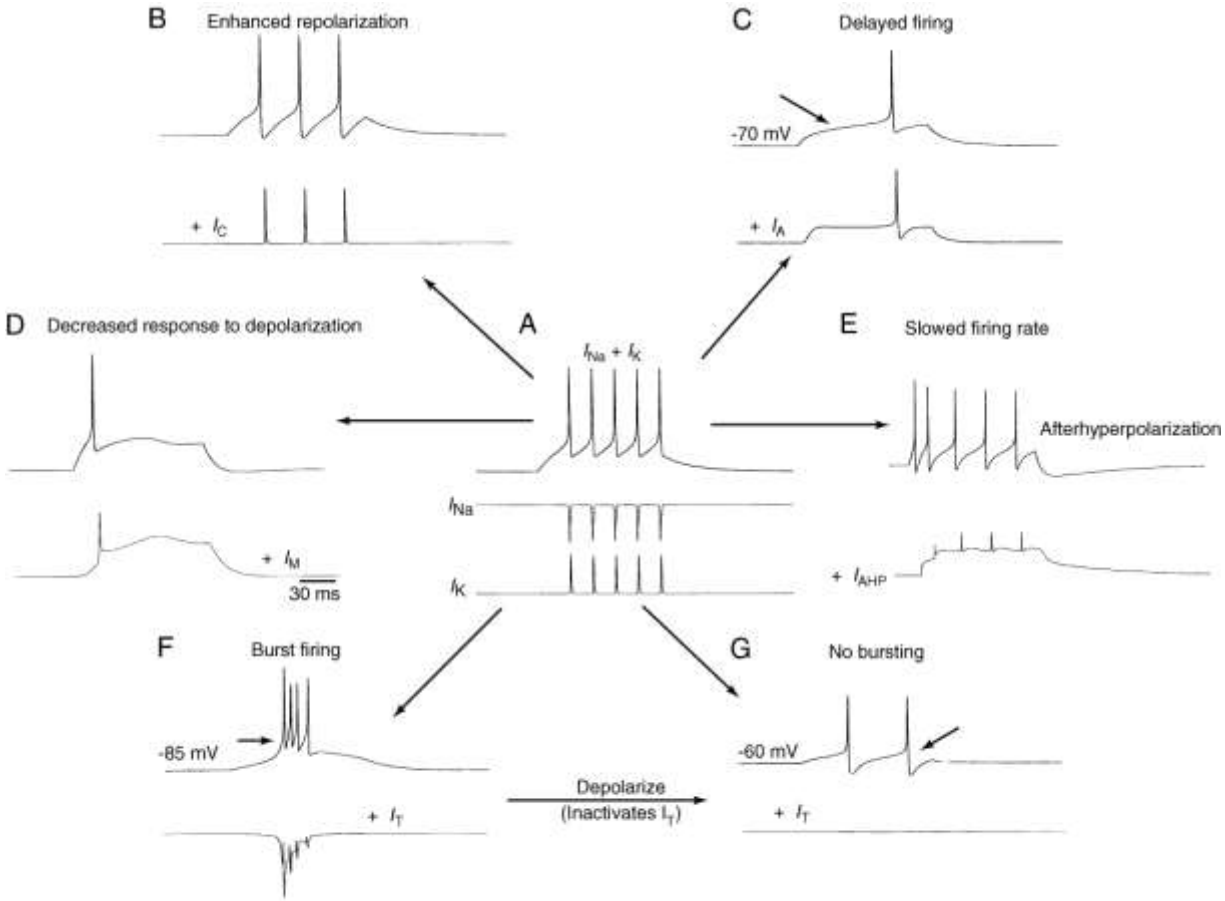


FIGURE 12 Simulation of the effects of the addition of various ionic currents to the pattern of activity generated by neurons in the mammalian CNS. (A) The repetitive impulse response of the classical Hodgkin–Huxley model (voltage recordings above, current traces below). With only *I*Na and *I*K, the neuron generates a train of five action potentials in response to depolarization. Addition of *I*C (B) enhances action potential repolarization. Addition of *I*A (C) delays the onset of action potential generation. Addition of *I*M (D) decreases the ability of the cell to generate a train of action potentials. Addition of *I*AHP (E) slows the firing rate and generates a slow afterhyperpolarization. Finally, addition of the transient Ca2+ current *I*T results in two states of action potential firing: (F) burst firing at -85 mV and (G) tonic firing at -60 mV. From Huguenard and McCormick (1994).