Typical electrophysiological measurements and information they provide

Miraslau Barabash

Lancaster University

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Example: ion channel family in the Jurkat cells

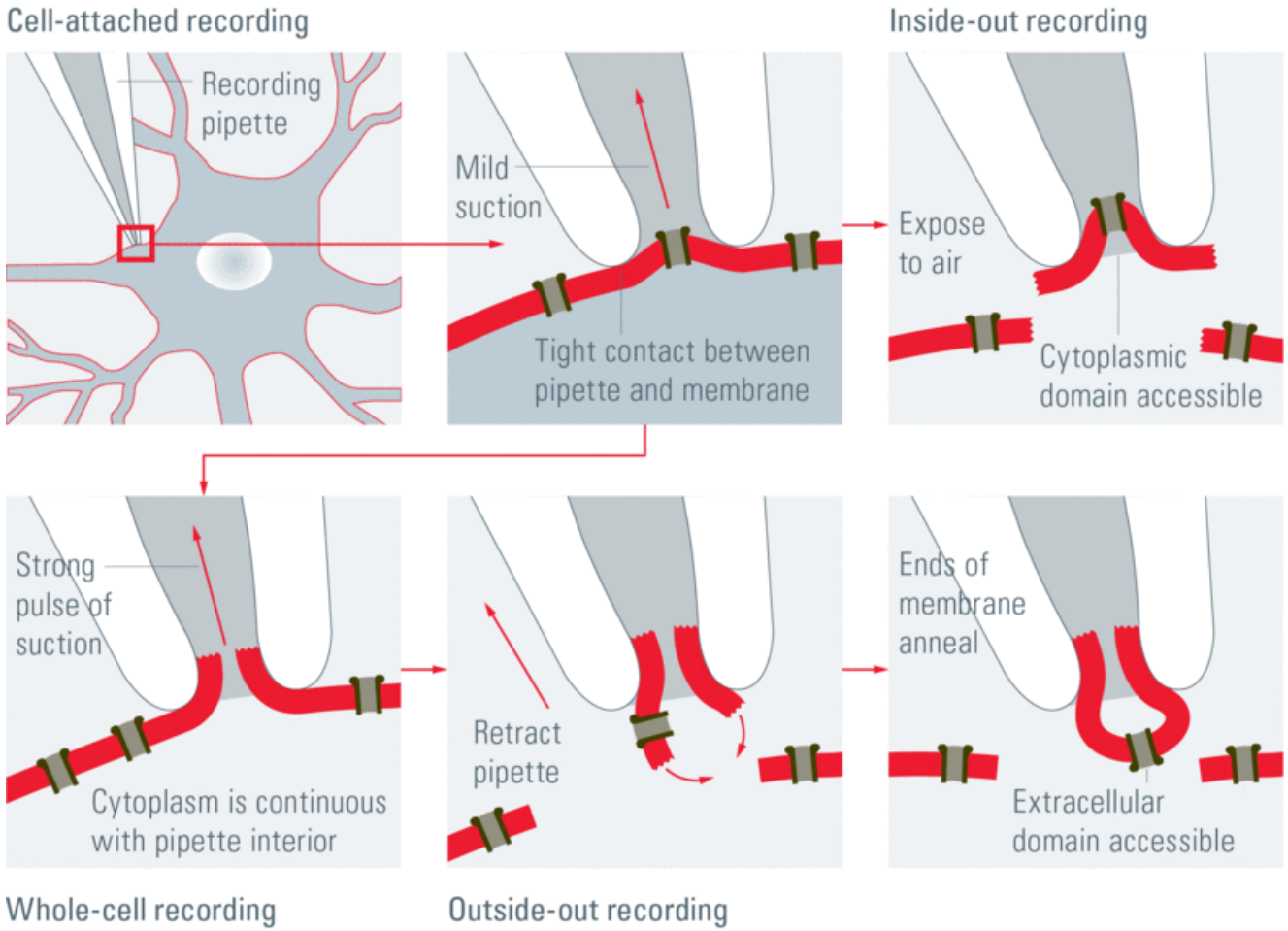
Channels (passive transporters)					
Channel	Selectivity	Conductance [pS]	Activation parameter	Function in Jurkat TL	Note
Kv1.3	K	3-5 (10-22?)	V (depolarization, Fig. 2) [2,3]	V maintainance, Volume regulat	100-400 ICs/cell. main efflux pathway in T cells. Delayed rectifier. Unusually broad sensitivity to pharmacological agents. Raising $[Ca]_i$ accelerates inactivation. Not the main pathway in our case [4].
KCa3.1	K	33-50	Ca^{2+}, Fig. 2 [2,3]. Adenosine inhib [5]	V maint; T cell activation; cytokine production; autoimmune colitis	10-20 channels. Opens rapidly when Ca^{2+} increases. [slow inactivation, delayed rectifier characteristics???]
Cl (mini)	Cl	1-2	Ca-activ?, (osmotic) pressure, hypotonic intra, ATP presence, swelling [2,3,6–8]	Volume regulation	$\sim$ 10000 channels. Outward rectif. Current is induced with a $\sim 1min$ delay following cell swelling, current is sustained as long as cytosolic ATP is present. $f \sim 300Hz$. Average surface density 10-100 Cl channels/μm^2.
Cl (maxi)	Cl	300	V (hyperpolarisation)	Volume regulation	Time-dependent gating (inactivation). No evidence that these are activated by osmotic stress.[Lewis1993.Check!!!]
Cl	Cl	40	Ca, cAMP [9]	Volume regulation. Cytokine production by T cells?	No evidence that these are activated by osmotic stress.[Lewis1993.Check!!!]
	H ???		pH [10]		Speculative. Voltage increases by 47mV if $pH \to pH + 1$ ($c_H \times 10$)
TRPM4	Na, K. not Ca		Ca^{2+}-activated, V-gated [2,3]		
	Na		V		revealed by a small fraction (1-5%) of normal TL
TRPM7 (MIC)	Mg, Ca, K, Na		ATP enhanced. Mg, MgATP inhibited Mg^{2+} depletion [2,3]	Mg and Ca homeostasis during metabolic variations	Outward rectifying, nonspecific to mono- and divalent cations.
$P2X_7$	Ca, Na, other cations?		ext ATP [11]	Ca homeostasis	
Ca_V	Ca		V [2]		debatable, not well documented
	Ca	~ 7	mitogen-stimulated [1,12]		$f^{-1} = 0.4 - 0.5s$. Not measurably V-dependent.
CRAC	Ca	9-24 fS	Ca^{2+} depletion in ER [2,3]		low conductance, inward rectif
IP_3R (in ER)	Ca		IP_3	Ca release from ER	In ER's membrane

Example: ion channel family in the Jurkat cells

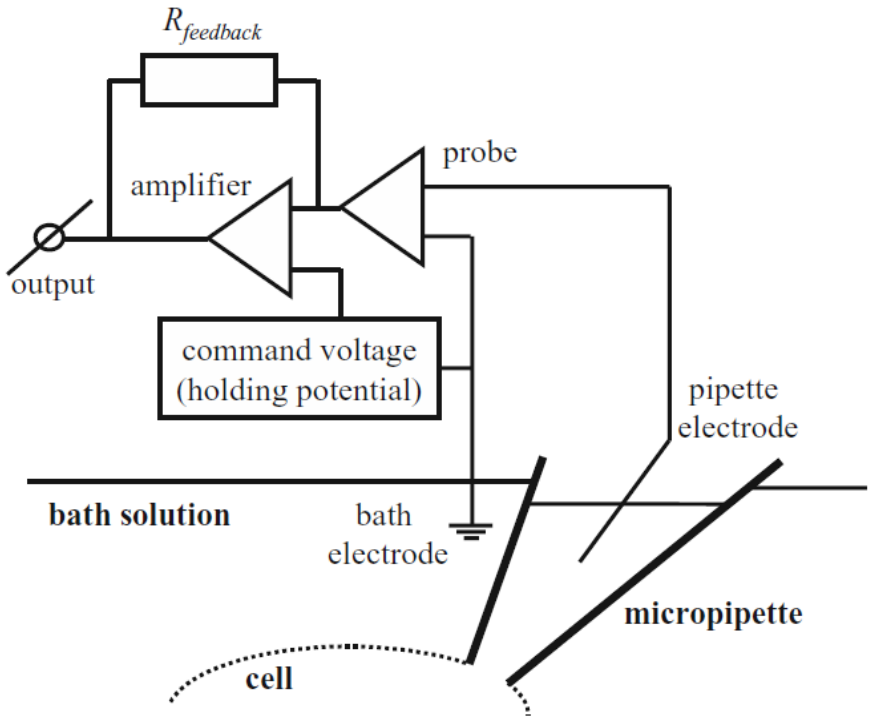
Pumps (active transporters)					
Na/K-ATPase	Na/K		ATP [13, 14]	Na, K maintenance	3 Na out, 2 K in.
Plasma membrane Ca ATPase	Ca		ATP [15]	Ca extrusion from cytosol	
SERCA	Ca		ATP		Ca: from cytosol to the lumen of the ER
Proton pump	H		ATP (exist in our case???)		H out
Exchangers (secondary active transport)					
Na/H	Na, H		ATP regulated [16–18]	major pH_i regul. Cellular vol	
Cl/HCO_3	Cl, HCO_3		[18]	Volume regul	
Na/K/2Cl cotransporter	Na, K, 2Cl		??? [18]	Volume regul	Na:K:2Cl. Energy from Na-ElChem grad (secondary active transport)
Other conductors					
Aquaporin	H_2O		[19]		
GLUT-1	glucose		[20, 21]	glucose transport effector	
Pannexin-1	ATP		[22]	T-cell activation at the immune synapse	

Patch-clamp: major electrophysiological tool

1. Whole-cell
2. Perforated patch
3. Cell-attached
4. Loose patch
5. Inside-out patch
6. Outside-out



Whole-cell patch-clamp technique



Chemical	ECS concentration (mM)	ICS concentration (mM)
Na^+	126	5
K^+	6	147
Mg^{2+}	2.5	1.2
Ca^{2+}	1.2	0
Cl^-	125	150
GTP	0	0.1
ATP	0	5
HEPES	10	20
Glucose	11	11
Sucrose	67	0

Properties

Peculiarities:

1. large pipette
2. $V_b \gg V_p \gg V_c$
3. perfusion
4. Dialysis

Assumptions:

1. Constant temperature
2. Equal osmolarities
3. Equal pH
4. Equal hydraulic pressure
5. No hardware filtering

Advantages:

1. Quick assertion of ion channel populations
2. Cytosolic environment is controlled
3. Extracellular side can be perfused

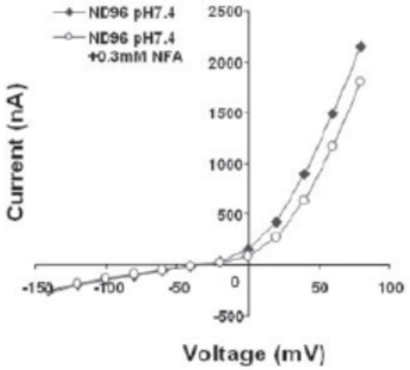
Disadvantage: Washout of cytosolic factors (Dialysis).

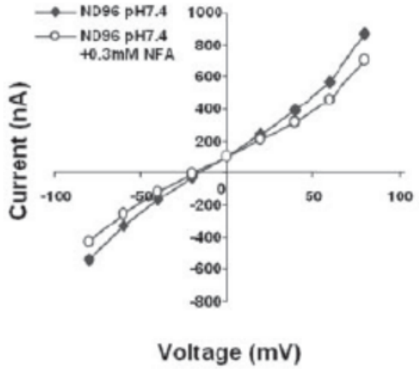
Extracted information

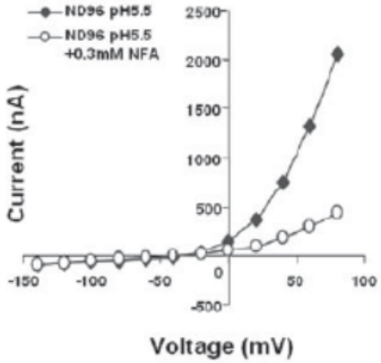
Separation of required current:

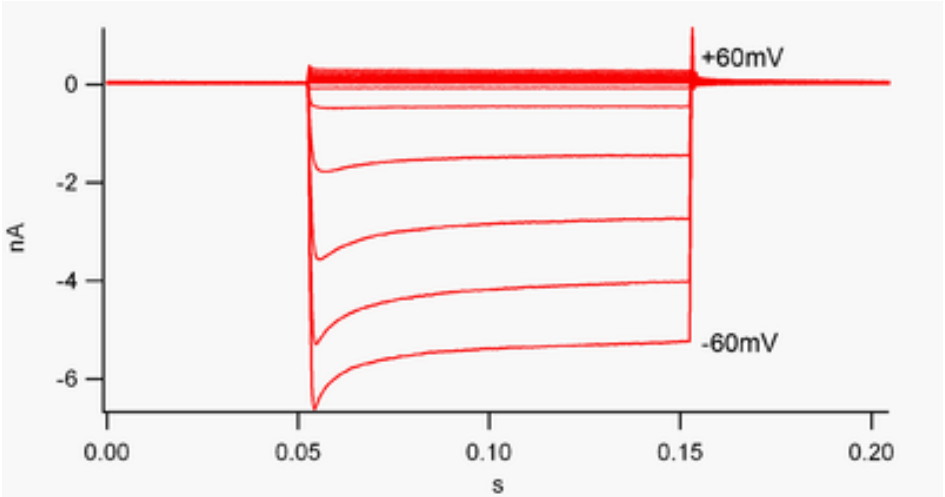
1. Shape of the current response
2. Drugs (blockers for specific channels and pumps)
3. Manipulation of ion concentration. Problem with an unknown channel.

Conductance



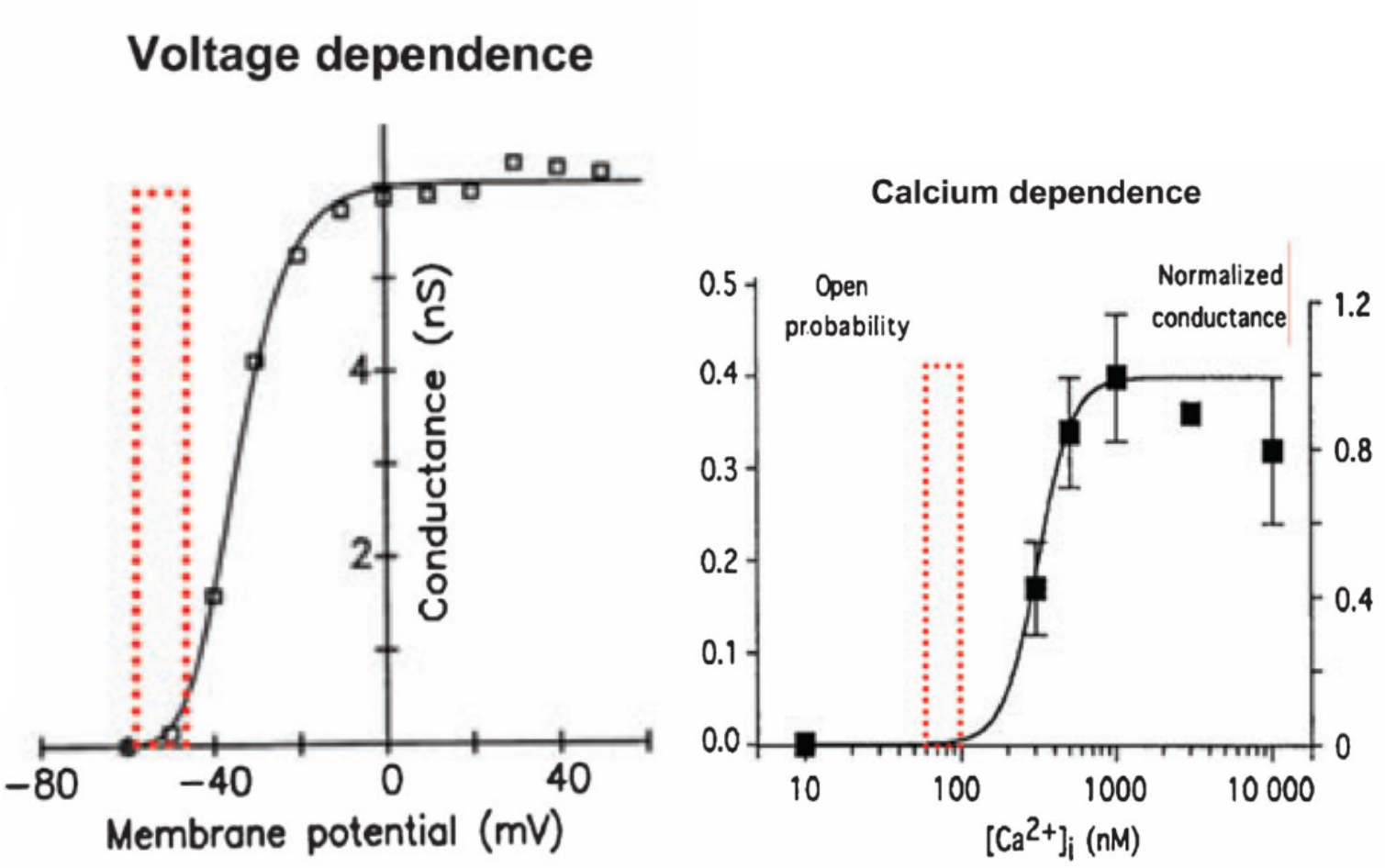




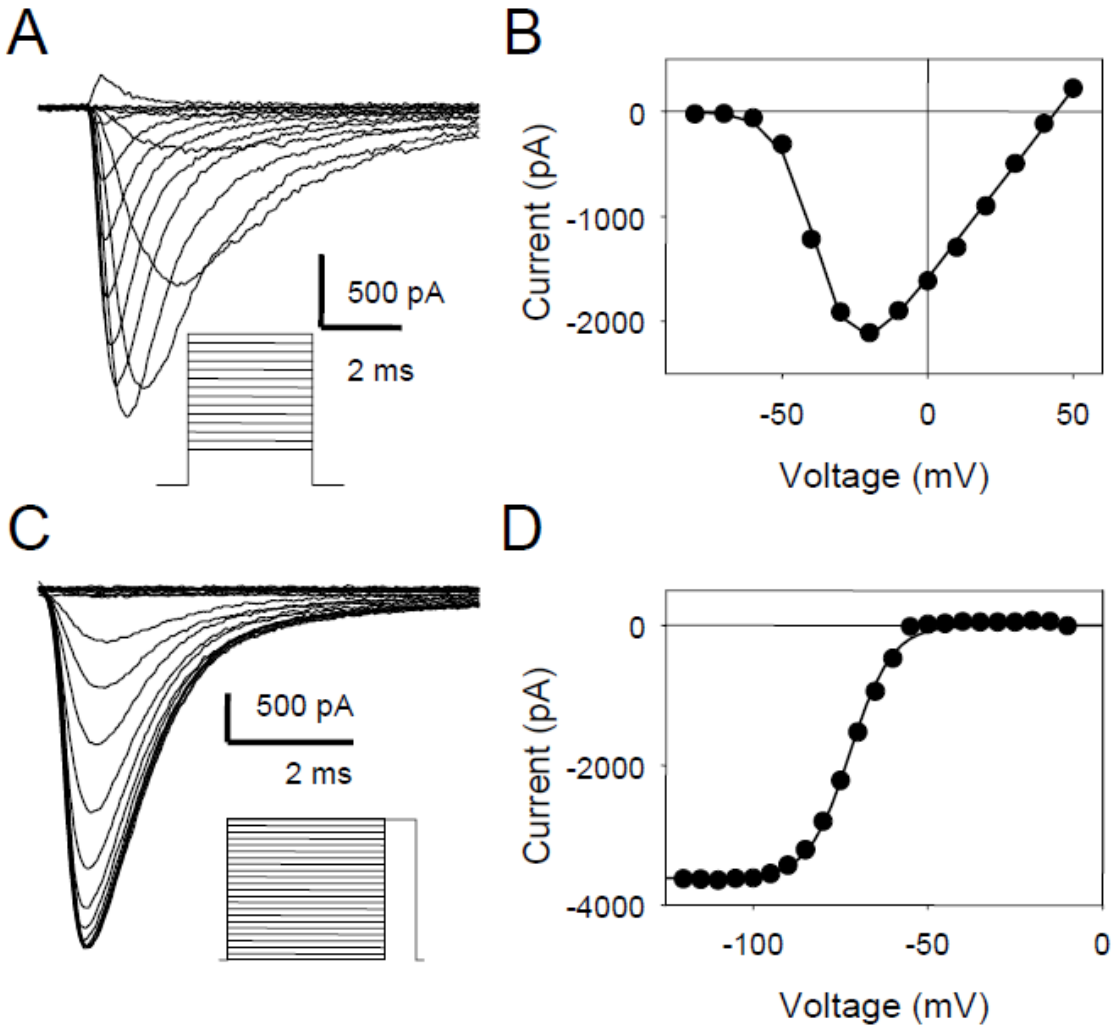


Conductance

f(V) or f(Ca, etc)

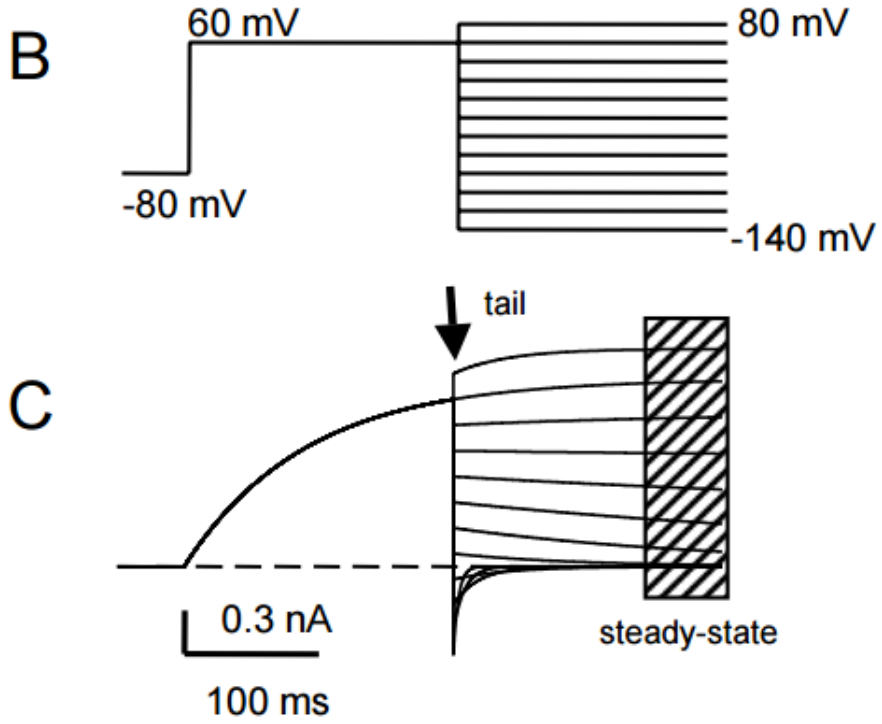


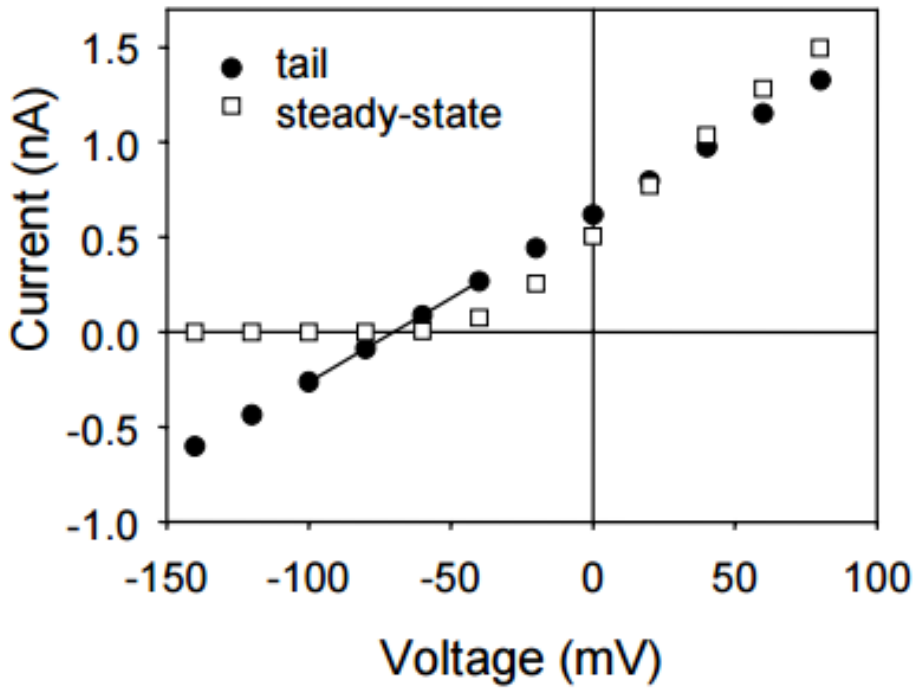
Peak currents



$$I(V) = G_{Na}(V - E^{rev})\frac{1}{1 + exp[z_g(V_{1/2} - V)F/RT]}$$

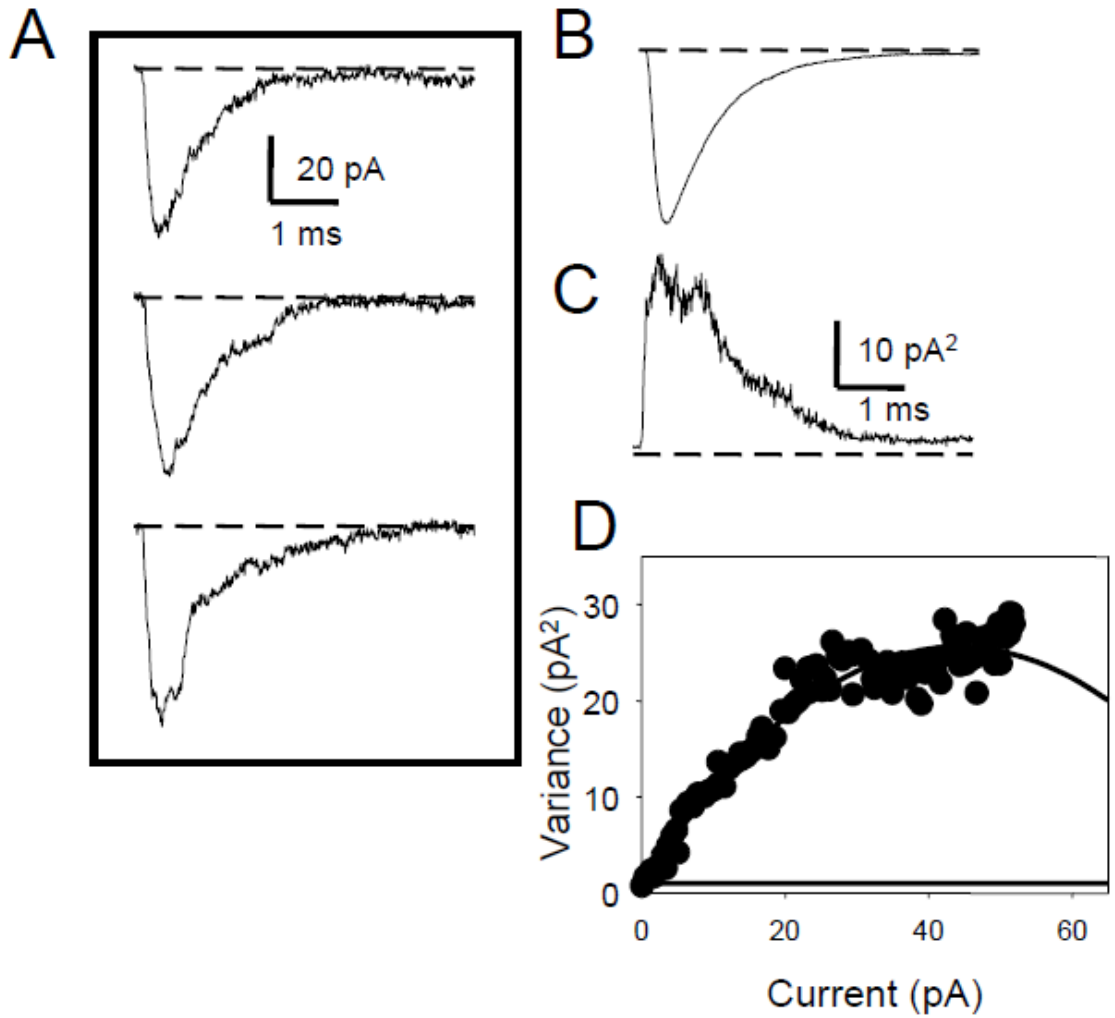
Tail-current analysis





Equilibrium (Nernst) potential: $E^{rev} = \frac{RT}{zF} \ln(\frac{c^e}{c^i})$

Non-stationary noise analysis



$$I(t) = \sum a_m \mathbf{e}^{-t/\tau}$$

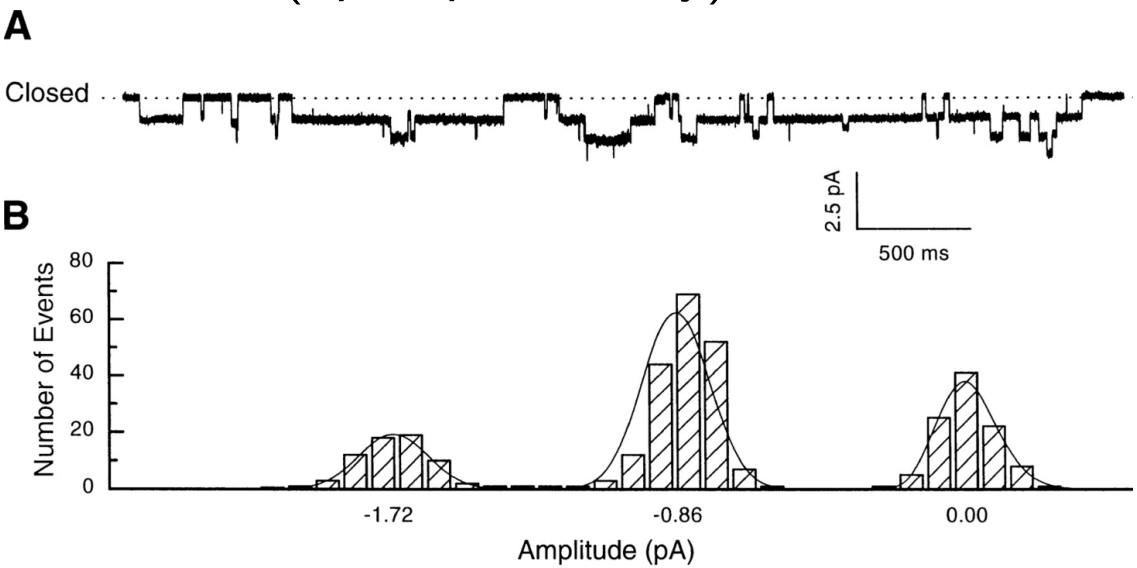
$$\sigma^2 = iI - I^2/N$$

Single-channel recordings

conduction

rectification

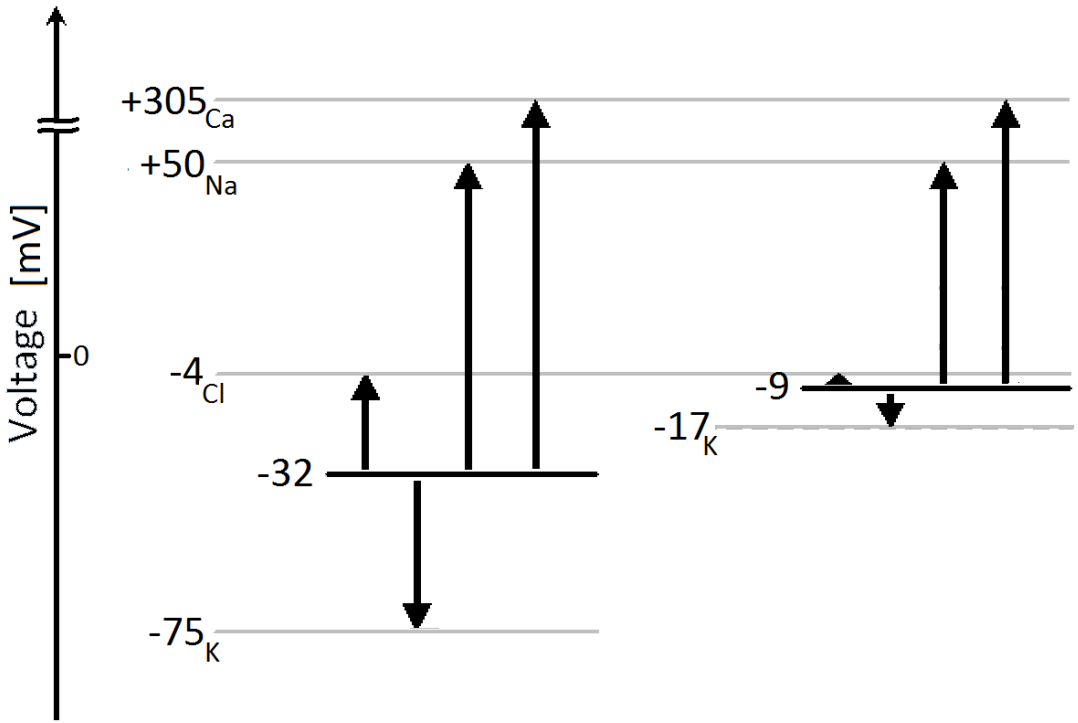
dwell time (open probability)



GHK: V(C)

Easy and therefore common

$$\phi = \frac{RT}{F} \ln \left[\frac{P_K c_K^e + P_{Na} c_{Na}^e + P_{Cl} c_{Cl}^i}{P_K c_K^i + P_{Na} c_{Na}^i + P_{Cl} c_{Cl}^e} \right],$$



GHK

Assumptions:

1. constant electric field
2. ions move under the influence of diffusion and the electric field
3. concentrations of ions at the edges of the membrane are directly proportional to those in the aqueous solutions.
4. The ions access the membrane instantaneously from the intra- and extracellular solutions.
5. The membrane is a homogeneous substance
6. The permeant ions do not interact.

Disadvantages:

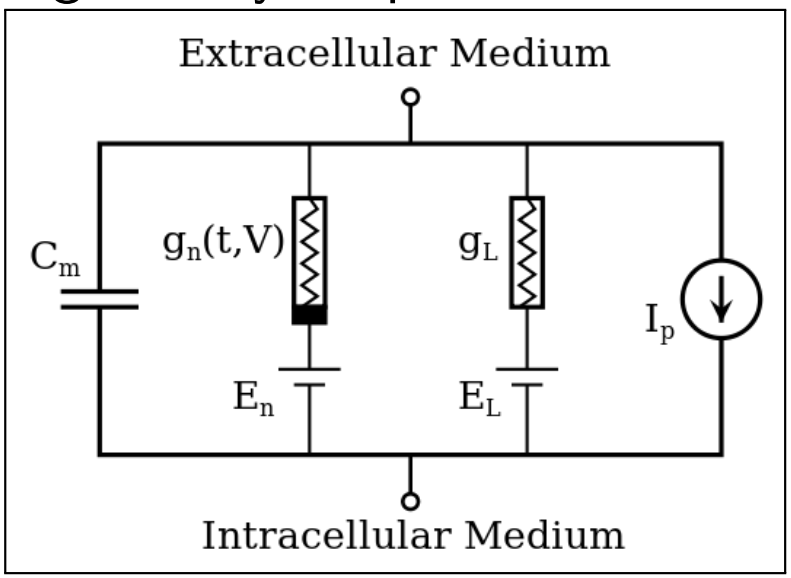
1. Constant field contradicts Poisson's eq.
2. Steady. Fixed bulk concentrations.

Phenomenological models

Polynomial equations to describe ionic current kinetics
Functions are more intimately associated with physical realities
Many parameters (usually obtained from experimental fit)
HH
FitzHugh-Nagumo

Hodgkin-Huxley model

Ingeniously simple.



$$I = I_p + C_m \frac{\mathrm{d}V_m}{\mathrm{d}t} + \bar{g}_\mathrm{K} n^4 (V_m - V_K) + \bar{g}_\mathrm{Na} m^3 h (V_m - V_{Na}) + \bar{g}_l (V_m - V_l),$$

$$\frac{dn}{dt} = \alpha_n(V_m)(1-n) - \beta_n(V_m)n$$

$$\frac{dm}{dt} = \alpha_m(V_m)(1-m) - \beta_m(V_m)m$$

$$\frac{dh}{dt} = \alpha_h(V_m)(1-h) - \beta_h(V_m)h$$

HH: pros and cons

Current balance equation obtained using Kirchoff's law and relaxation equations for the components in the ionic conductances.
Accurate assumption: K_v^+ channels contain four identical voltage-sensor domains that can activate largely independently.
Assumptions:

- Ionic currents obey Ohm's law
- The channel contains four independent and identical voltage sensors that activate in two steps.
- Channel opening (CO) represents a concerted conformational change that follows but is distinct from voltage-sensor activation.

Limitation:
Does not capture correctly the kinetics of the Na^+ channel.
Cannot account for the stochastic response to current injection (discrete nature of ion channels).
Spatial domain effects
Hydration
No C-V coupling

Questions

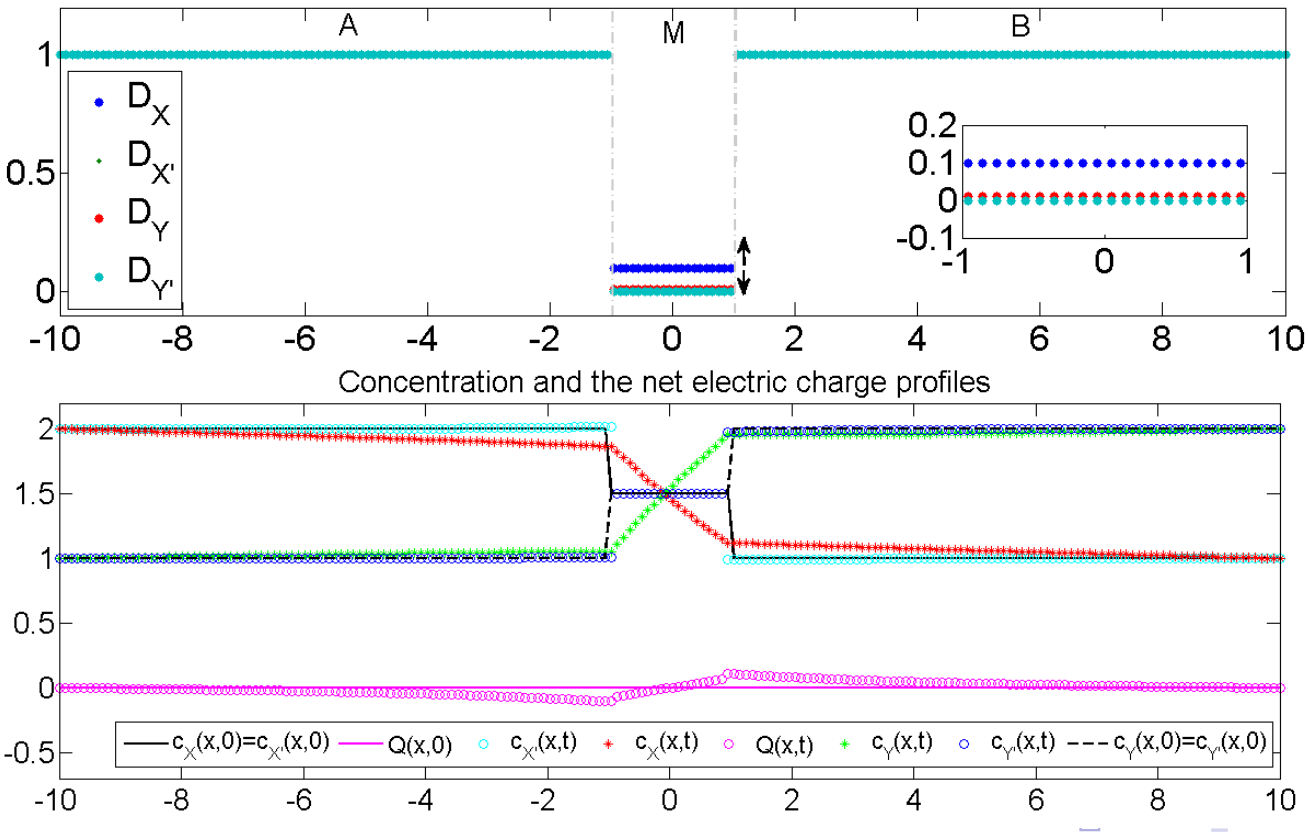
- How the activity of ion channels alters voltage properties?
- Why do fluctuations appear?
- What defines their magnitude?
- How the observed spectrum can be explained?

Briefly: theory is needed.

Simple model: Poisson-Nernst-Planck

$$\frac{\partial c_m}{\partial t} = -\nabla \mathbf{J}_m, \ \mathbf{J}_m = -D_m \left(\nabla c_m - \frac{z_m e}{k_B T} c_m \mathbf{E} \right) + \mathbf{j}_m,$$

$$\nabla \varepsilon \mathbf{E} = -\nabla(\varepsilon \nabla \phi) = 4\pi \varrho = 4\pi e \sum_m z_m c_m,$$



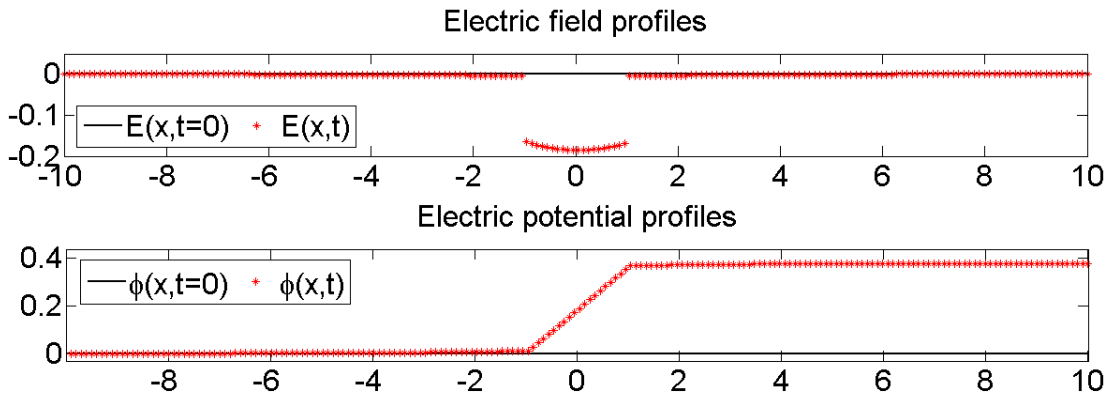
PNP: Pros and cons

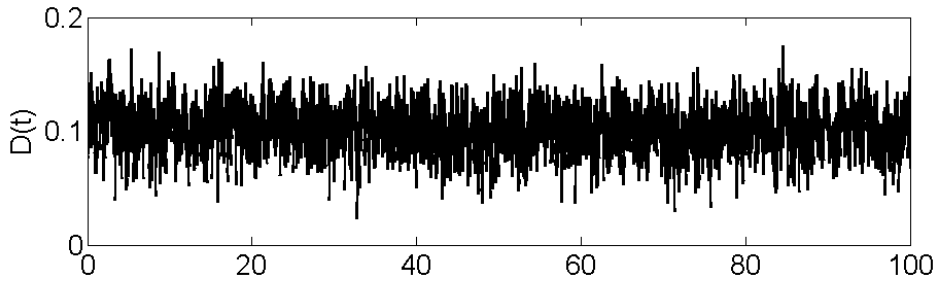
Advantages:

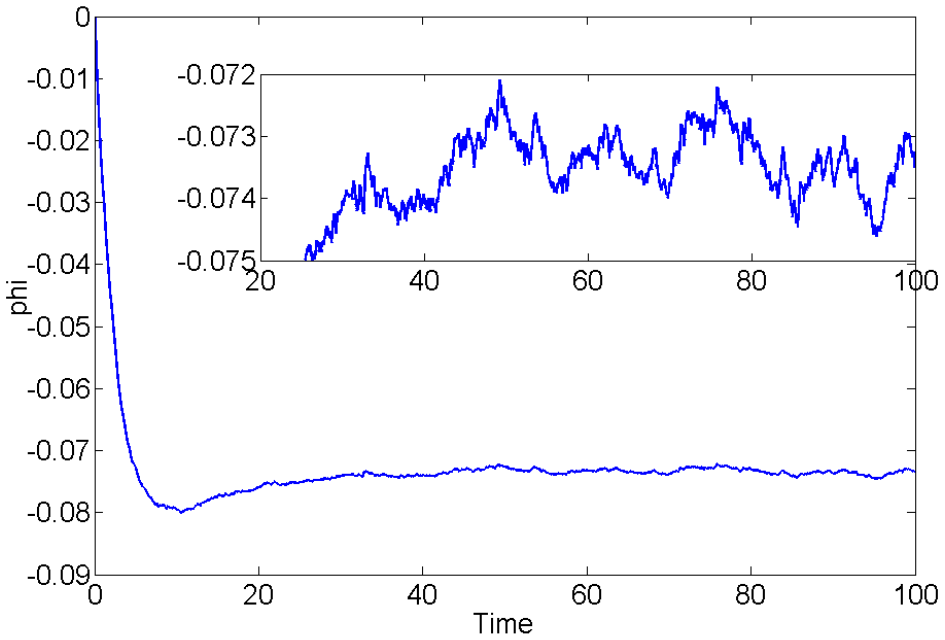
1. from profound physical principles
2. self-consistent
3. computationally cheap
4. I-V curves. Detection of a given ion species.

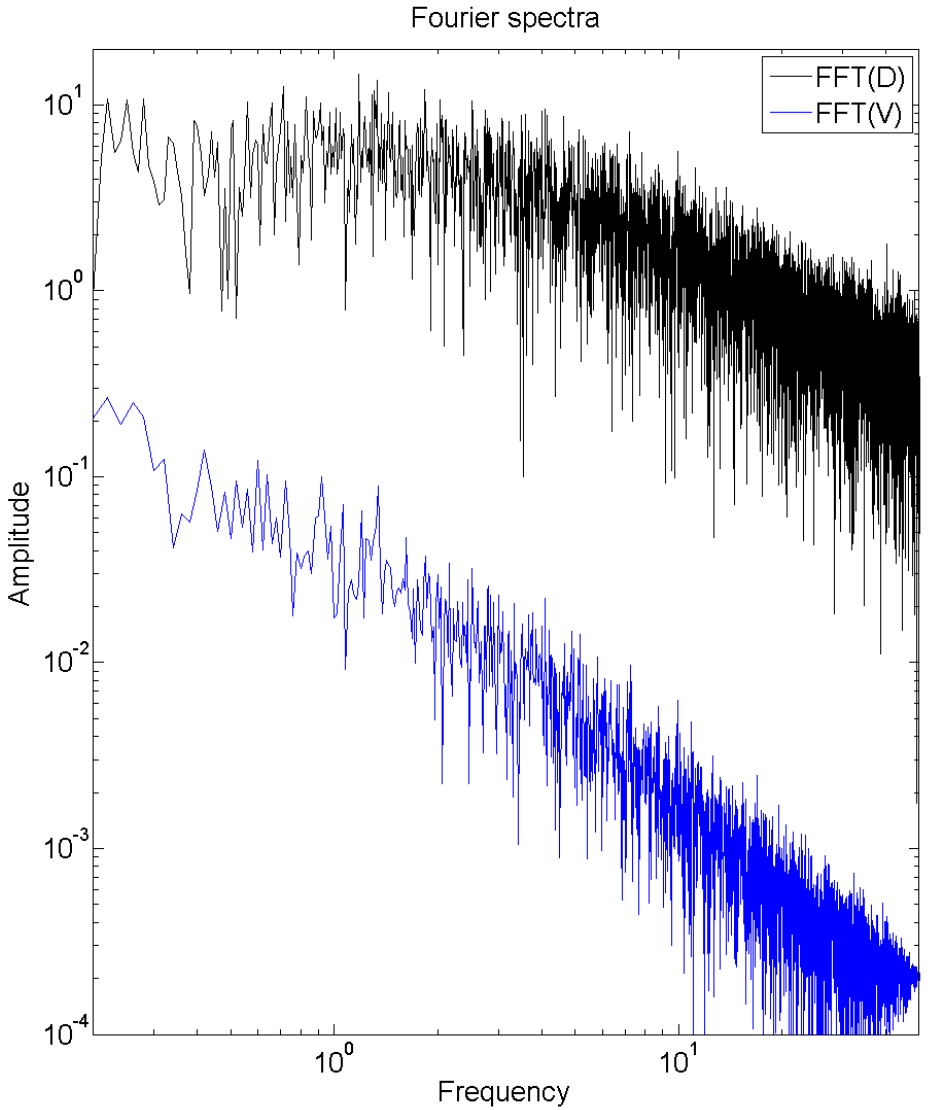
Faults:

1. Continuous. Not applicable in narrow channels.
2. No saturation
3. No self-energy barrier
4. Spontaneous gating only. No voltage gating.




D(t)
0.2
0.1
0
0
20
40
60
80
100


phi
Time
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
-0.072
-0.073
-0.074
-0.075
20
40
60
80
100


Fourier spectra
FFT(D)
FFT(V)
Amplitude
Frequency
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}

PNP: main result