

Neuroscience 201A

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Single Channels

- Noise
- Gigohm Seals/Patch pipettes
- Technical Challenges
- P_{open}
- Rate constants of opening, closing
- Channel states
- Bursting

Single Channels History

- HH were not aware of the existence of channels; rather, they spoke of conductance.
- The presence of channels was inferred by the fact that increased noise in current traces was evident under conditions when channels are opening/closing.
- It was not until the work of Neher and Sakmann, beginning in the 1970s, on the patch clamp method, that we “saw” channels for the first time.
 - What changed?
 1. Sample a small number of channels (small amount of membrane)
 2. High gain, low noise

Channel openings/closings increase noise

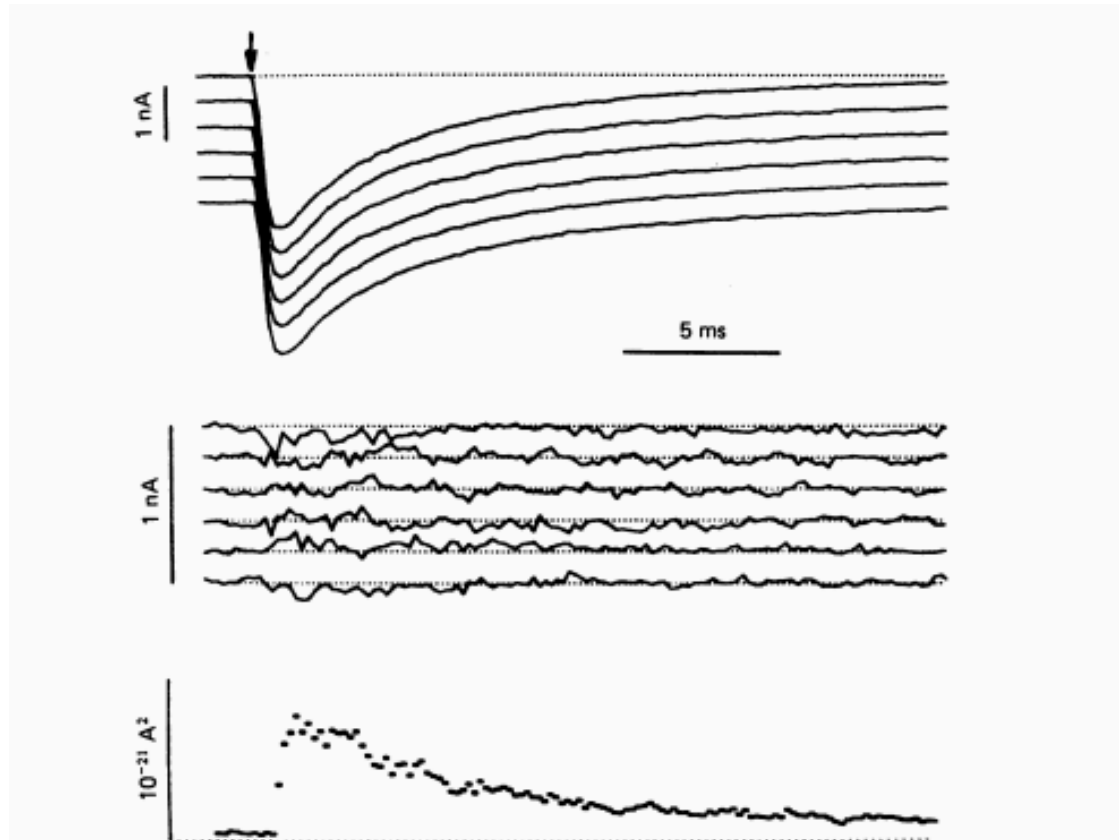


Fig. 3. Illustration of the ensemble variance calculation. *A*, six successive current records Y_{jk} from depolarizations to -5 mV, aligned at the start of the depolarizations (arrow). Linear leak subtraction was employed. *B*, residual current x_{jk} after subtracting the mean of a group of records. (Normally, groups consisted of four, six or eight records. Here, however, the mean of twelve records was used.) Note the larger deviations near the time of peak current. *C*, the variance at each sample point, computed from sixty-five groups of six records. Node 33.

Current noise produced by acetylcholine at the neuromuscular junction

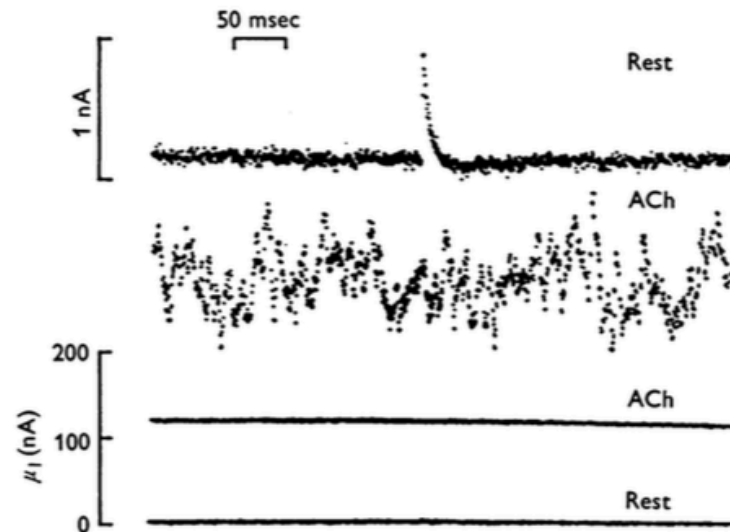


Fig. 2. Digitalized time sweep of membrane currents in voltage clamped end-plate of ethylene glycol treated muscle at 8° C. High gain, records filter below 1 Hz (40 db/decade) are presented above and low gain d.c. coupled traces below. The control trace includes a spontaneous miniature end-plate current. Iontophoretic application of ACh [current = 10 nA] produced a mean end-plate current of 120 nA and increase in R.M.S. noise from 0.07 to 0.25 nA (measured with 500 Hz, low pass active filter). The upper two traces are shown on the same scale. $V = -100$ mV.

Breakthrough

Single-channel currents recorded from membrane of denervated frog muscle fibres

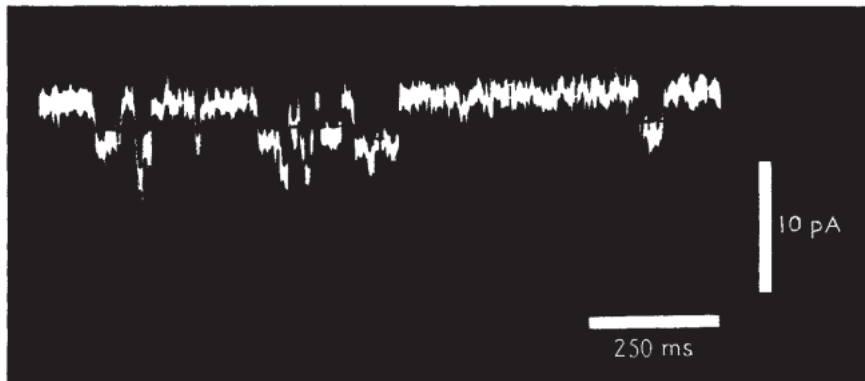
ERWIN NEHER

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Department of Physiology,
New Haven, Connecticut 06510*

BERT SAKMANN

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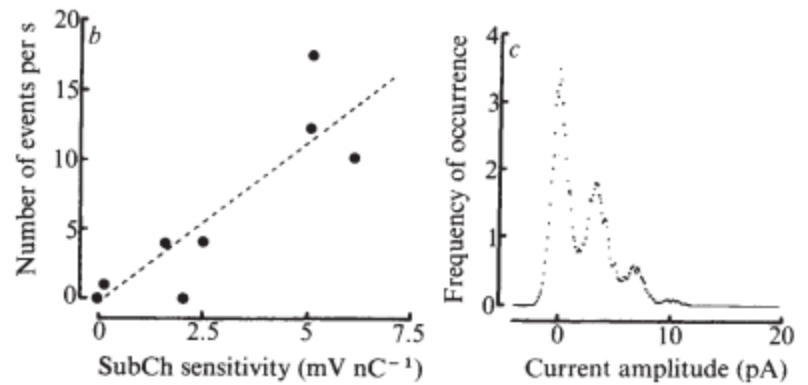
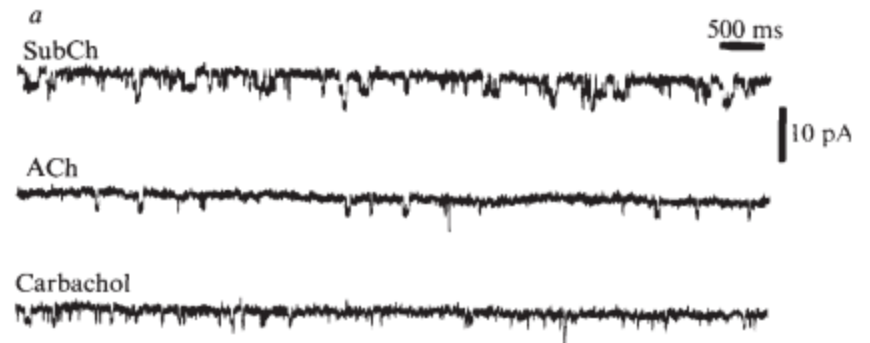
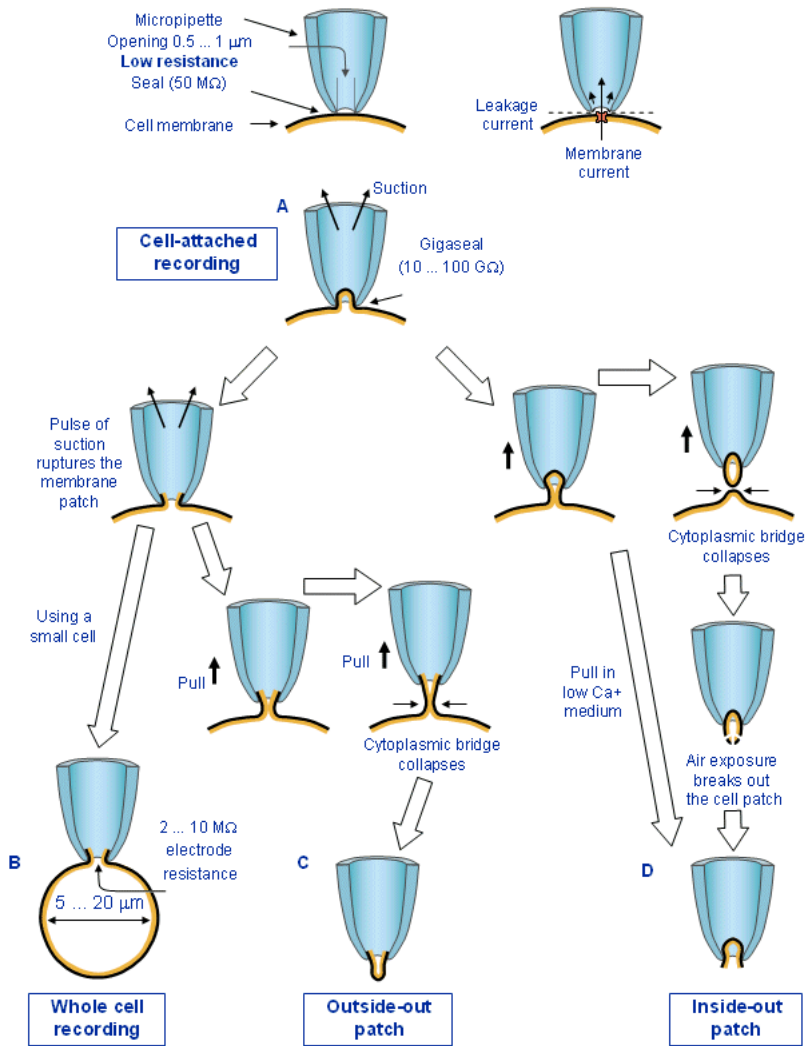
Received January 26; accepted March 1, 1976.



The key to the high resolution in the present experiments lies in limiting the membrane area from which current is measured to a small patch, and thereby decreasing background membrane noise. This is achieved by applying closely the tip of a glass pipette, 3–5 μm in diameter, on to the muscle surface, thus isolating electrically a small patch of membrane (Fig. 1). This method has been applied pre-

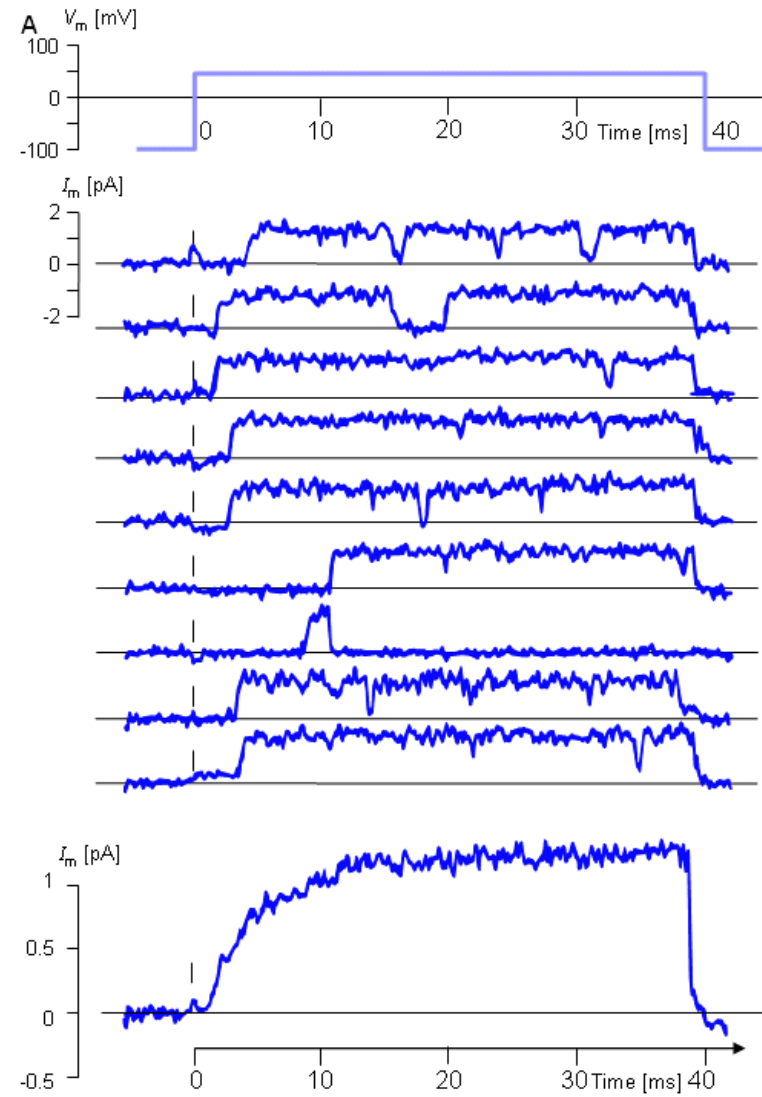
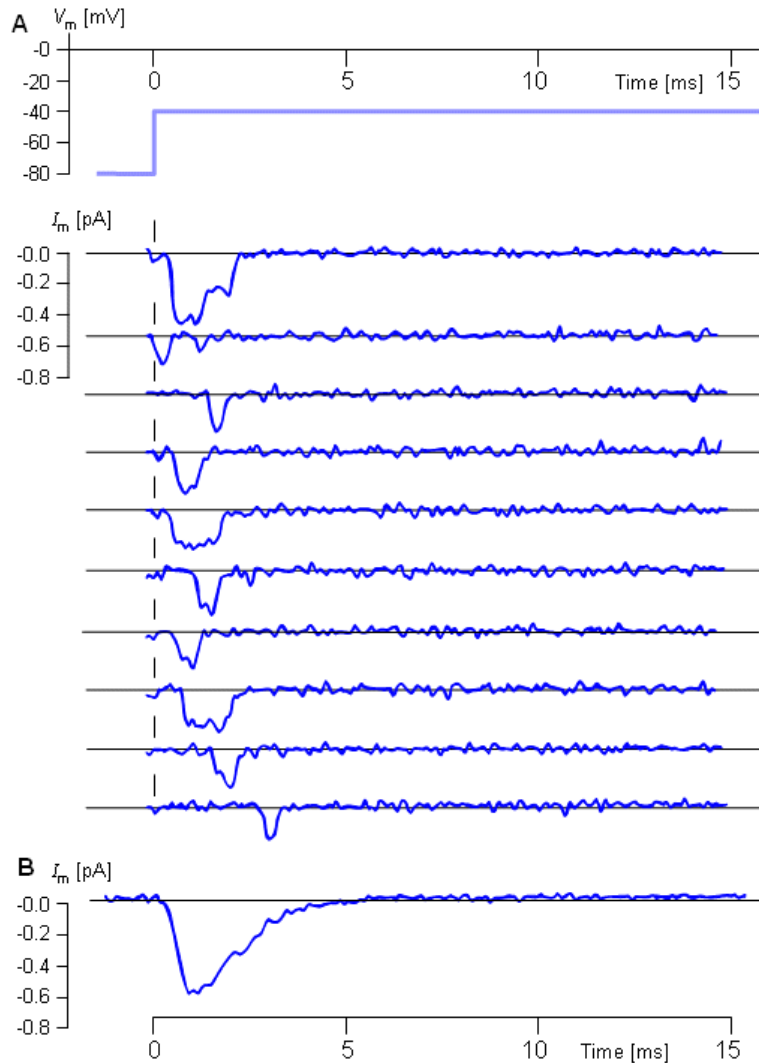
The dominant source of background noise in these measurements was the leakage shunt under the pipette rim between membrane and glass. It was constantly monitored by measuring the electrical conductance between pipette interior and bath. Discrete conductance changes could be resolved only when the conductance between pipette interior and bath decreased by a factor of four or more after contact between pipette and membrane. To minimise the leakage conductance, the muscle was treated with collagenase and protease⁹. This enzyme treatment digested connective tissue and the basement membrane, thereby enabling closer contact between glass and membrane. At the

Gigohm (10^9 ohm) seal recordings



Neher and Sakmann (1976) Nature 260: 799-801.

Single channel records of sodium and potassium channels



Single Channels Topics

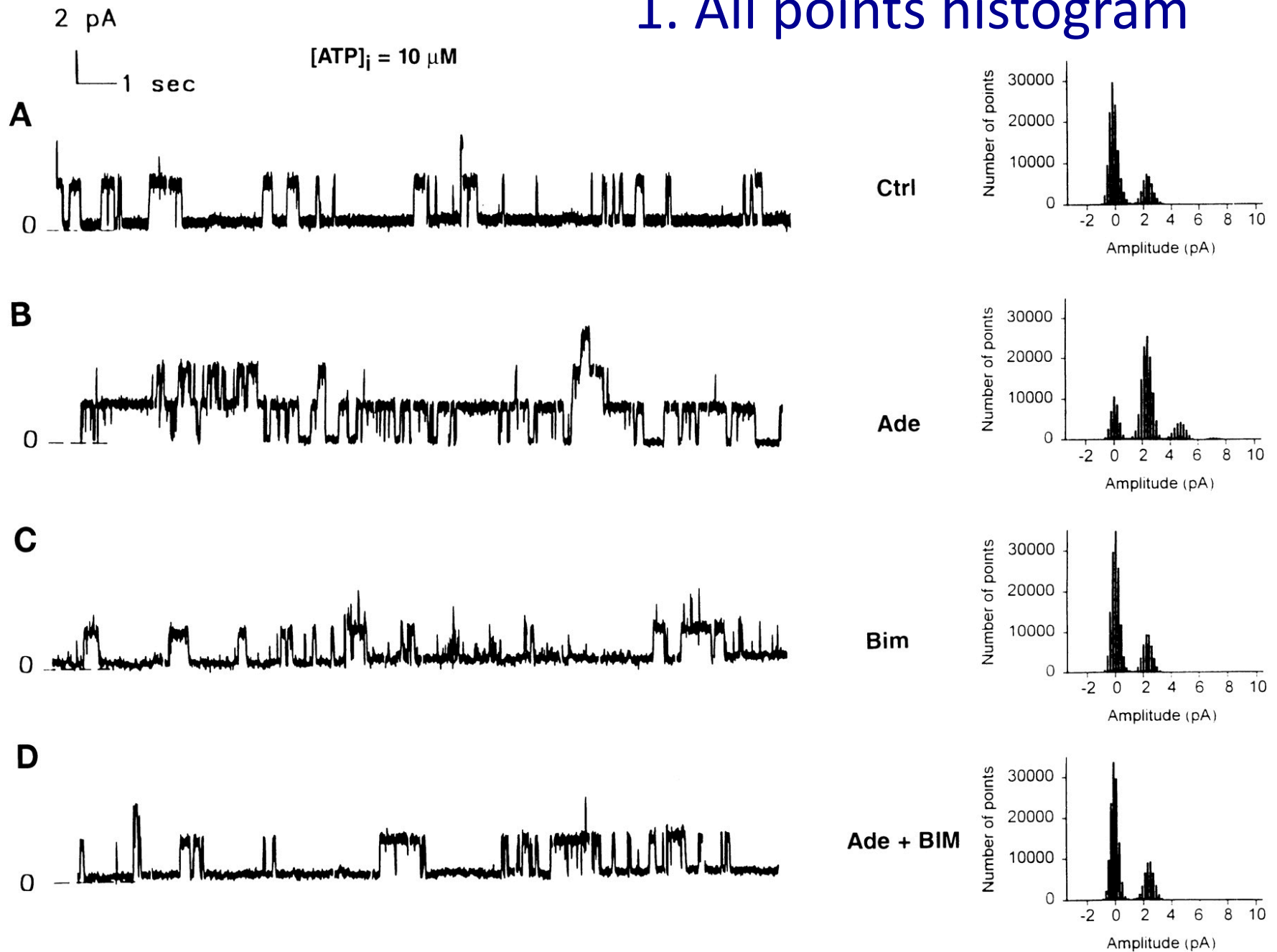
- Technical Issues
 - How many channels are in the patch?
 - Are the channels all of the same type? (if there are many)
 - How do you analyze the data?
- Are there degrees of opening (“more/less open”)?
- What are the rate constants for opening, closing?
- How many channel states are there?

How many channels are in the patch?

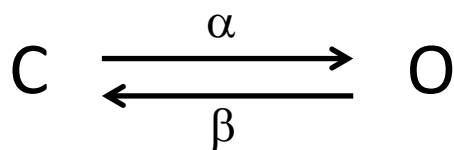
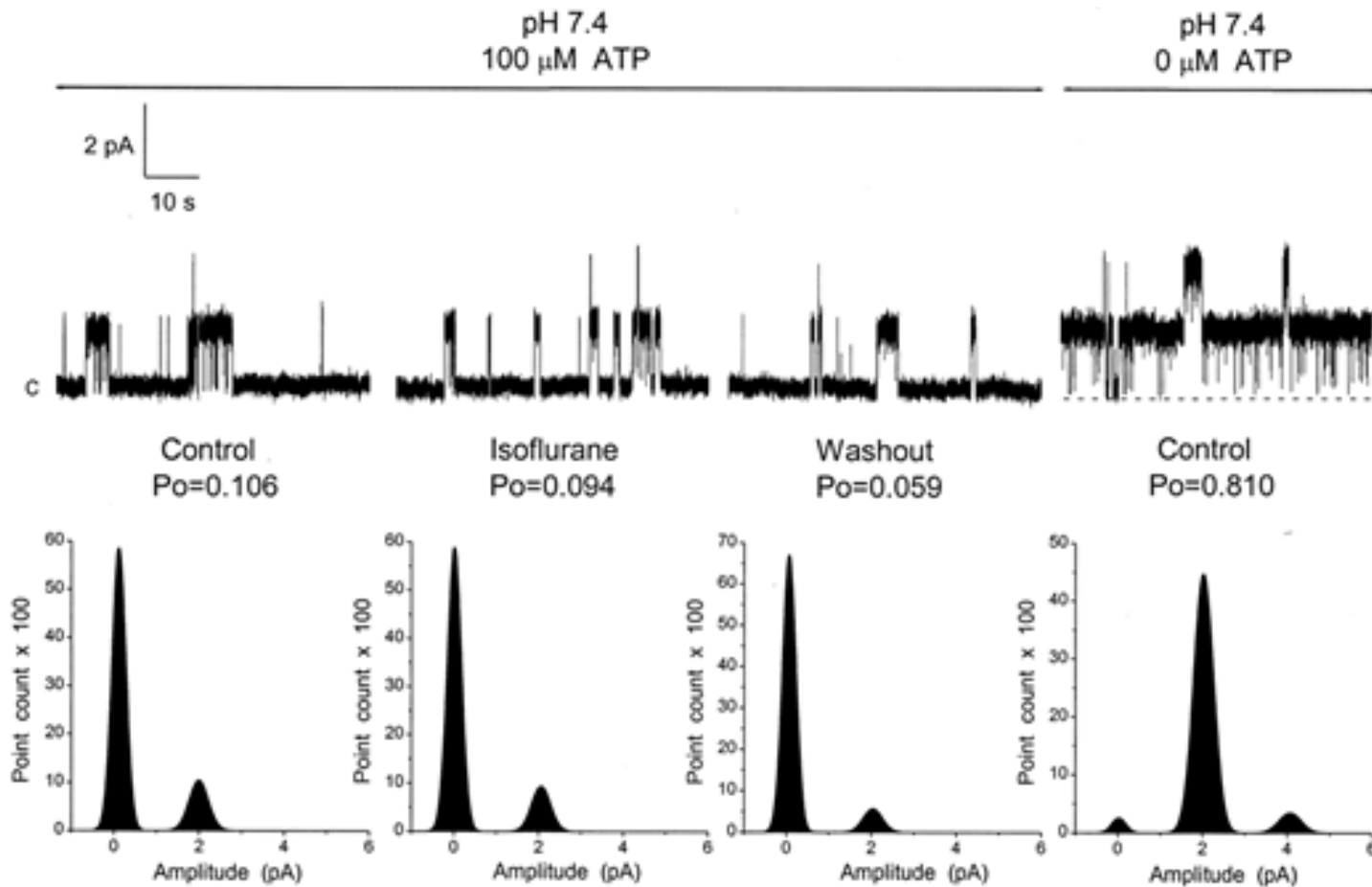


What if you never see more than one channel open at a time? Is it safe to assume that there's only one channel in the patch?

1. All points histogram



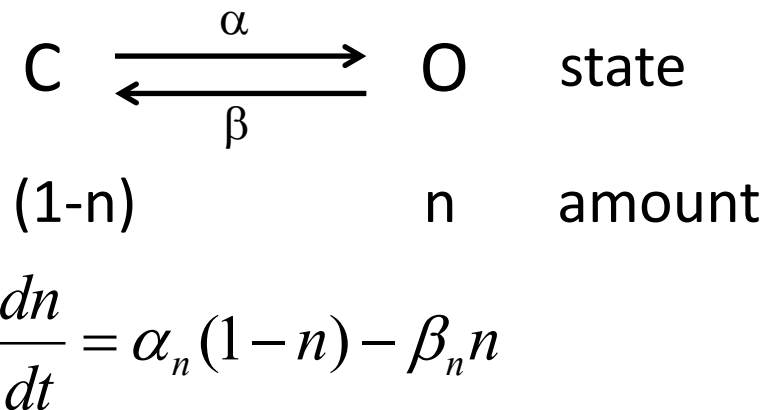
2. p_{open}



$$P_{open} = \frac{\alpha}{\alpha + \beta}$$

3. Kinetic Analysis

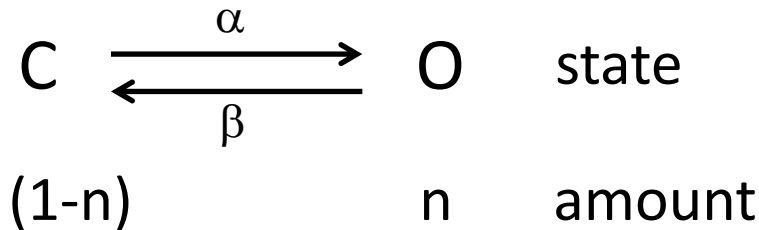
- Consider two states, closed and open



- The law of mass action: the rate of a reaction is proportional to the concentration of reactants
- Rate constants (α , β) have the units of s^{-1} (e.g., $200 s^{-1}$)
- We can convert rate constants to probabilities by multiplying by Δt (provided that Δt is small); thus, $P(C \rightarrow O)$ in Δt is $\alpha^* \Delta t$, and $P(O \rightarrow C)$ in Δt is $\beta^* \Delta t$.

Kinetic Analysis (cont.)

- Consider two states, closed and open



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

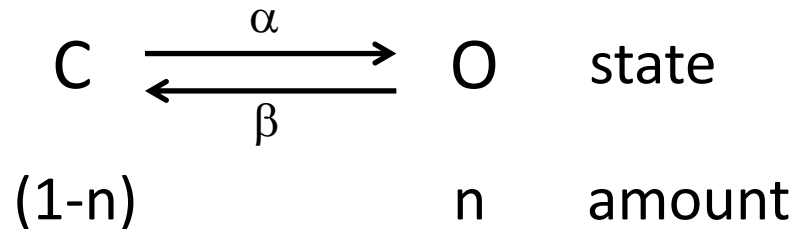
- A random/stochastic process, like a coin toss
- The past history of [C] or [O] is of no importance. Markov model. All that's needed to predict the future is the present! This means that the probability that an open channel will remain open for an additional time Δt , is the same regardless of how long it's already been opened.



Andrey Markov
1856 - 1922

Kinetic Analysis (cont.)

- Consider two states, closed and open



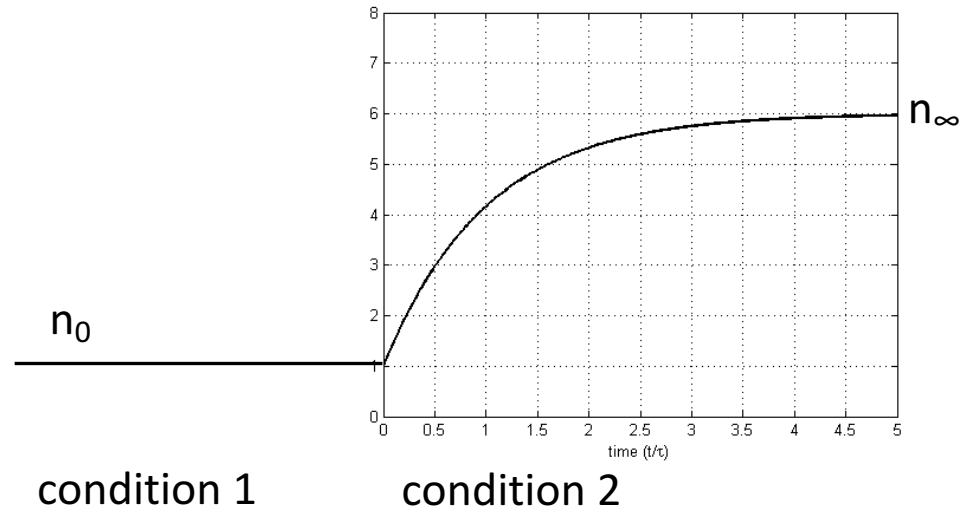
$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

$$n = n_\infty - (n_\infty - n_0) \exp(-t / \tau_n)$$

At equilibrium ...

$$n_\infty = \alpha_n / (\alpha_n + \beta_n)$$

$$\tau_n = 1 / (\alpha_n + \beta_n)$$

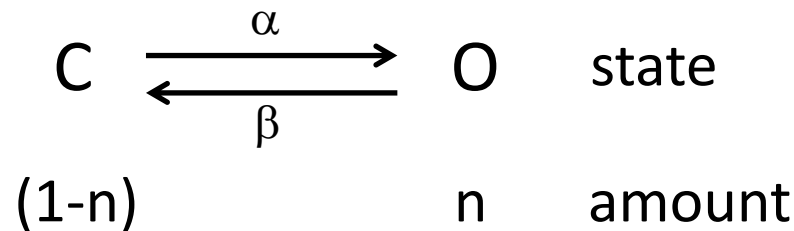


e.g. $V_{\text{hold}} = -65 \text{ mV}$
 e.g. $[glu]_o = 0 \text{ mM}$

$= 0 \text{ mV}$
 $= 100 \text{ } \mu\text{M}$

Distribution of Shut Times

(simpler; the τ has only one rate constant)

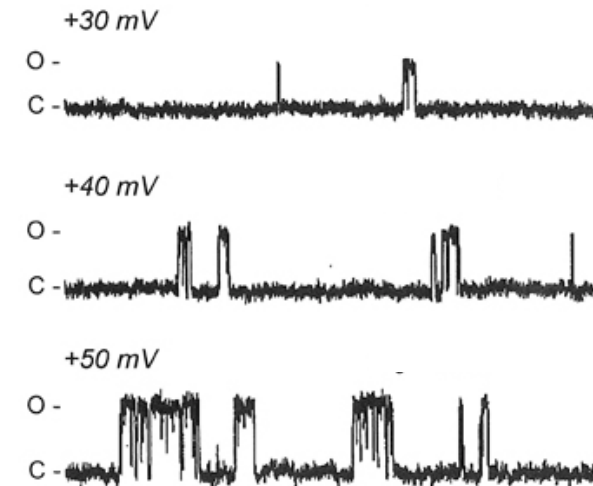
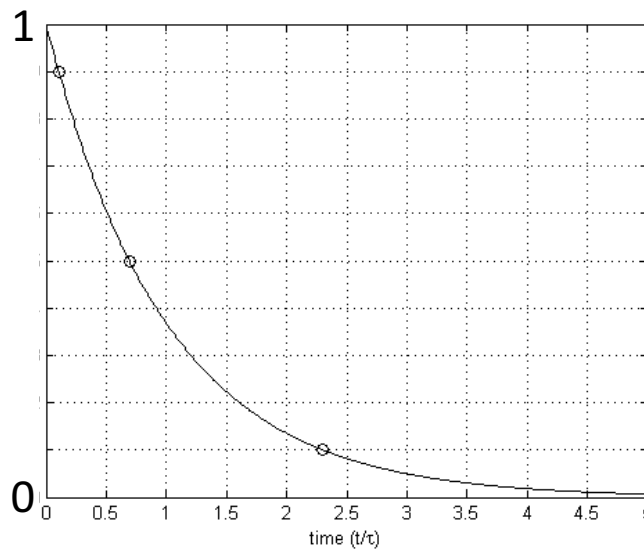


$$F(t) = \exp(-\alpha t)$$

$$F(t) = \exp(-t/\tau)$$

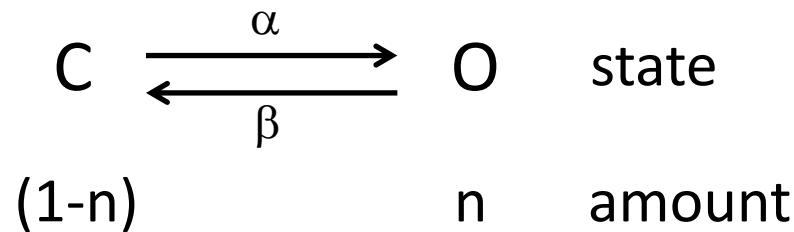
where $\tau = 1/\alpha$

$$\text{Avg. shut time} = 1/\alpha$$



Distribution (probability density function: pdf) of shut times

Distribution of Open Times

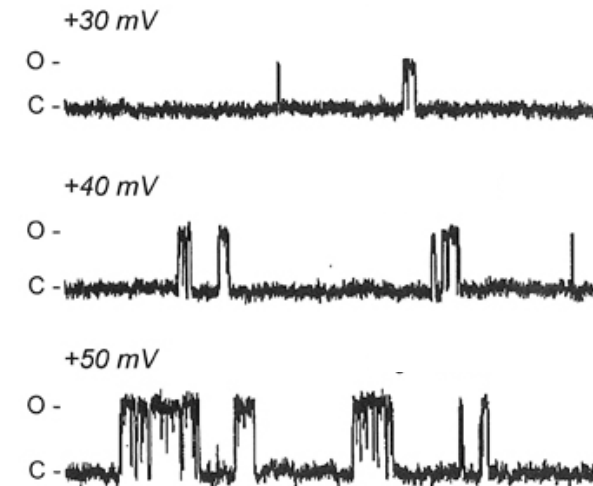
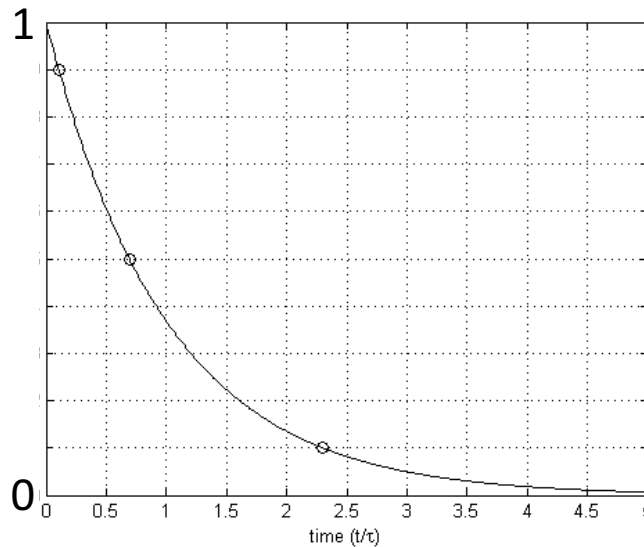


$$F(t) = \exp(-\beta t)$$

$$F(t) = \exp(-t/\tau)$$

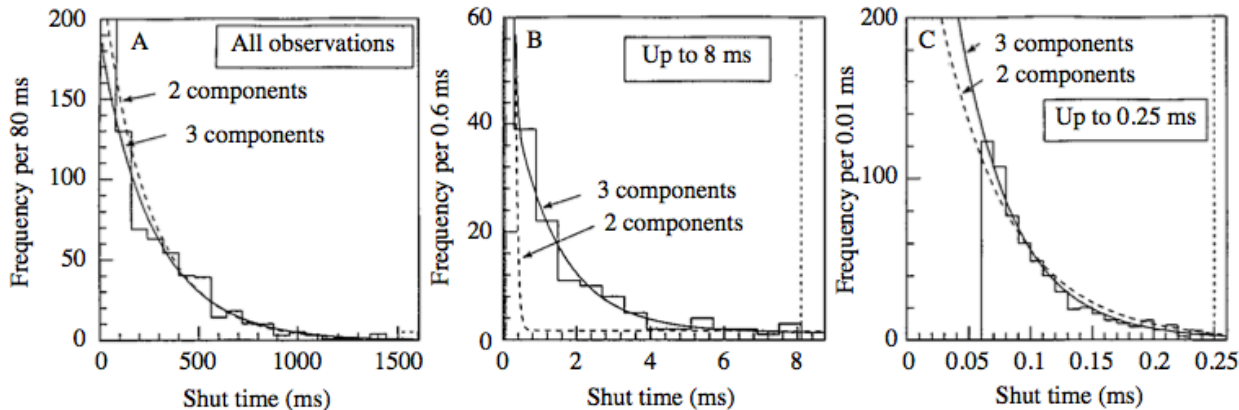
where $\tau = 1/\beta$

$$\text{Avg. shut time} = 1/\beta$$

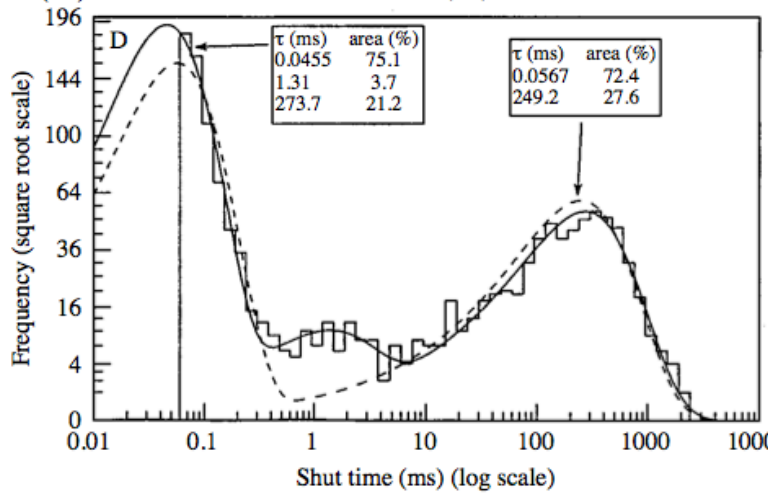


Distribution (probability density function: pdf) of open times

Actual Analysis

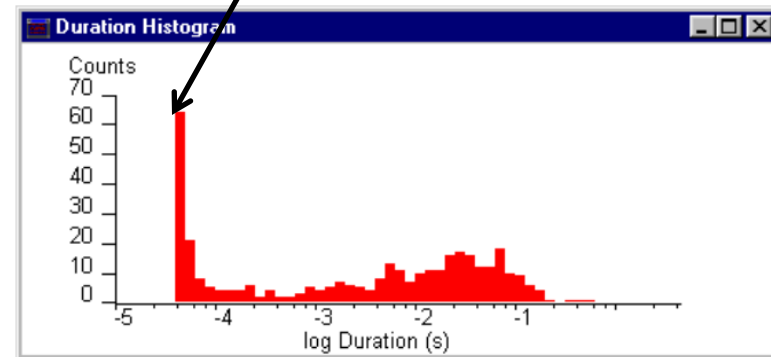


There are (at least) three shut states, not one!



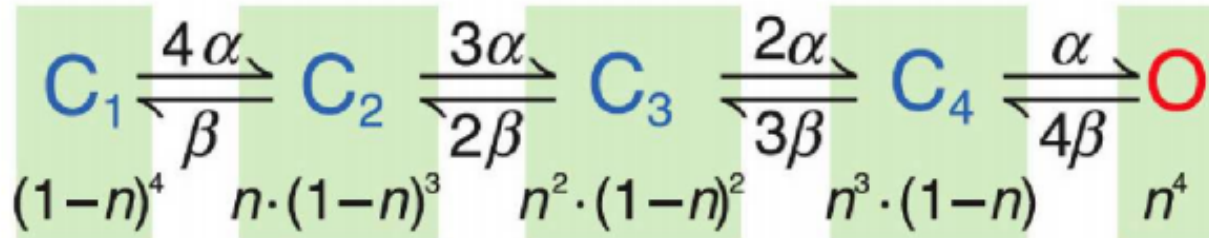
What's happening here?

Another example



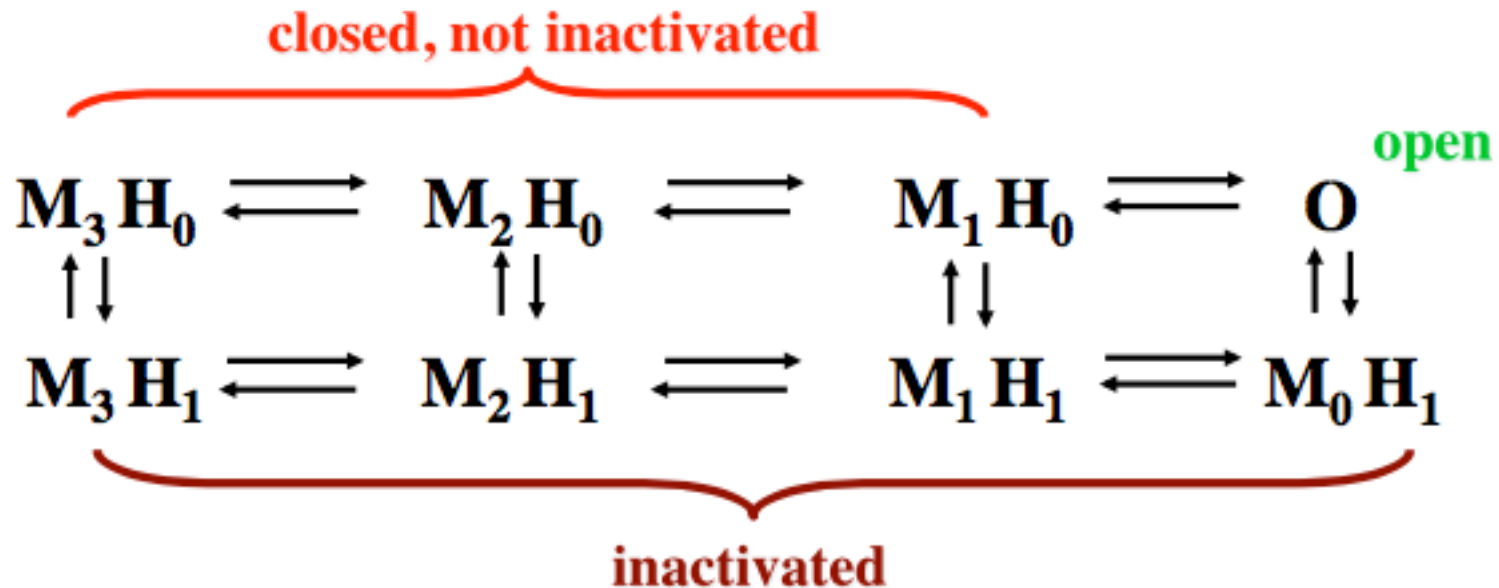
Colquhoun D in Microelectrodes book

The Hodgkin Huxley potassium channel: how many states?



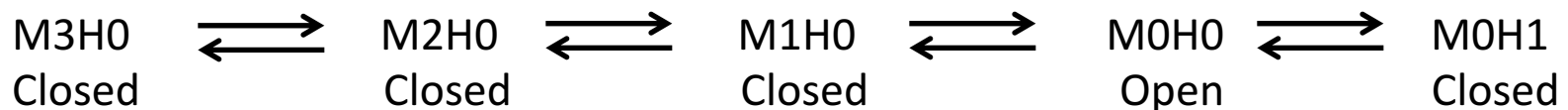
There are likely to be many “hidden” states: ones that are functionally indistinguishable.

HH Sodium Channel: how many states?

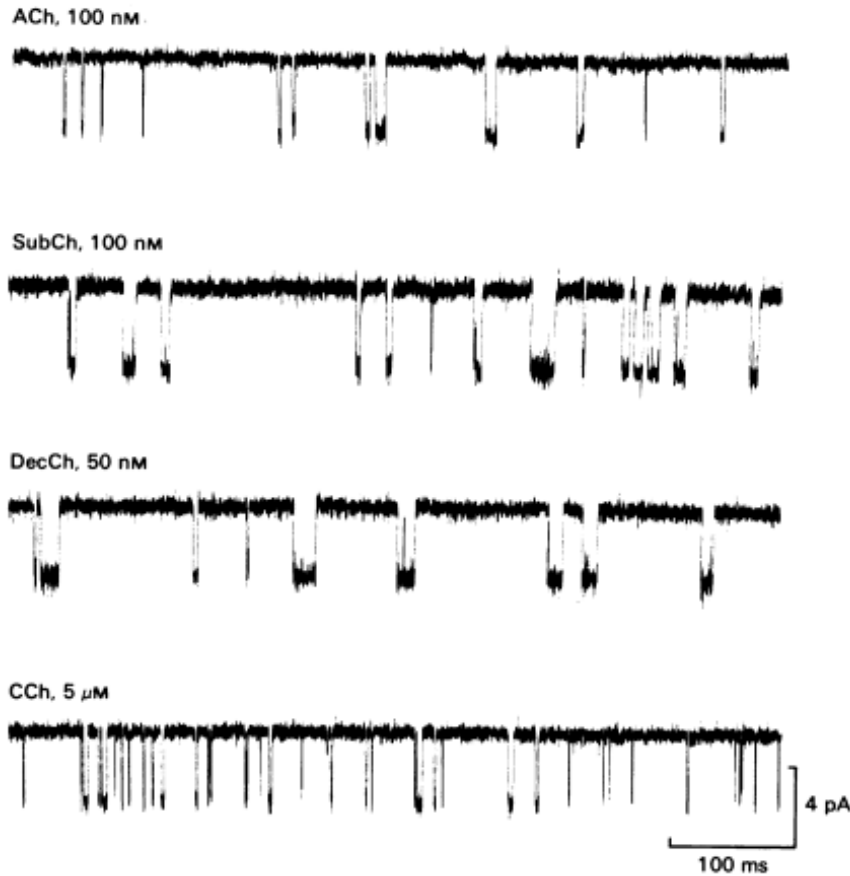


8 states? Seven closed and one open?

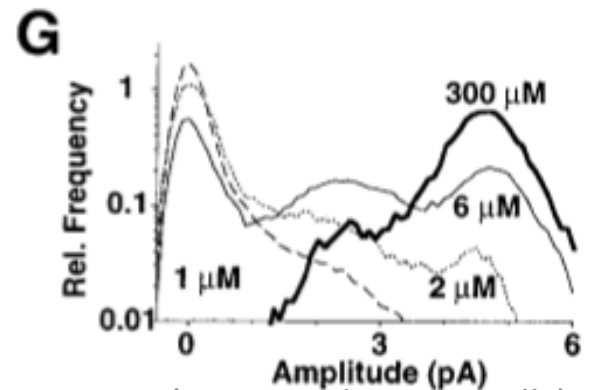
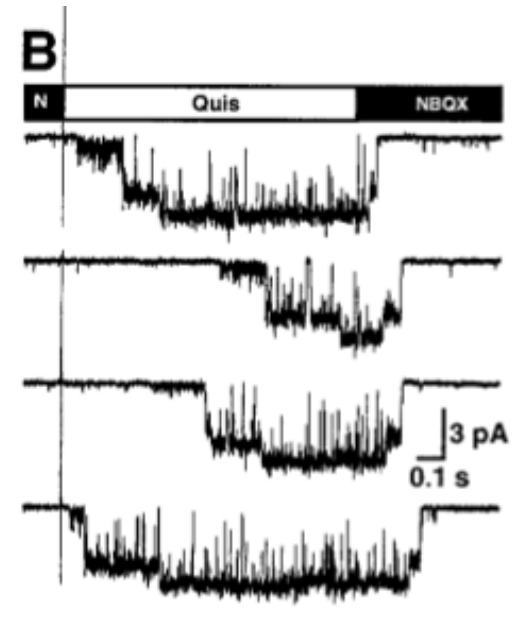
Subsequence work suggests that inactivation proceeds more rapidly from activated channels.



Degrees of “open-ness?”

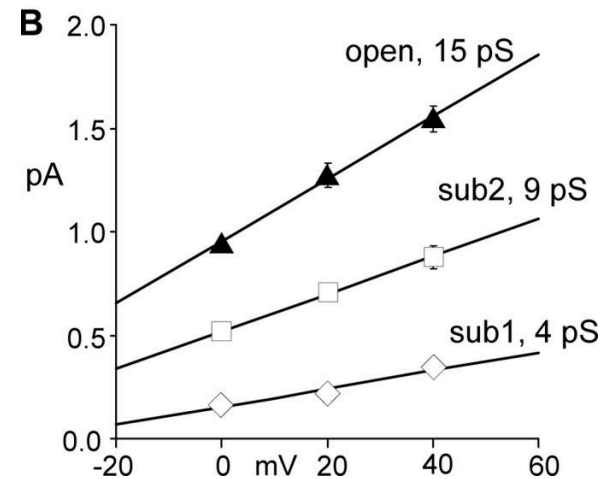
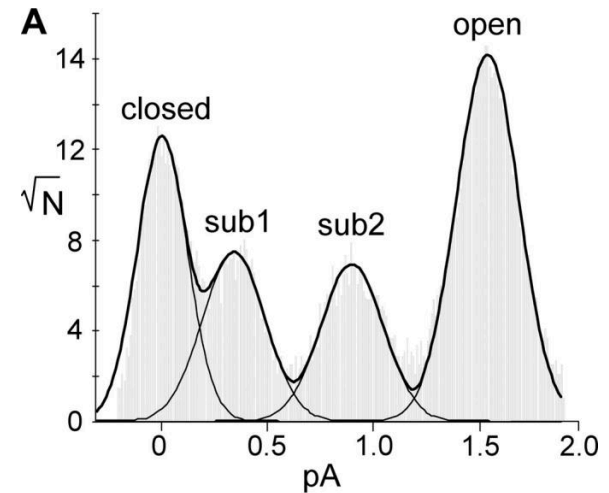
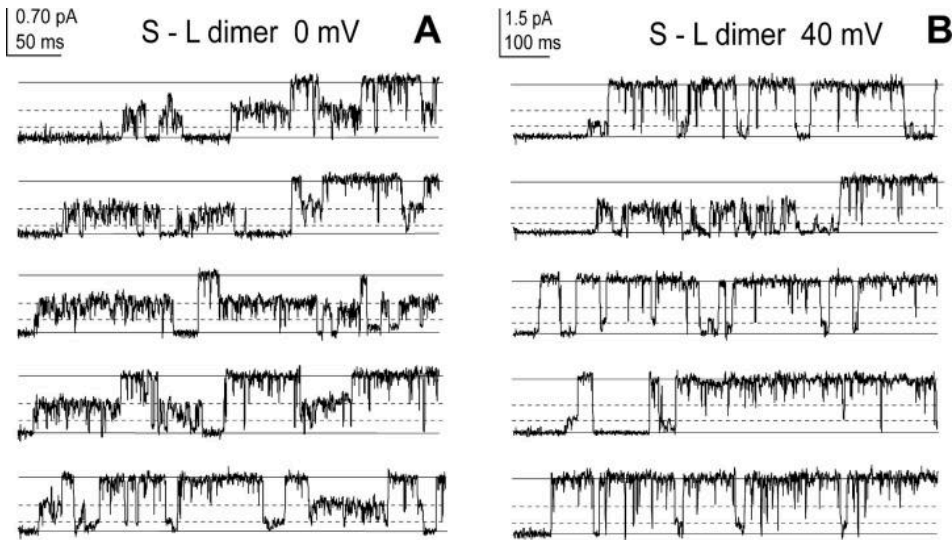


Frog muscle nicotinic receptors
Colquhoun D, Sakmann B (1985) *J. Physiol.*
369: 501-557.



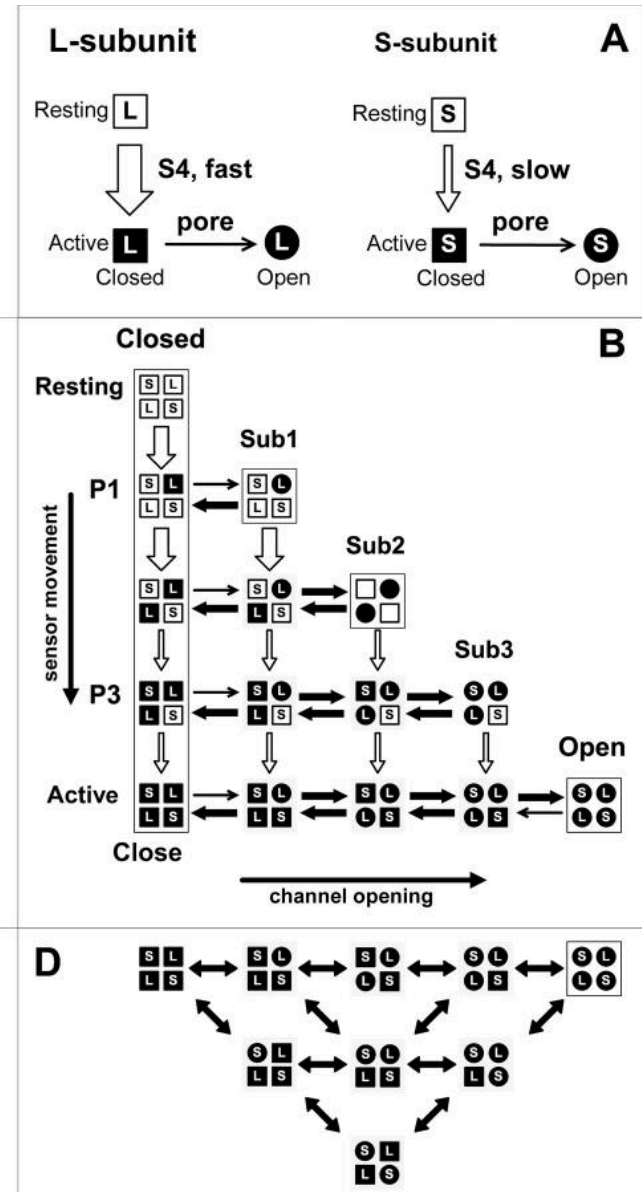
AMPA receptors (expressed in HEK cells)
Rosenmund C, Stern-Bach Y, Stevens CF (1998)
Science 280: 1596-1598.

Subconductance states for a K_v channel



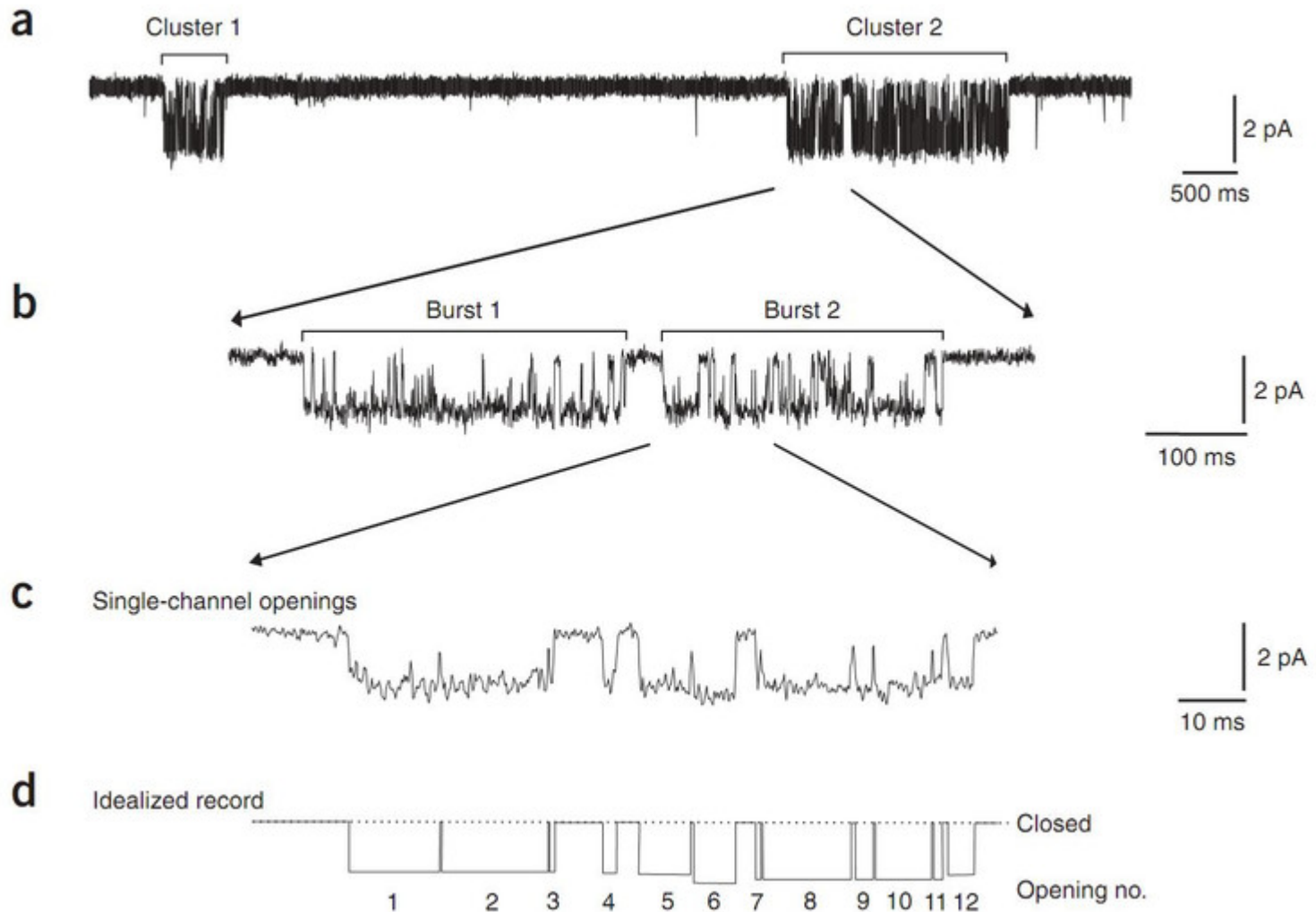
Chapman ML, VanDongen AM (2005) K channel subconductance levels result from heteromeric pore conformations. *J Gen Physiol* 126: 87-103.

15 (-35) state model!

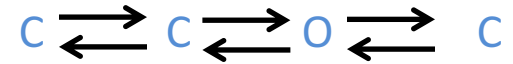
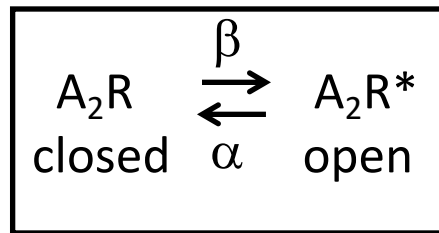
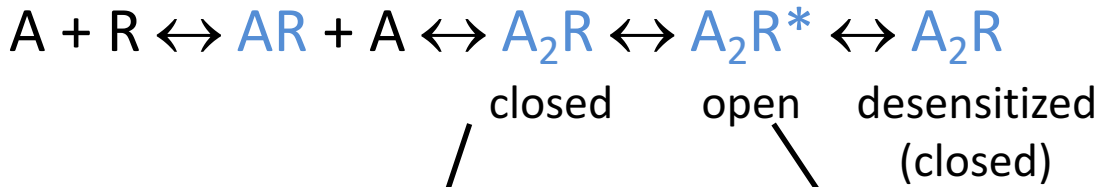


Chapman ML, VanDongen AM (2005) K channel subconductance levels result from heteromeric pore conformations. *J Gen Physiol* 126: 87-103.

What once appeared to be simple openings became “bursts” when the methodology improved

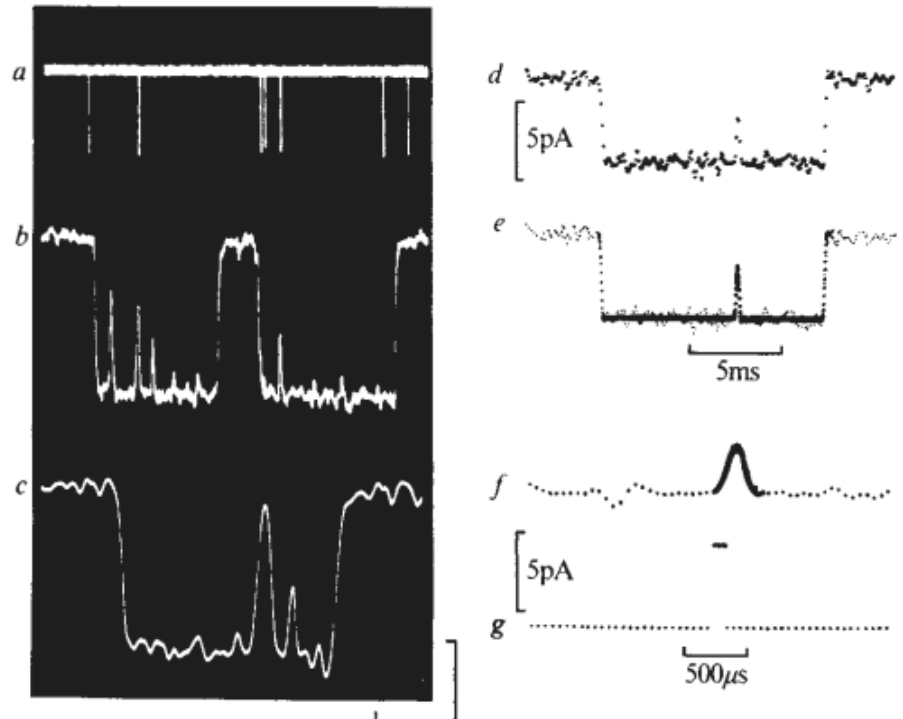


Bursting



Lifetime (open) = $1/(\text{sum of rate constants transitioning to other states})$

Colquhoun and Sakmann (1981) Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. Nature 294: 464-466.



Effects of an allosteric regulator

