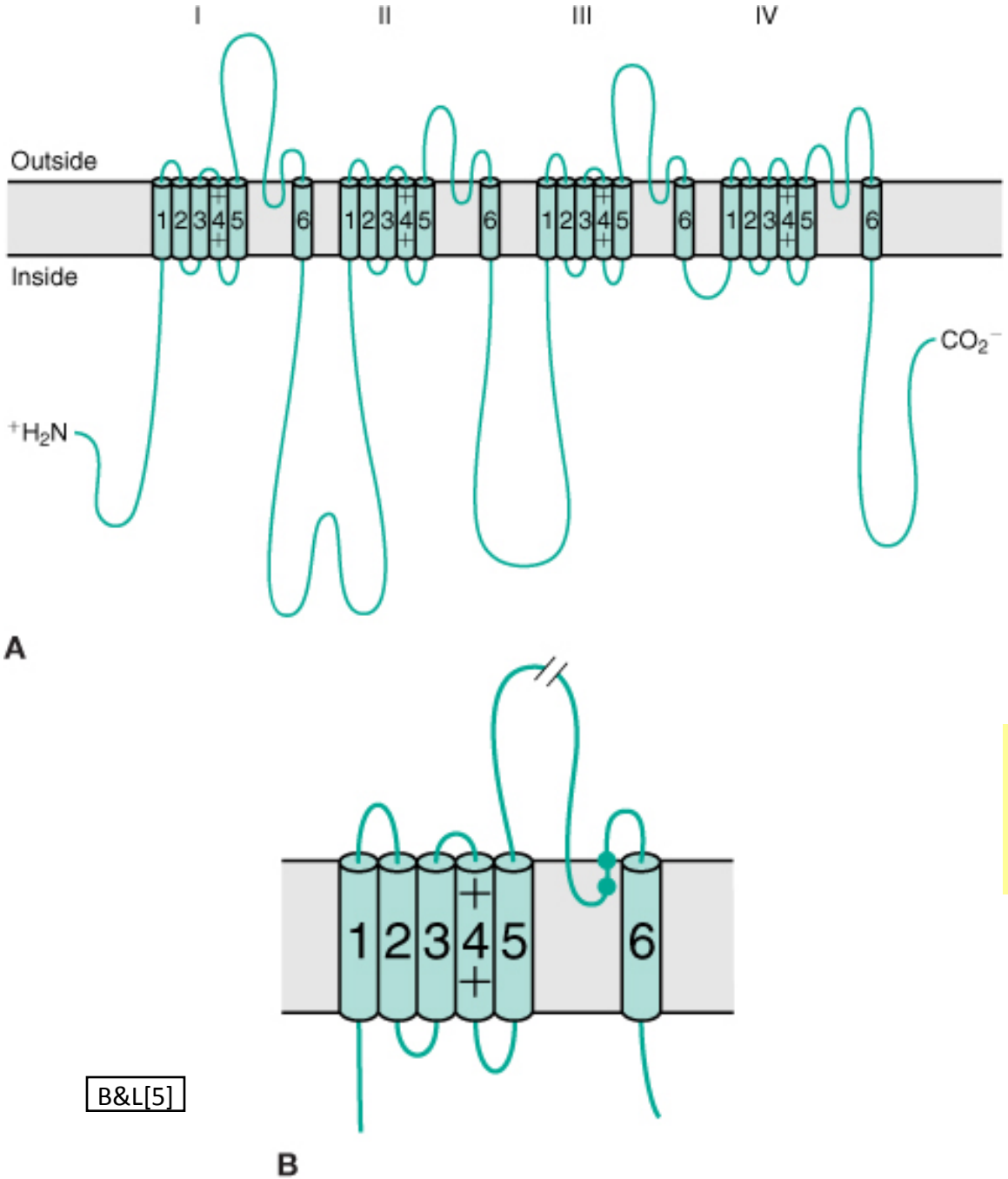


B&L[5]

Figure 3-6 Model of the voltage-dependent Na^+ channel protein. A, Two-dimensional model. The cylinders represent transmembrane α-helices. There are four repeats of six-cylinder domains of homologous α-helices. The S4 helices, marked with plus signs, function as voltage sensors, and movements of these helices are responsible for activation (opening) of the channel. The intracellular loop connecting domains III and IV functions as the inactivation gate: after depolarization, with a slight delay, this loop apparently swings up into the mouth of the channel to block ion conduction. B, Domain IV. The part of the extracellular loop that connects helices 5 and 6 and that dips into the membrane as the "pore loop" that helps form the selectivity filter of the channel. The residues indicated by solid circles are key determinants of the ionic selectivity of the channel. (B Redrawn from Catteral W: J Bioenerg Biomembr 28:219, 1996.)

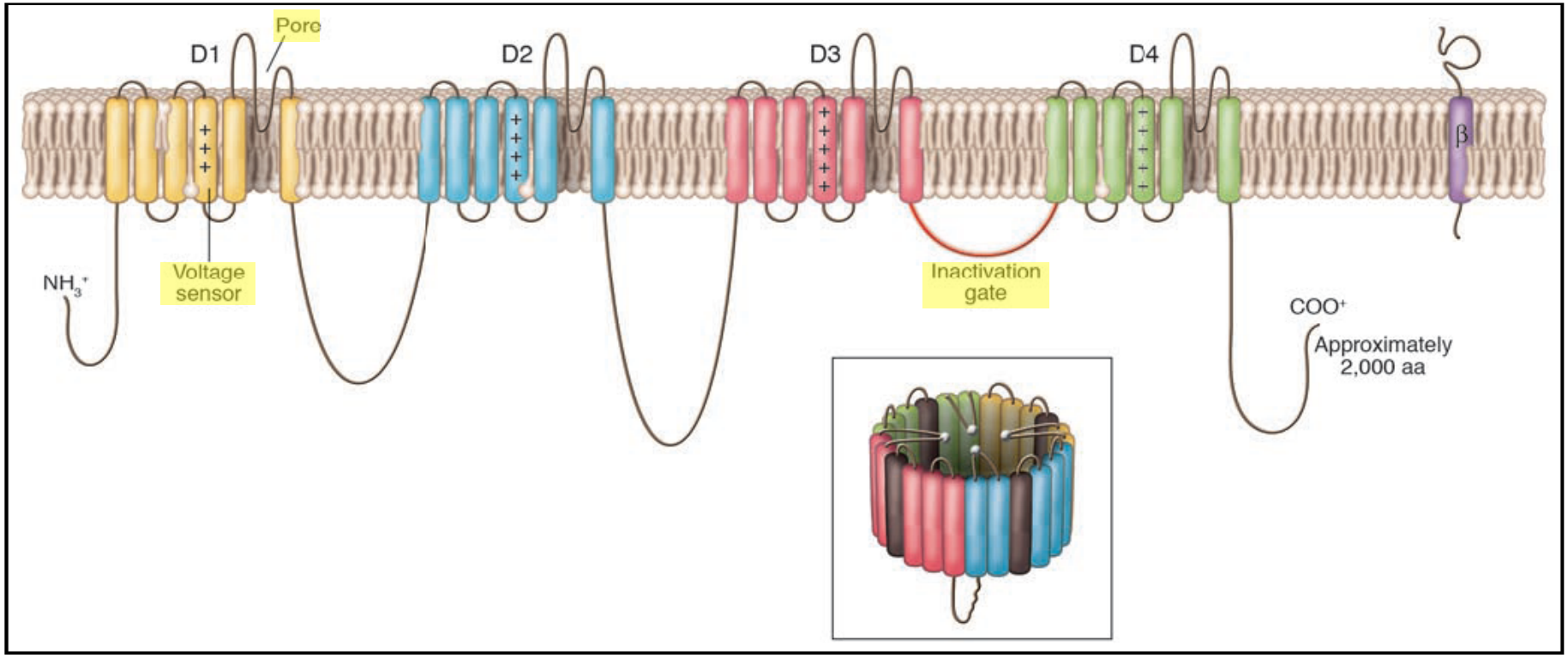
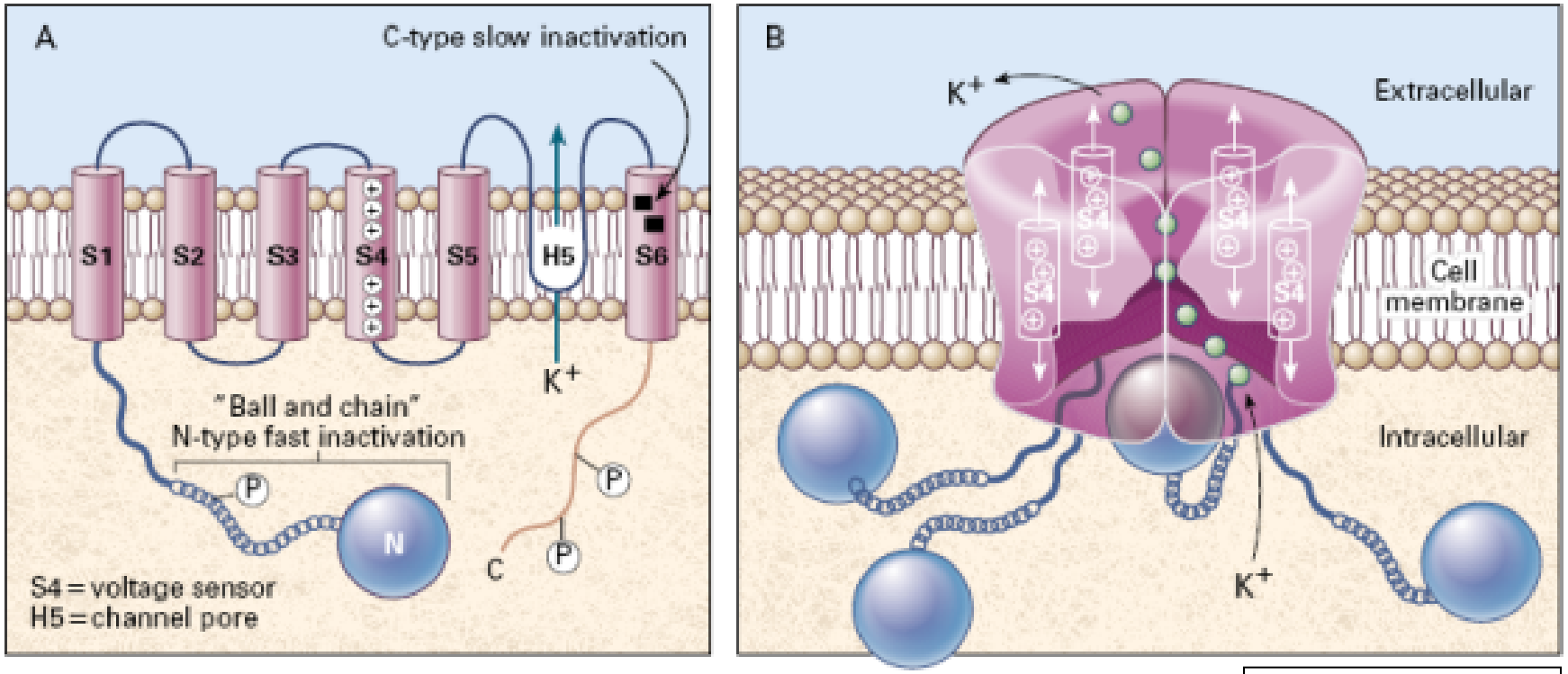


Figure 1 The sodium channel α and β subunits are transmembrane proteins. The 4 homologous domains of the α subunit are represented in different colors. The transmembrane segments associate in the membrane to form an Na^+-permeable pore lined by the re-entrant S5–S6 pore-loop segments (inset). From Meisler MH and JA Kearney, J. Clin. Invest. 2005 115(8): 2010-17.

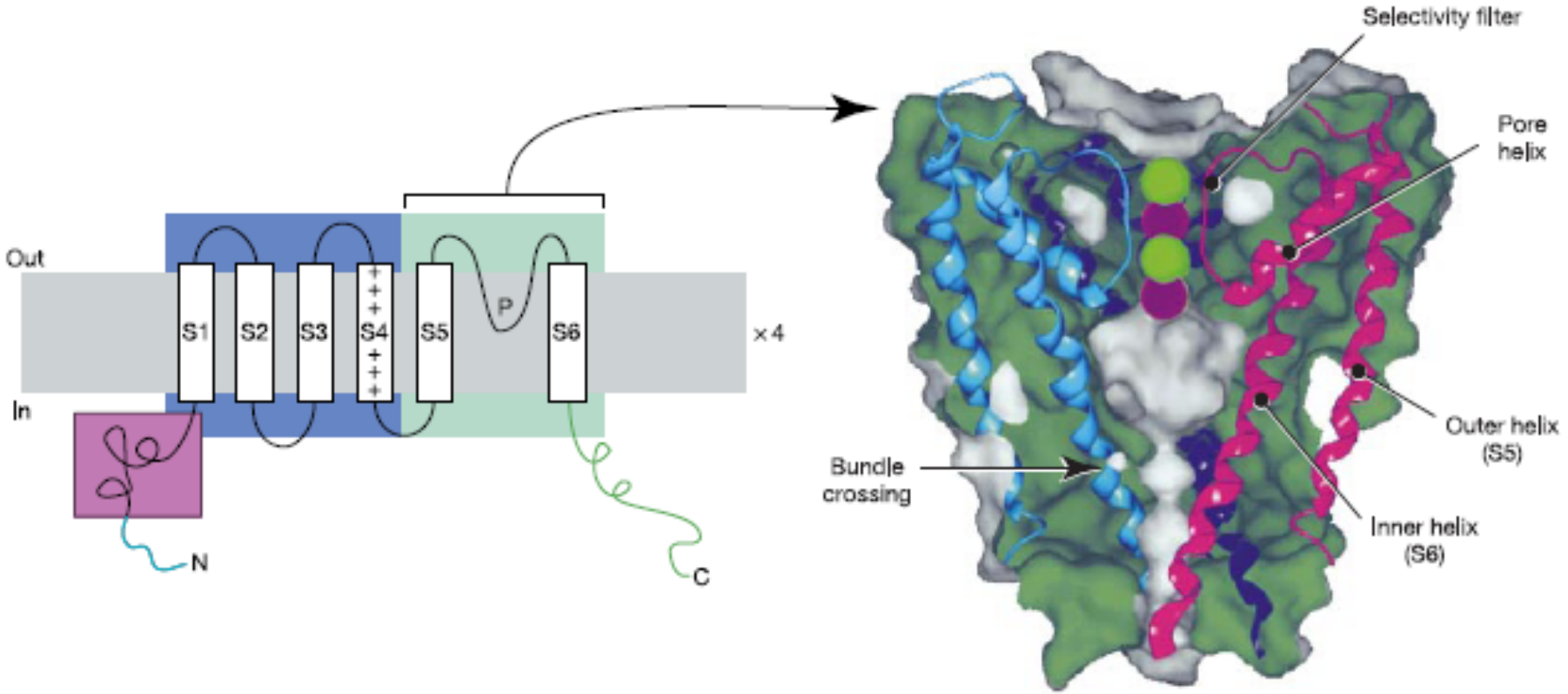


Ackerman et al., NEJM (1997) 336(22):1575

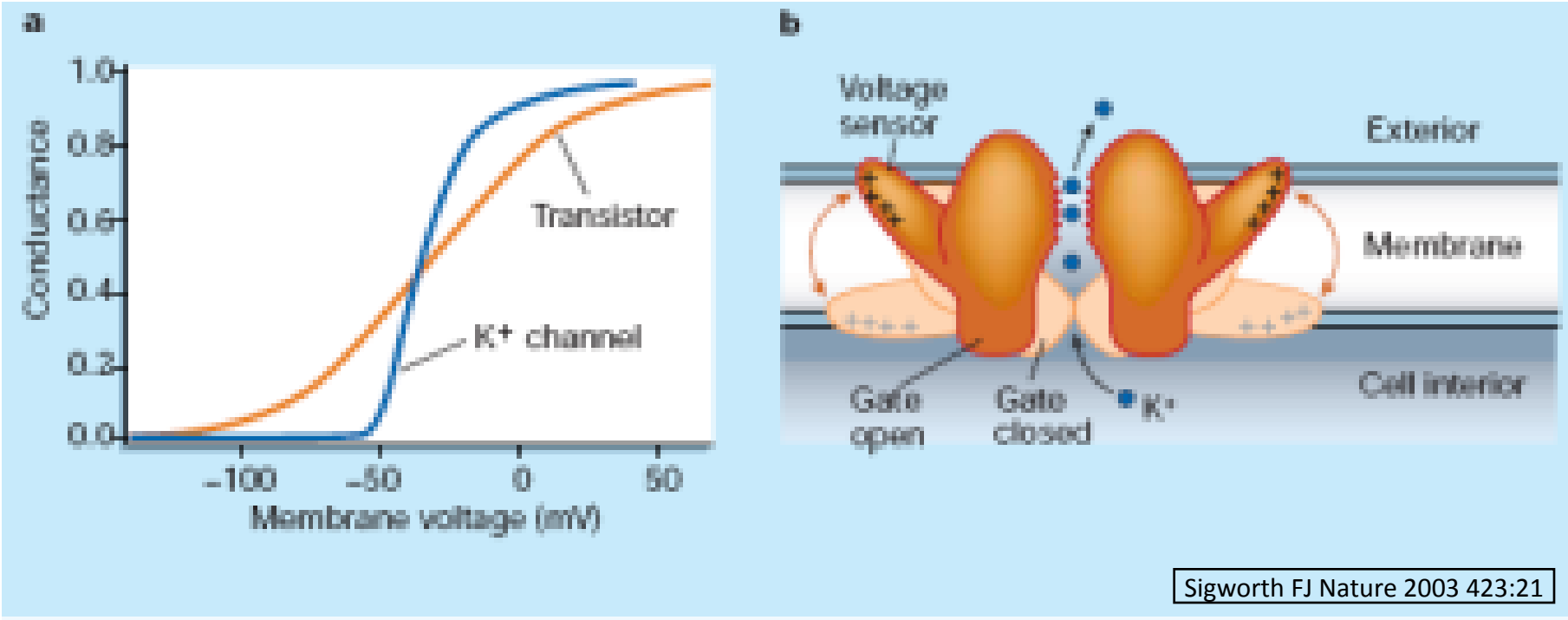
Figure 3. Structure of Ion Channels.

Panel A shows a subunit containing six transmembrane-spanning motifs, S1 through S6, that forms the core structure of sodium, calcium, and potassium channels. The "ball and chain" structure at the N-terminal of the protein is the region that participates in N-type "fast inactivation," occluding the permeation pathway. The circles containing plus signs in S4, the voltage sensor, are positively charged lysine and arginine residues. Key residues lining the channel pore (H5) are found between S5 and S6. The genes for sodium and calcium channels encode a protein containing four repeats of this basic subunit, whereas the genes for voltage-activated potassium channels (Kv) encode a protein with only a single subunit. The genes for Kir channels encode a simple subunit structure containing only an H5 (pore) loop between two transmembrane-spanning segments. P denotes phosphorylation.

Panel B shows four such subunits assembled to form a potassium channel. Although no mammalian voltage-dependent ion-channel structure has been revealed at high resolution by x-ray crystallography, the dimensions of the pore region shown here were derived by using high-affinity scorpion toxins and their structures (as determined by nuclear magnetic resonance imaging) as molecular calipers.[12] The pore region appears to have wide intracellular and extracellular vestibules (approximately 2.8 to 3.4 nm wide and 0.4 to 0.8 nm deep) that lead to a constricted pore 0.9 to 1.4 nm in diameter at its entrance, tapering to a diameter of 0.4 to 0.5 nm at a depth of 0.5 to 0.7 nm from the vestibule.



Yellen G. Nature 2002 419:35-42



Sigworth FJ Nature 2003 423:21

Figure 1 Voltage sensing in a potassium ion channel. a, The control of ion flow through voltage-gated channels is very sensitive to the voltage across the cell membrane. By comparison, an electronic device such as a transistor is much less sensitive to applied voltage. b, MacKinnon and colleagues[1,2] have found that the voltage sensors in a bacterial potassium channel are charged 'paddles' that move through the fluid membrane interior. Four voltage sensors (two of which are shown here) are linked mechanically to the channel's 'gate'. Each voltage sensor has four tethered positive charges (arginine amino acids); the high sensitivity of channel gating results from the transport of so many charges, 16 in all, most of the way across the membrane.

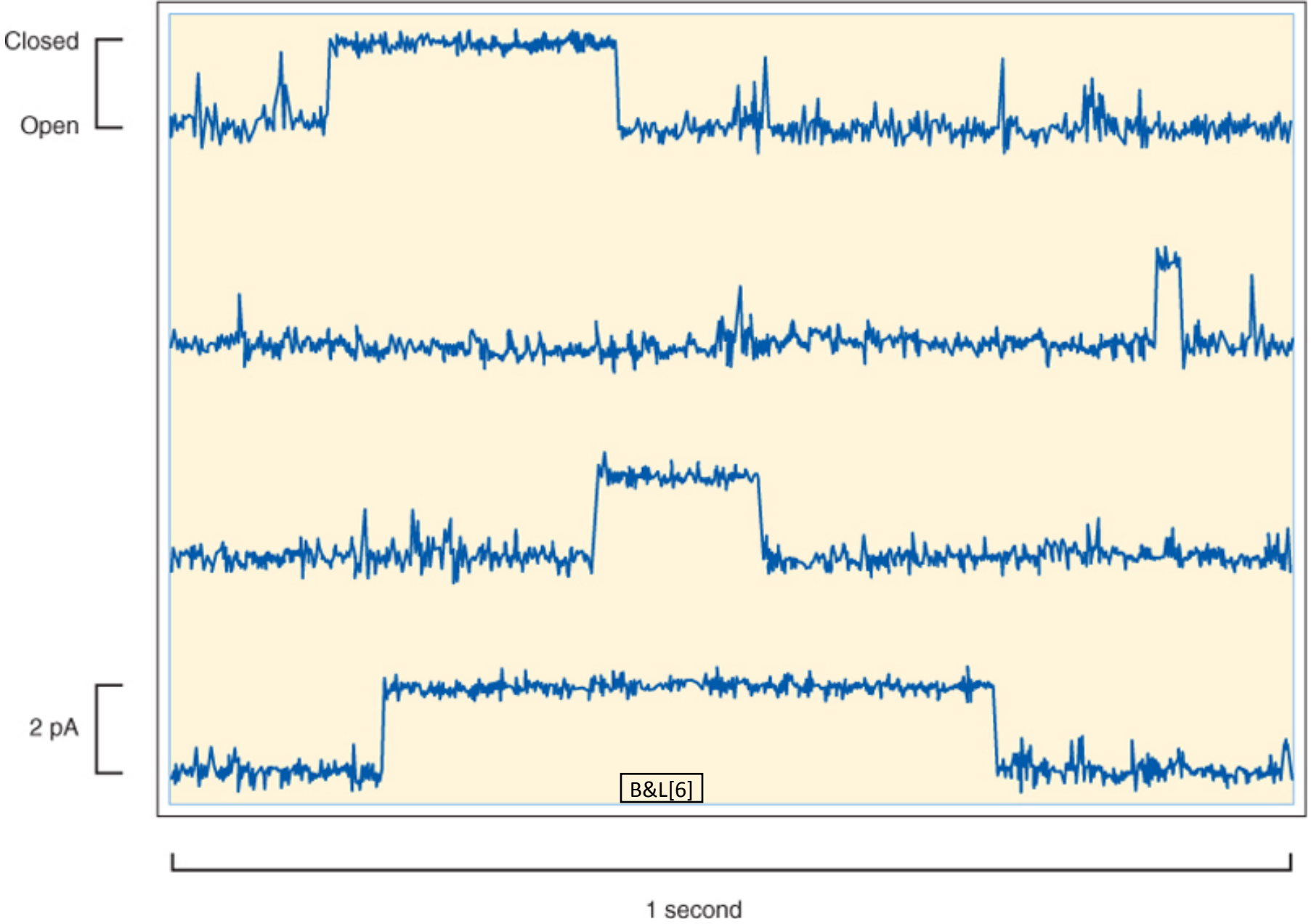


Figure 1-4 Recording of current flow through a single K^+-selective ion channel. The channel spontaneously fluctuates between an open and closed state. The amplitude of the current is approximately 2 pA ($2 \bullet 10^{-12}$ amps), or 12.5 million ions cross the membrane per second.

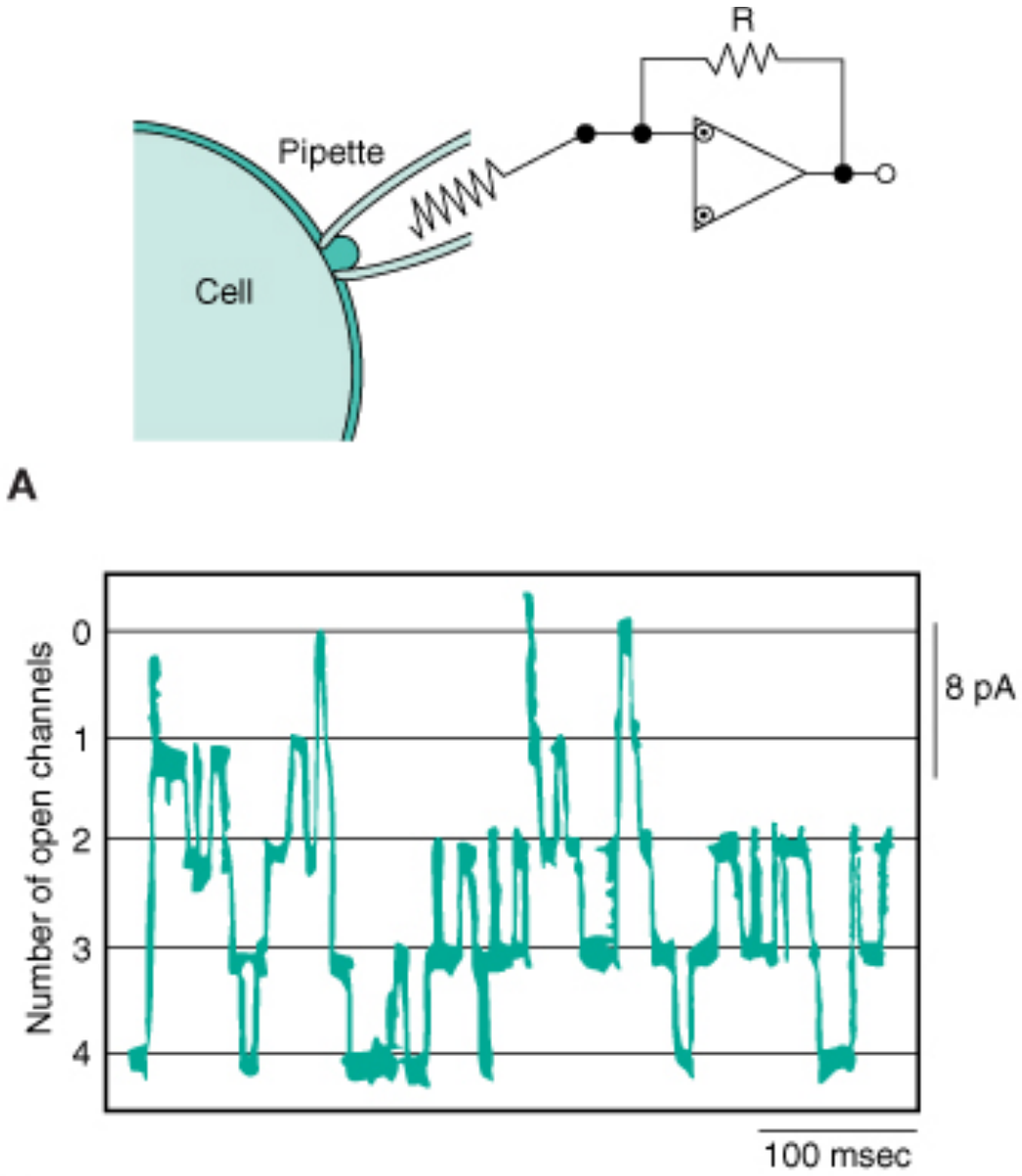
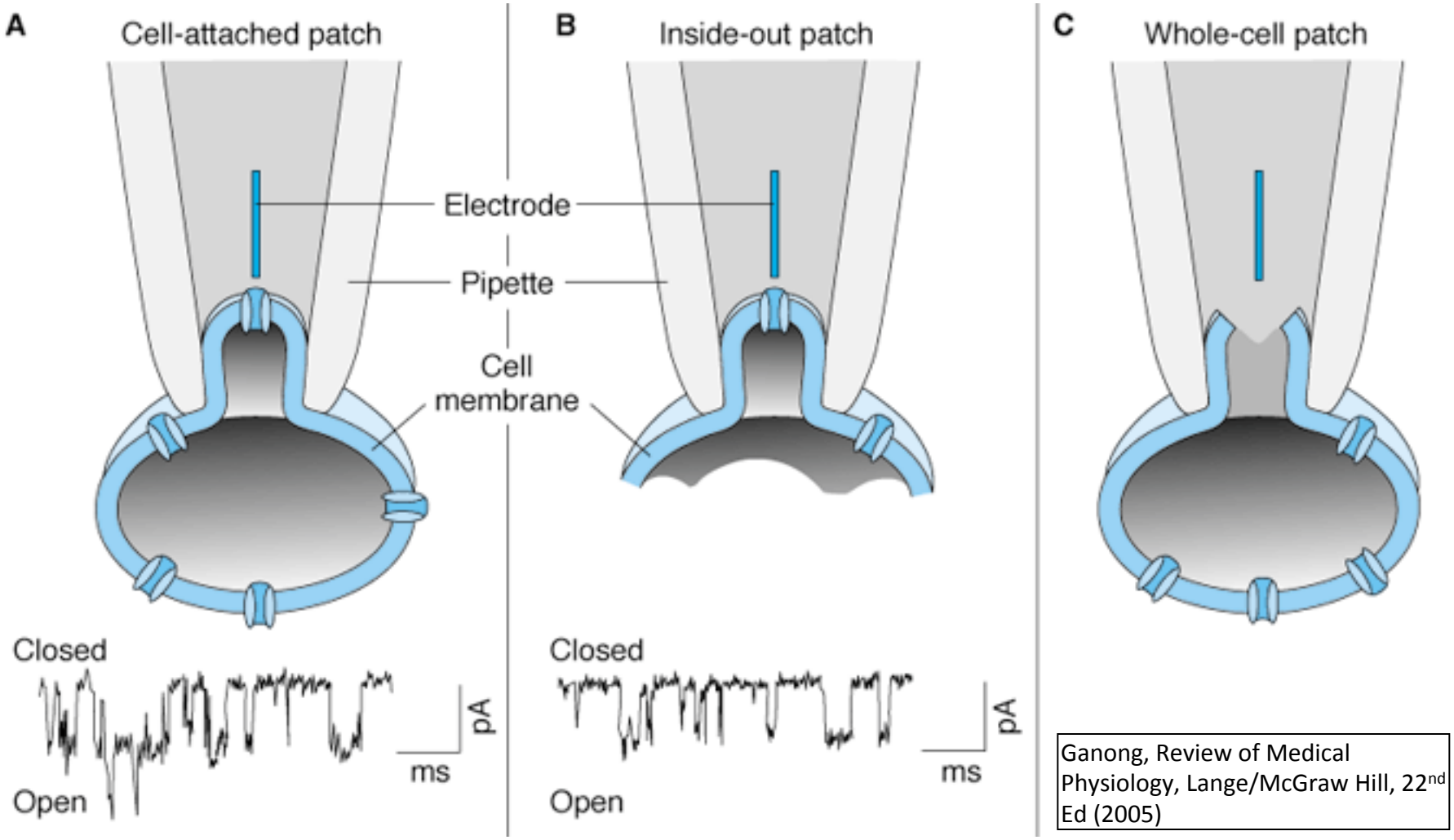


Figure 3-9 A, Patch electrode and circuitry required to record the ionic currents that flow through the small number of ion channels isolated in the electrode patch. B, Current recording from a patch electrode on the plasma membrane of a skeletal muscle cell. The five current levels show that this particular patch contains four different ion channels, each opening and closing independently of the others. (A Redrawn from Sigworth FJ, Neher E: Nature 287:447, 1980; B Redrawn from Hammill OP et al: Pflügers Arch 391:85, 1981.)



Ganong, Review of Medical Physiology, Lange/McGraw Hill, 22nd Ed (2005)

Figure 1-28. Types of patch clamps used to study activity of ion channels across a cell membrane. In A and B, the changes in membrane current with time are also shown. (Modified from Ackerman MJ, Clapham DE: Ion channels: Basic science and clinical disease. N Engl J Med 1997;336:1575.)

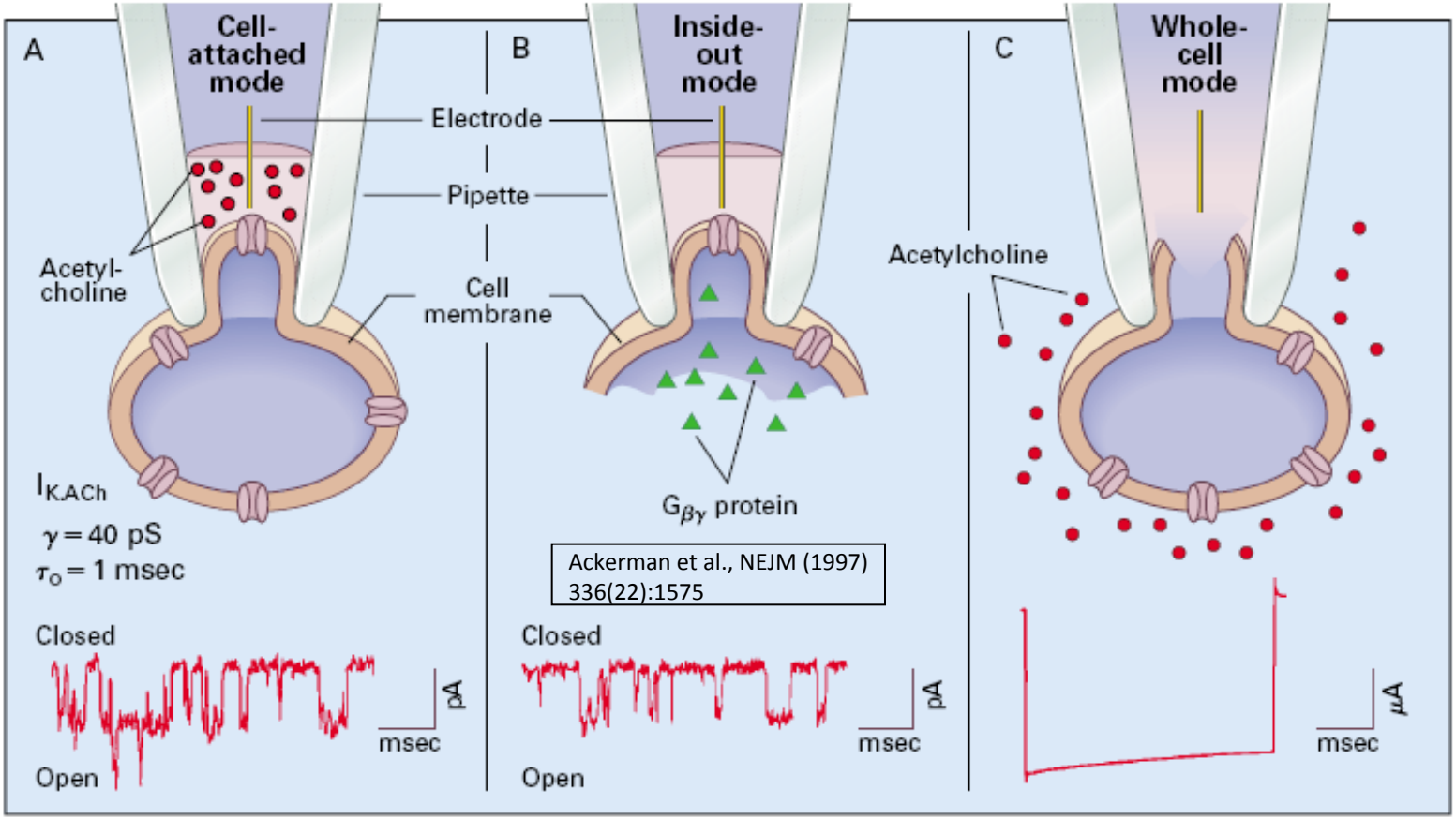


Figure 2. Patch-Clamp Measurement of Ion-Channel Activity, with the Acetylcholine-Sensitive Potassium Channel ($I_{K,ACh}$) Used as an Example.

In the "cell-attached" mode (Panel A), a pipette is pressed tightly against the cell membrane, suction is applied, and a tight seal is formed between the pipette and the membrane. The seal ensures that the pipette captures the current flowing through the channel. In the cell-attached membrane patch, the intracellular contents remain undisturbed. Here, acetylcholine in the pipette activates the $I_{K,ACh}$, which has a characteristic open time (τ_0) of 1 msec and a conductance (γ) of 40 picosiemens.

In the inside-out mode (Panel B), after a cell-attached patch has been formed, the pipette is pulled away from the cell, ripping off a patch of membrane that forms an enclosed vesicle. The brief exposure to air disrupts only the free hemisphere of the membrane, leaving the formerly intracellular surface of the membrane exposed to the bath. Now the milieu of the intracellular surface of the channels can be altered. In this figure, adding purified $G_{\beta\gamma}$ protein to the exposed cytoplasmic surface activates the $I_{K,ACh}$.

In the whole-cell mode (Panel C), after a cell-attached patch has been formed, a pulse of suction disrupts the membrane circumscribed by the pipette, making the entire intracellular space accessible to the pipette. Instead of disrupting the patch by suction, a pore-forming molecule, such as amphotericin B or nystatin, can be incorporated into the intact patch, allowing ions access to the interior of the cell but maintaining a barrier to larger molecules. In this figure, the net current ($I_{K,ACh}$) after the application of acetylcholine is shown.

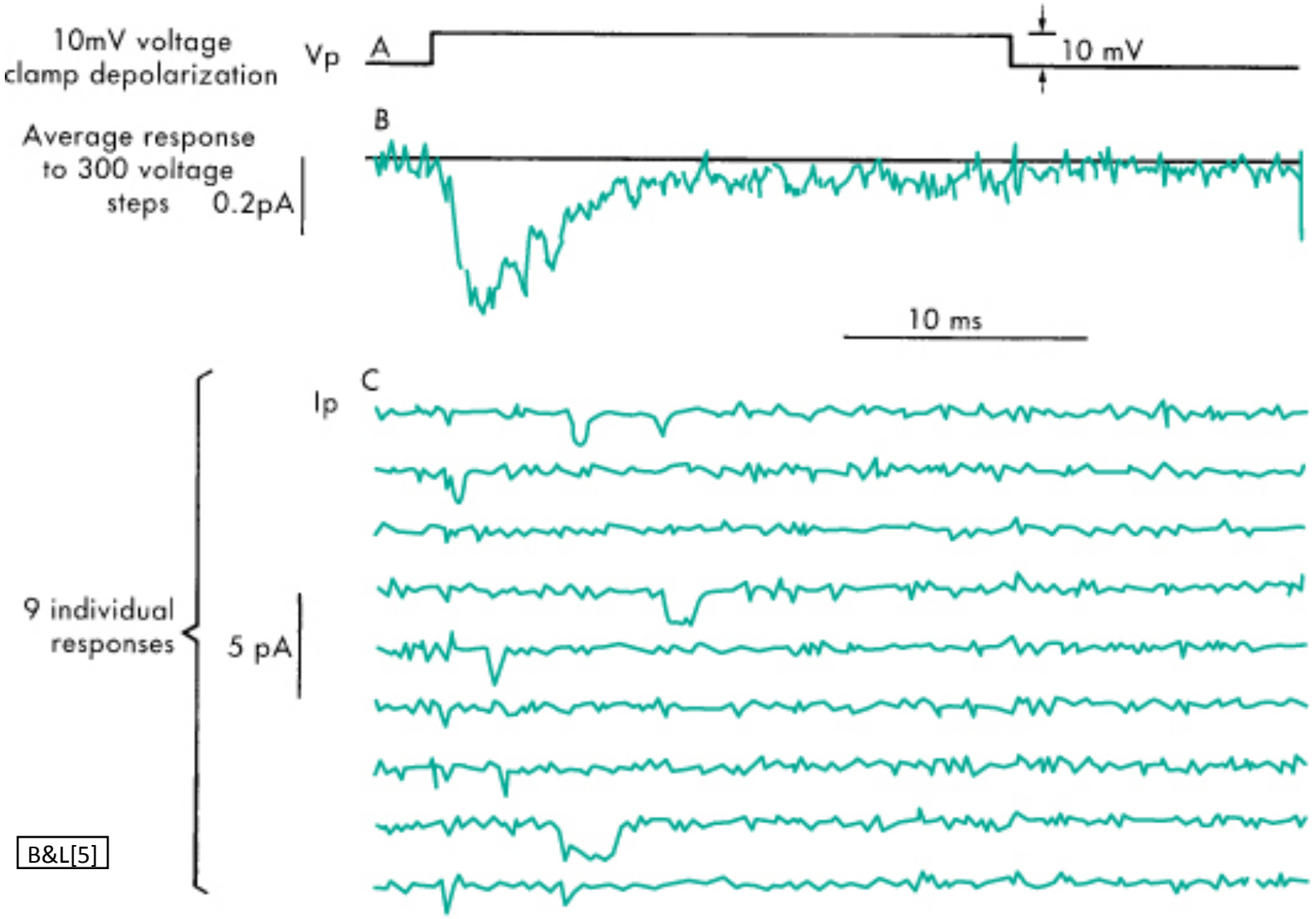


Figure 3-10 A patch electrode recorded the currents that flowed in small patches of rat muscle membrane in response to a 10 mV depolarization (trace A). TEA was used to block K^+ channels that might have been present in the patch. The traces in C show responses to 9 individual depolarizations. B is the average of 300 individual responses. Note that this average response resembles the summed response of thousands of sodium channels, as seen in measurements of whole cell Na^+ currents. Redrawn from Sigworth and Neher, Nature, 1980, 287: 447.

END

Video 2, Module 2