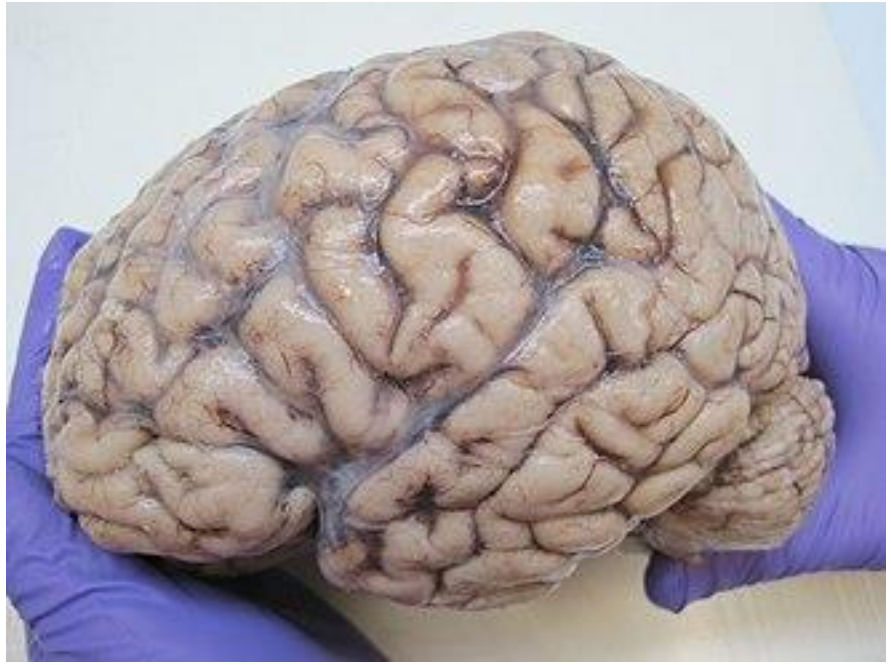
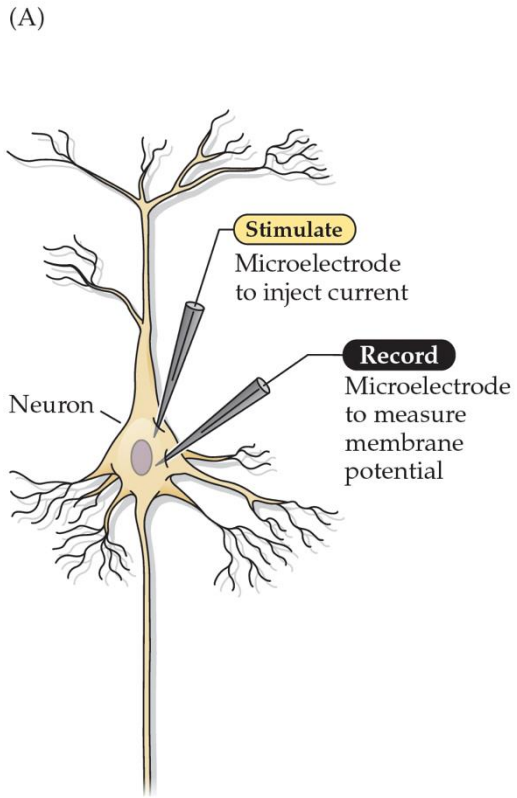
Unit 2: Brain Anatomy & the Action Potential



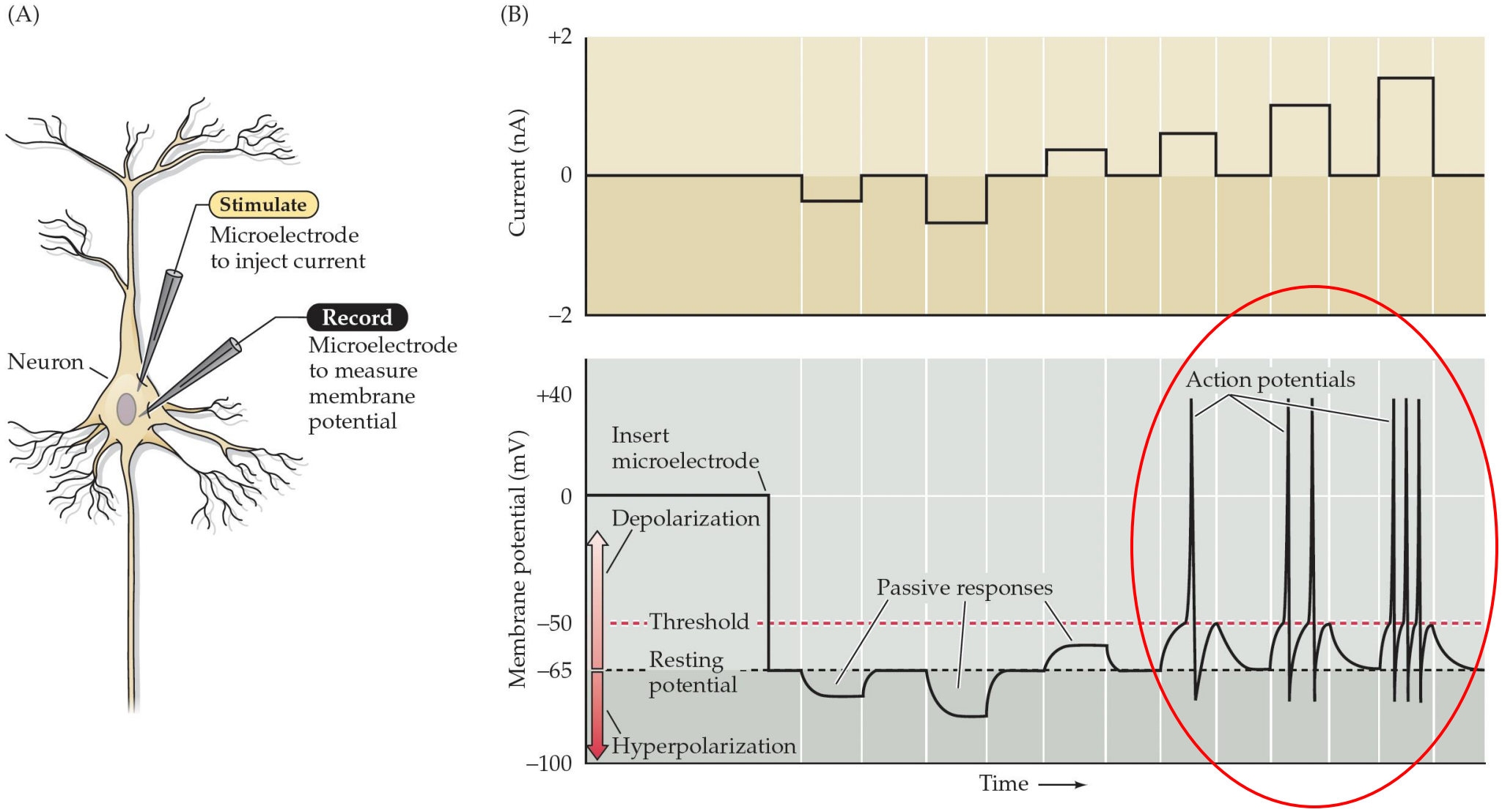
Wikipedia



Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs and tears. Through it, in particular, we think, see, hear, and distinguish the ugly from the beautiful, the bad from the good, the pleasant from the unpleasant. . . . It is the same thing which makes us mad or delirious, inspires us with dread and fear, whether by night or by day, brings sleeplessness, inopportune mistakes, aimless anxieties, absent-mindedness, and acts that are contrary to habit. These things that we suffer all come from the brain, when it is not healthy, but becomes abnormally hot, cold, moist, or dry, or suffers any other unnatural affection to which it was not accustomed. Madness comes from its moistness. When the brain is abnormally moist, of necessity it moves, and when it moves neither sight nor hearing are still, but we see or hear now one thing and now another, and the tongue speaks in accordance with the things seen and heard on any occasion. But when the brain is still, a man can think properly.

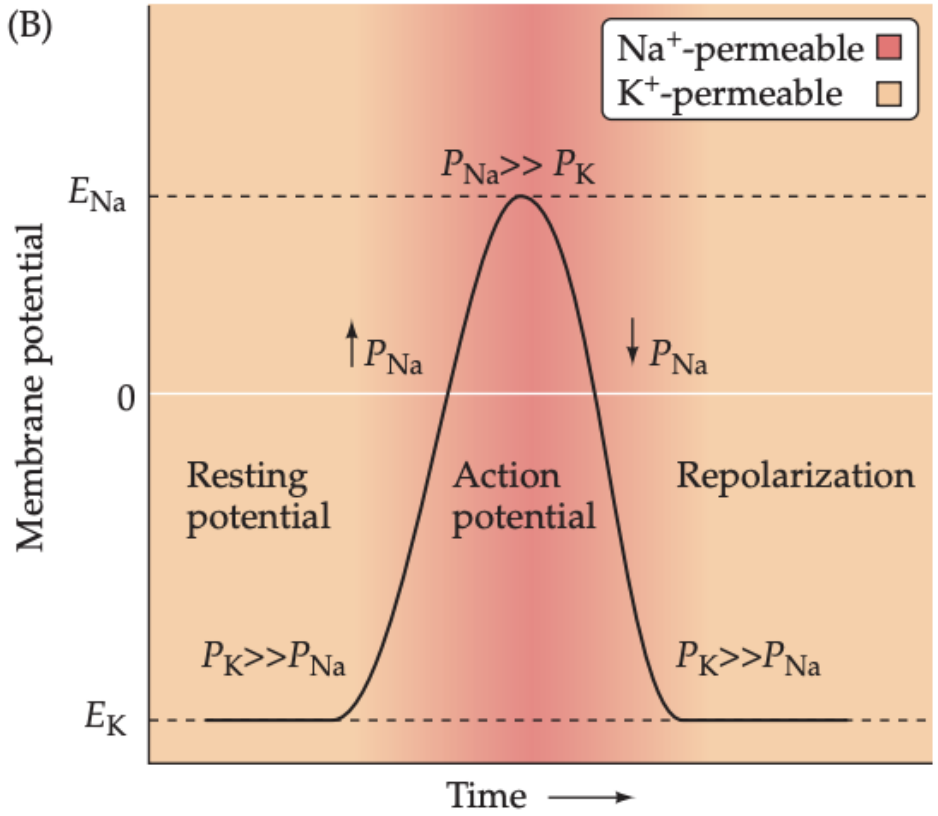
attributed to Hippocrates
Fifth Century, B.C.

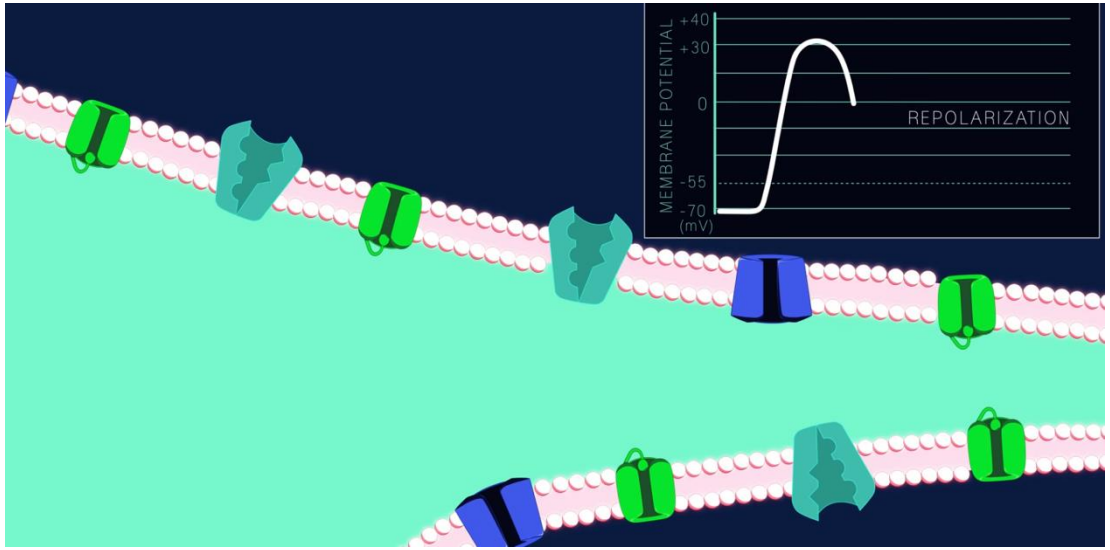
The action potential, an active membrane potential response



Purves, Figure 2.2

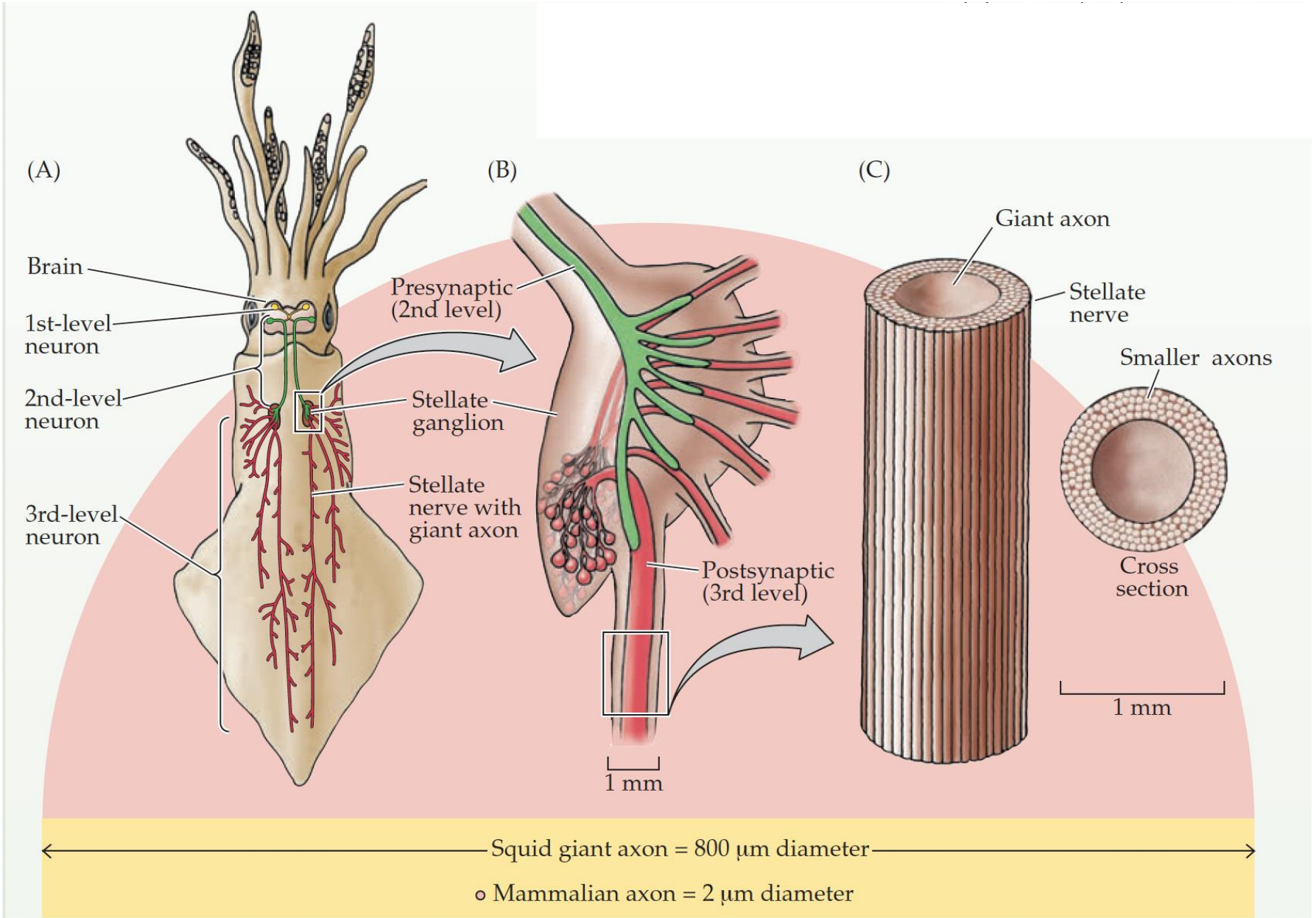
The sodium-potassium (Na^+/K^+) pump establishes an electrochemical gradient





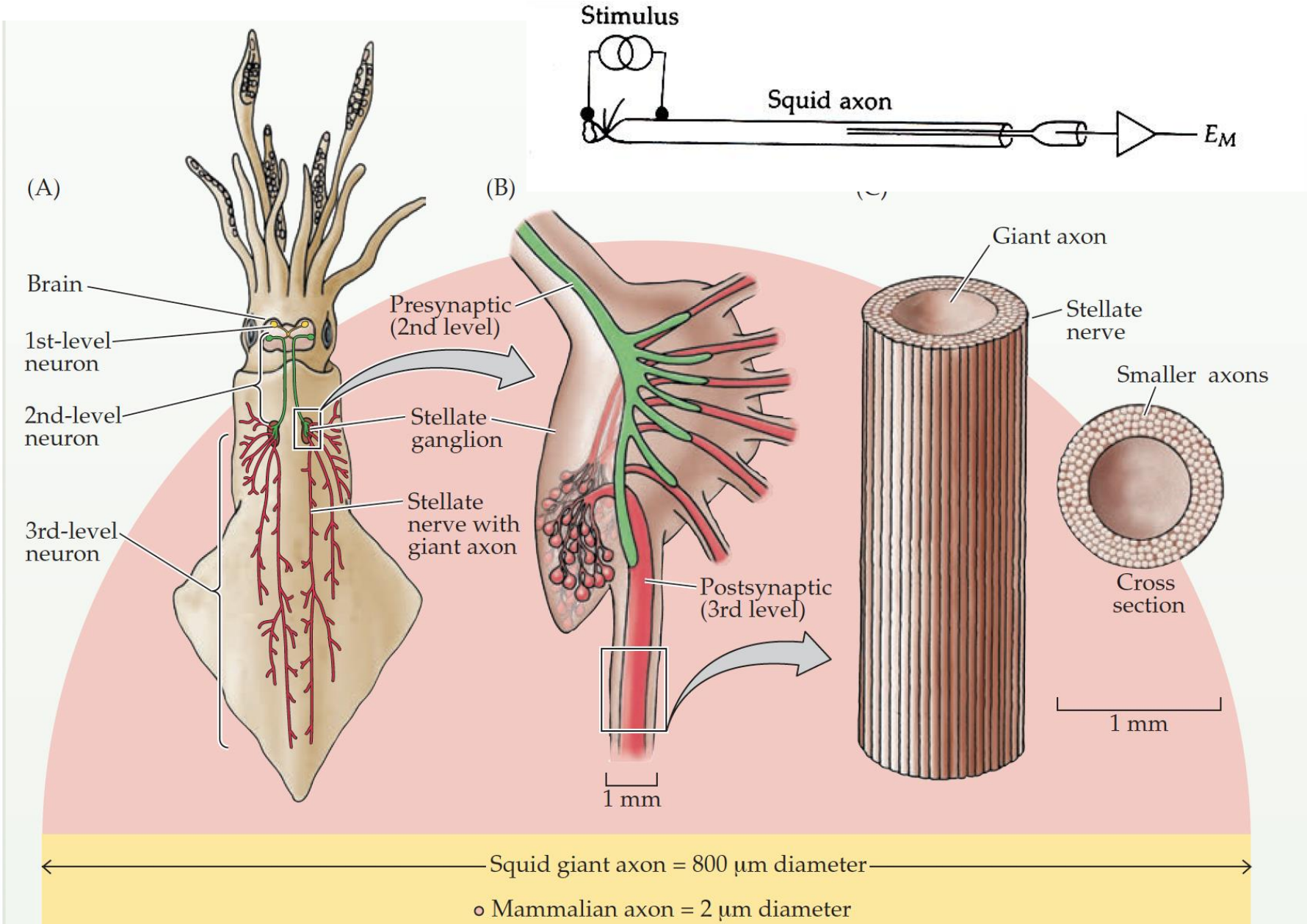
https://www.youtube.com/watch?v=oa6rvUJlg7o

Insights drawn from the squid's 'giant axon'

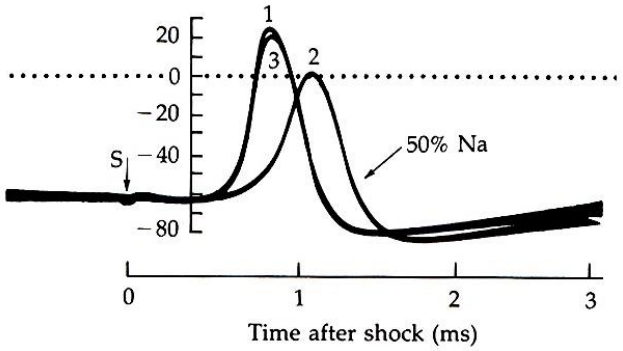


Purves, Box 2A

Insights drawn from the squid's 'giant axons'



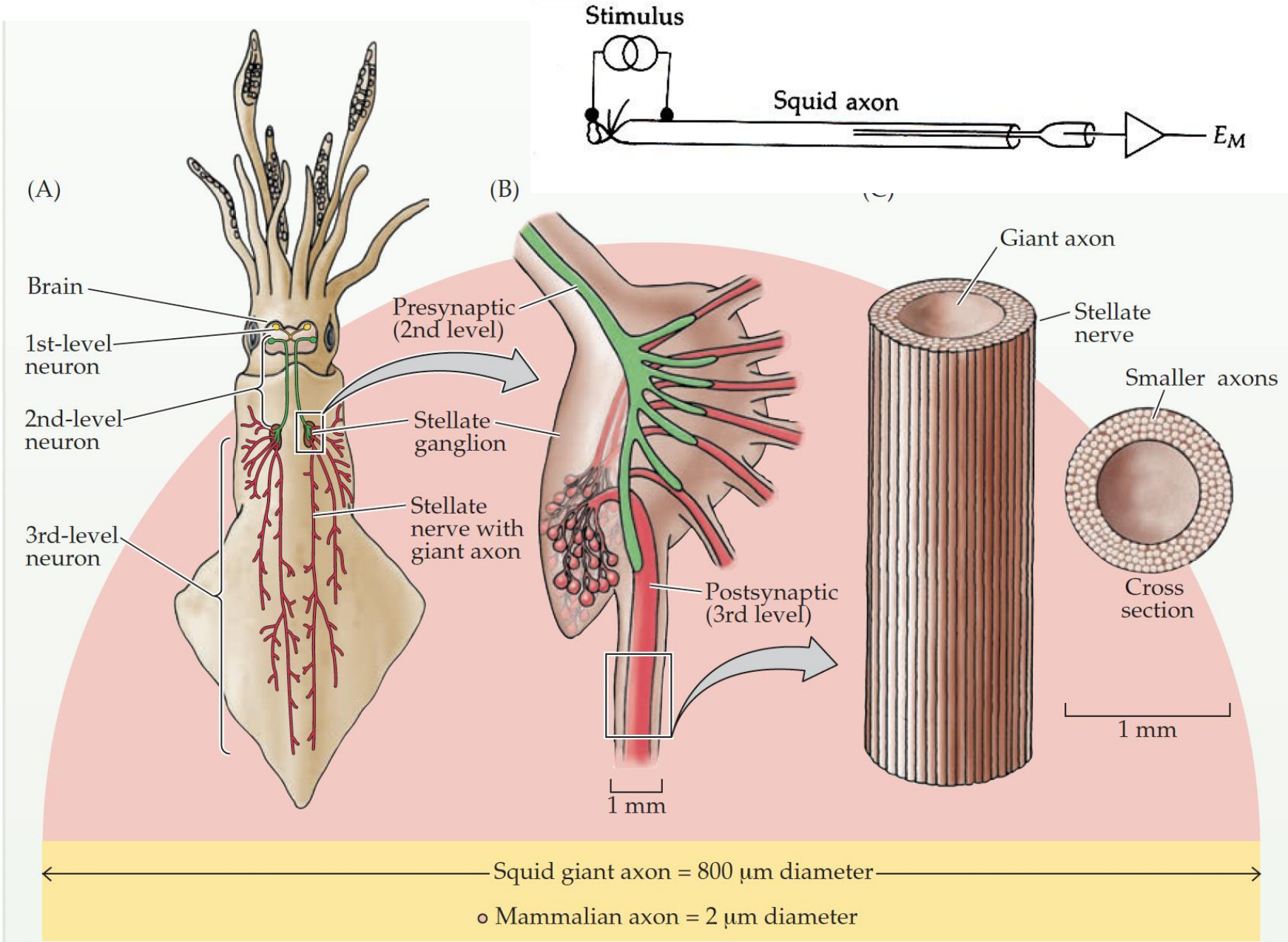
- Intracellular recording of V_m
- An example of "current clamp"



Hille, Figure 2.4

Purves, Box 2A

Insights drawn from the squid's 'giant axons'



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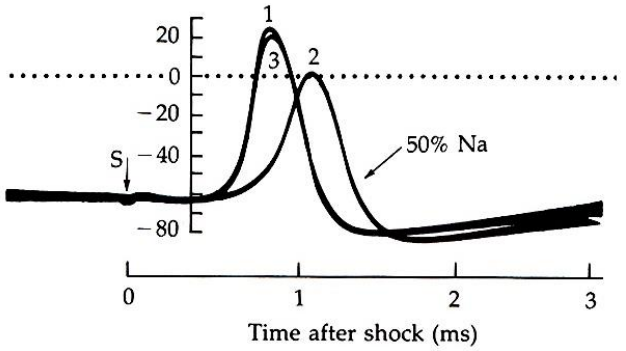
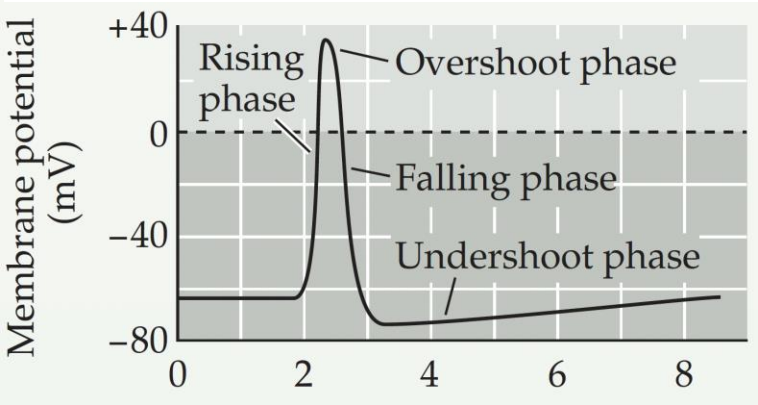


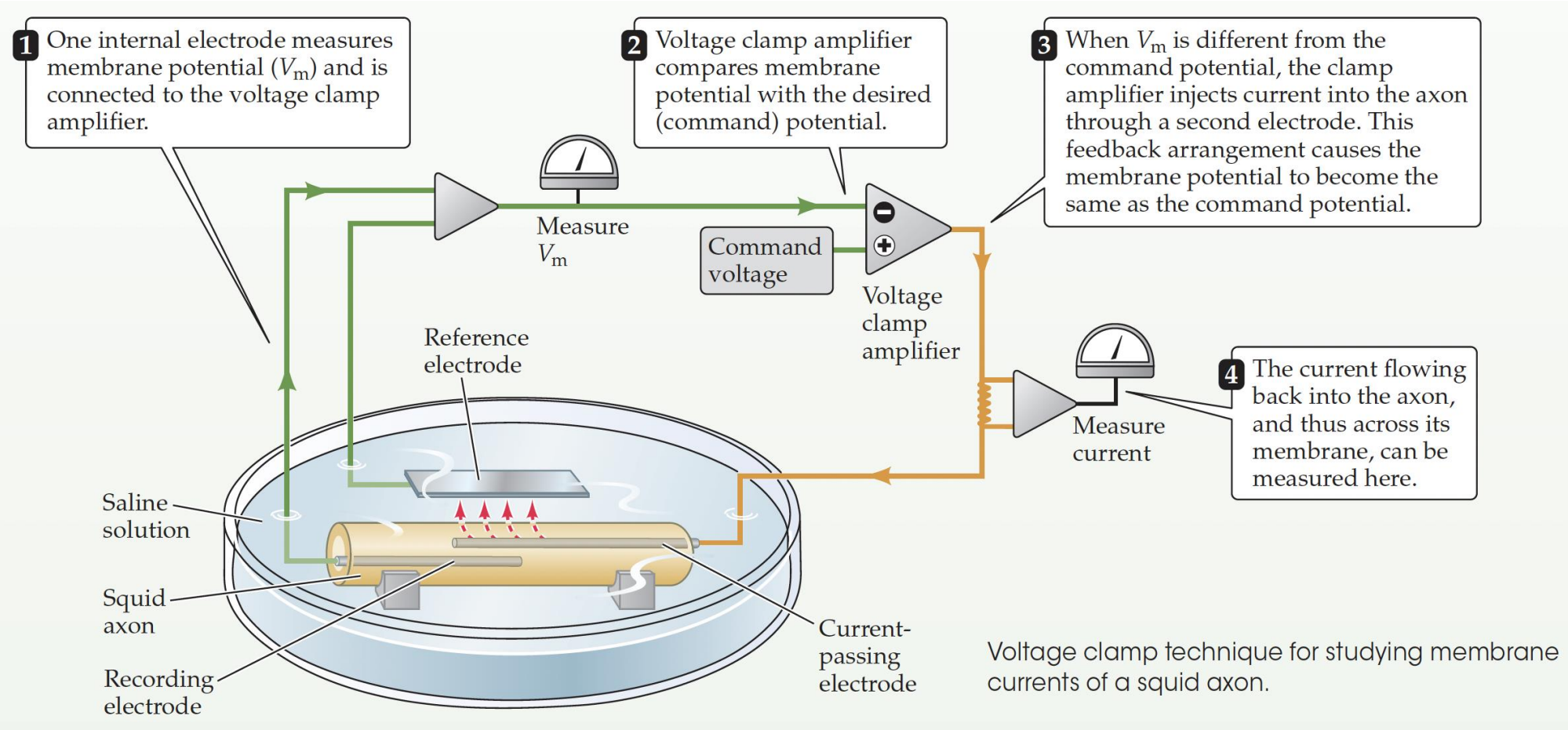
Hille, Figure 2.4



Purves, Box 2A

Purves, Box 2B

How do we uncover the currents giving rise to the action potential?

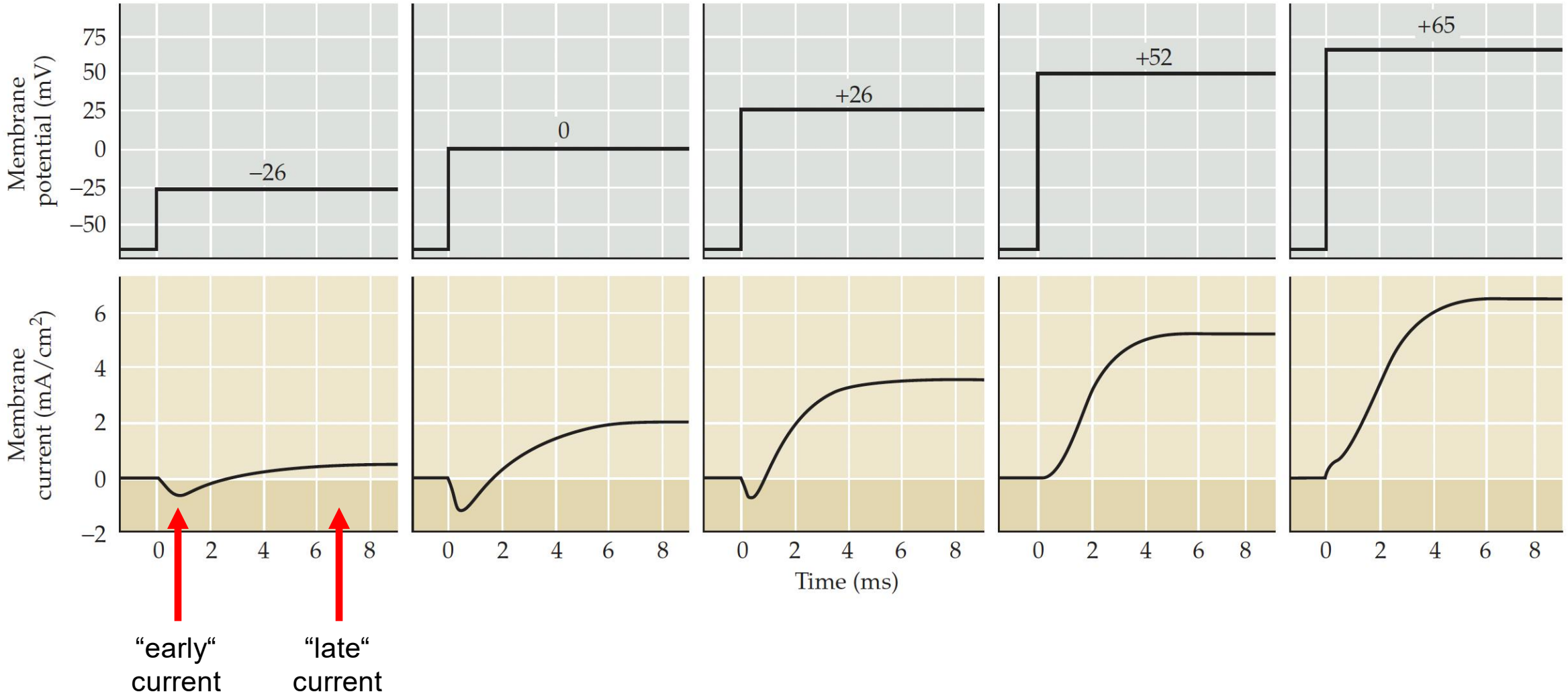


Voltage clamp technique for studying membrane currents of a squid axon.

- Measurement of *membrane potential* , V_m, with an intracellular electrode
- A second electrode "injects" current by a feedback amplifier <u>to keep V_m constant</u>
- Voltage clamp offers control over a key variable (V_m) that determines channel gating

Purves, Box 3A

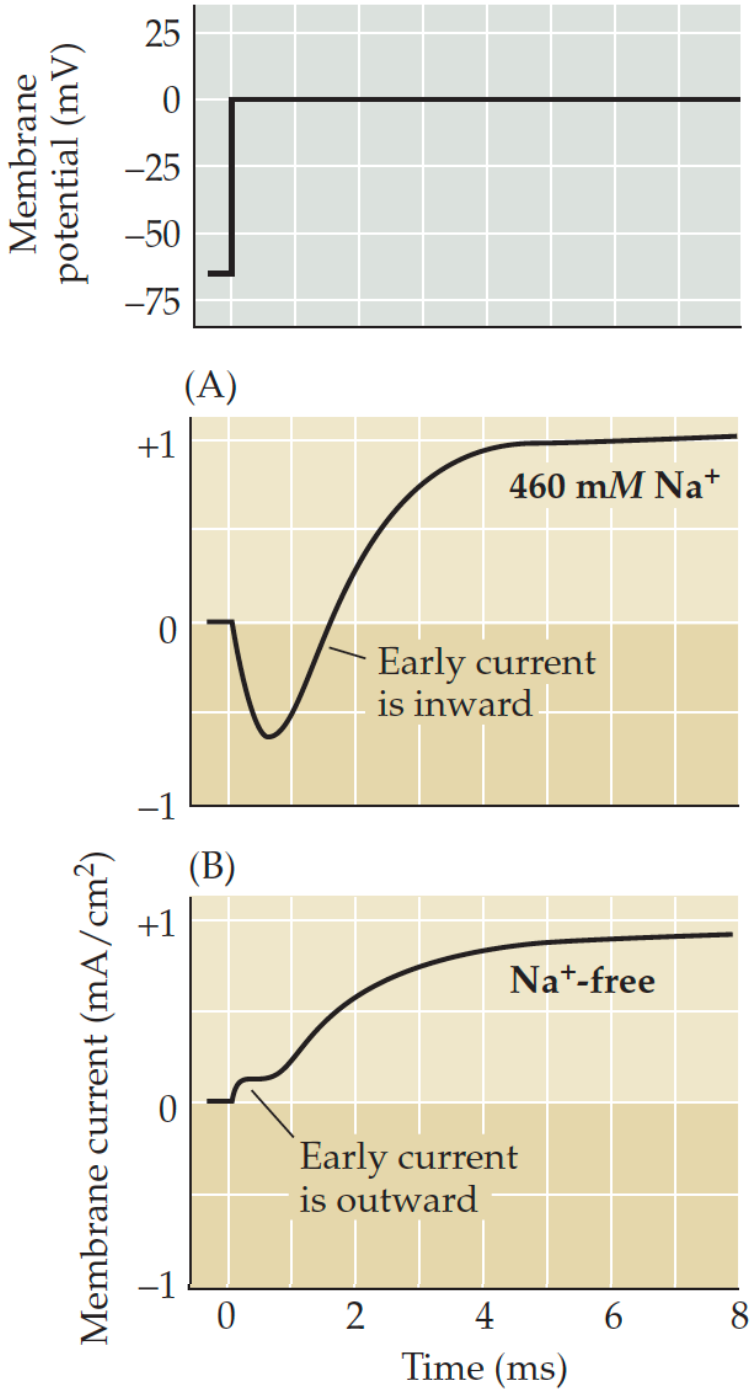
Currents measured with voltage-clamp in the squid giant axon



current drawn downward is called "inward" current and would depolarize the cell

Purves, Figure 3.2

The early current requires extracellular Na^+

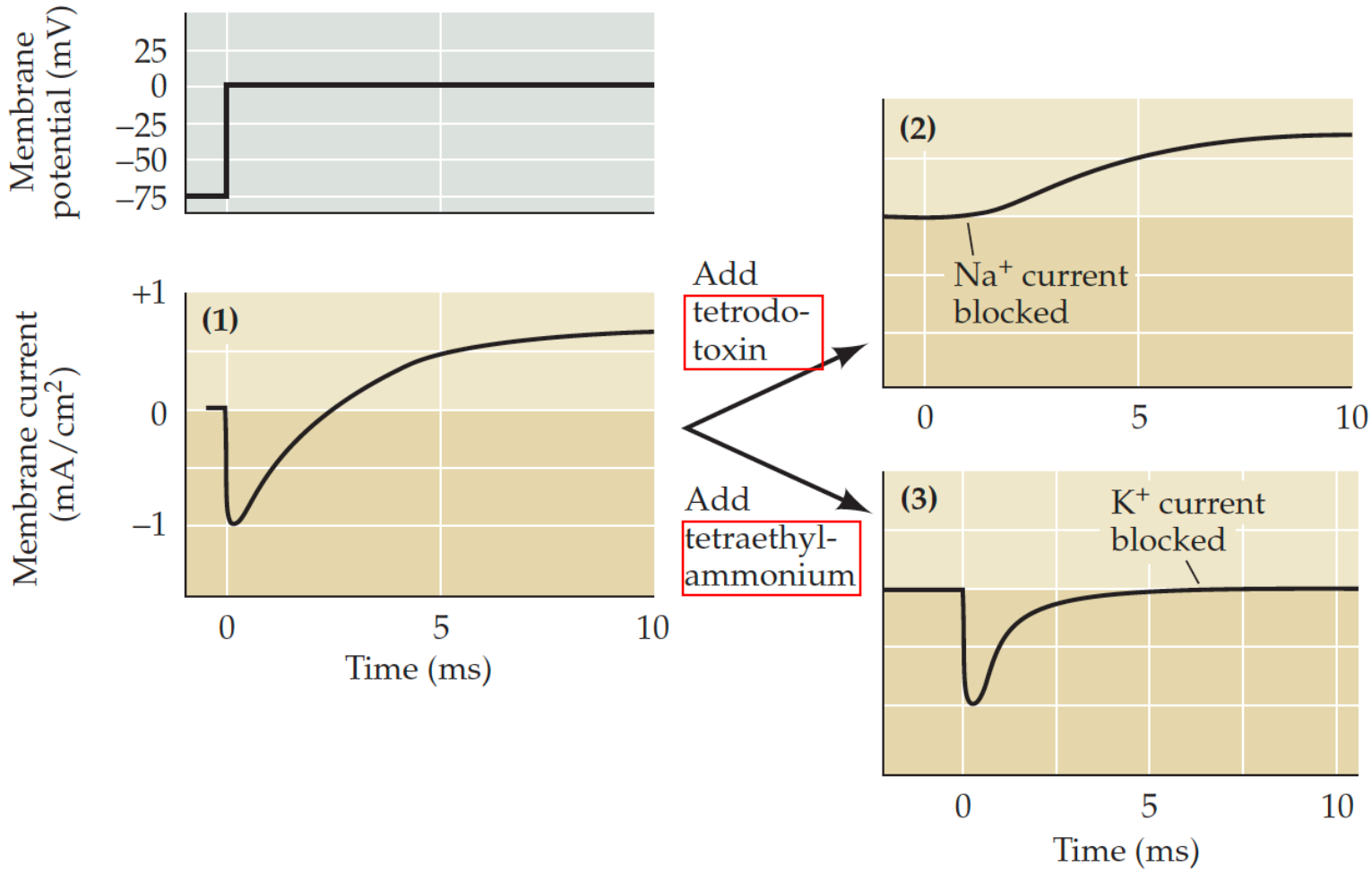


Note: 460 mM Na^+ is because the squid is a seawater organism.
There is higher [Na^+] in the extracellular fluids of these animals.

- The early current must be a movement of Na^+ ions from outside to inside (i.e., inward current)

Purves, Figure 3.4

One can independently block either the early or the late current



- Hodgkin-Huxley proposed that separate conductances for Na^+ ions and K^+ ions are activated during depolarization (there is also a leak conductance)

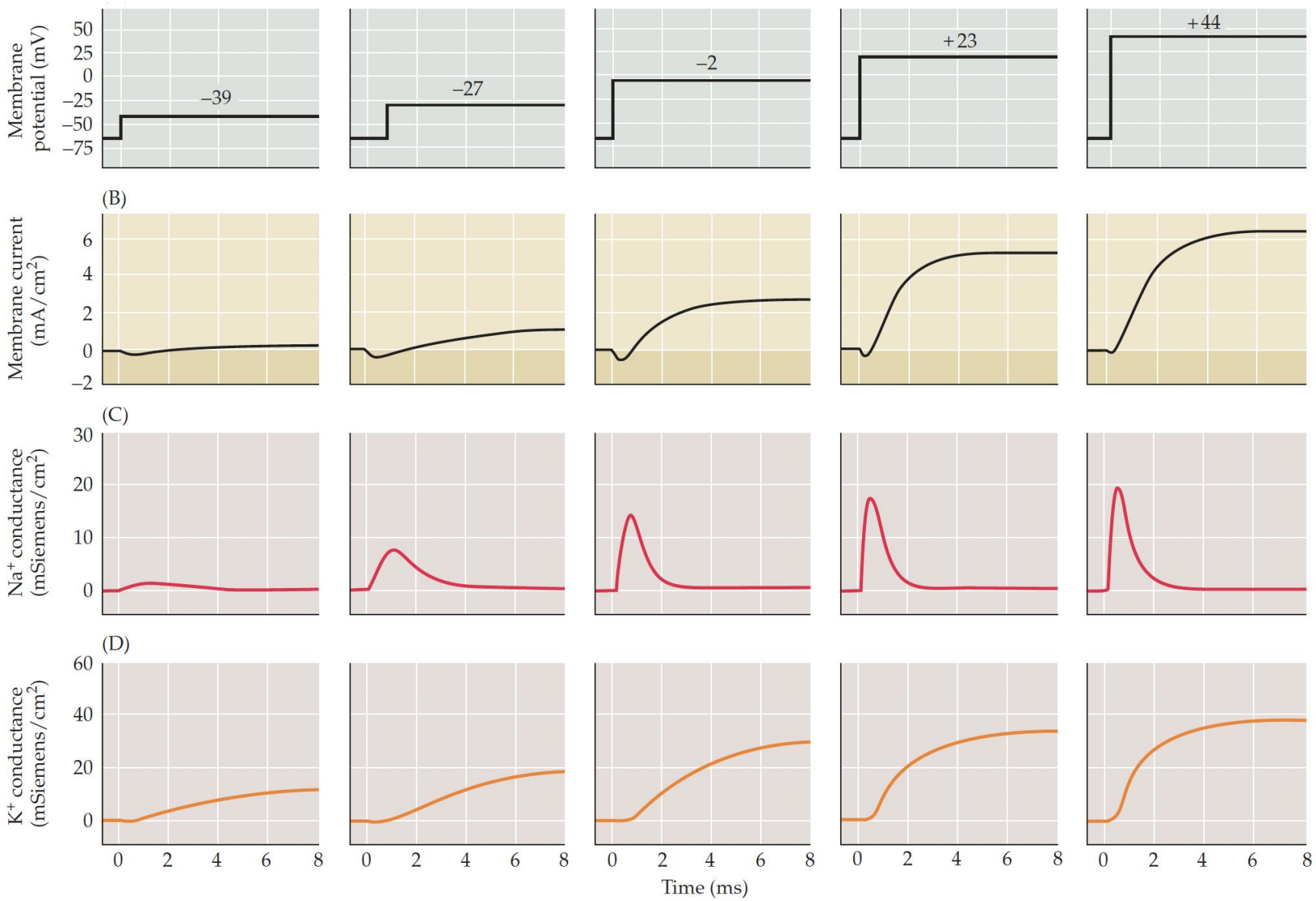
Purves, Figure 3.5

Action potential Na^+ and K^+ conductances depend on voltage and time

Holding potential

Na^+

K^+



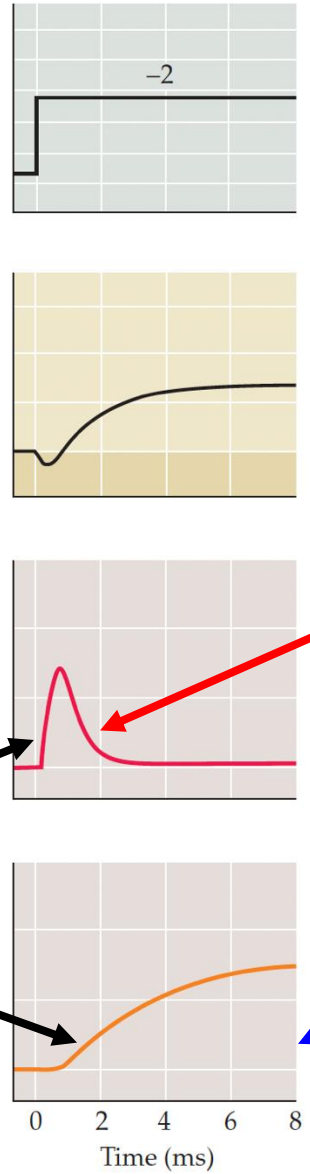
Purves, Figure 3.6

Three important concepts for voltage-dependent ion channel gating

Holding potential

Na^+

K^+



1. **Activation**
There is an increase in conductance (channels open), *usually* upon depolarization

2. **Inactivation**
There is a decrease in conductance (channels close), despite ongoing depolarization

ONLY in Na^+ channels

3. **Deactivation**
Channels close when the voltage step ends (not visible in this schema)

Purves, Figure 3.6

Activation – The time-dependent **opening** of ion channels in response to a stimulus, typically membrane depolarization (*or agonist pulse in ligand-gated ion channels*)

Inactivation - The time-dependent **closing** of ion channels *despite maintained depolarization* (V_m step for voltage-gated channels)

Deactivation - channel **closes** upon repolarization (not visible for I_{Na} because most channels are already inactivated)

Aside - 1952, a remarkable year for Hodgkin & Huxley

Experimental

Measurement of current-voltage relations in the membrane of the giant axon of Loligo.
HODGKIN AL, HUXLEY AF, KATZ B. *J Physiol*. 1952 Apr;116(4): **pp. 424-448**.

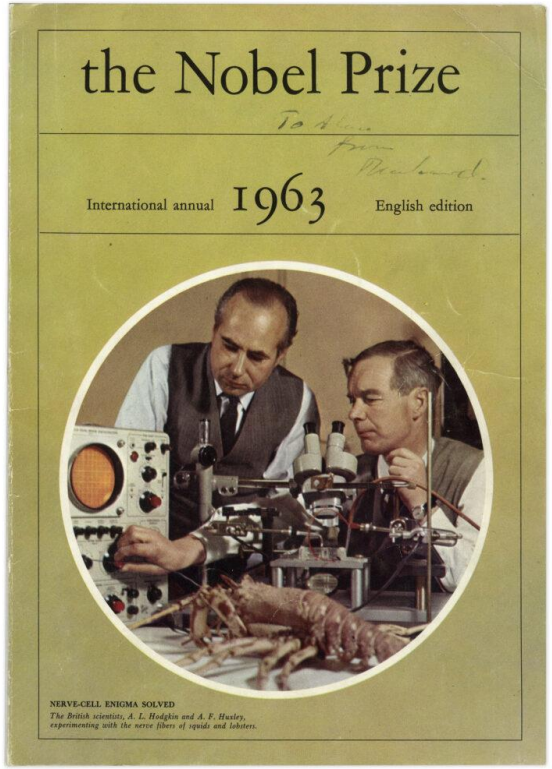
Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 449-472**.

The components of membrane conductance in the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 473-496**.

The dual effect of membrane potential on sodium conductance in the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 497-506**.

Mathematical modeling study

A quantitative description of membrane current and its application to conduction and excitation in nerve.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Aug;117(4): **pp. 500-544**.

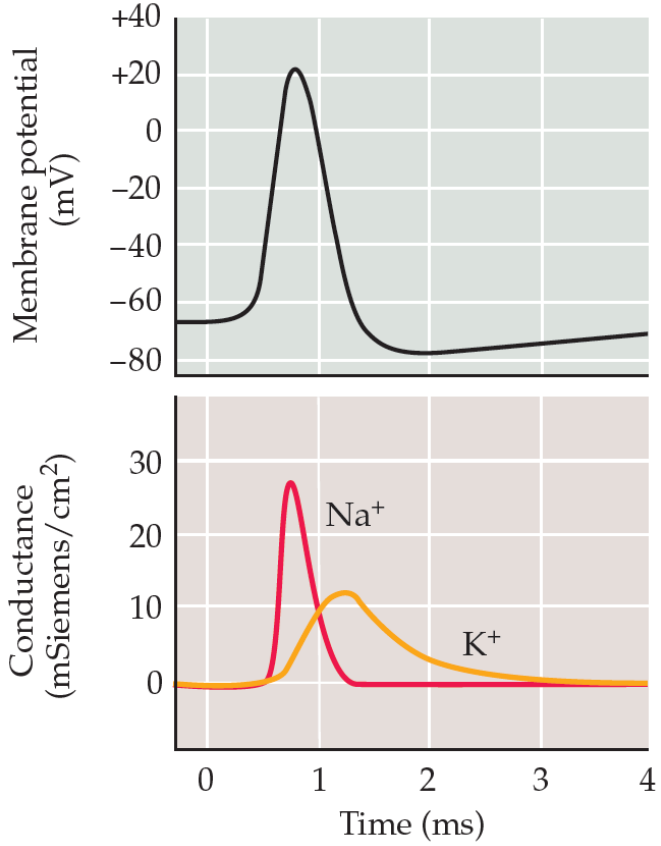


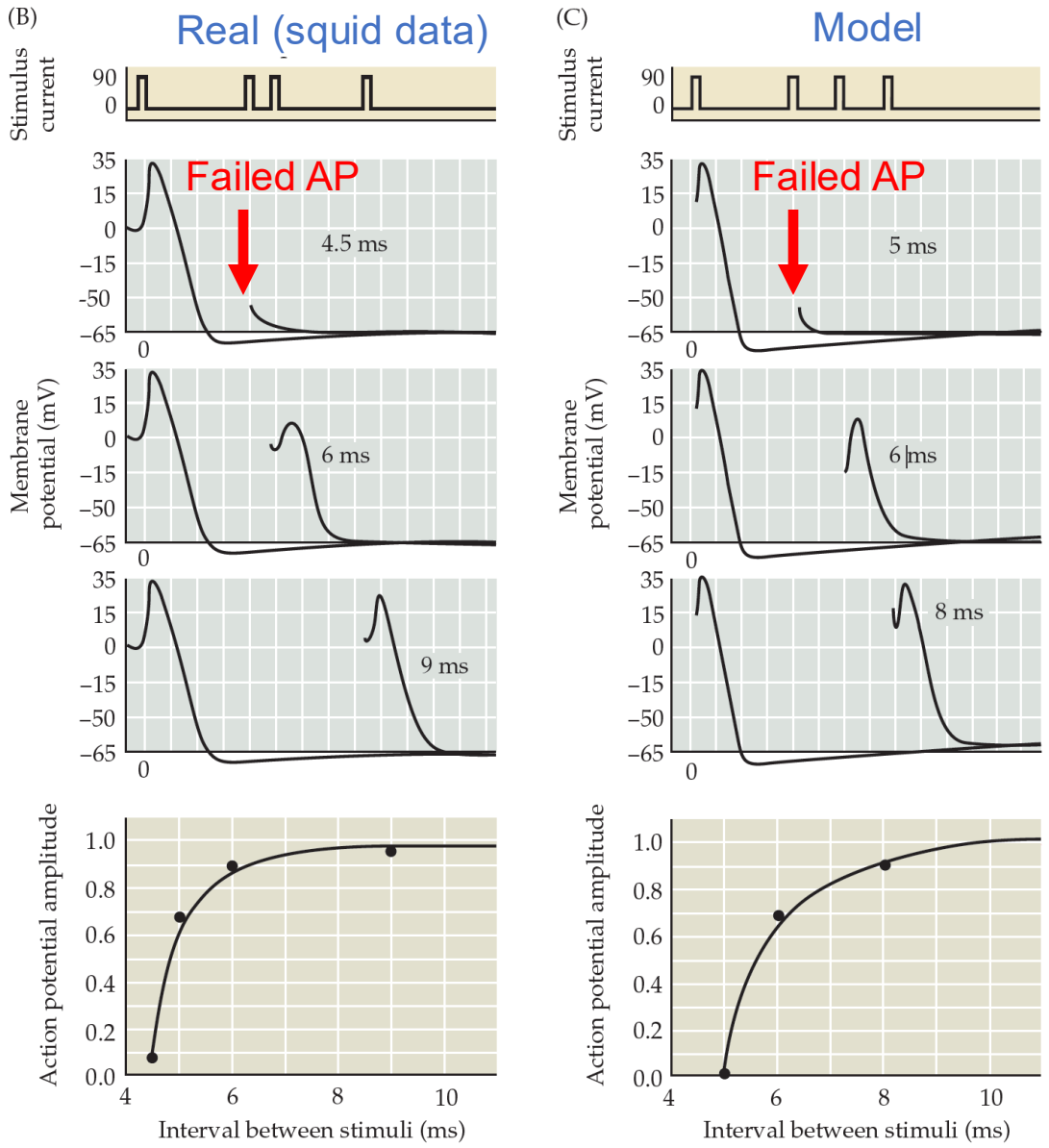
Hodgkin & Huxley

"This article concludes a series of papers concerned with the flow of electric current through the surface membrane of a giant nerve fibre. Its general object is to discuss the results of the preceding papers (Part I), to put them into mathematical form (Part II) and to show that they will account for conduction and excitation in quantitative terms (Part III)."

Hodgkin's Nobel Prize lecture: https://www.nobelprize.org/prizes/medicine/1963/hodgkin/lecture/

Mathematical reconstruction of the action potential

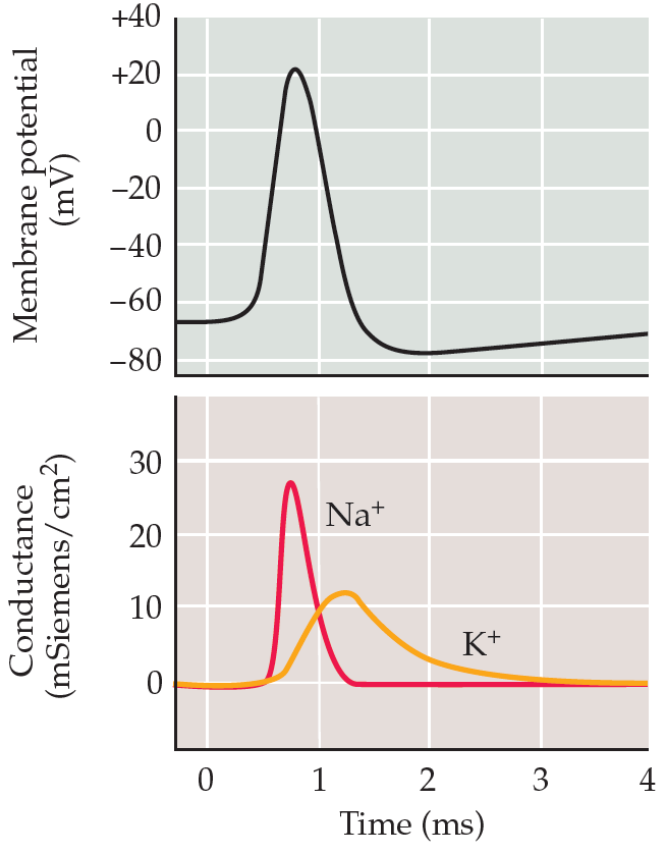


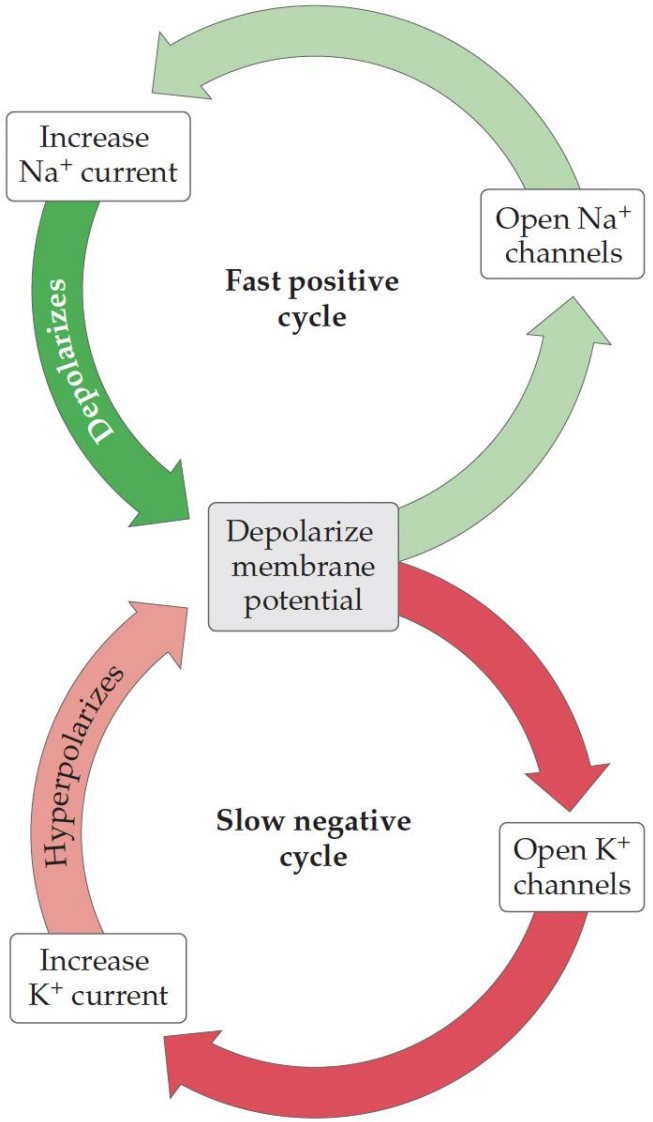


In 1952, H&H did not know yet about "ion channels" or other transmembrane proteins. They concluded that there are separate membrane conductances for Na^+ and K^+ and predicted these might be ion channels

Purves, Figure 3.8

The action potential includes positive and negative feedback loops

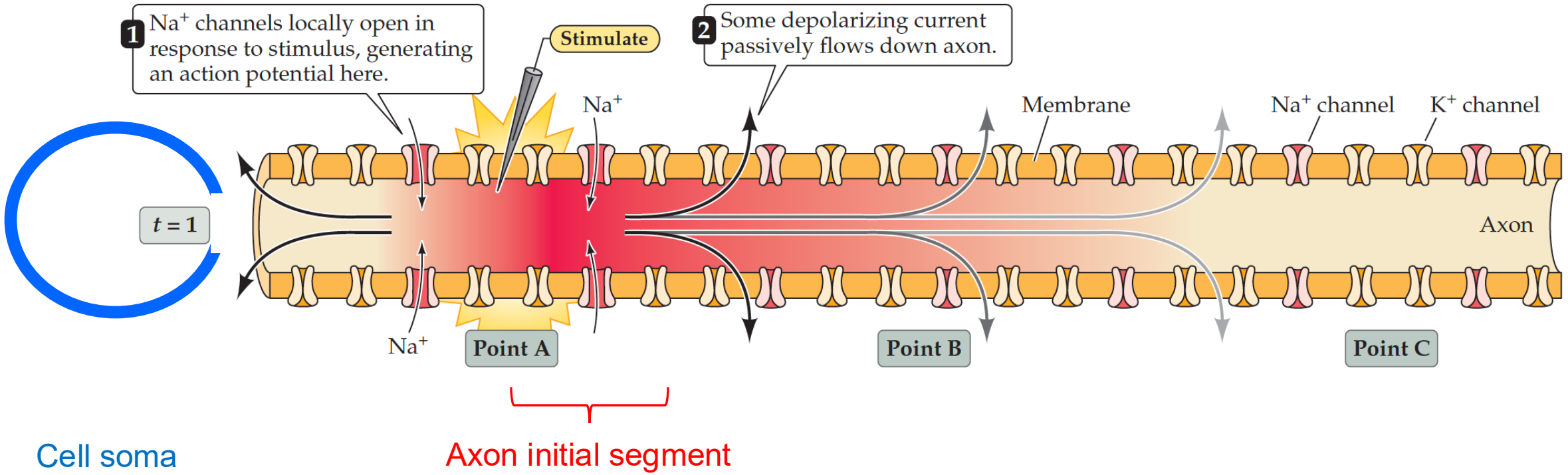




Na⁺ currents are ***positive*** feedback
K⁺ currents are ***negative*** feedback

Purves, Figure 3.9

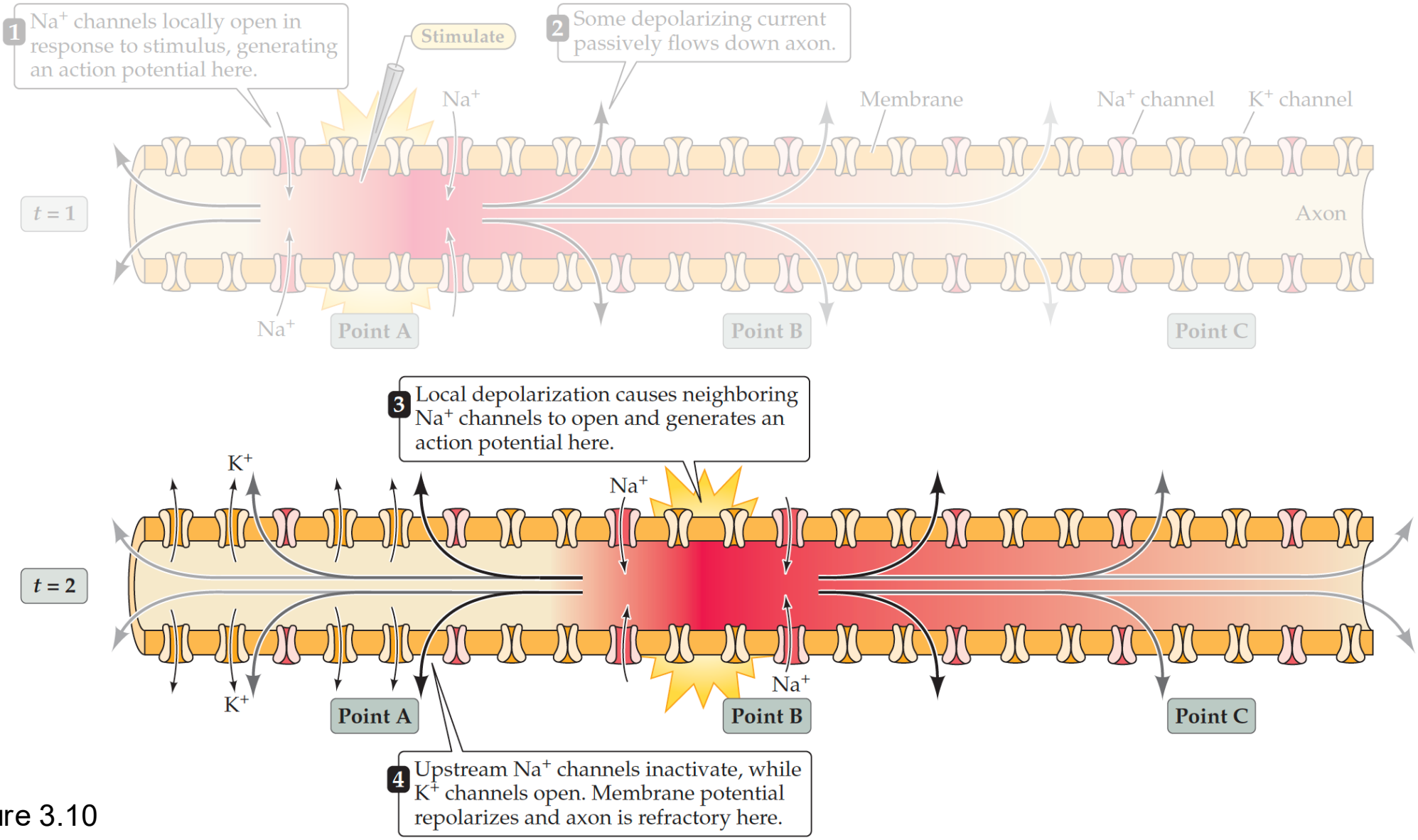
Action potentials are generated at the axon initial segment (near the soma) when the threshold V_m is reached



"*Stimulate*" refers to a strong EPSP / membrane depolarization at the soma/dendrites, reaching the **threshold V_m of** ~ -50 mV

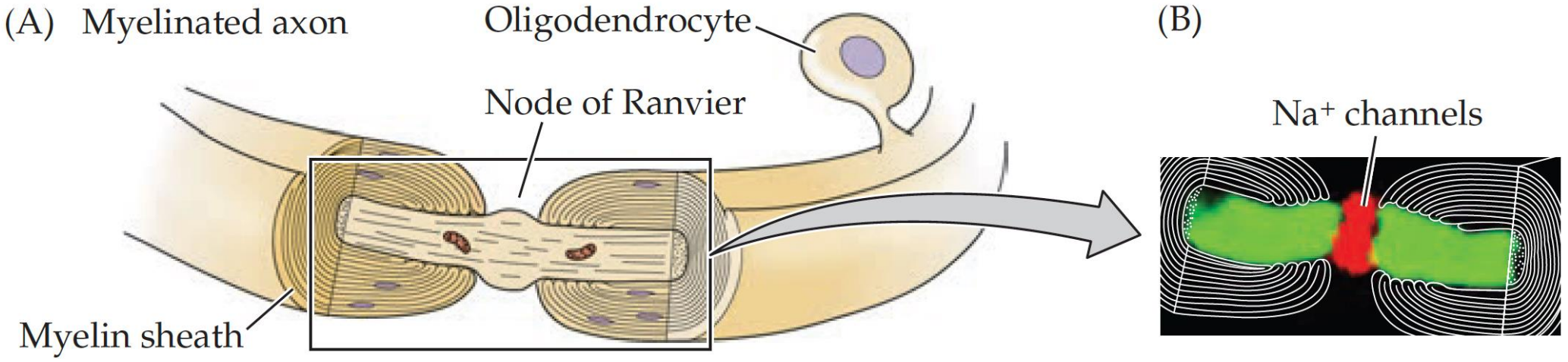
Purves, Figure 3.10

Action potentials propagate by *passive flow* and *asymmetric Na^+ inactivation*



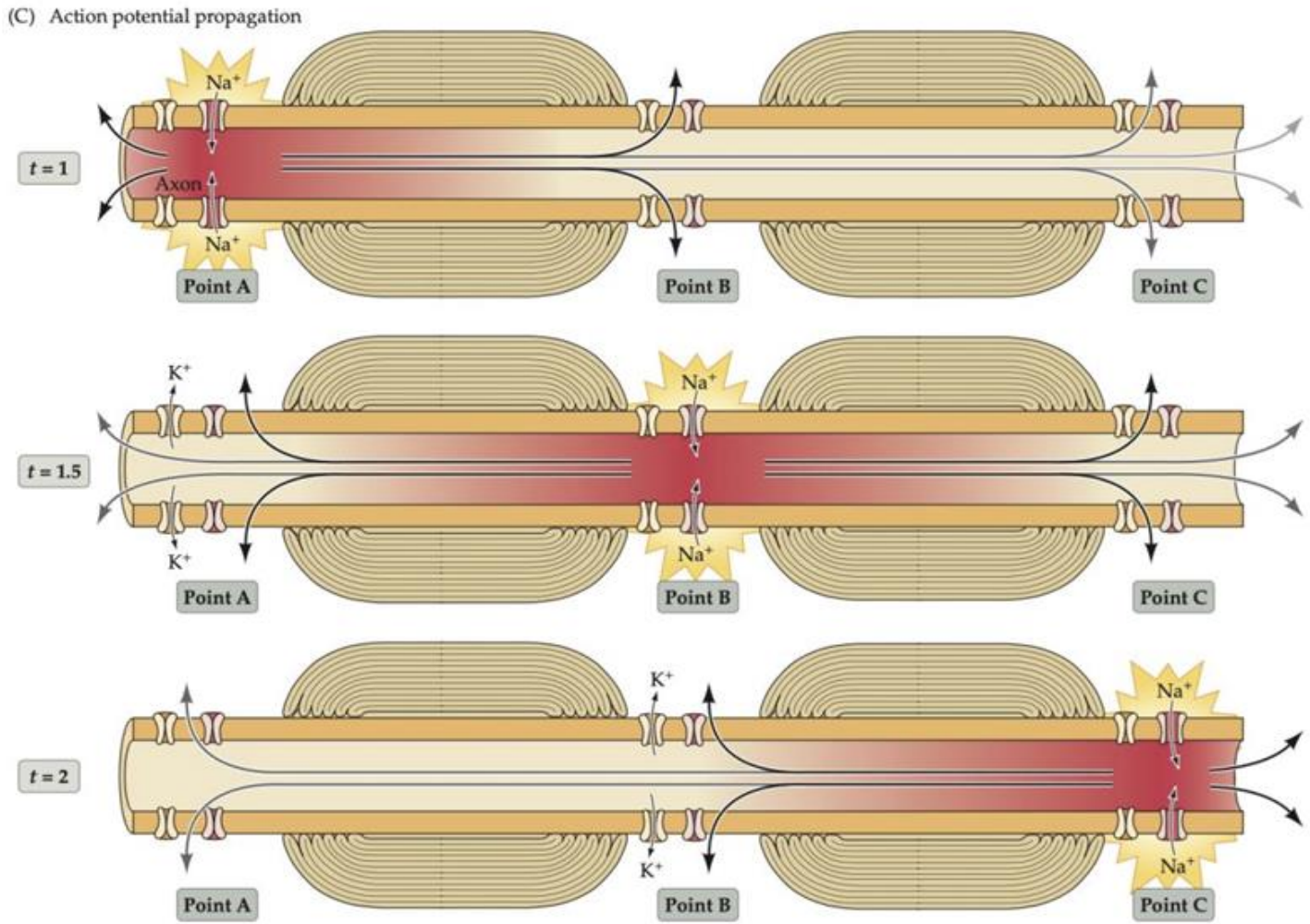
Purves, Figure 3.10

Saltatory ("jumping") conduction of action potentials in myelinated axons



Purves, Figure 3.11

Saltatory (“jumping“) conduction of action potentials in myelinated axons



Insulating layers around the axons called myelin sheath. These are glial cells.

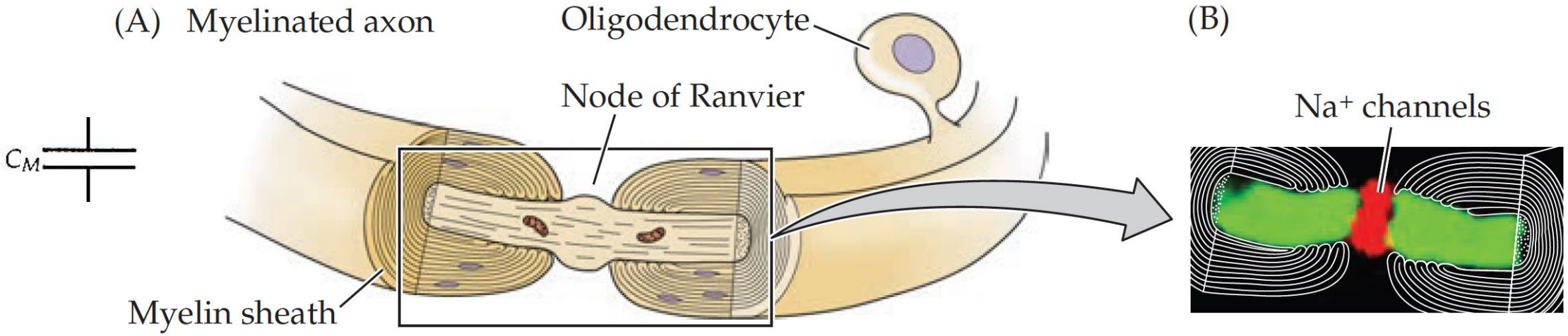
No ion channels along the axon except at gaps called Nodes of Ranvier.

Faster propagation because:

- Effectively thicker membranes → lower capacitance
- Less current leak through the membrane → passive current can trigger action potential further down the axon (at the next Node of Ranvier)

Purves, Figure 3.11

Action potential conduction is faster in myelinated axons



Larger distance **d** through membrane in myelinated region:
Causes a ↓ C_m (see eqn)

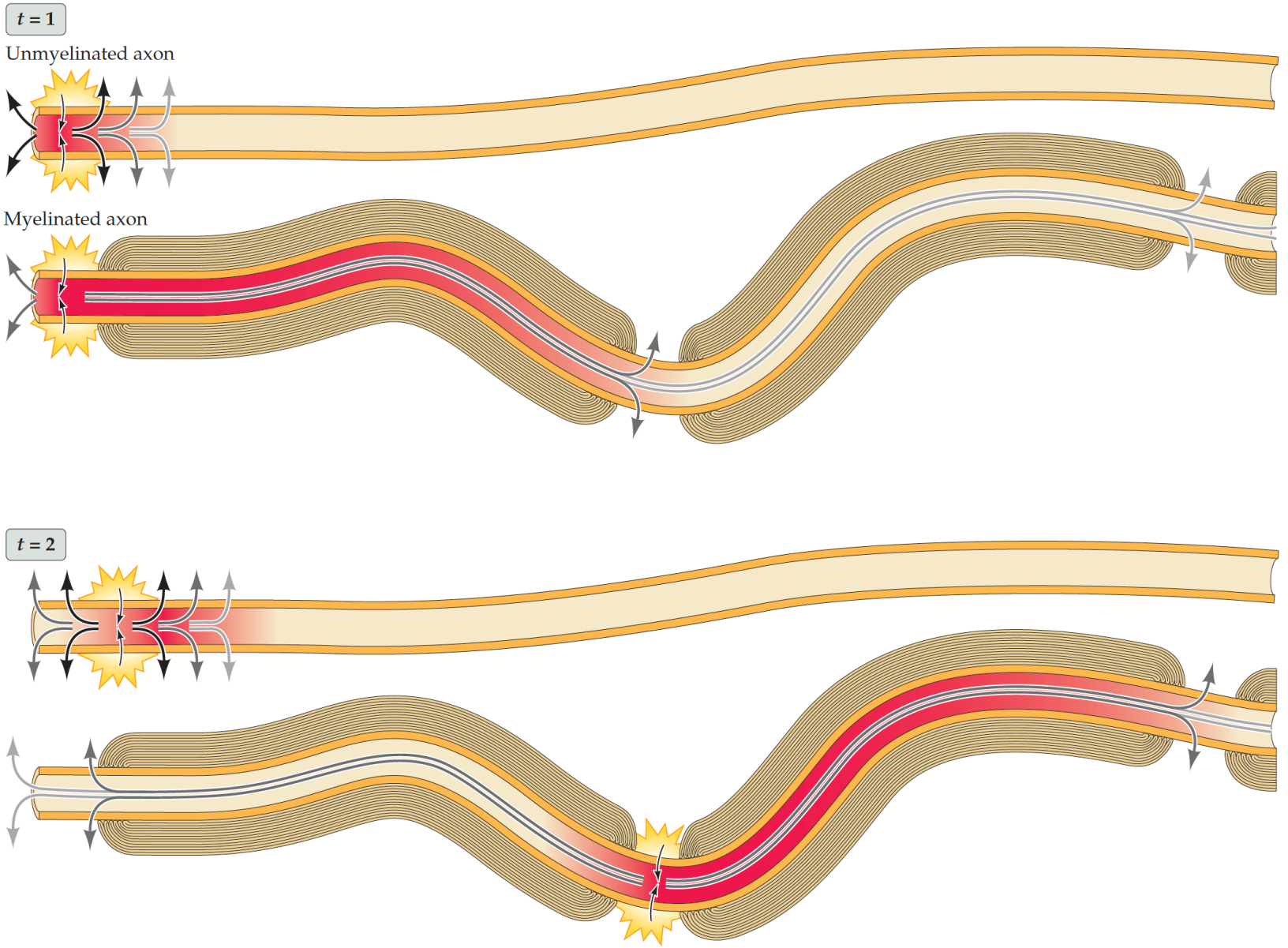
$$C = \frac{\varepsilon \cdot \varepsilon_0 \cdot A}{d}$$

Few ion channels in myelinated region:
Causes a ↑ R_m

Less ***loss*** by capacitative and resistive currents along the axons leads to ***faster*** conduction!

Purves, Figure 3.11

Action potential conduction is faster in myelinated axons



Purves, Figure 3.12

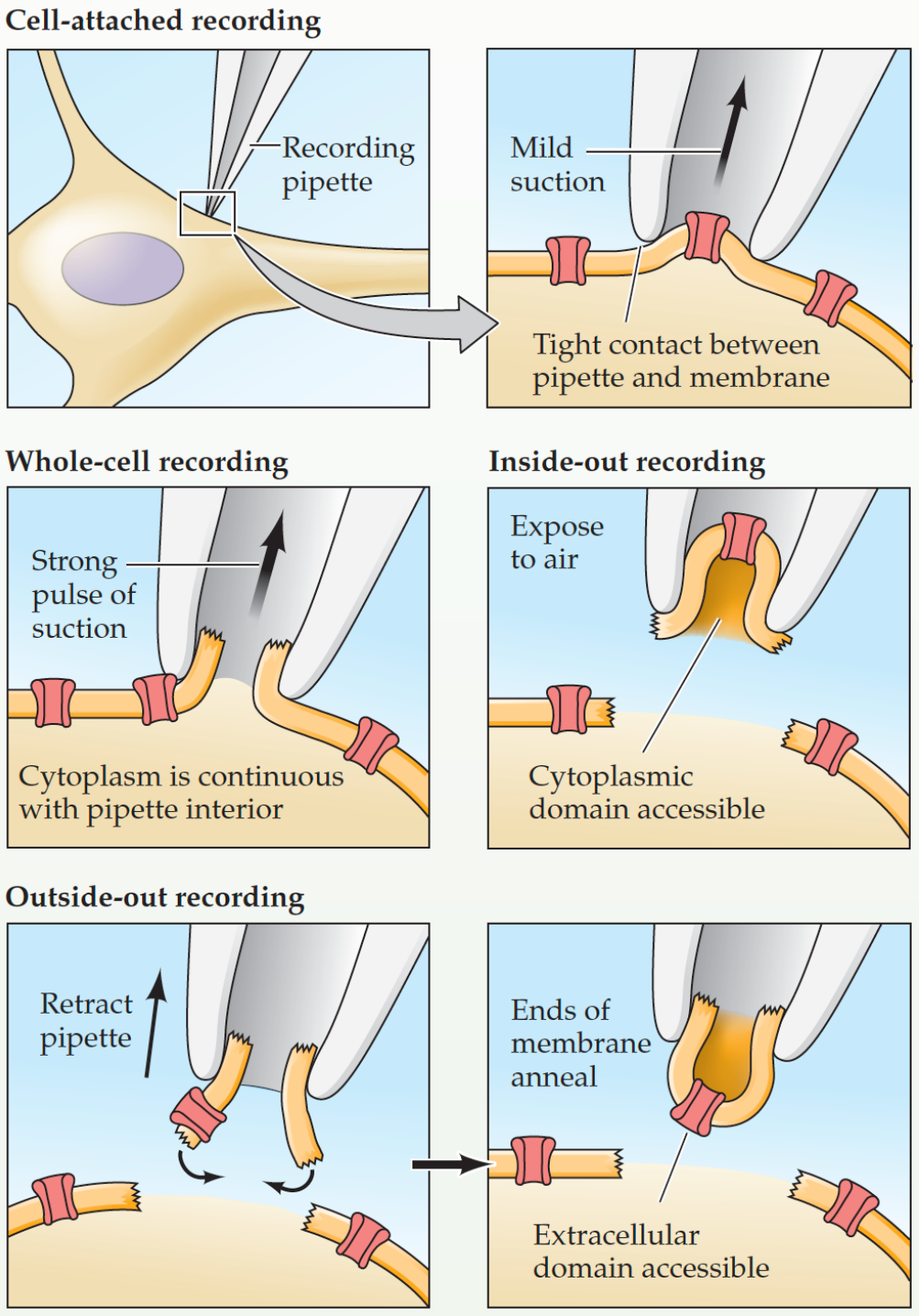
We now know that Na^+ and K^+ currents are mediated by separate ion channels.

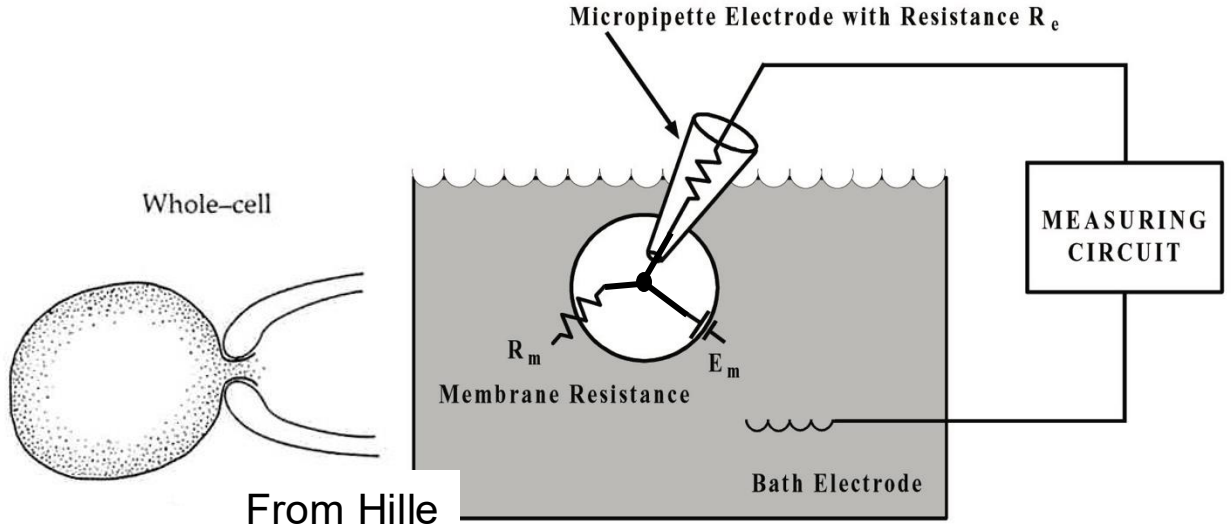
Goal: How can we learn more about the properties of these ion channels?

One approach: Single-channel “patch-clamp” recordings (early 1980's)

Multiple configurations of patch-clamp recordings

- **Cell-attached** recordings: high resistance contact
- **Whole-cell** recordings: break-in to cell interior with patch pipette filled with “intracellular solution“
- **Inside-out** recordings: channel interior becomes accessible
- **Outside-out** recordings: channel exterior becomes accessible to e.g., test channel ligands



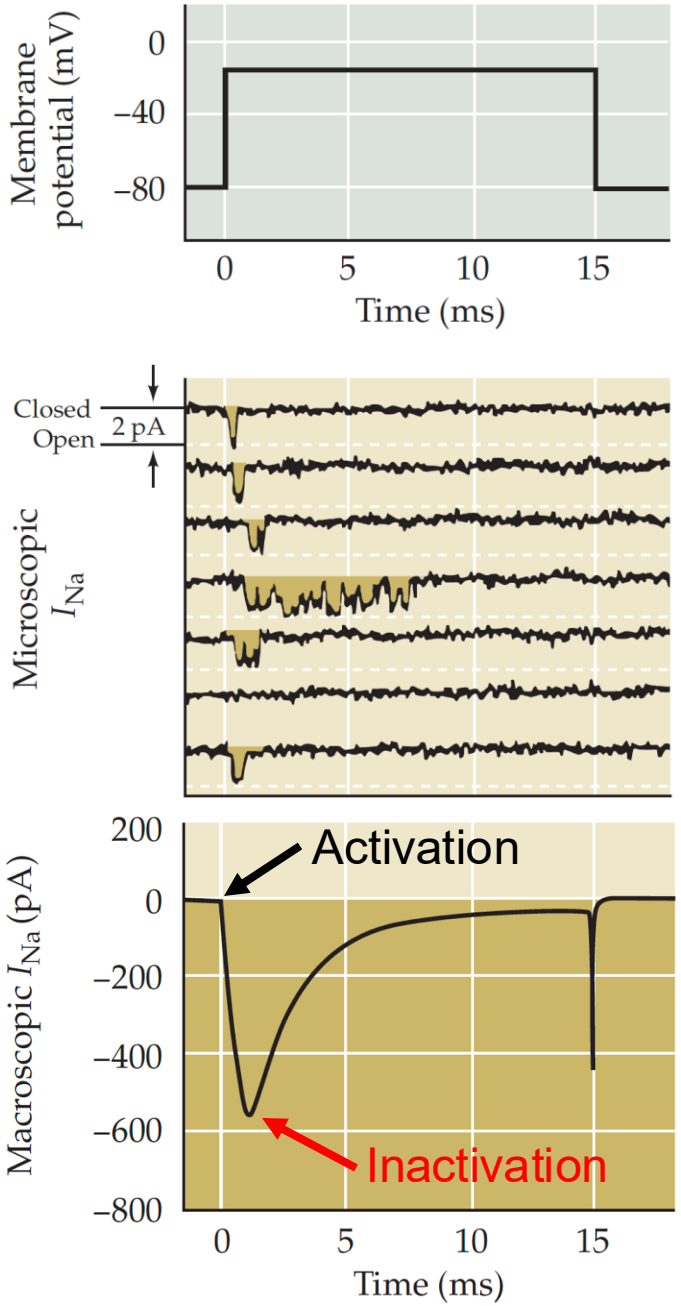


From Hille

Purves, Box 4A

From single-channels to macroscopic currents: voltage-gated Na^+ channels

Single voltage-gated **Na^+-channels** in a membrane "patch" give early **inward** depolarizing currents



Note that single-channel gating is *stochastic* despite repeated identical voltage-steps

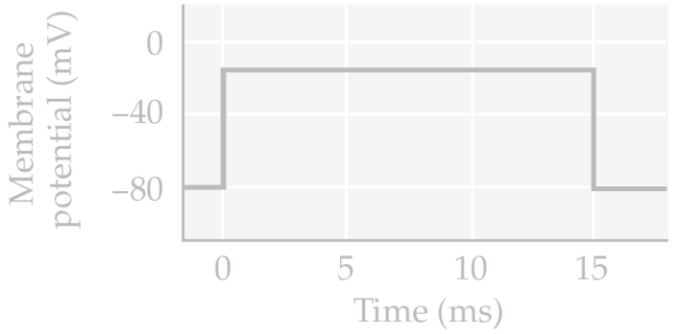
Total "ensemble" current given by:

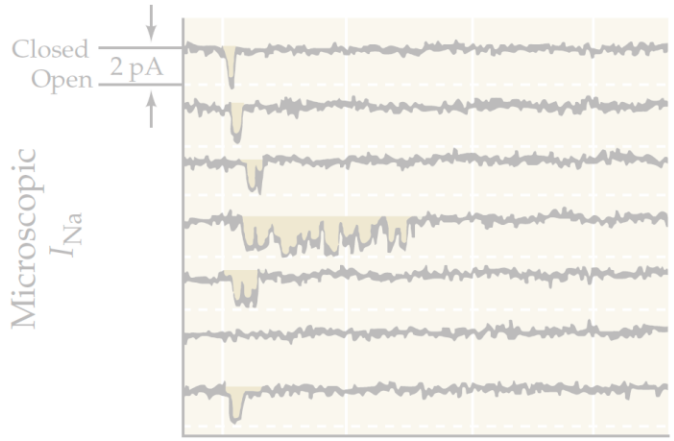
$$I_{total} = i_{single\ channel} \times N_{channels} \times p_{open}$$

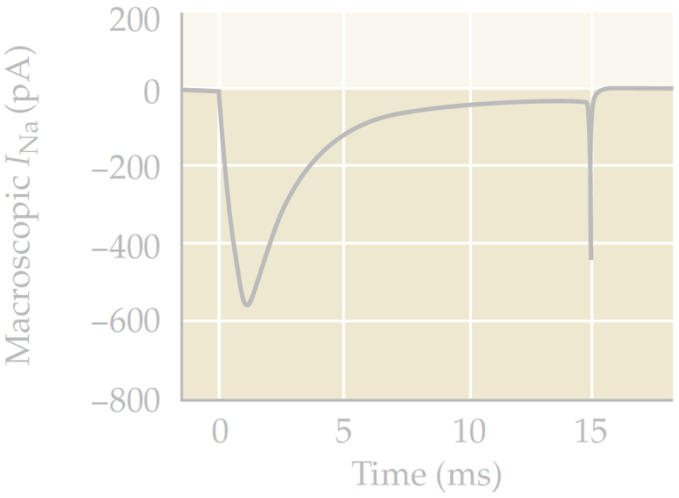
Purves, Figure 4.1

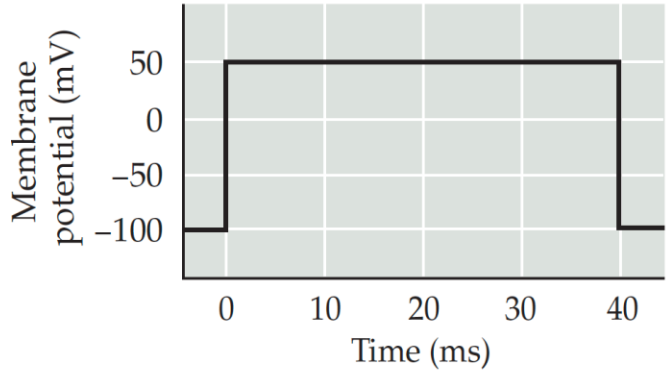
From single-channels to macroscopic currents: voltage-gated K^+ channels

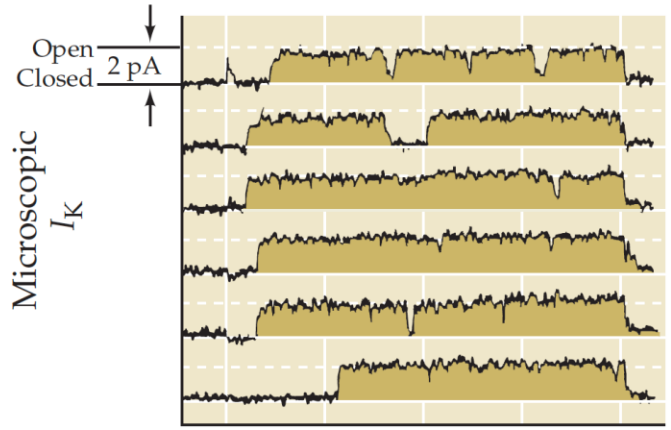
Single voltage-gated **Na^+-channels** in a membrane "patch" give early **inward** depolarizing currents



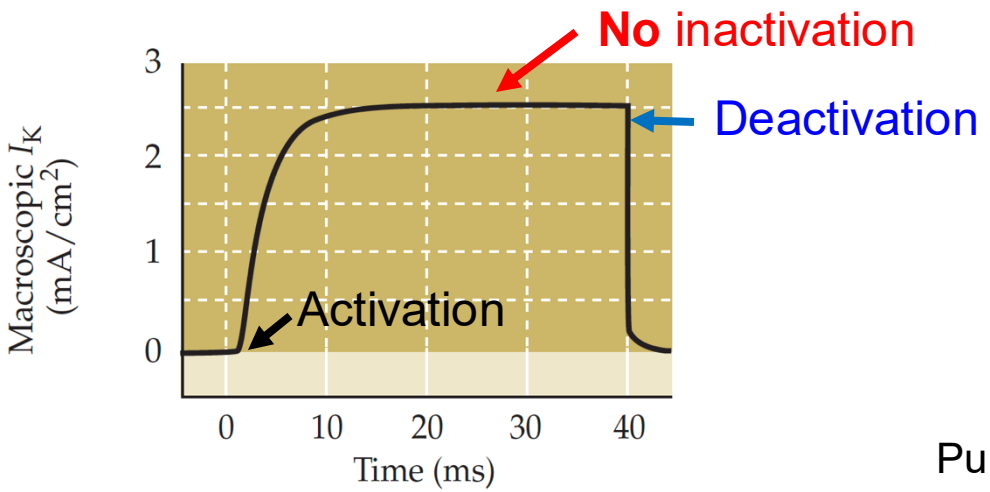






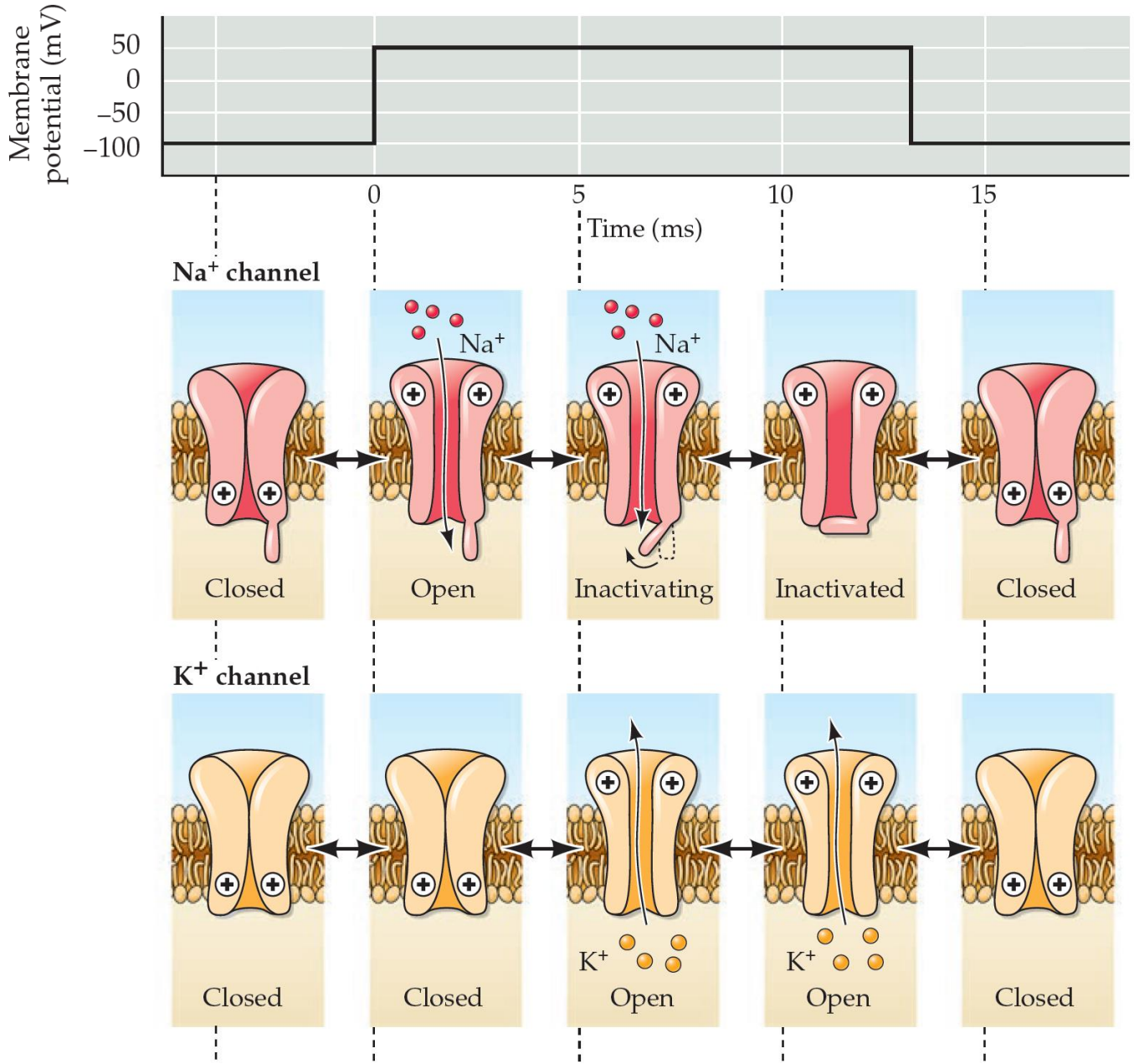


Single voltage-gated **K^+-channels** in a membrane "patch" give more slowly activating, **outward** hyperpolarizing currents



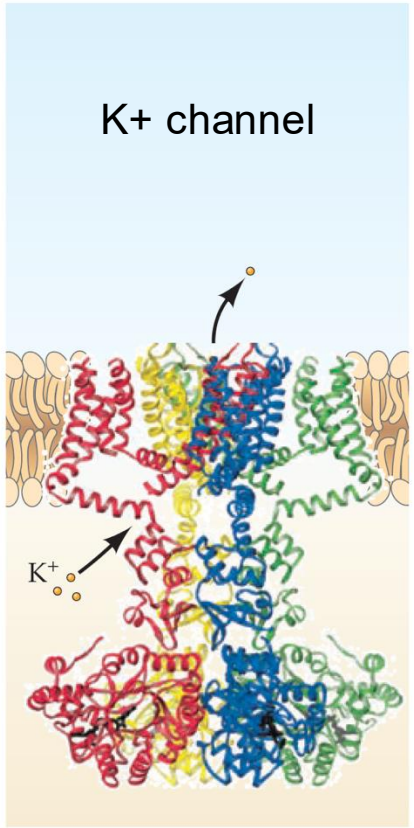
Purves, Figure 4.2

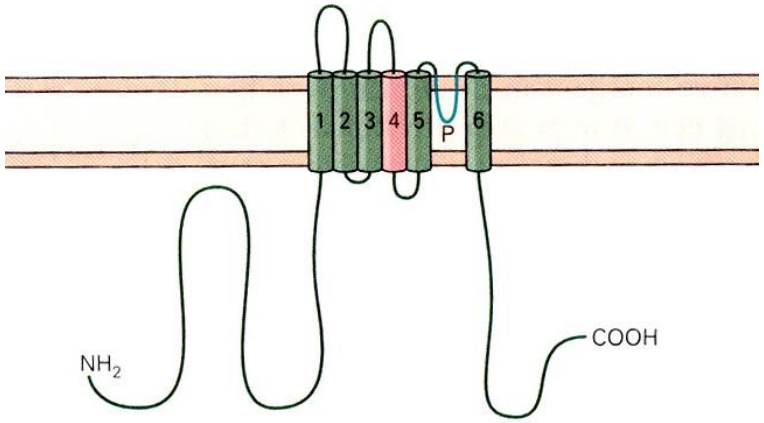
Schema of voltage gated ion channels

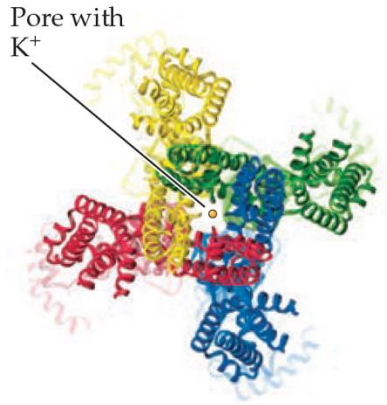


Purves, Figure 4.3

Crystallography structures of voltage-gated K+ ion channels





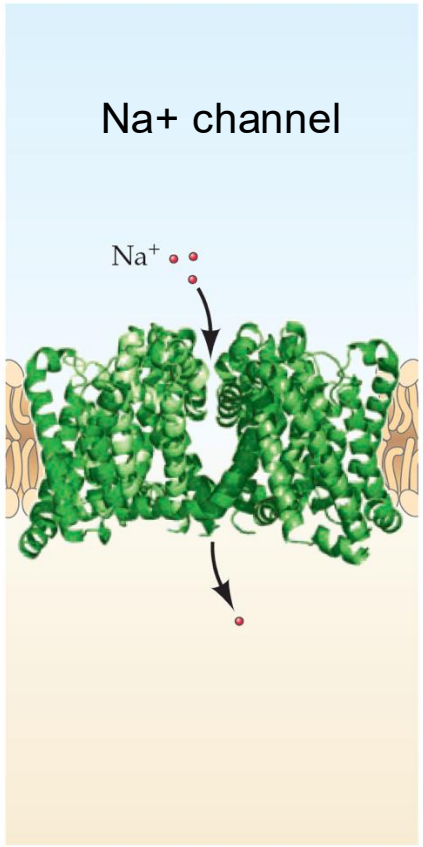


- N-terminal and C-terminals intracellular
- 6 membrane-spanning domains (S1-S6)
- S4 contains positively-charged residues (arginines, lysines) acting as a voltage-sensor for gating
- K^+-channels are tetramers (4 subunits form a channel)
- Accessory β-subunits

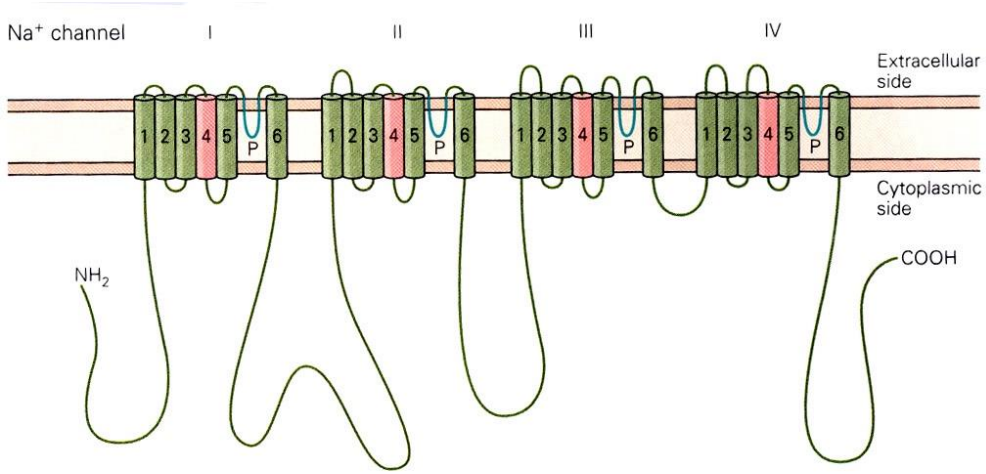
Purves, Figure 4.6

Kandel, Figure 7-14

Crystallography structures of voltage-gated Na+ ion channels

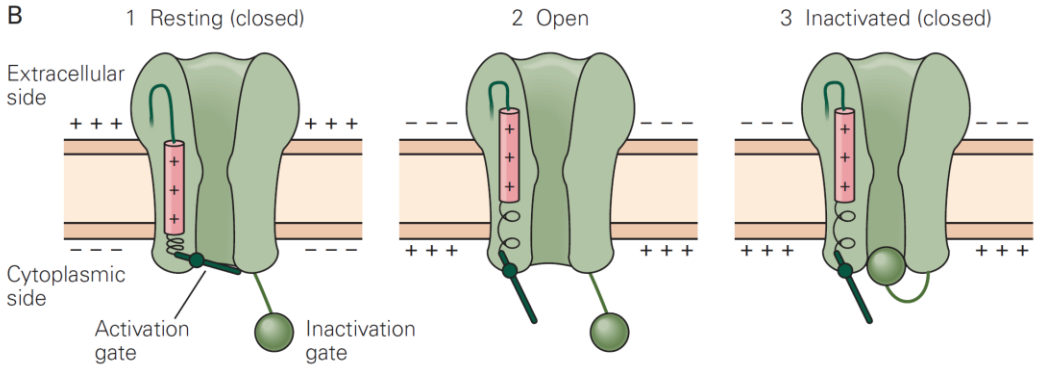


Purves, Figure 4.6



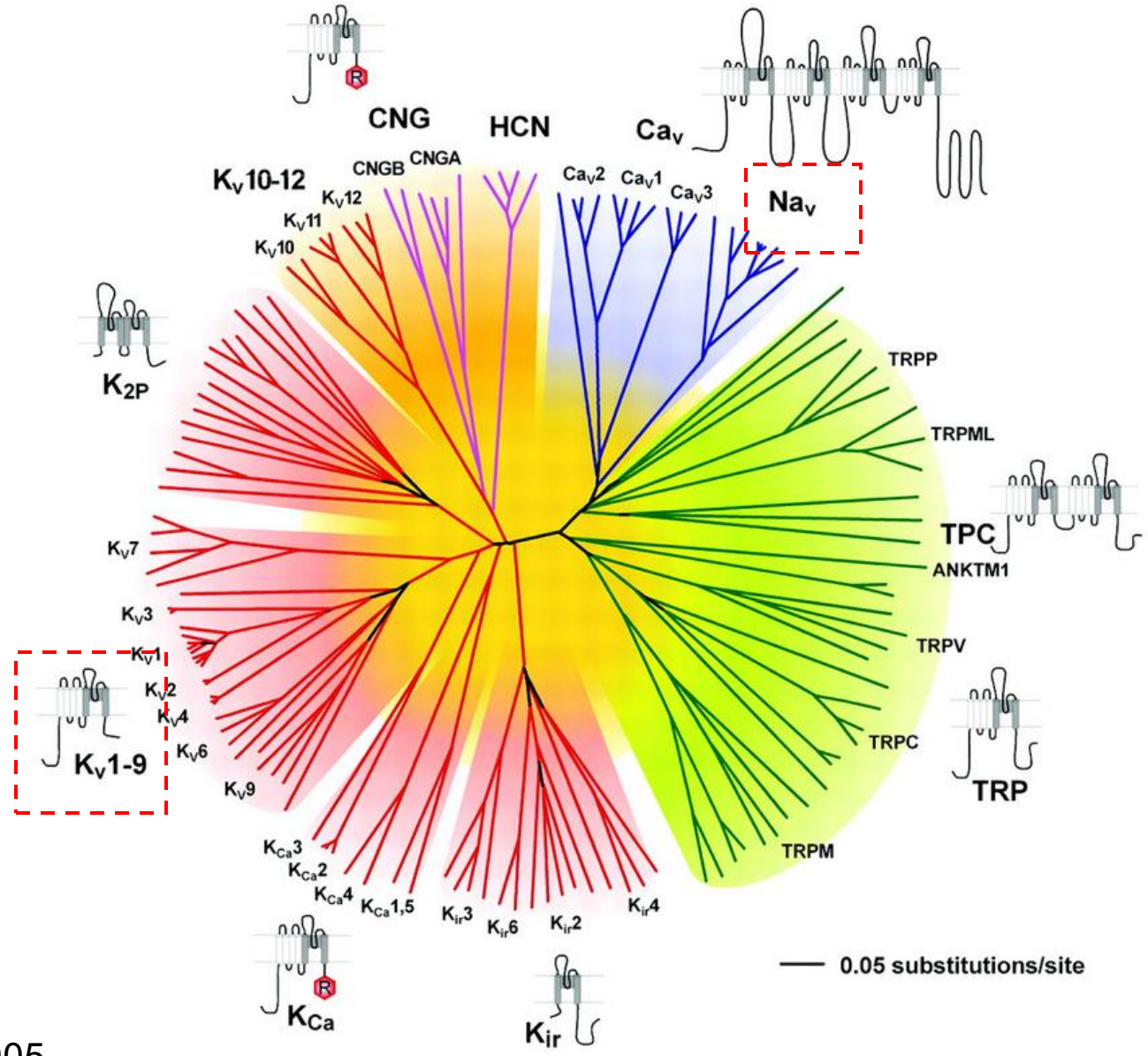
Kandel, Figure 7-14

- 4 "repeats" of K^+ channel-like structure
- Accessory β-subunits
- Separate activation and inactivation gates:



Kandel, Figure 7-13

A large number of genes code for voltage-gated and related ion channels



Na_V - voltage-gated Na^+ channels

K_V - voltage-gated K^+ channels

Ca_V - voltage-gated Ca^{2+} channels

TRP - "transient receptor potential" channels (touch, nociception)

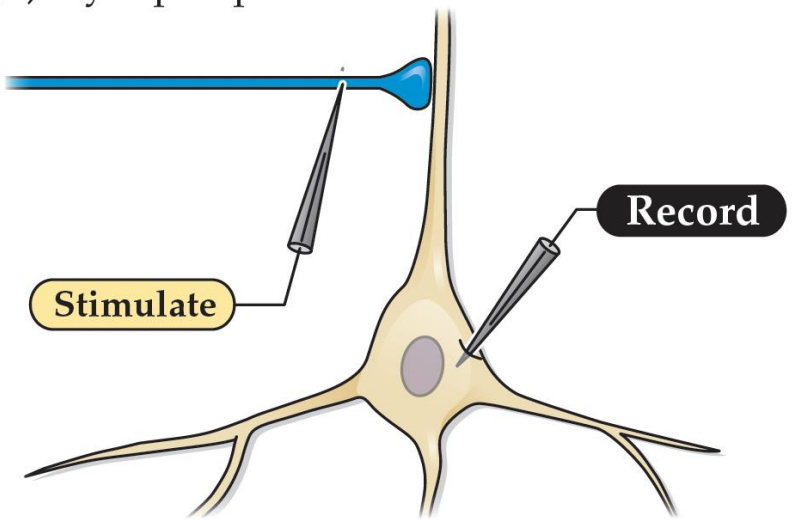
CNG - cyclic nucleotide gated channels (olfaction, vision)

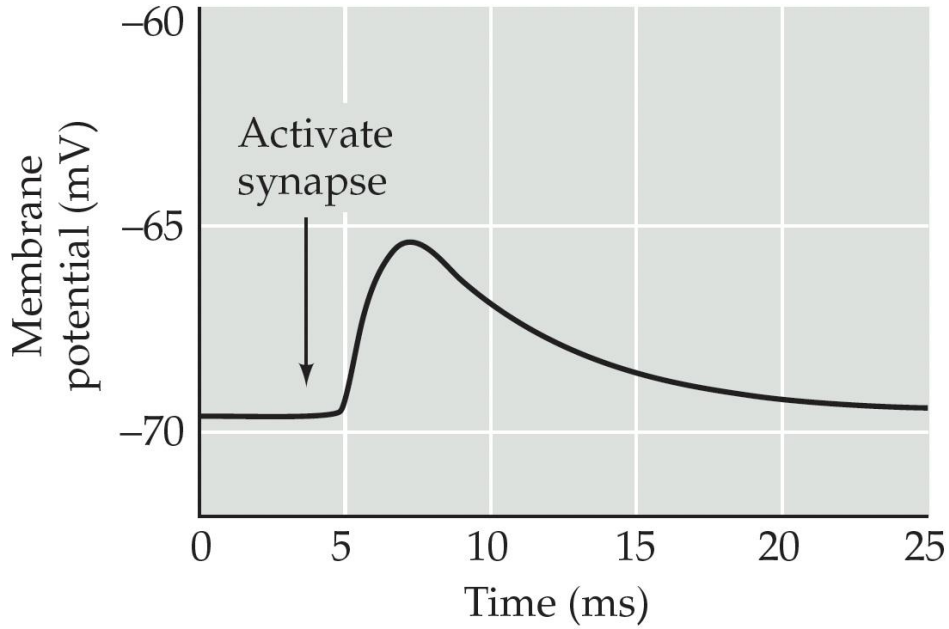
K_{Ca} - Ca^{2+} activated K channels

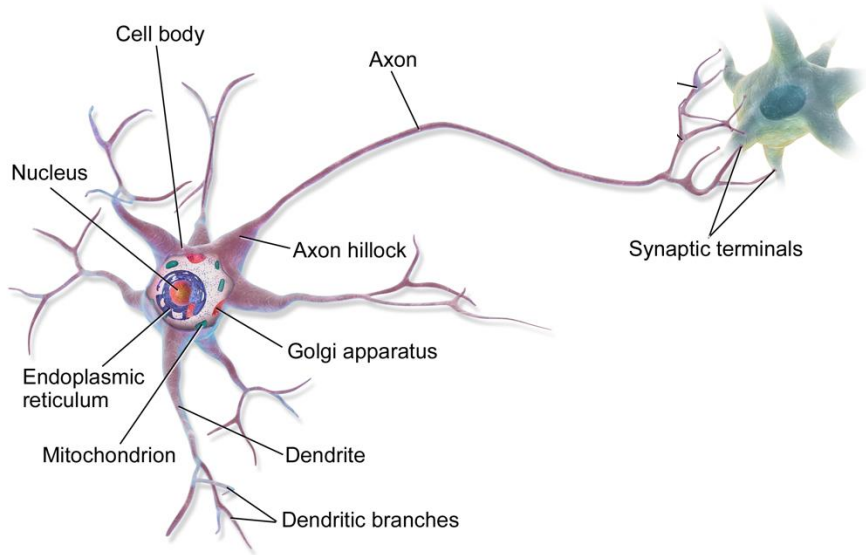
… and more …

Yu & Catterall 2005

(B) Synaptic potential







Brain anatomy: an early functional map

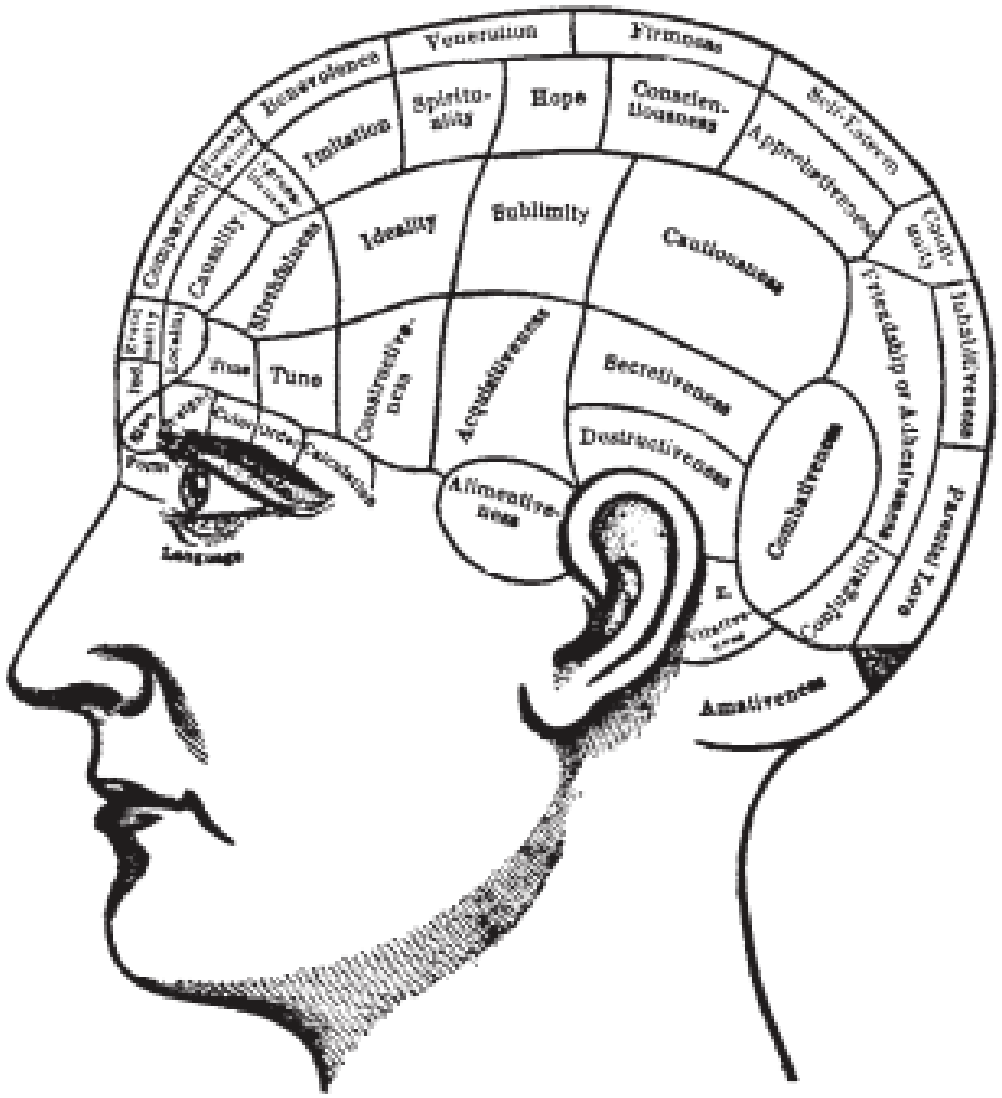


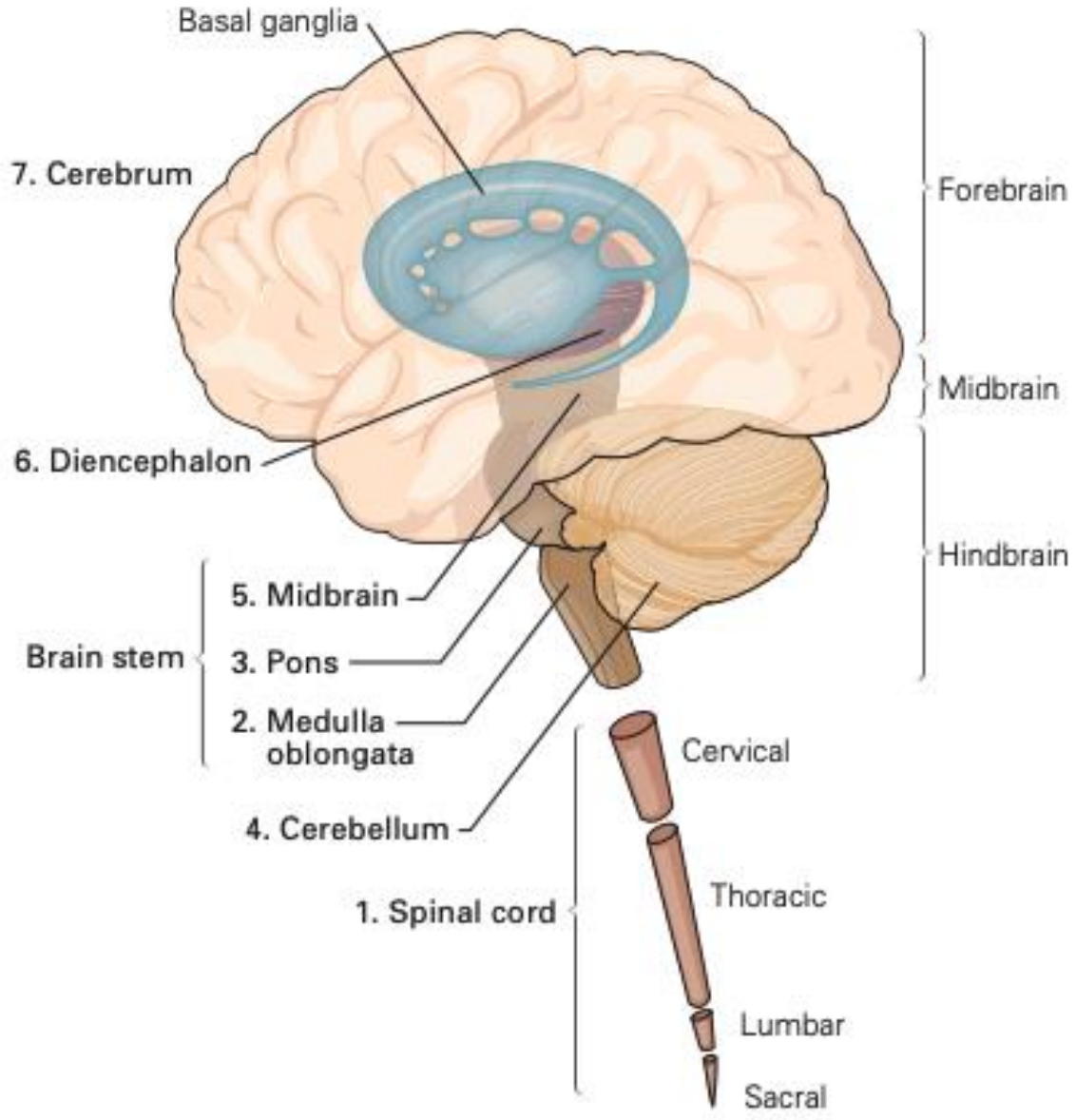
Figure 1–1 An early map of functional localization in the brain. According to the 19th century doctrine of phrenology, complex traits such as combativeness, spirituality, hope, and conscientiousness are controlled by specific areas in the brain, which expand as the traits develop. This enlargement of local areas of the brain was thought to produce characteristic bumps and ridges on the overlying skull, from which an individual's character could be determined. This map, taken from a drawing of the early 1800s, purports to show 42 intellectual and emotional faculties in distinct areas of the skull and the cerebral cortex underneath.

Franz Joseph Gall, ~1800

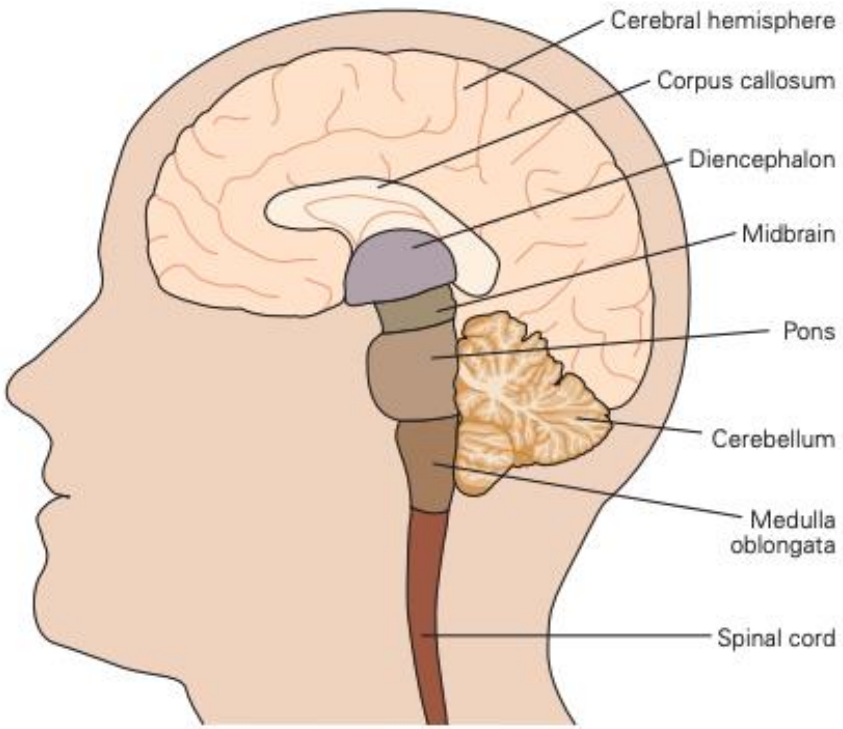
Brain anatomy: 7 main functional parts

The Central Nervous System Has Seven Main Parts

- spinal cord
- brain stem
- medulla oblongata
- pons
- cerebellum
- midbrain
- diencephalon
- cerebrum



Brain anatomy: divisions are clear down the midline



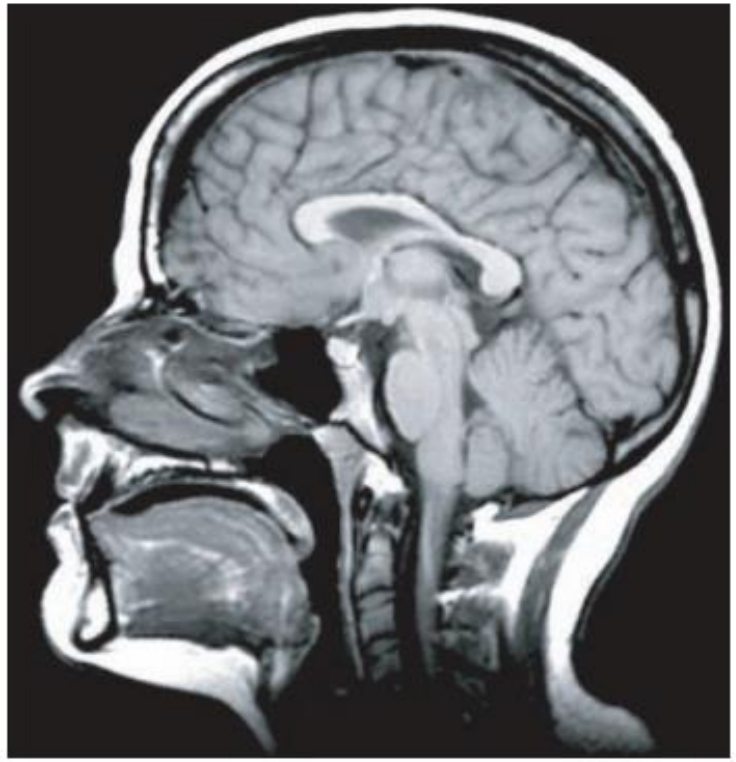


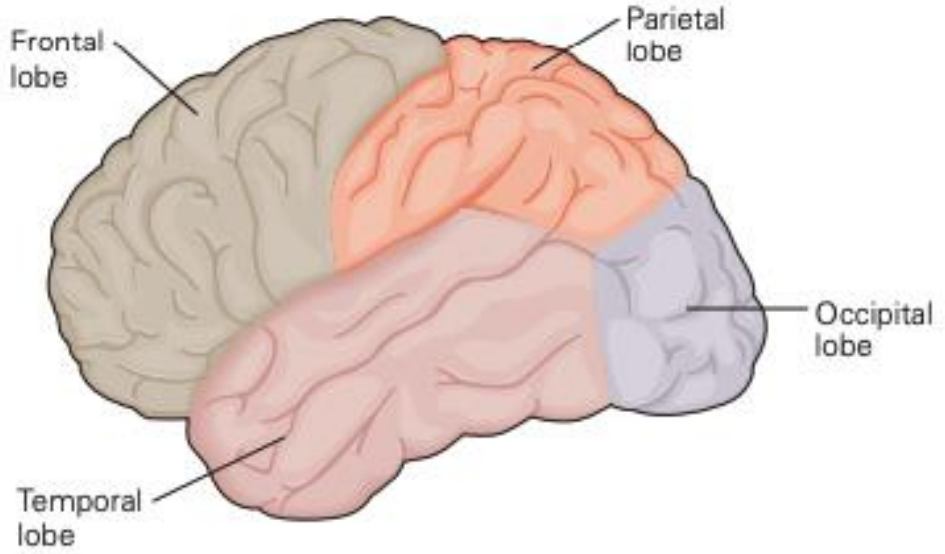
Figure 1–3 The main divisions are clearly visible when the brain is cut down the midline between the two cerebral hemispheres.

A. This schematic drawing shows the position of major structures of the brain in relation to external landmarks. Students of brain anatomy quickly learn to discern the major internal landmarks, such as the corpus callosum, a large bundle of nerve fibers that connects the left and right hemispheres.

B. The major brain divisions drawn in **A** are also evident in a magnetic resonance image of a living human brain.

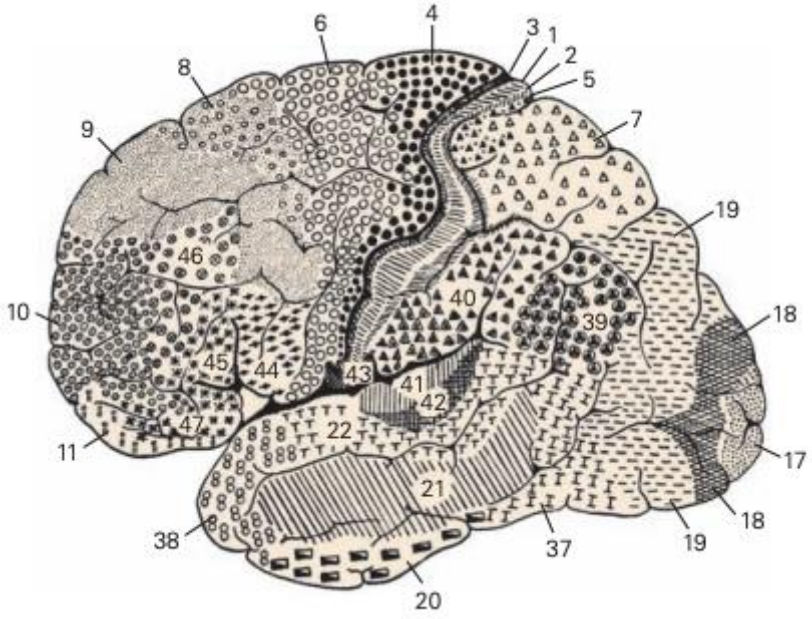
Brain anatomy: cerebrum

- Two cerebral hemispheres
- Outer **cerebral cortex** (frontal, parietal, occipital, temporal lobes)
- Deep structures:
  - **Basal ganglia** – motor regulation
  - **Hippocampus** – memory storage
  - **Amygdala** – emotional/autonomic responses

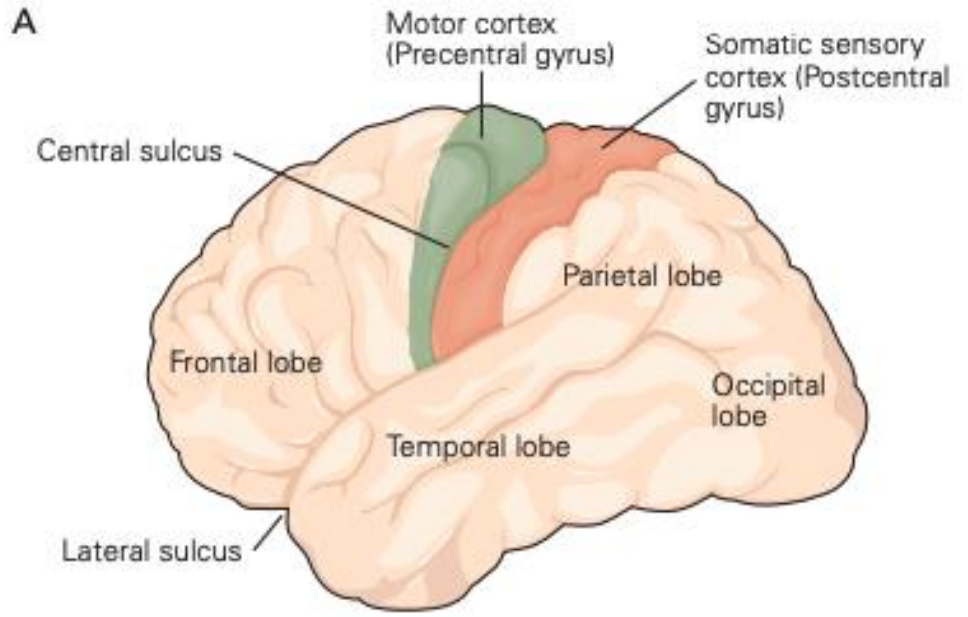


The four lobes of the cerebral cortex

Brain anatomy: functional & “named” brain areas



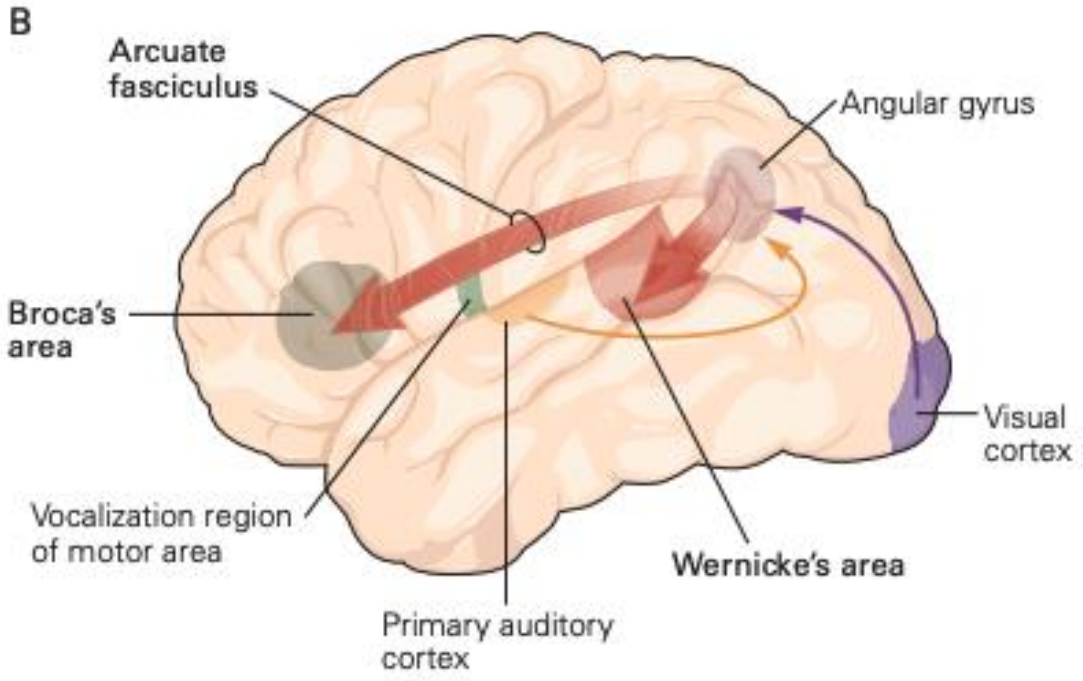
Brodmann’s division of the human cerebral cortex into 52 discrete functional areas. Brodmann identified these areas on the basis of distinctive nerve cell structures and characteristic arrangements of cell layers.



The four lobes of the cerebral cortex. The motor and somatic sensory areas of the cortex are separated by the central sulcus

Brain anatomy: “circuits”

Example: areas involved in language



Wernicke’s area processes auditory input for language and is important for understanding speech.

- It lies near the primary auditory cortex and the angular gyrus, which combines auditory input with information from other senses. ‘

Broca’s area controls the production of intelligible speech.

- It lies near the region of the motor area that controls the mouth and tongue movements that form words.

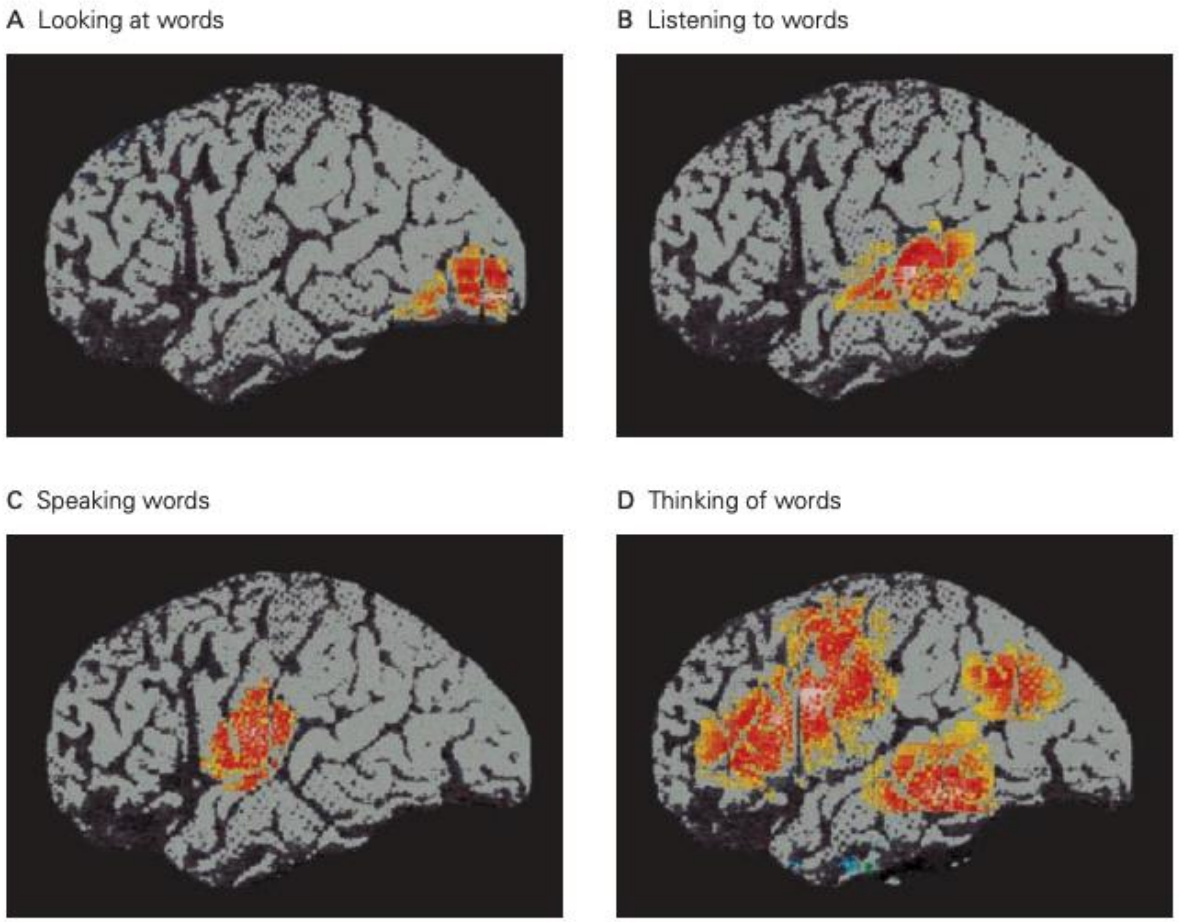
Wernicke’s area communicates with Broca’s area by a bidirectional pathway, part of which is made up of the arcuate fasciculus.

Kandel 5th edition Figure 1-4. (Adapted, with permission, from Geschwind 1979.)

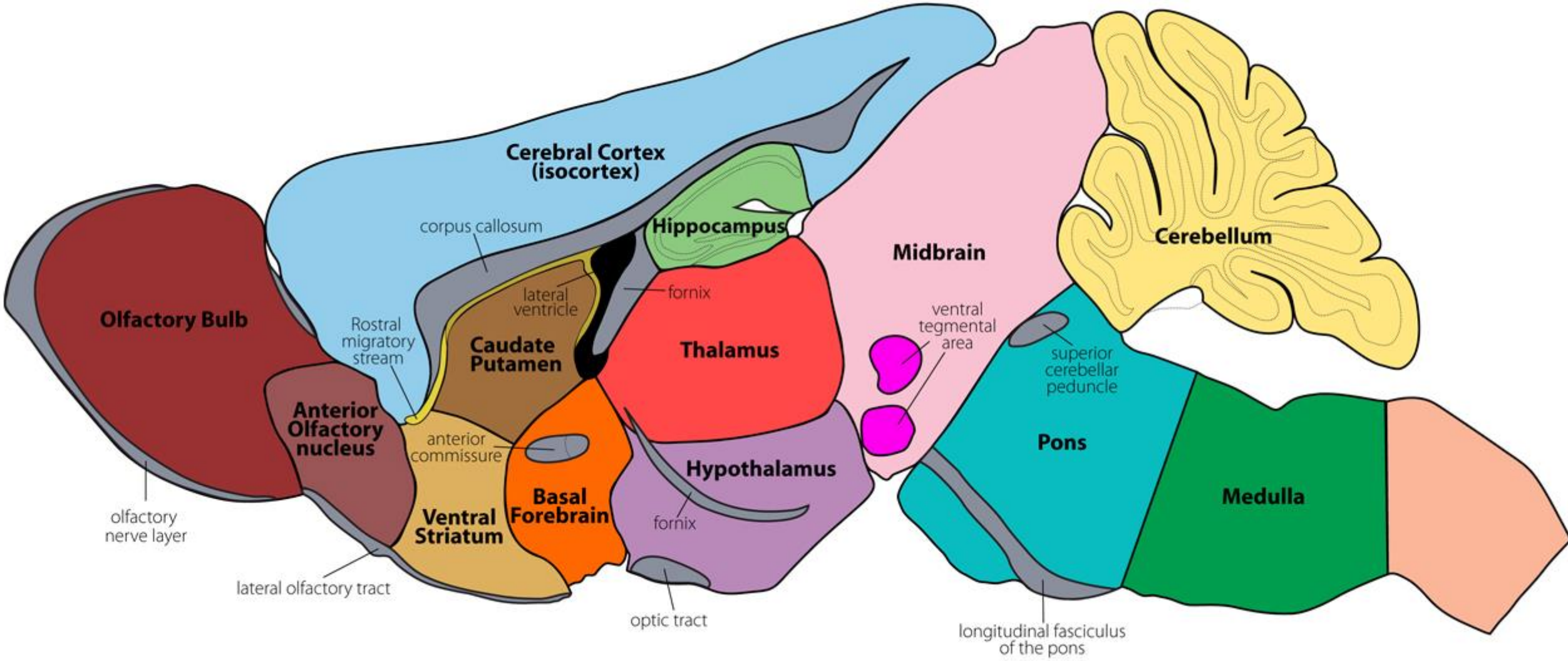
Brain anatomy: “circuits”

Example: areas involved in language, functional mapping (PET)

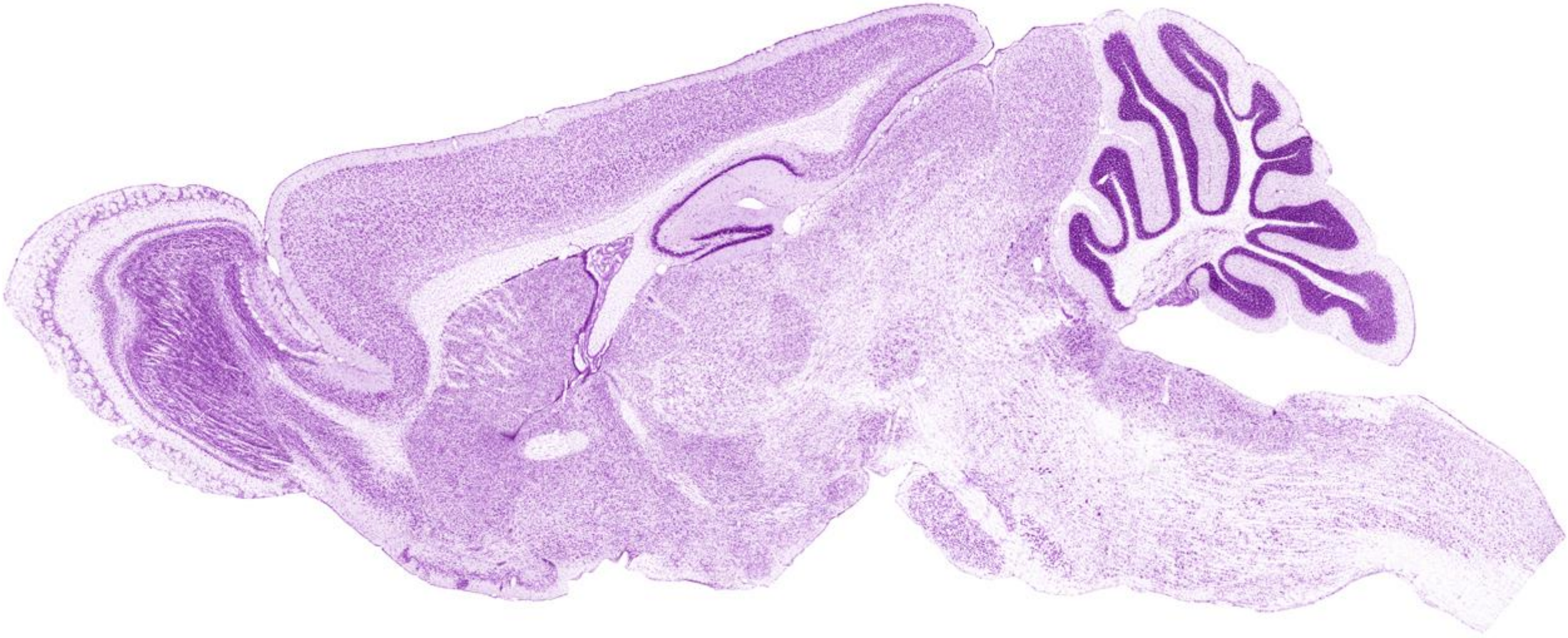
Specific regions of the cortex involved in the recognition of a spoken or written word can be identified with positron emission tomography (PET).



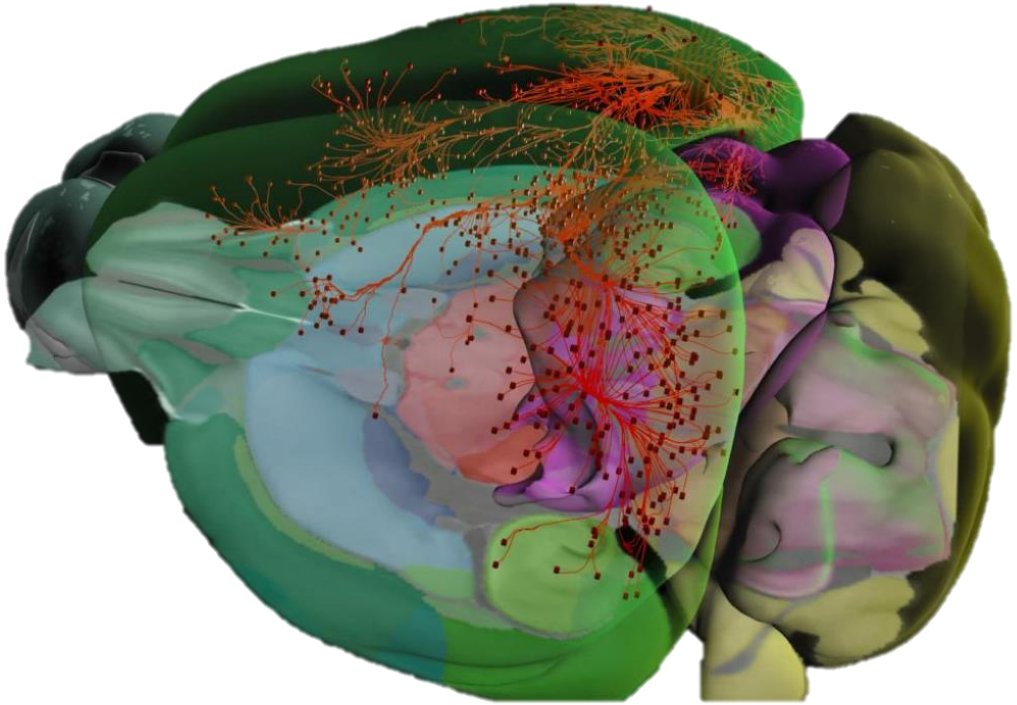
Brain anatomy: the mouse

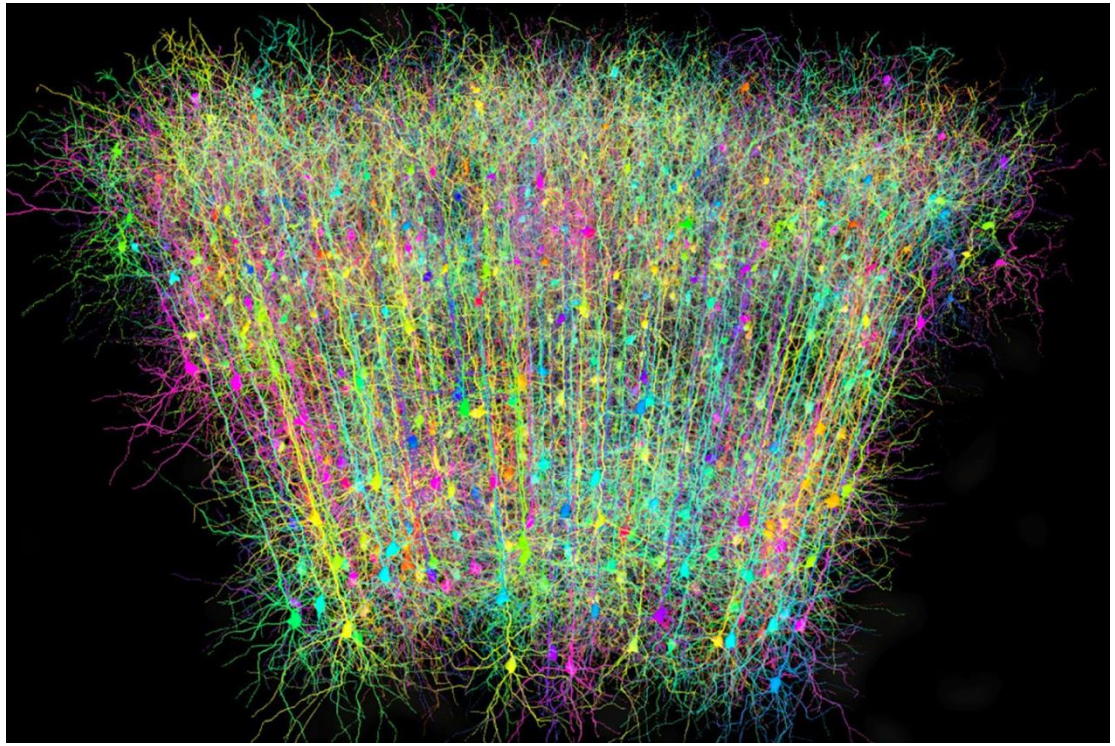


Brain anatomy: the mouse

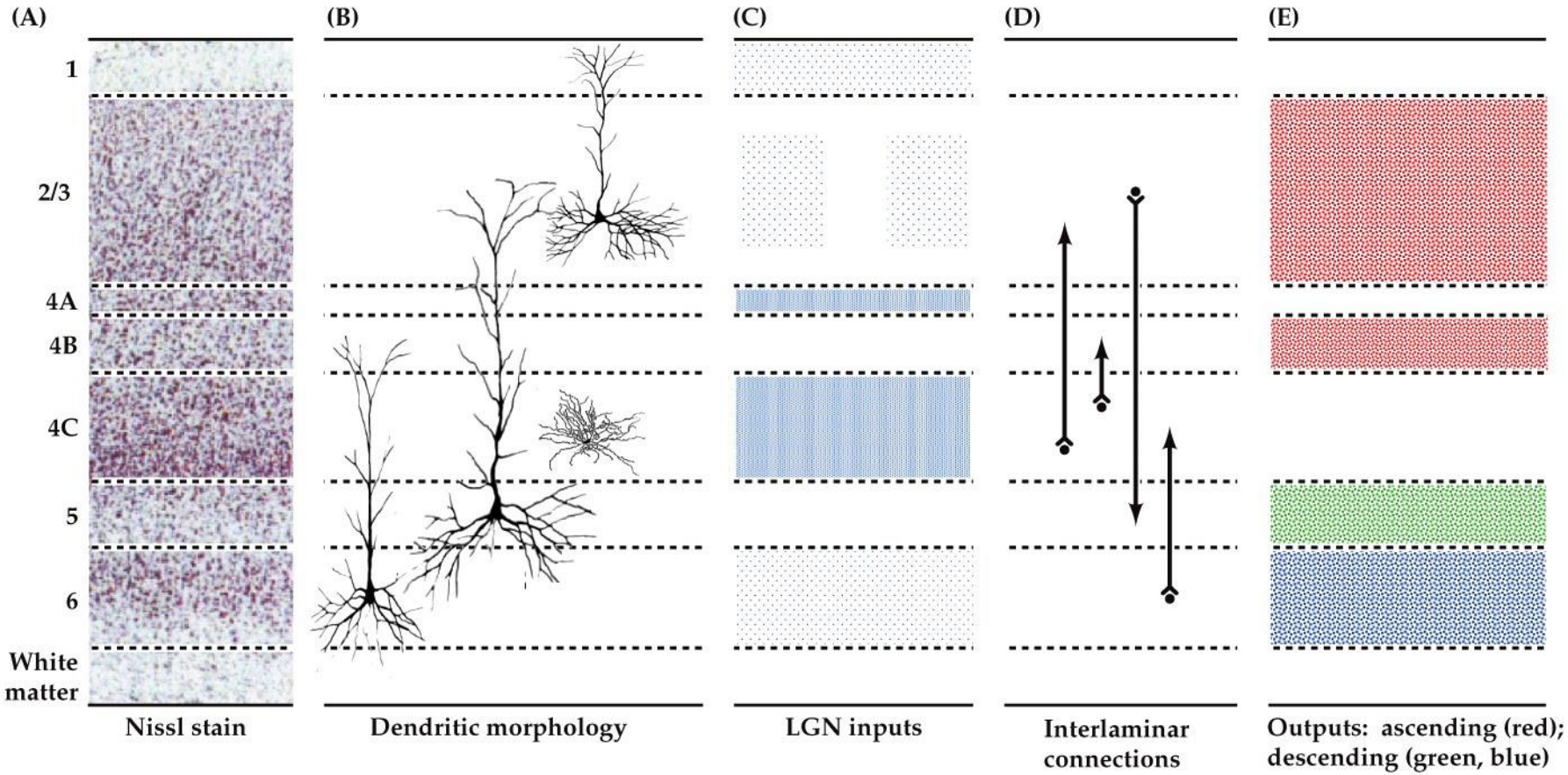


Brain anatomy: the mouse



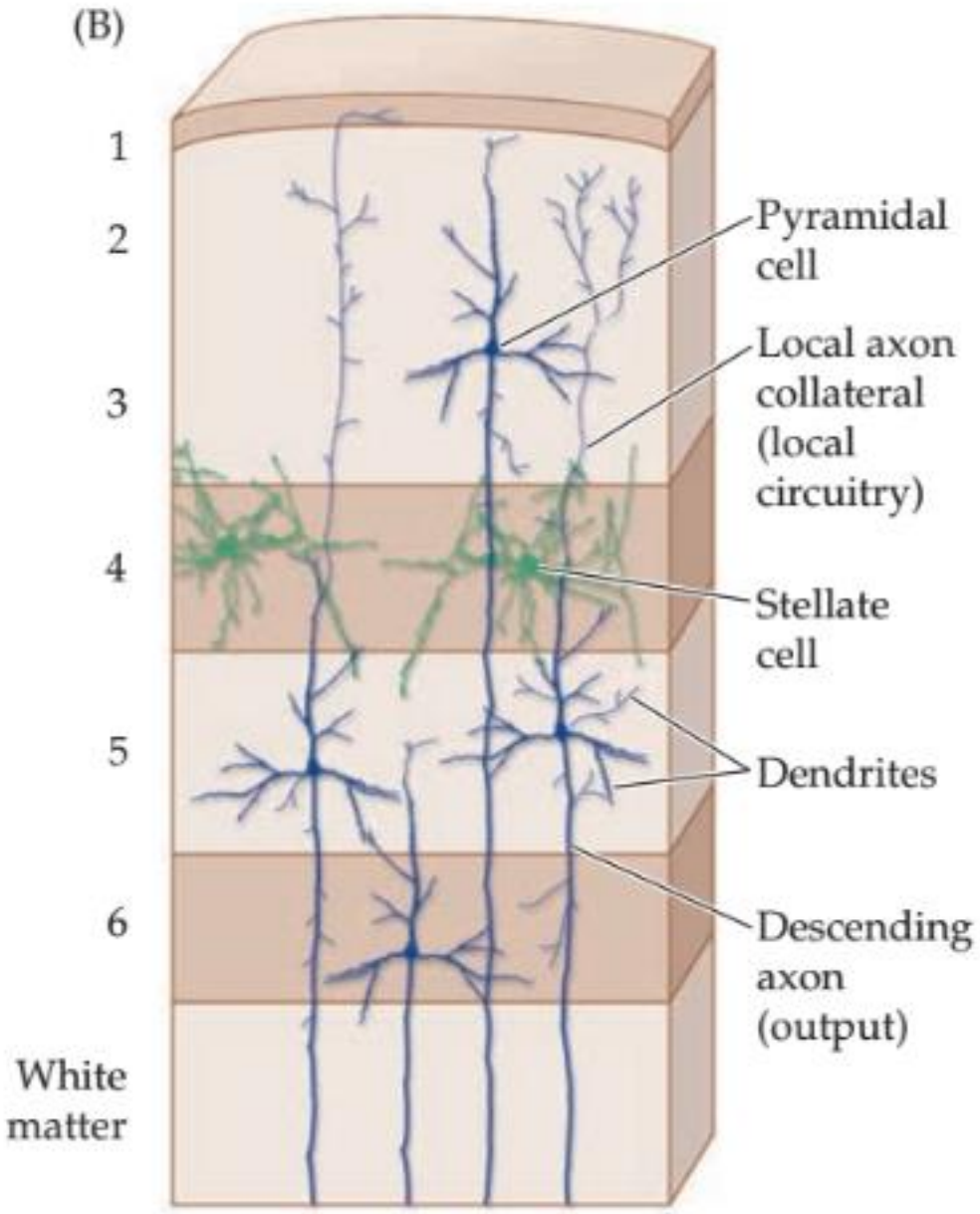


Layered organization of the **cerebral cortex** (a typical primary sensory cortex)



- **inputs from thalamus: to Layer 4**, and "patchy" to L2/3, L1
- in-between layer connections: **L4 → L2/3** **L2/3 → L5**
- outputs:
  - L2/L3 "ascending" ; "associational"; that is to higher cortical areas
  - L5 "descending"
  - L6 "cortico-thalamic"

Layered organization of the cortex (a typical primary sensory cortex)



- **inputs from LGN: to Layer 4**, and "patchy" to L2/3, L1

- in-between layer connections: **L4 → L2/3**
  L2/3 → L5

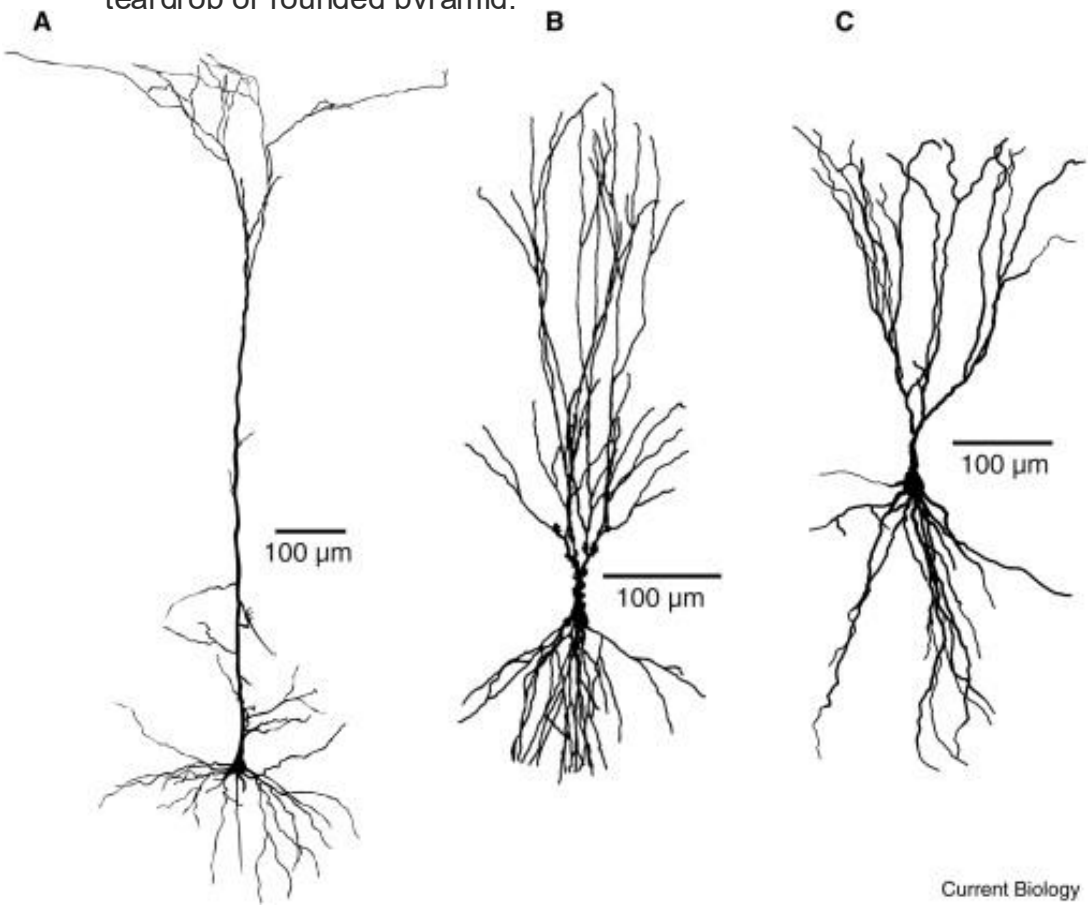
- outputs:
- L2/L3 "ascending" ; "associational"; that is to higher cortical areas (e.g., V2)
- L5 "descending" to superior colliculus (!)
- L6 "cortico-thalamic" back to visual thalamus

2 broad classes of neurons:
- Spiny (pyramidal & stellate) GLUT
- Aspinous (smooth) GABA

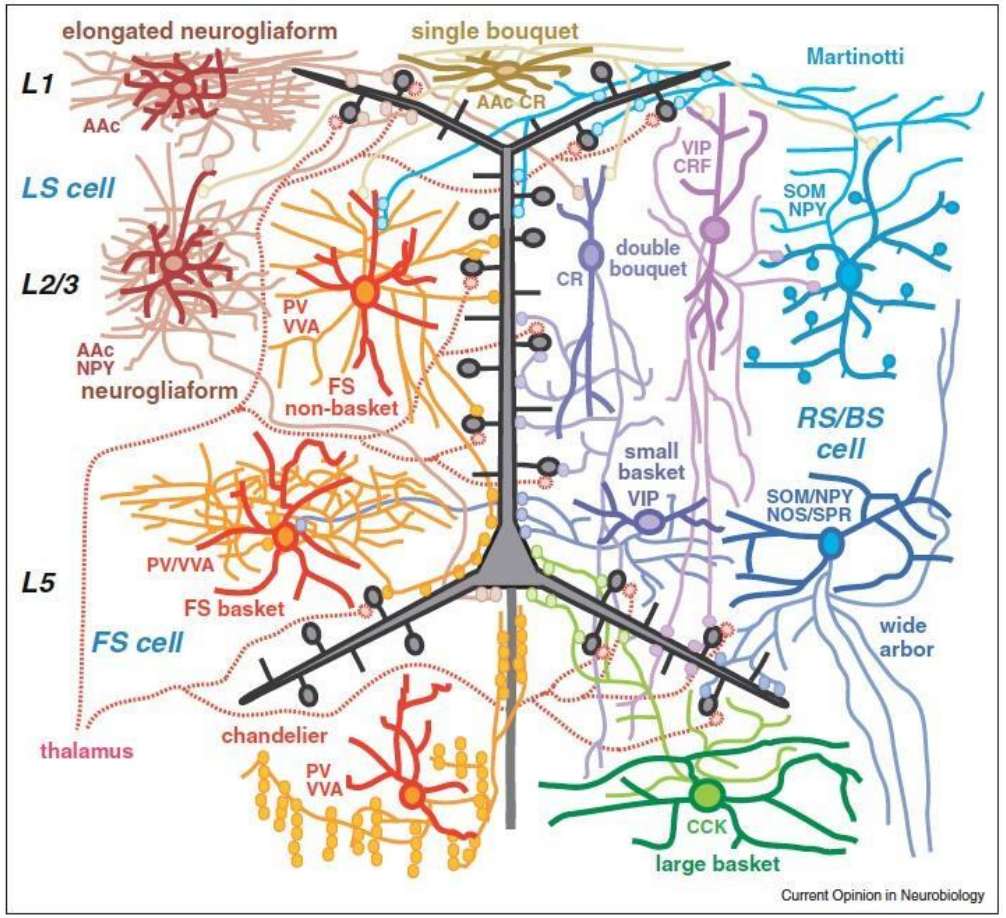
2 broad classes of neurons:

- Spiny (pyramidal & stellate) GLUT “excitatory”
- Aspinous (smooth) GABA “inhibitory”

(Excitatory) pyramidal: They are named for their shape- typically, they have a soma (cell body) that is shaped like a teardrop or rounded pyramid.



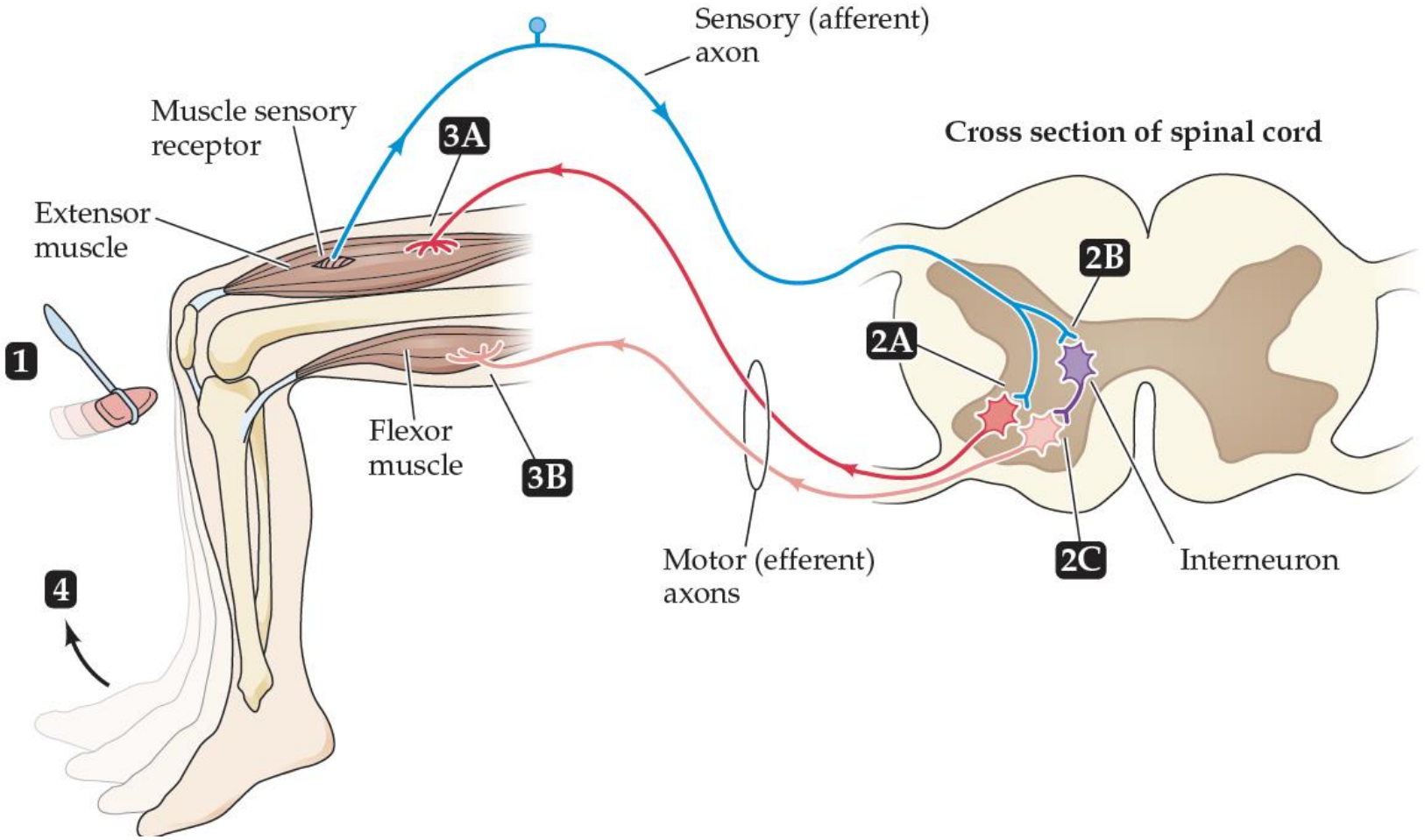
(Inhibitory) Interneurons:

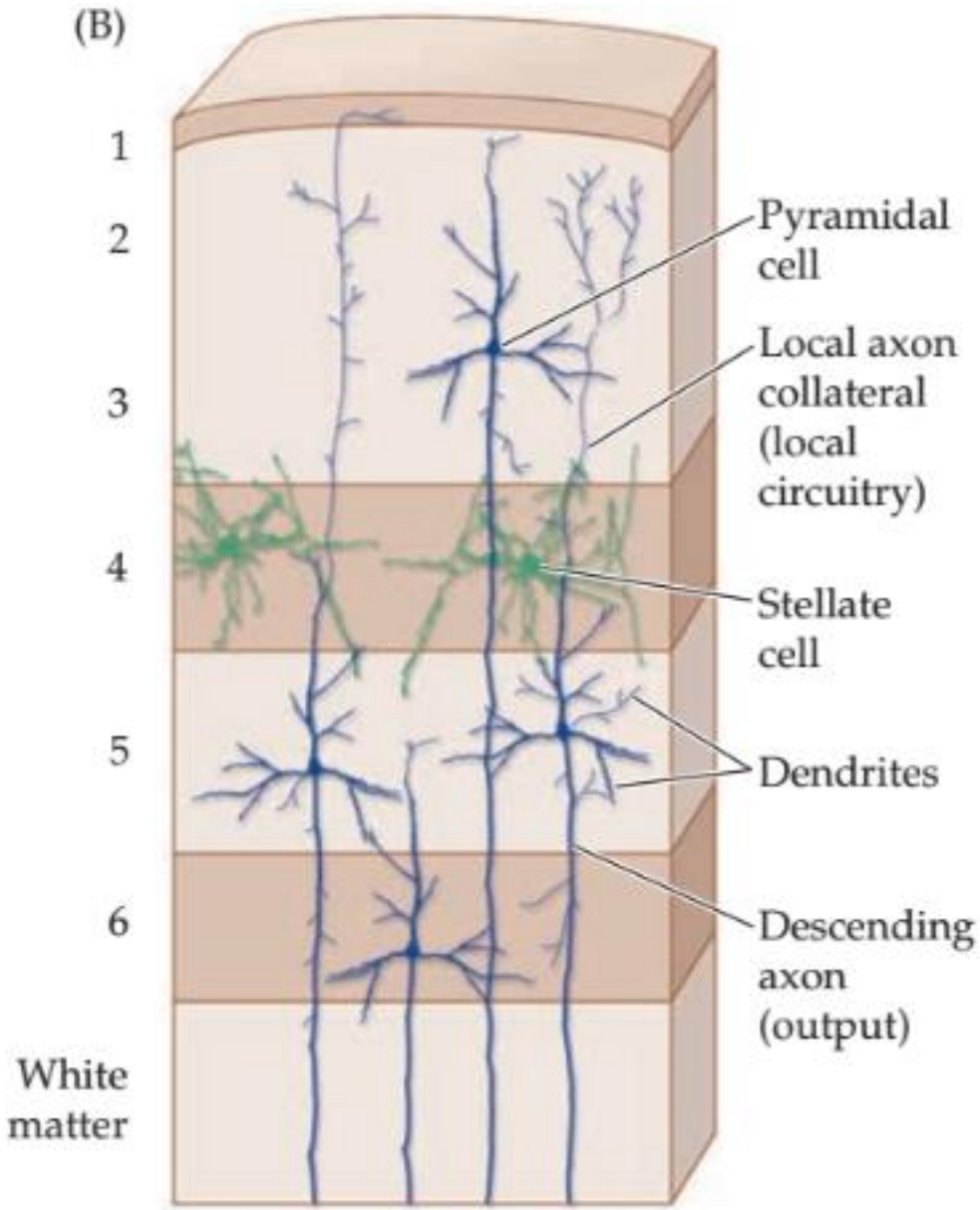


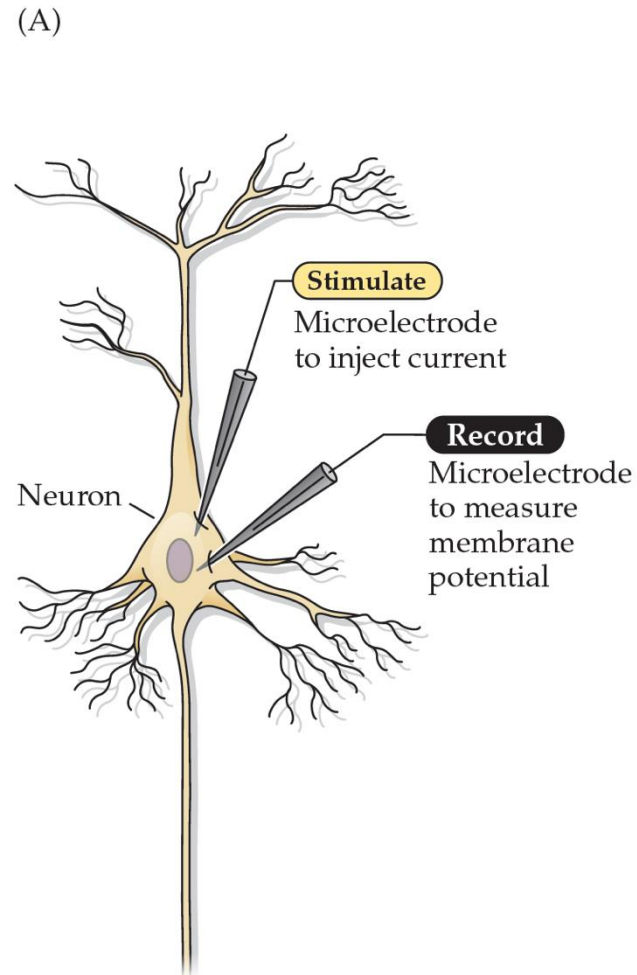
Cortical microcircuit of inhibitory targets. Abbreviations: DiS, dually innervated spine; L5, layer V; AA2, alpha actinin 2; CCK, cholecystokinin; CR, calretinin; CRF, corticotrophin releasing factor; NOS, nitric oxide synthase; NPY, neuropeptide Y; PV, parvalbumin; SOM, somatostatin; SPR, substance P receptor; VIP, vasoactive intestinal polypeptide; 5HT3AR, 5-HT3A receptor. (adapted from (Kubota et al., 2016))

https://www.cell.com/current-biology/fulltext/S0960-9822%2811%2901198-5

A excitatory & inhibitory classical circuit




(B)
1
2
3
4
5
6
White matter
Pyramidal cell
Local axon collateral (local circuitry)
Stellate cell
Dendrites
Descending axon (output)


(A)
Stimulate
Microelectrode to inject current
Record
Microelectrode to measure membrane potential
Neuron

Summary: Important concepts and keywords

- Brain anatomy:
  7 divisions, the cortex, the 6 layers of cortex, “circuits"

- Measurement techniques:
  2-electrode voltage clamp
  patch-clamp techniques

- Definition of current clamp and voltage clamp measurements

- Voltage-gated membrane currents, early & late currents, and their ionic basis (Na^+ and K^+)

- Measurements and consequences of single Na^+ and single K^+ channel conductances (different proteins)

- Sequence of events during the action potential

- Voltage-gated ion channels:
  Principal topology of Na^+ and K^+ channels