Biochemistry- Retina, RPE:

Biochemical basis of electroretinogram

August 17, 2011

Xiangyun Wei
weix@upmc.edu

Outline

What is electroretinogram

Molecular basis of membrane potentials

Retinal circuitry and biochemical bases of major ERG waves

Some questions to think about

Recommended readings:

"Neuroscience" by Alan Longstaff
Sections B, C, D, G

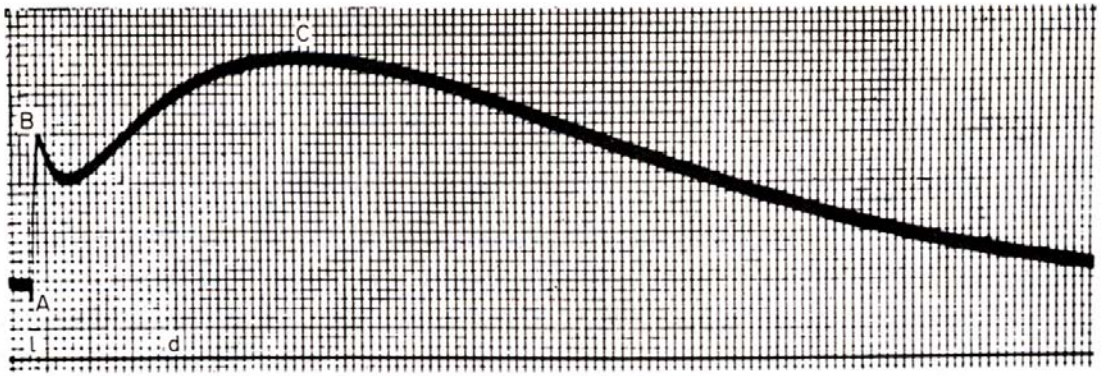
"Principles and Practice of Clinical Electrophysiology of Vision"
by Heckenlively & Arden
Chapters 4, 5, 6, 7, 8, 9, 12

Light stimulation evokes electrical current

Fig. 1-1. Recording the electroretinogram from the frog. This figure shows how Dewar and M'Kendrick (1877) recorded the electroretinogram without removing the eye from the animal. The recording method is essentially the same as might be used today. A wick electrode, E, is placed on the cornea of the animal; and a reference electrode, E, is inserted in a wound on its back. When the eye is stimulated, current flows through the circuit, causing the galvanometer, G, to deflect. K is a key or switch which may be opened or closed as desired. The mechanism for stimulating the eye is not shown.

"The Electroretinogram" by John Armington 1974

Examples of electroretinogram



The first ERG

Fig. 1-7. The components of the electroretinogram according to Einthoven and Jolly (1908). This is one of the first recordings to show the well-known A, B, and C waves. The stimulus was a green light, which was switched on at l. Each division on the ordinate equals 10 μV across the eye. Each division on the abscissa is equal to .5 sec.

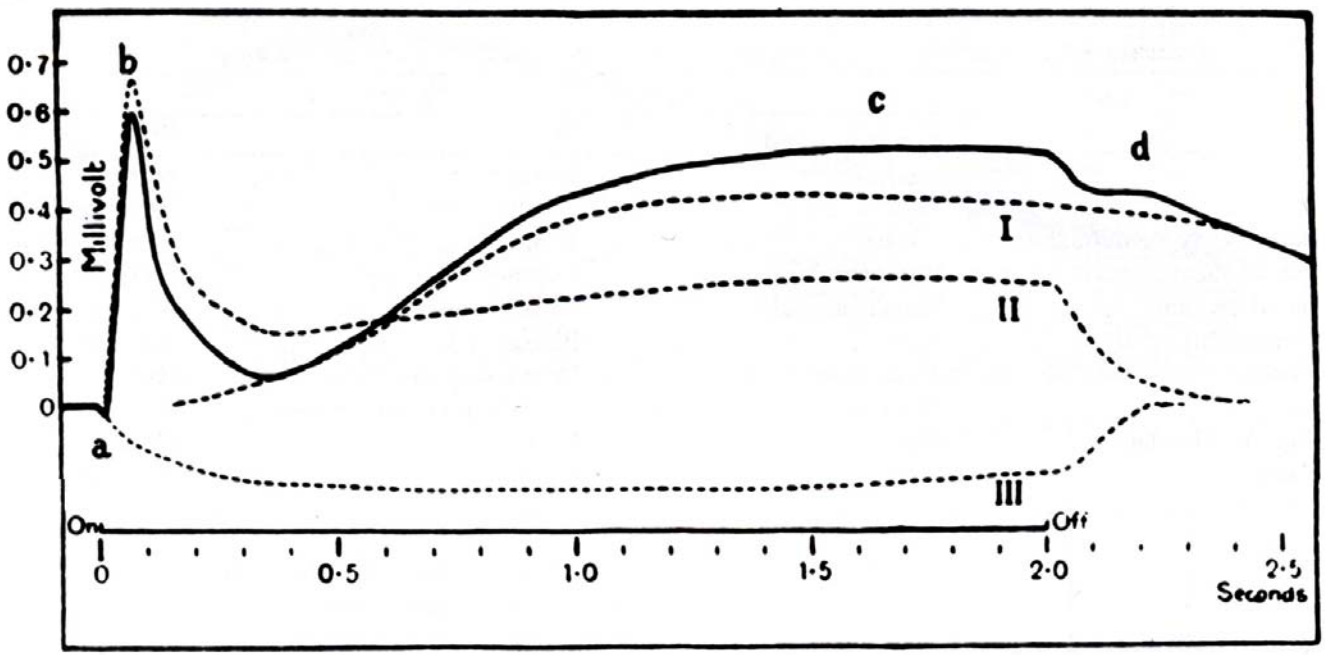


FIGURE 1.4 Analysis of the E-ERG (cat) at 14 mL . The a-wave has been broadened slightly out of proportion to demonstrate its derivation more clearly. (From Granit R: *J Physiol* 1934; 81:1–28.)

Dynamics of membrane potentials: the molecular basis of electrophysiology

Intracellular recording is a basic way to directly measure membrane potentials of a cell

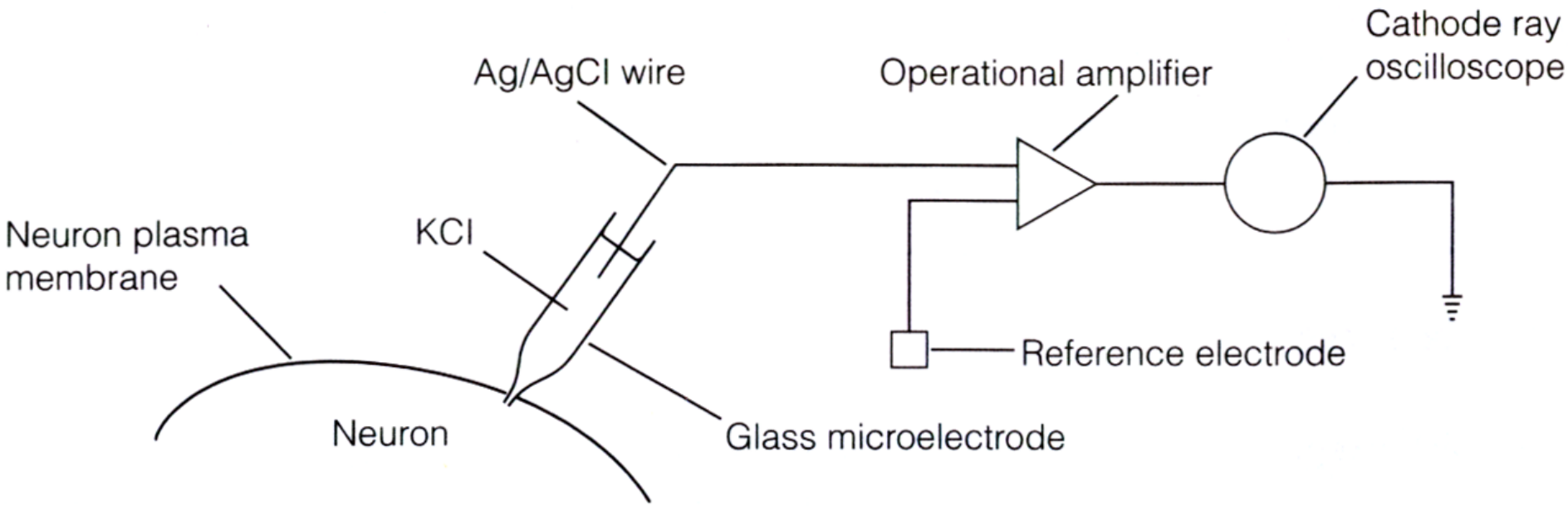


Fig. 1. The circuitry used for intracellular recording.

"Neuroscience" Longstaff, 2005

Resting membrane potentials and polarization

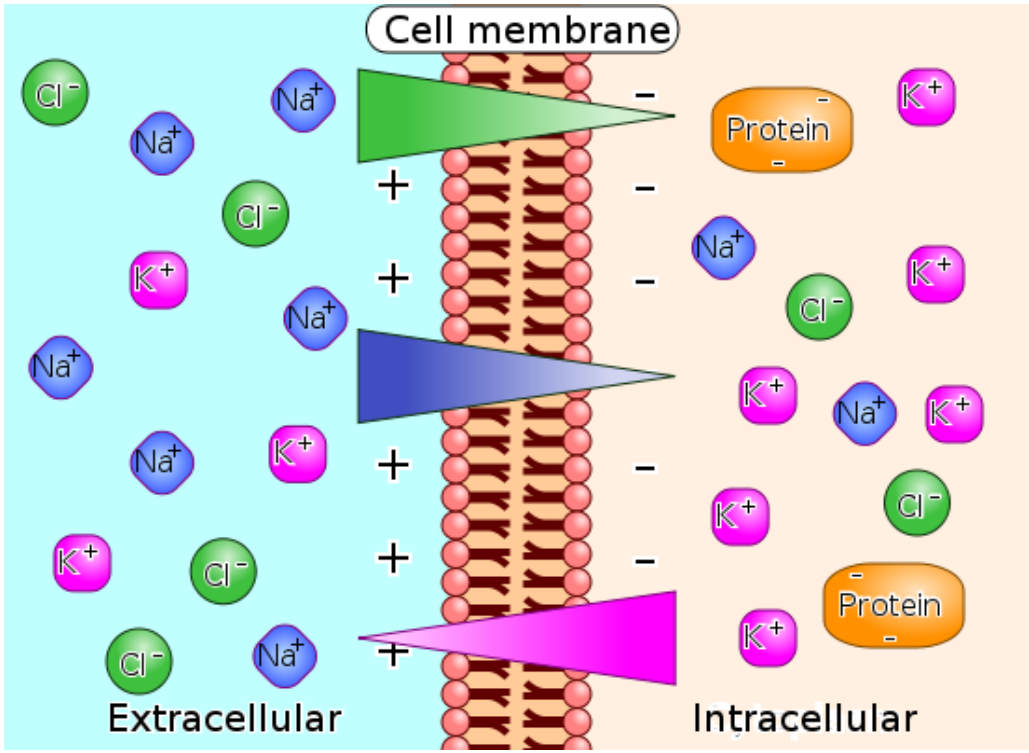


Table 1. Ionic concentrations across mammalian membranes (mmol l^{-1})

Ion	Extracellular fluid	Axoplasm
K^+	2.5	115
Na^+	145	14
Cl^-	90	6

Resting potential (polarization):
The membrane potentials when a cell not stimulated.
Expressed as inside relative to outside (the outside is taken to be zero)
-60~-90 mV (polarized)

The plasma membrane acts as a capacitor to Store energy in the electrostatic field.

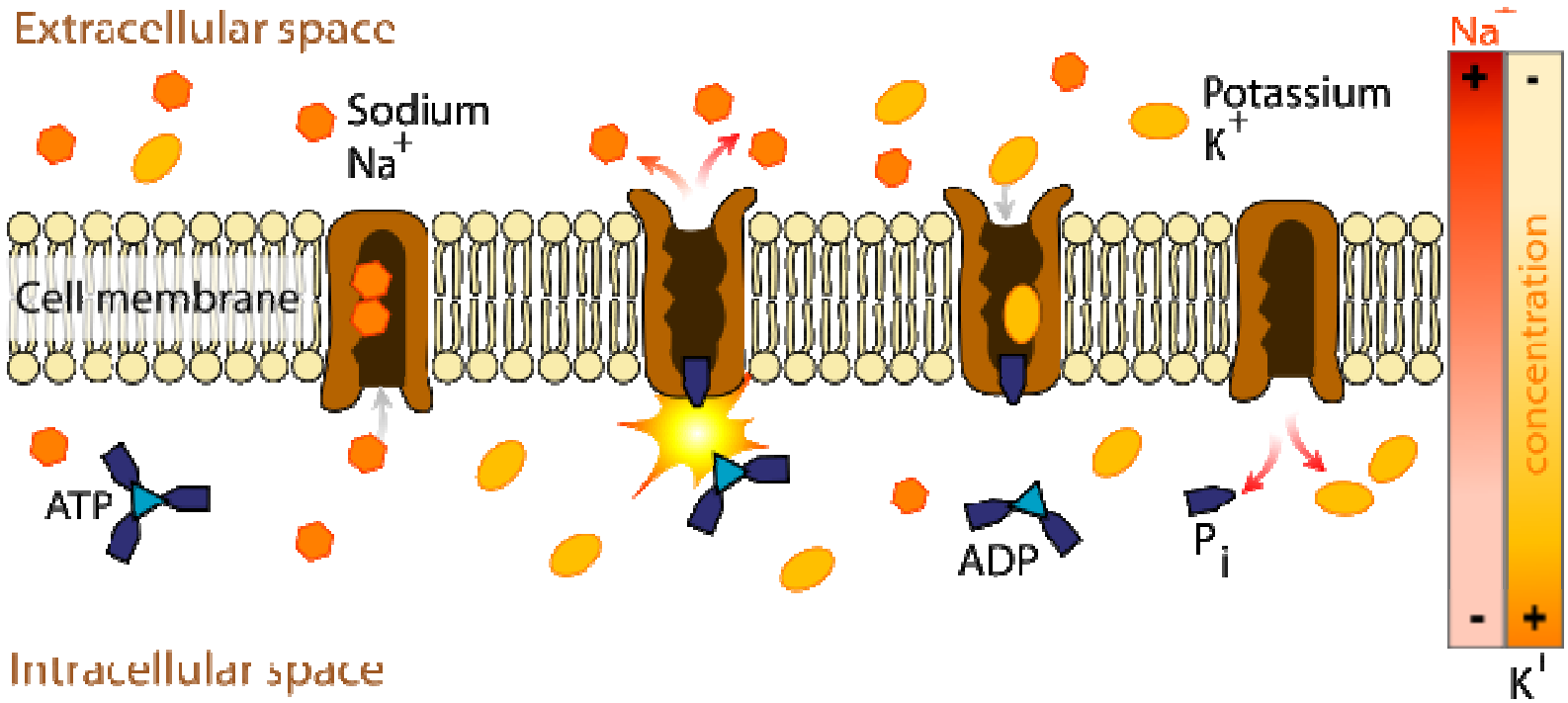
Depolarization:
The reduction of membrane potential (the inside gets less negatively charged).

Hyperpolarization:
The inside of a cell gets more negative.

The concentrations of different ions on each side are different.

"Neuroscience" Longstaff, 2005

Energy is used to establish membrane potentials



sodium-potassium exchange pump.
Use ATP to exchange ions.
3 Na+ going out, and 2 K+ coming in
Higher Na+ concentration in the extracellular space.
Higher K+ concentration in the intracellular space. The membrane is permeable to K+.
Generate a cross membrane electric potential: positive outside, and negative inside.

Membrane potential regulation:
Selective permeability, diffusion force, and electrostatic force

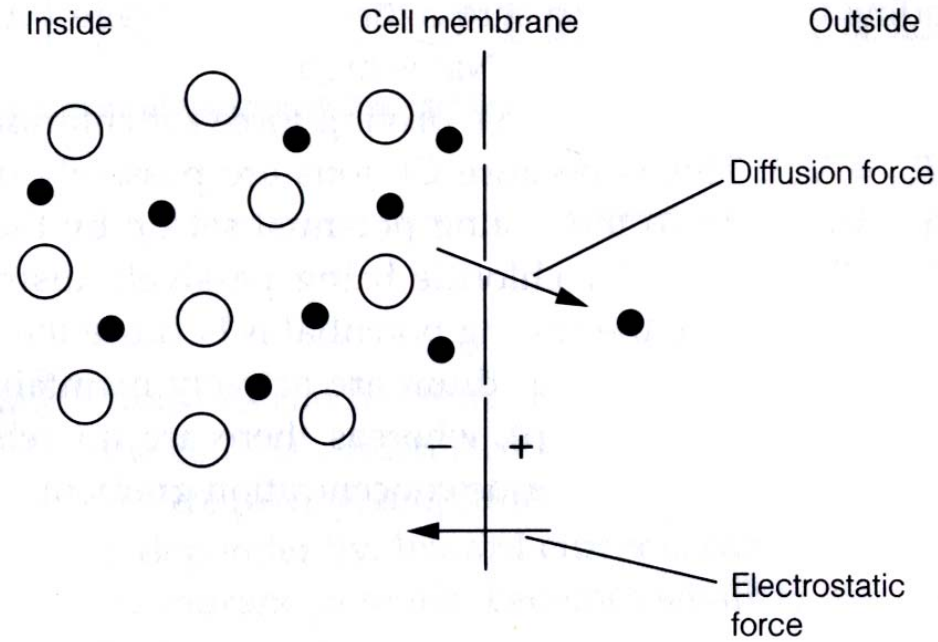


Fig. 2. Illustration of how a potassium equilibrium potential is formed. A small potential exists across the membrane when the diffusional force equals the electrostatic force. Small filled circles represent K^+ ions, large open circles represent anions.

Membrane permeability is regulated by ligand-gated or voltage-gated ion channels:

- **Selective for specific ions**
- **Transient activation**

"Neuroscience" Longstaff, 2005

An action potential is the consequence of the temporary opening of the Na+ and K+ channels

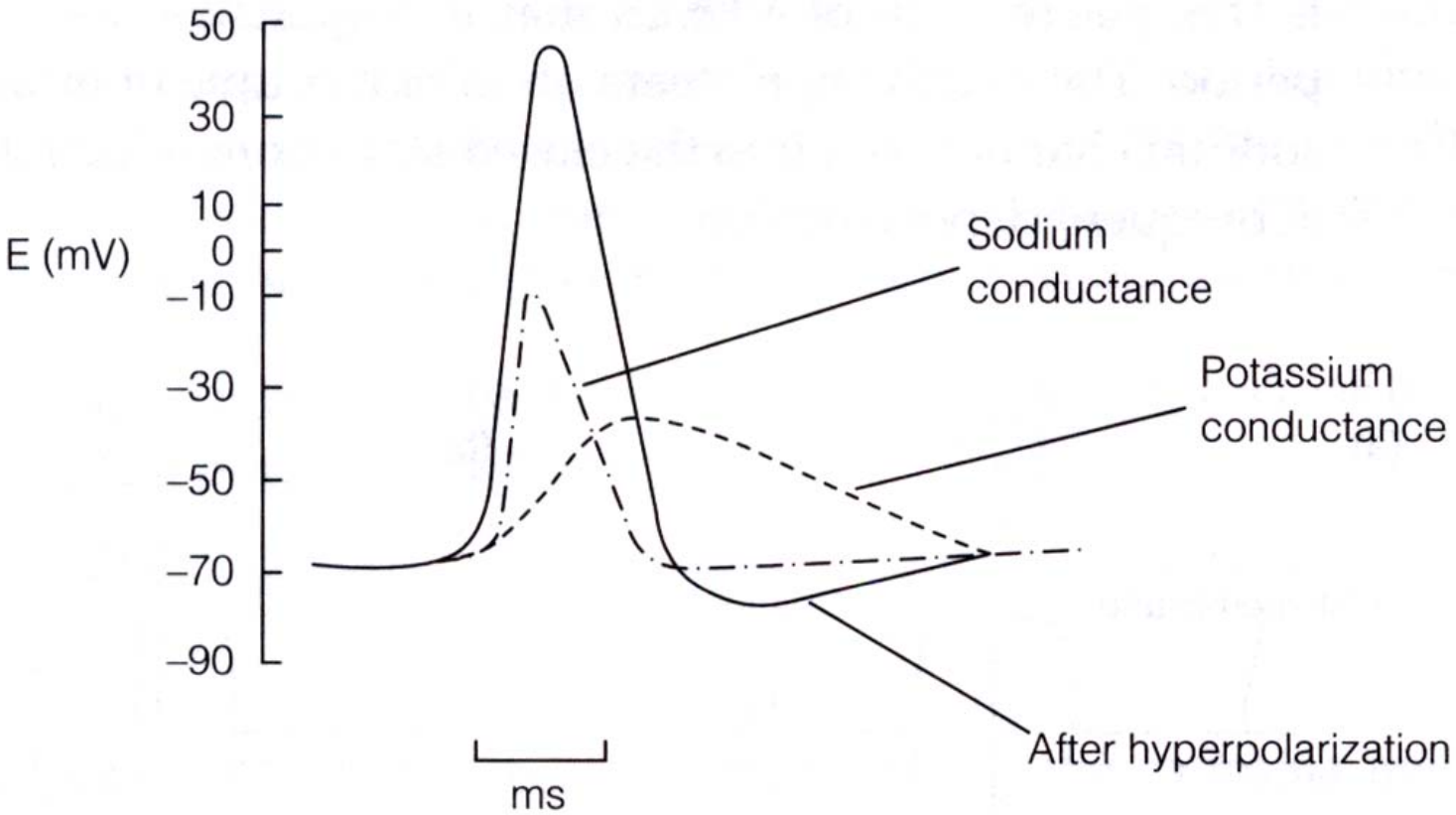


Fig. 2. Changes in ion conductance during the action potential.

Voltage-dependent sodium channels

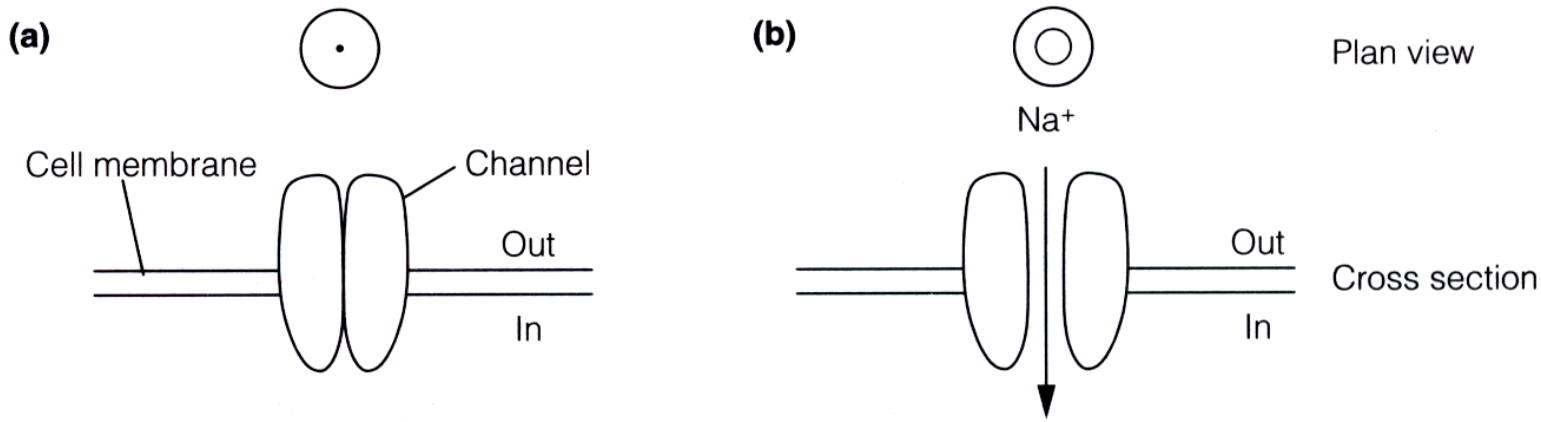


Fig. 1. Behavior of a voltage-dependent sodium channel (a) at rest when it is in the closed state and (b) during the spike of an action potential, when it is activated.

Glycoproteins
Closed, at resting potentials
Threshold depolarization => confirmation changes => channel open
Take 10 μs to open
Remain open for 0.5 ~ 1 ms => 6,000 Na+ to cross the channel
A few channels open => further depolarization => other channels open
=> action potential

Closed Open Inactivated (closed, absolute refractory period) Closed

Voltage-dependent potassium channels

Delayed outward rectifier (the opening of K+ channels lags behind Na+ ones)

Closed Open (relative refractory period) Inactivated Closed

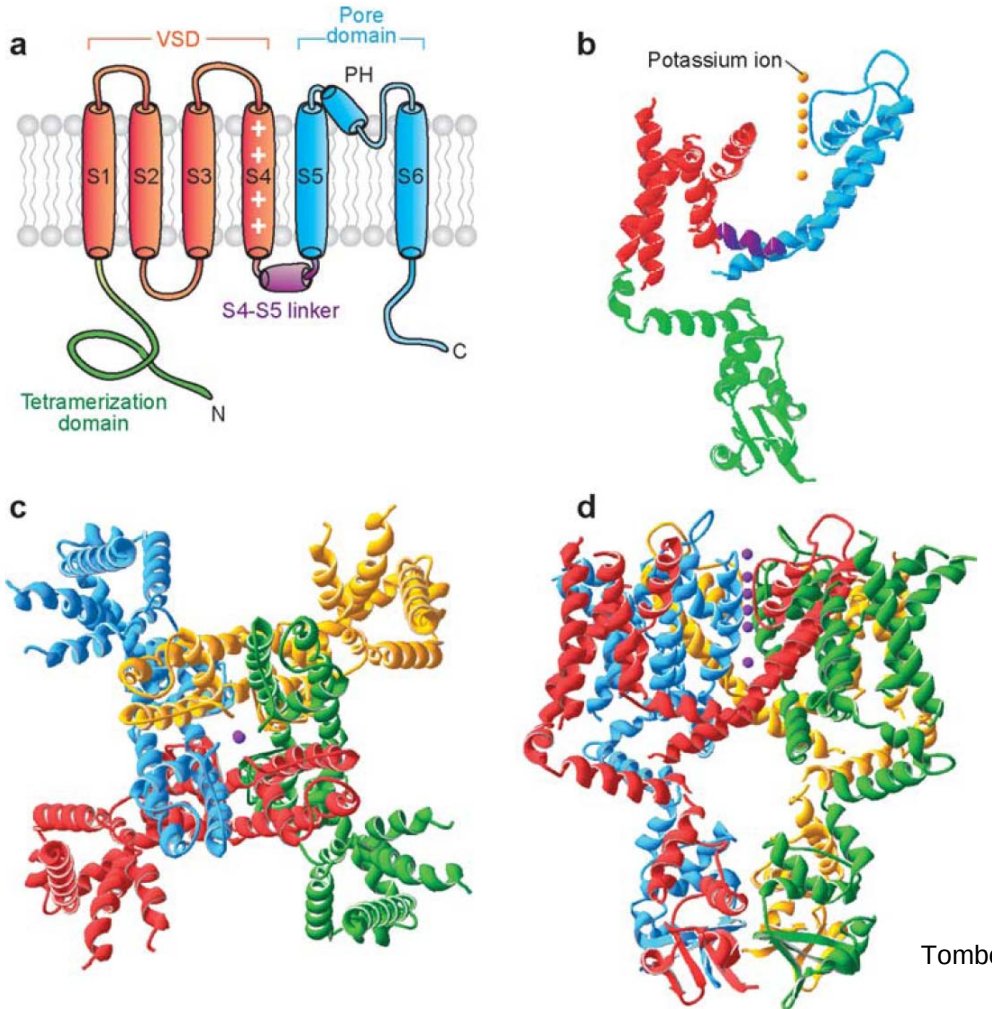


Figure 1

(*a*) The architecture of a Kv channel subunit. Cylinders are helical segments. The pore domain is shown in blue, the voltage-sensing domain (VSD) in red, the S4-S5 linker in purple, and the tetramerization domain in green. (*b*) A single Kv1.2 subunit color coded as in *a*. Potassium ions are colored yellow. The Kv1.2 tetramer (*c*) top view (extracellular side) and (*d*) side view. Each subunit is shown in a different color. Potassium ions are colored purple. Coordinates from Long et al. (2005a), PDB ID 2A79. All the molecular drawings have been created using Swiss-Pdb viewer (**http://www.expasy.org/spdbv/**).

Tombola et al., Annu Rev Cell Dev Biol. 2006;22:23-52.

Propagation of an action potential
0.5 - 2 m/second

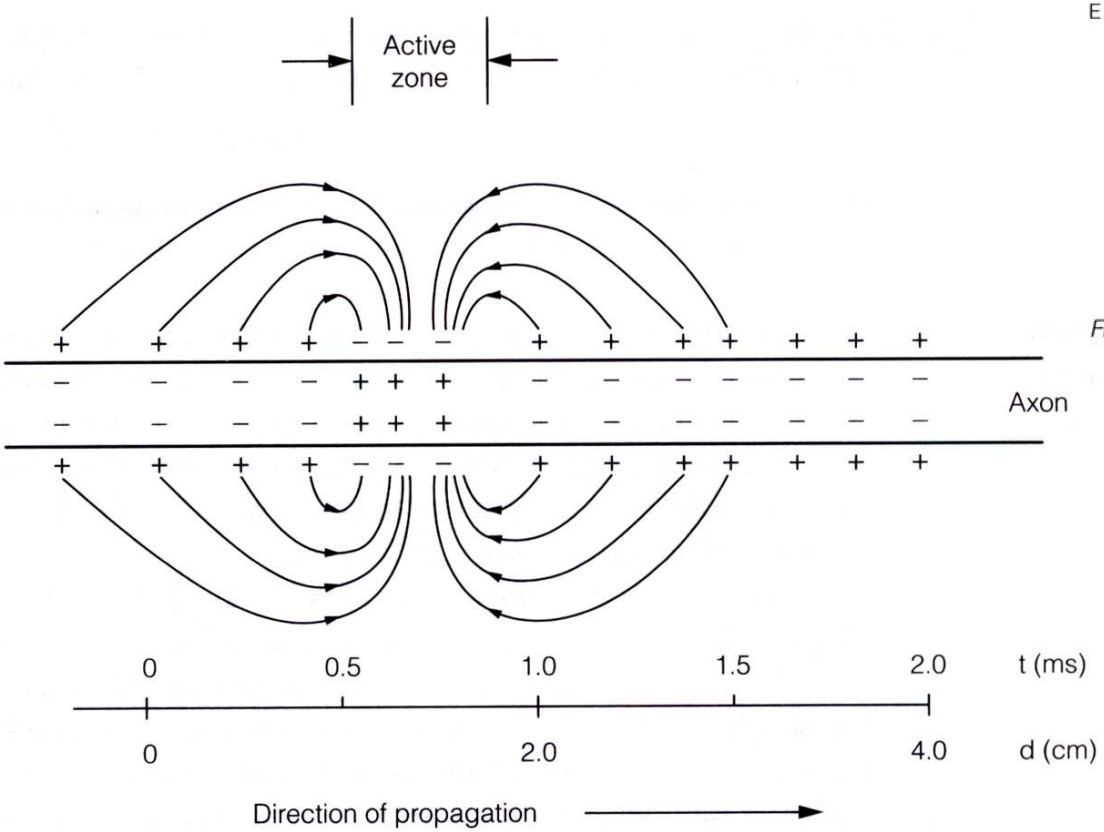


Fig. 1. Local circuit currents involved in the conduction of an action potential. For clarity currents inside the axon are omitted. The action potential is depicted as travelling from left to right along the axon, and the leading edge of the spike (active zone) is 2 cm from the origin (lower scale) after 1 ms (upper scale). t, time; d, distance.

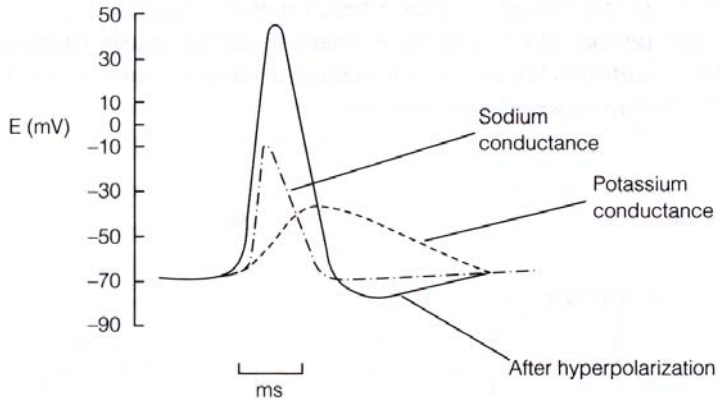
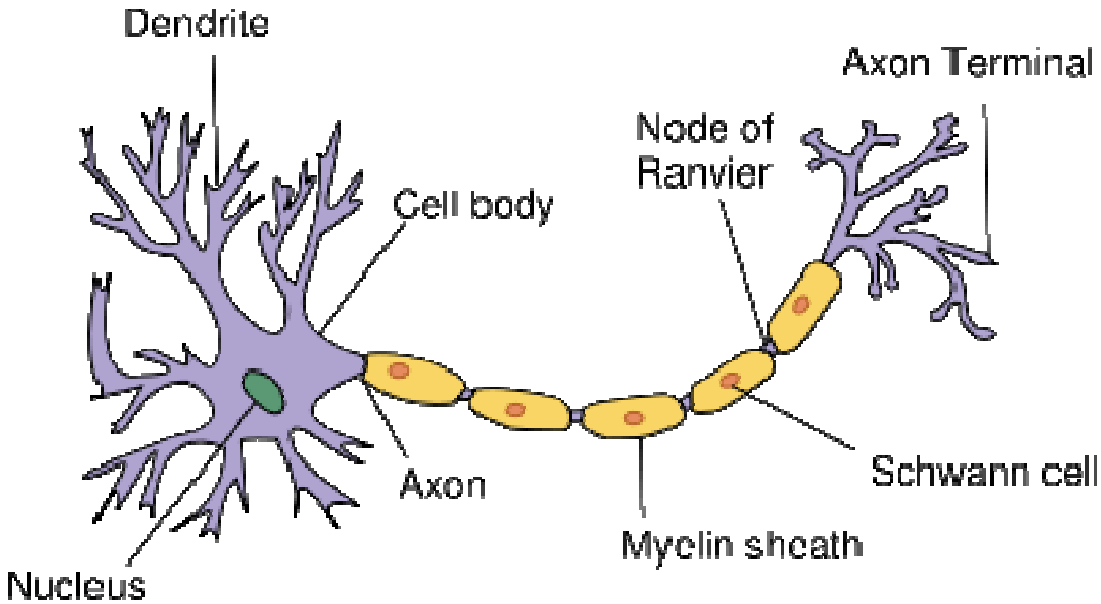
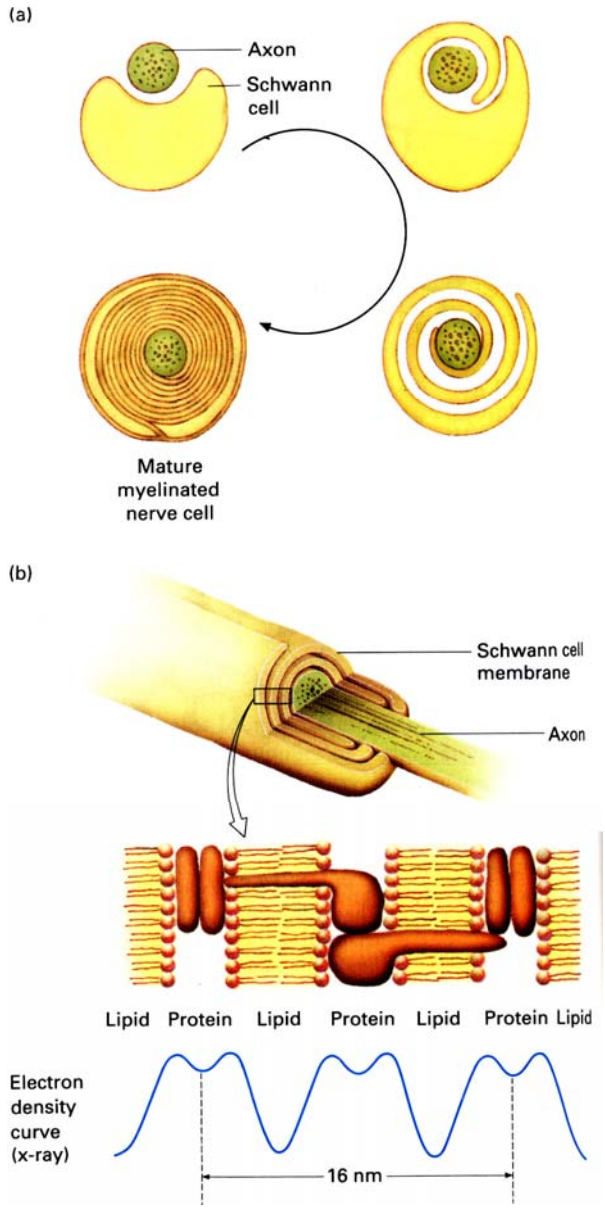


Fig. 2. Changes in ion conductance during the action potential.

"Neuroscience" Longstaff, 2005

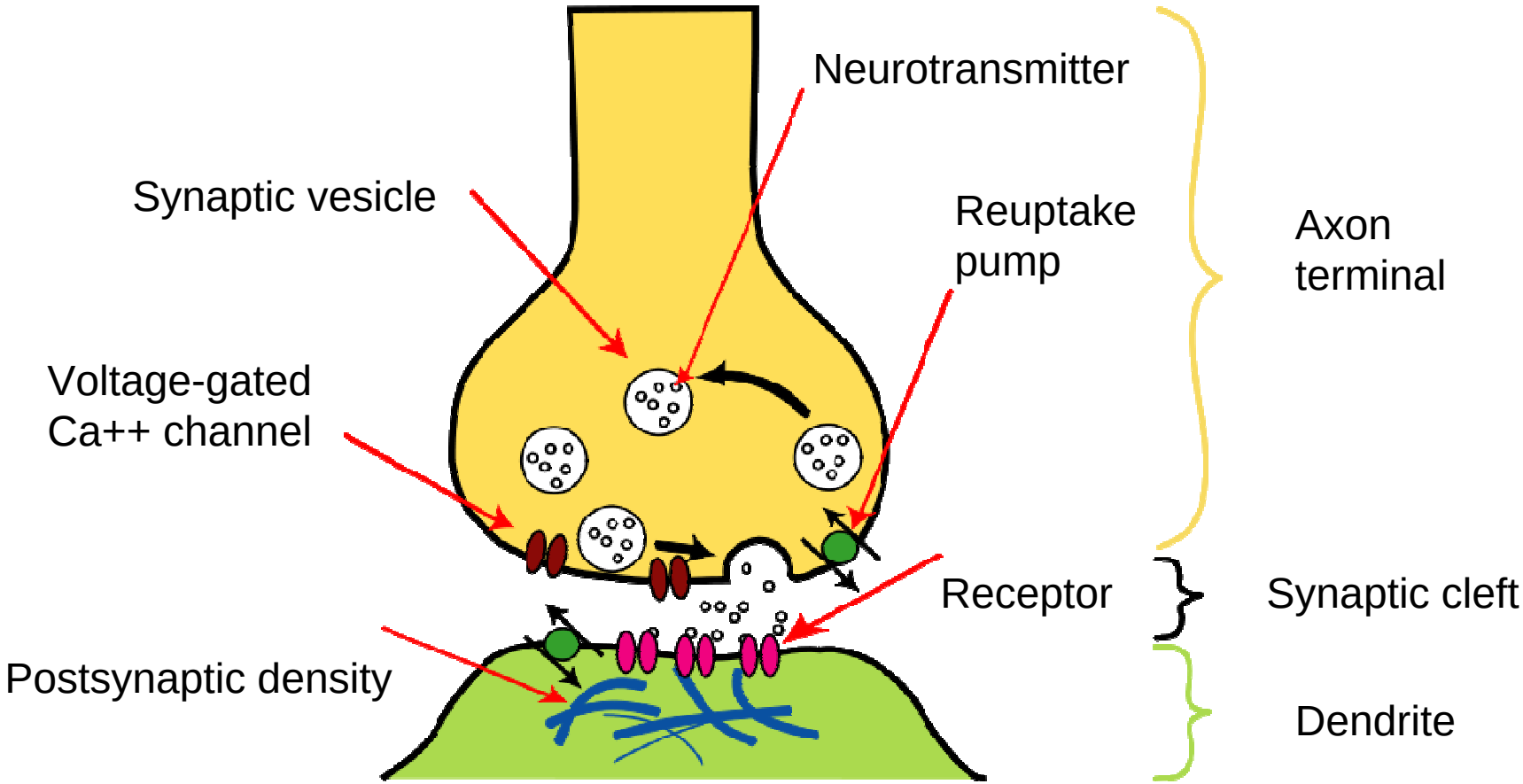
Saltatory conduction of action potential
7 -100 m/second





- Action potential is triggered at the axon hillock
- Reduced amount of charge stored across the membrane => less time to depolarize it.
- Only the nodes of Ranvier are to be activated => action potentials jump from node to node.

Relay of electrical signals via neurotransmitters at the synapses



http://en.wikipedia.org/wiki/Neurotransmitter

Various neurotransmitters

Table 1. Key central nervous system neurotransmitters

Classical	Amino acids	Glutamate	
		Aspartate	
		γ-aminobutyrate	
		Glycine	
	(Mono)amines	Acetylcholine	
		Dopamine	catecholamines
		Norepinephrine	catecholamines
		Epinephrine	catecholamines
		Serotonin (5-hydroxytryptamine) indolamine	
Peptides	Opioids	Dynorphins	
		Endorphins	
		Enkephalins	
	Tachykinins	Substance P	
	Hormones	Cholecystokinin	
		Somatostatin	

"Neuroscience" Longstaff, 2005

Ionotropic receptors

Ionotropic receptors (ligand-gated ion channel receptors):

opened or closed in response to the binding of a chemical messenger

ion-selective channels

binding of neurotransmitters directly affects the ionic permeability

Table 1. Ligand-gated ion channels

		permable to
Nicotinic receptor family	nAChR	Na^+ , K^+
	$GABA_A$	Cl-
	$GABA_C$	Cl-
	glycine	
	$5\text{-}HT_3$	
Glutamate receptor family	GluR1–GluR4	Na^+, K^+, Ca^+
	GluR6	Na^+, K^+, Ca^+
	NMDAR	Na^+, K^+, Ca^+

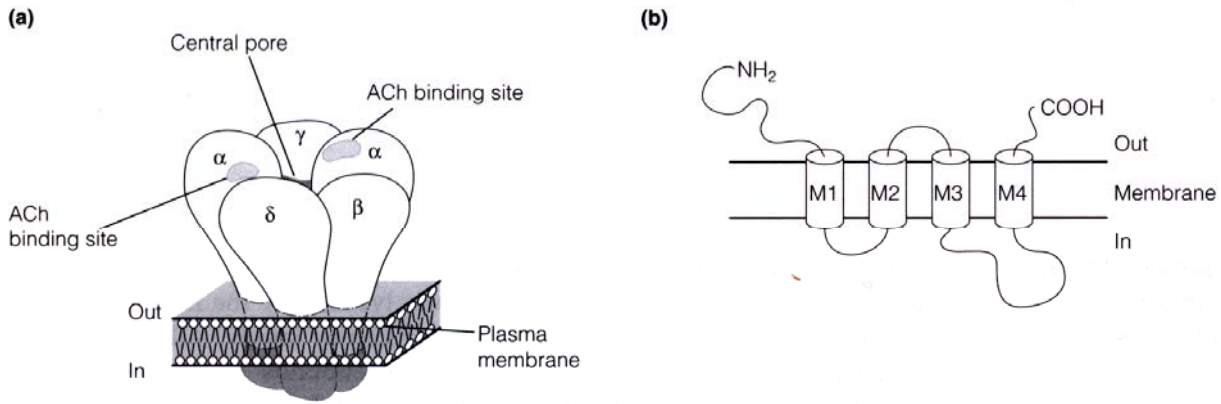
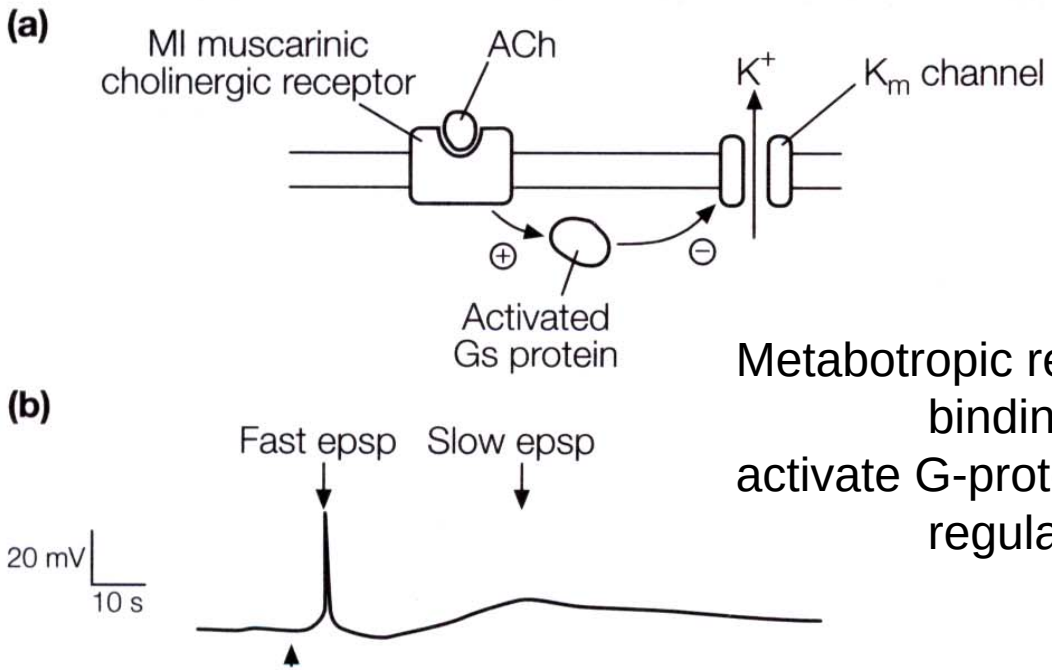


Fig. 1. The nicotinic receptor family: (a) pentameric arrangement of subunits; (b) cartoon of subunit secondary structure.

Metabotropic receptors

Affect ion channels indirectly,
By way of a cascade of events, which takes more time to turn on or off
Second messengers diffuse in the cell => receptor action not confined to synapses
Long lasting effects, may also affect gene expression.



Metabotropic receptors:
binding of neurotransmitter =>
activate G-protein =>
regulated ionic permeability

Fig. 4. Modulation of M-type (muscaninic) potassium (K_m) channels. (a) ACh binding to MI muscaninic receptors closes K_m channels. (b) Response of autonomic postganglionic neuron to firing of the preganglionic cell. The slow epsp is due to K_m channel closure by ACh. (The fast epsp is due to activation of nicotinic cholinergic receptors.)

Excitatory postsynaptic potentials

Release of excitatory neurotransmitters (such as glutamate) => open of cation channels => Depolarization

- It is technically hard to record individual epsps at vertebrate synapses, so generally the epsp resulting from the activation of *several* synapses is recorded at the cell body.
- They are small and graded in size, ranging from fractions of a millivolt to about 8 mV, depending on the number of afferent fibers being stimulated. The reason for this is that if more afferent fibers are stimulated, then more synapses are activated.
- There is a short delay of 0.5 to 1 ms between stimulating the afferents and the generation of an epsp. This is called the **synaptic delay**.
- They typically last for about 10–20 ms before decaying exponentially.

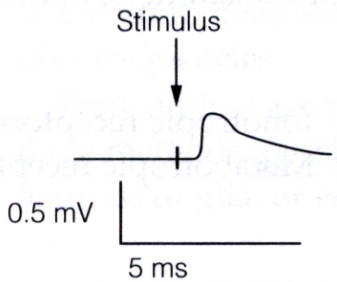


Fig. 1. Excitatory postsynaptic potentials in spinal motor neurons in response to stimulating a single sensory axon.

Inhibitory postsynaptic potentials

Release of inhibitory neurotransmitters (such as GABA, glycine) =>
open of Cl- channels =>
Move to chloride equilibrium =>
hyperpolarization

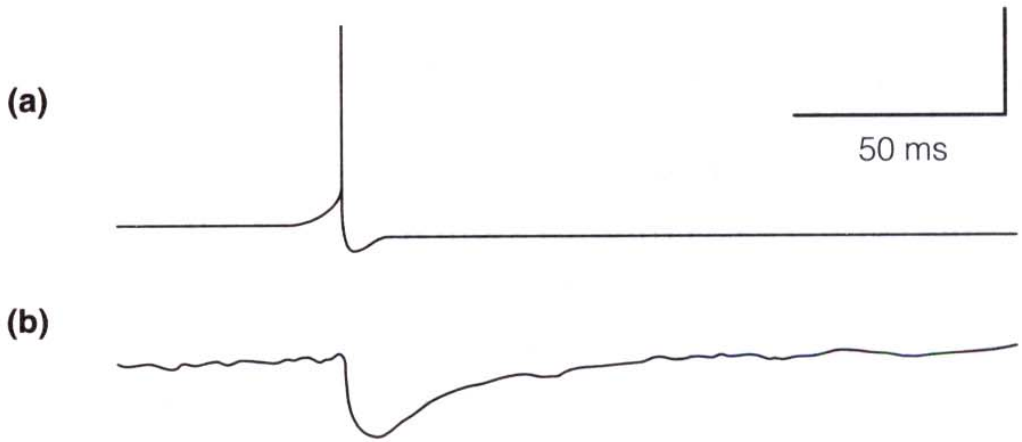


Fig. 2. Inhibitory postsynaptic potential in a pyramidal cell produced by GABA release from an inhibitory (basket) neuron: (a) presynaptic action potential in basket cell, vertical scale bar 25 mV; (b) postsynaptic potential in pyramidal cell, vertical scale bar 0.5 mV.

Inactivation of neurotransmitters

Enzymatic degradation

Transportation

Diffusion

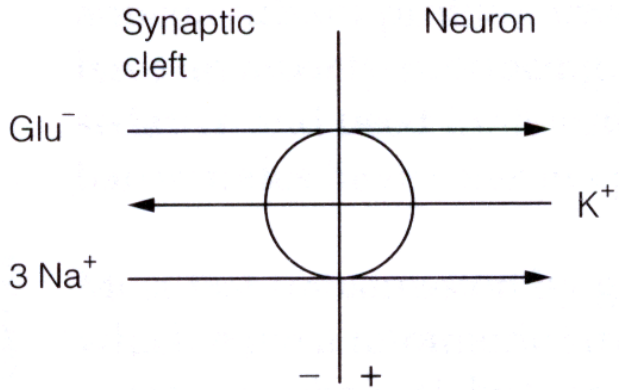


Fig. 1. Glutamate transport by a Na^+/K^+ cotransporter.

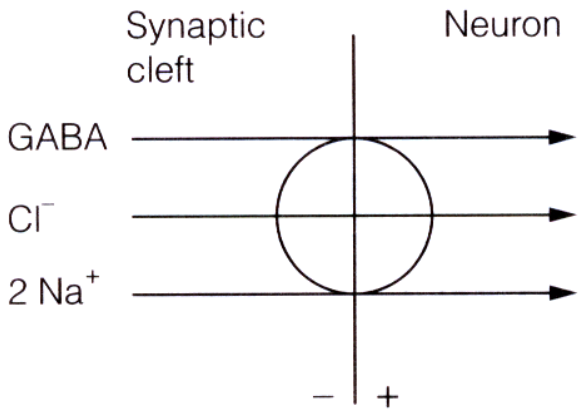


Fig. 2. GABA transport by a Na^+/Cl^- cotransporter.

Cellular layers of the retina

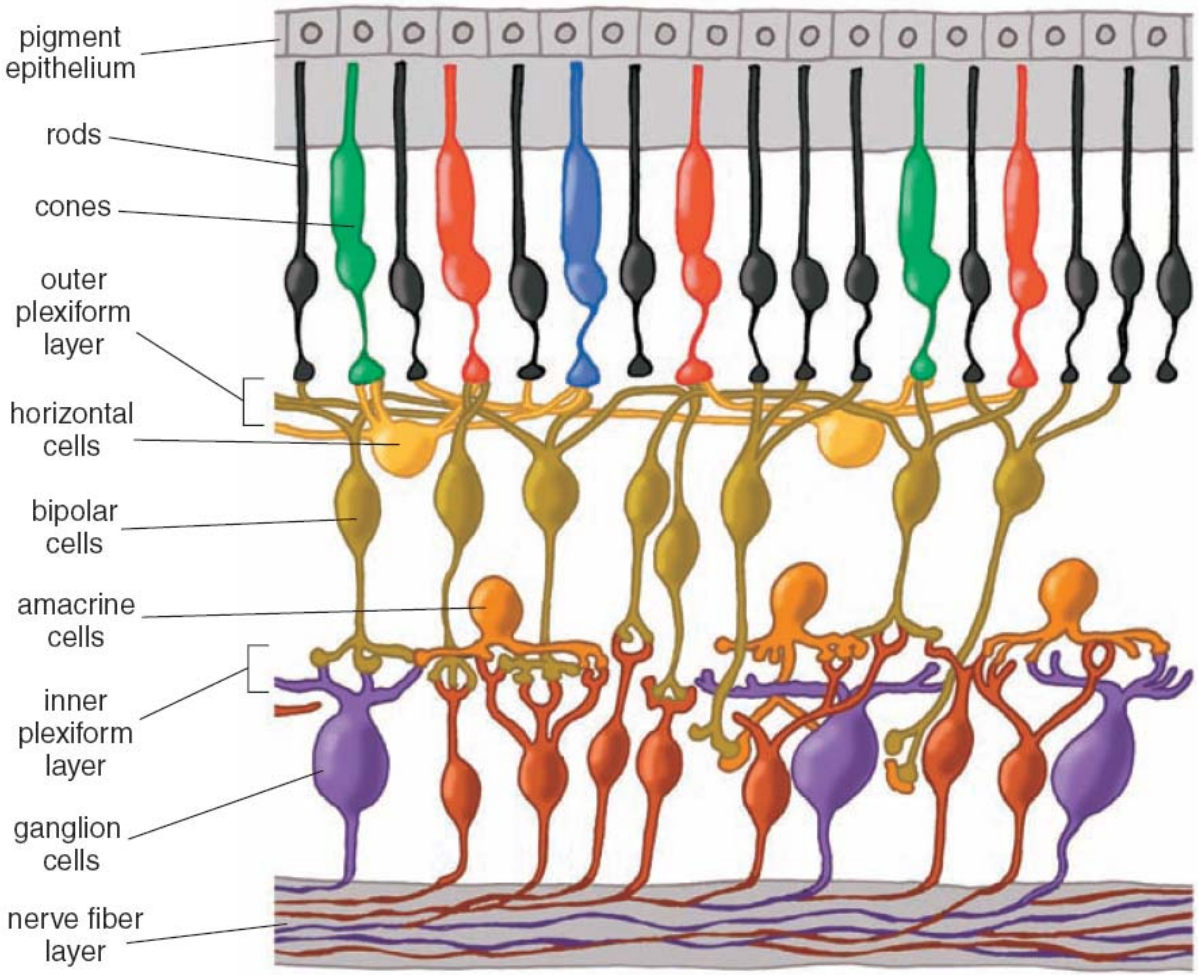


Figure 3. Cells in the retina are arrayed in discrete layers. The photoreceptors are at the top of this rendering, close to the pigment epithelium. The bodies of horizontal cells and bipolar cells compose the inner nuclear layer. Amacrine cells lie close to ganglion cells near the surface of the retina. Axon-to-dendrite neural connections make up the plexiform layers separating rows of cell bodies.

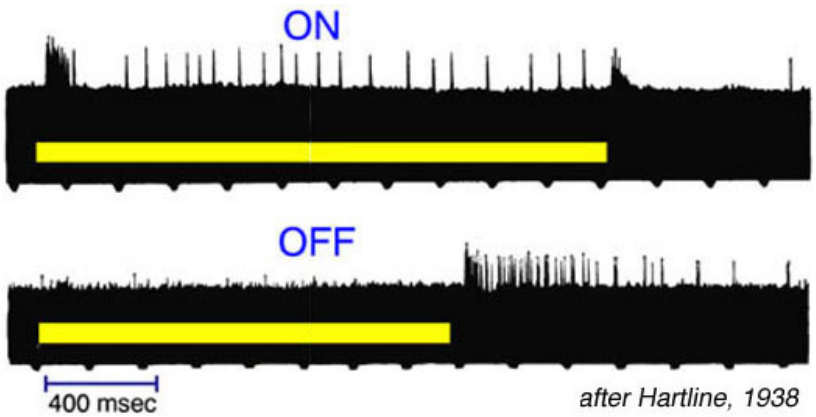
ON and OFF Pathways

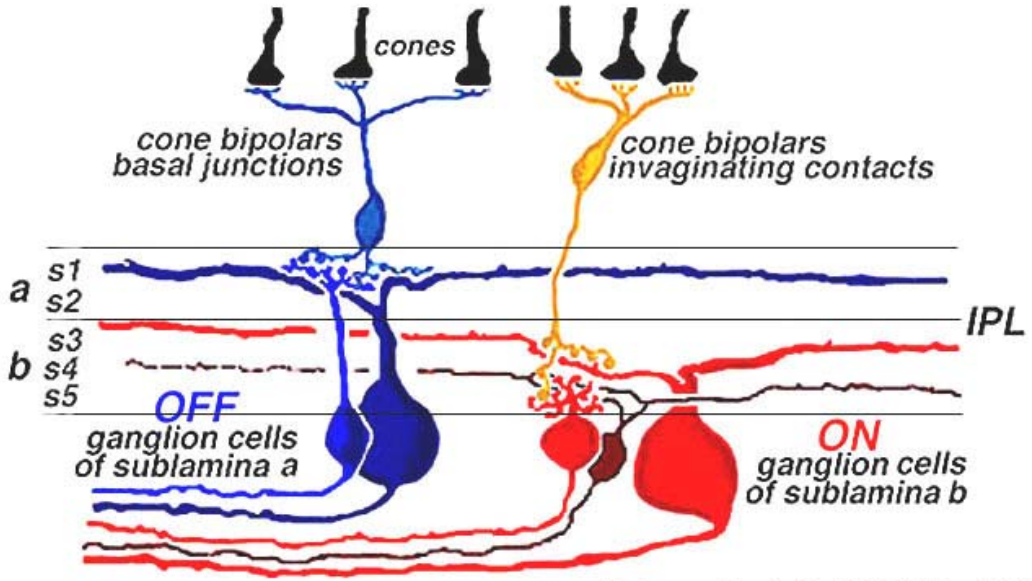
Haldan Hartline

Fig. 2. Ragnar Granit, 1967 Nobel Laureate.

Ragnar Granit

Hardline and Granit shared the 1967 Nobel prize in physiology and medicine for the first electrical recording of light responses from individual ganglion cells of the vertebrate retina.





Rod and Cone Pathways

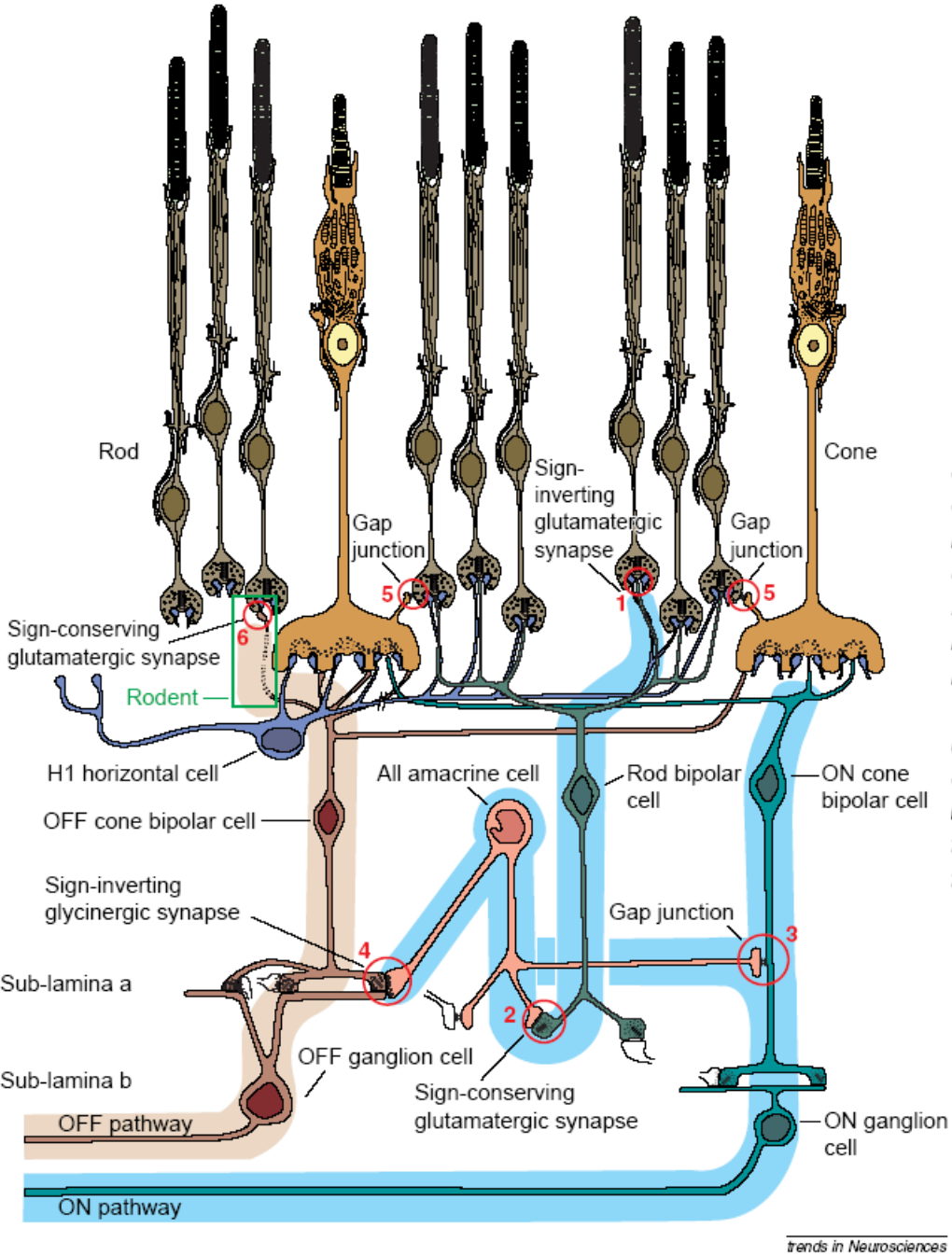


Fig. 1. ***Rod and cone pathways in the mammalian retina.*** *The retina is a complex neural tissue interweaving multiple circuits for transmitting photon signals from the light-sensitive rod and cone photoreceptors to the ON and OFF ganglion cells, the axons of which form the optic nerve. Integral to the circuits are bipolar, amacrine and horizontal cells, which maintain or enhance the linkage. The highly schematic retinal diagram depicted here (see also Refs 1,2) concentrates on the pathways available to the rods, all of which either infiltrate or superimpose upon the cone circuitry. The numbered circles highlight the six so-far identified or inferred regions of rod-signal transmission: (1) the rod–rod bipolar metabotropic (sign-inverting) glutamatergic synapse; (2) the rod bipolar–amacrine AII cell (sign-conserving) glutamatergic synapse; (3) the amacrine II–ON cone bipolar (sign-conserving) electrical gap junction; (4) the amacrine II–OFF cone bipolar (sign-inverting) glycinergic synapse; (5) the rod–cone (sign-conserving) electrical gap junction (shown twice, once each for the ON and OFF pathways); and (6) the inferred rod–OFF cone bipolar ionotropic (sign-conserving) glutamatergic synapse. Only the parasol ON (light green) and OFF (beige) pathways, which transmit the largest rod signals, are shown. The cone–cone gap junctions and H2 horizontal cells (the axons of which do not connect to rods) are not shown. The //, which cuts the axon of the H1 horizontal cell, indicates that the axon is much longer than depicted here.*

Lindsay T. Sharpe and Andrew Stockman *TINS* Vol. 22, No. 11, 1999

Phototransduction

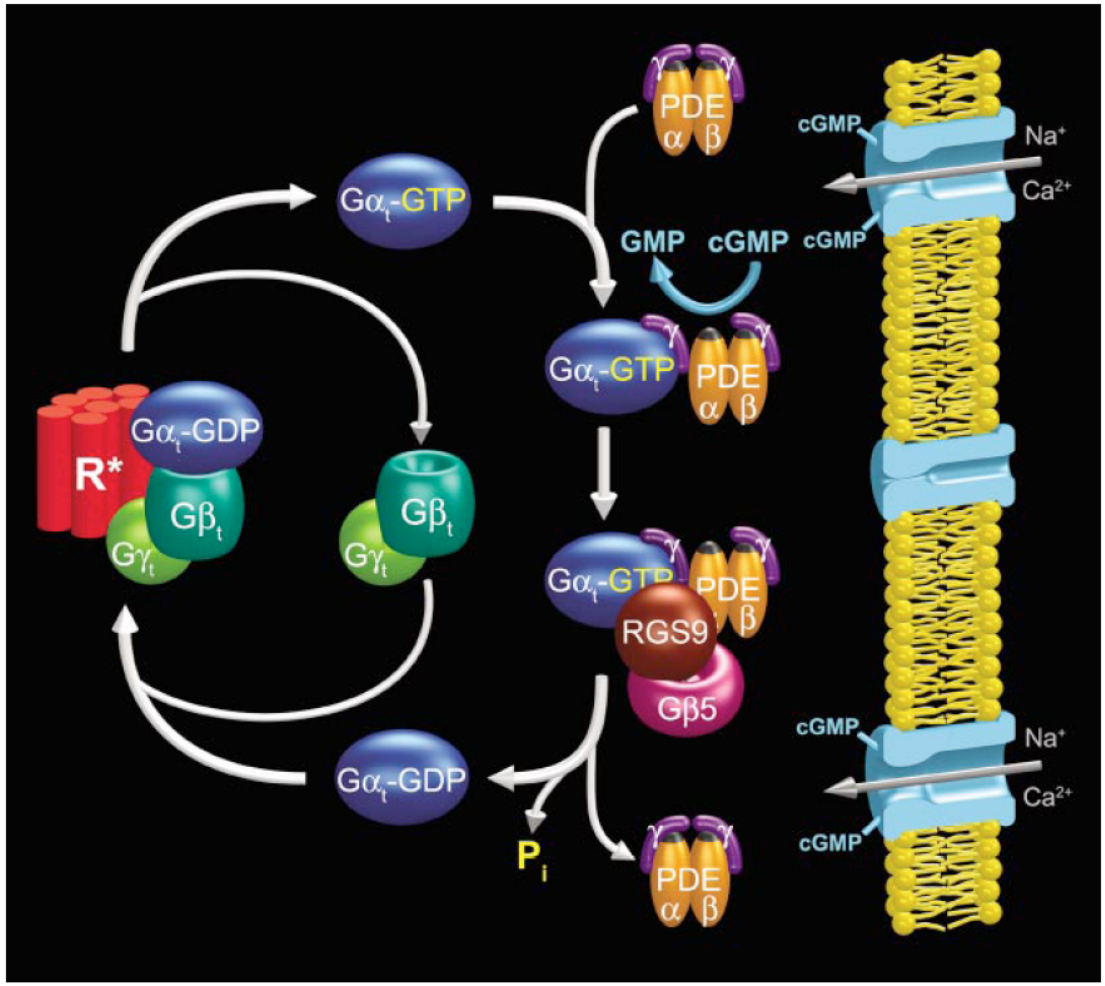
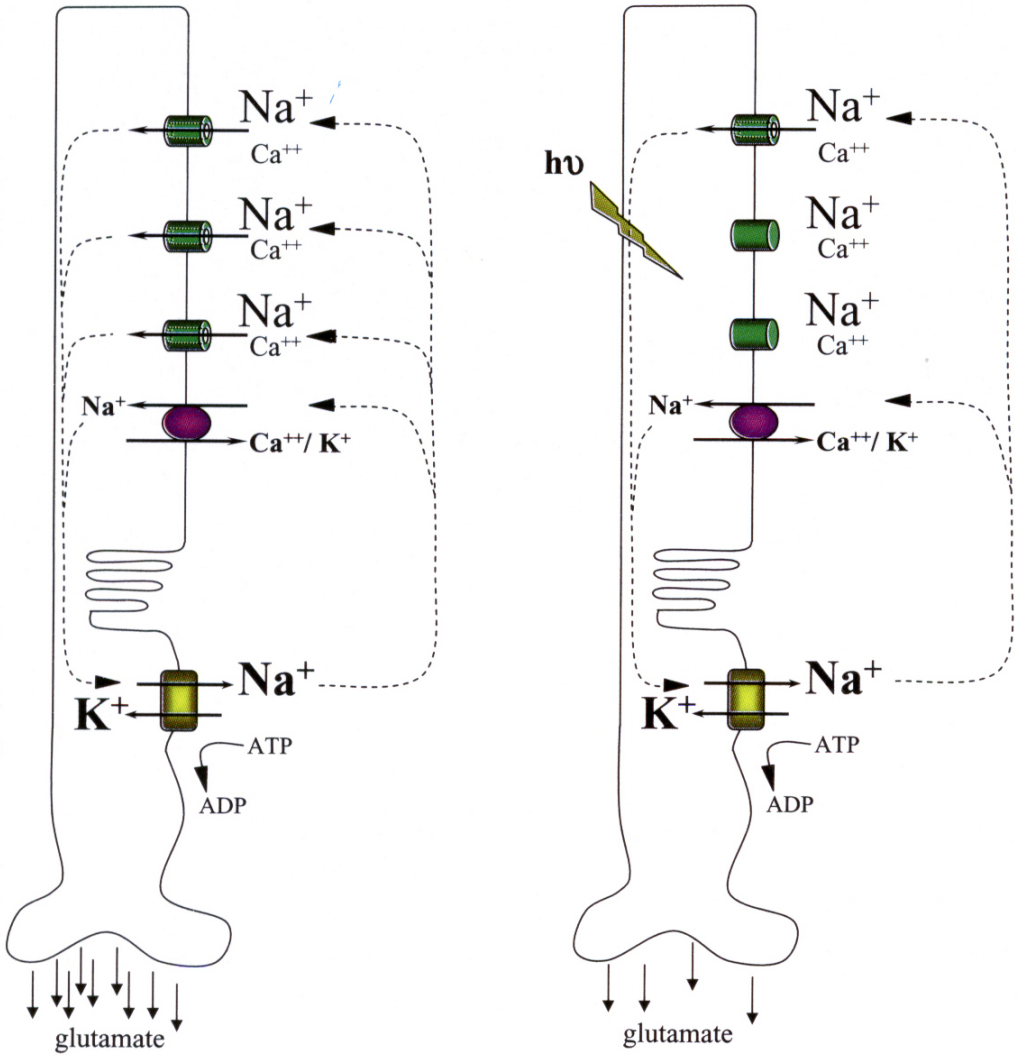


Figure 2 The cycle of G protein activation and inactivation in phototransduction.

Dark current, light-evoked hyperpolarization, and glutamate reduction



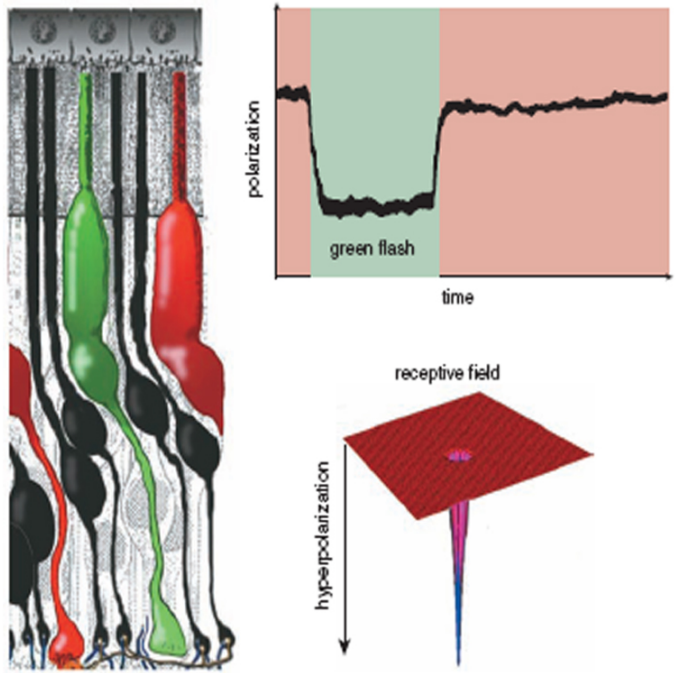


Figure 6. A single green-sensitive cone photoreceptor responds to the presence of green light by becoming hyperpolarized; that is, the membrane's electrical potential becomes more negative. The hyperpolarization lasts as long as the light flash *(top right)*. The cone only responds to light immediately directed to it, so its receptive field is very narrow *(bottom right)*.

PLATE 8 Ion circulation across the photoreceptor membrane. In the dark photoreceptor, cGMP-gated channels are open, allowing influx of Na^+ and Ca^{2+} ions. Calcium balance is maintained by the action of a Na^+/Ca^{2+} exchanger, which uses the Na^+ gradient to extrude Ca^{2+}. The sodium balance is maintained by a Na^+/K^+ pump, which uses ATP to return Na^+ against its ionic gradient. In response to light, one or more cGMP-gated channels are closed, resulting in a hyperpolarization of the cell membrane, since the Na^+/K^+ pump continues to operate. Membrane hyperpolarization causes a decrease in glutamate release from the synaptic terminal. (See figure 7.5.)

Photoreceptors are responsible for the a-Wave

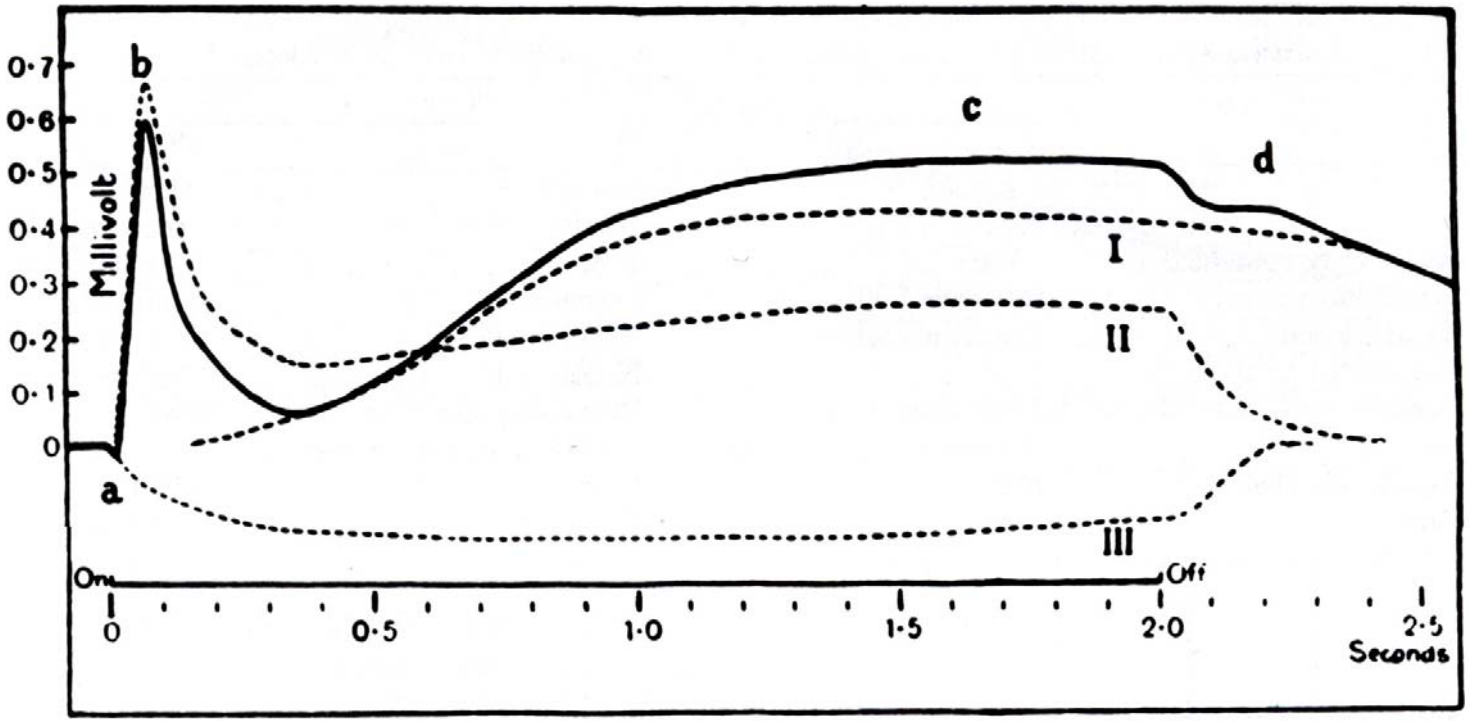


FIGURE 1.4 Analysis of the E-ERG (cat) at 14 mL . The a-wave has been broadened slightly out of proportion to demonstrate its derivation more clearly. (From Granit R: *J Physiol* 1934; 81:1–28.)

-mainly associate with photoreceptors
-also has some postreceptoral contributions

-scotopic: dark adapted a-wave <= mainly by rods

-photopic: light adapted (rod-saturating) a-wave <= mainly by cones

-a-wave <= suppression of dark current of photoreceptors

-postreceptoral contributions to certain amplitude of the a-wave

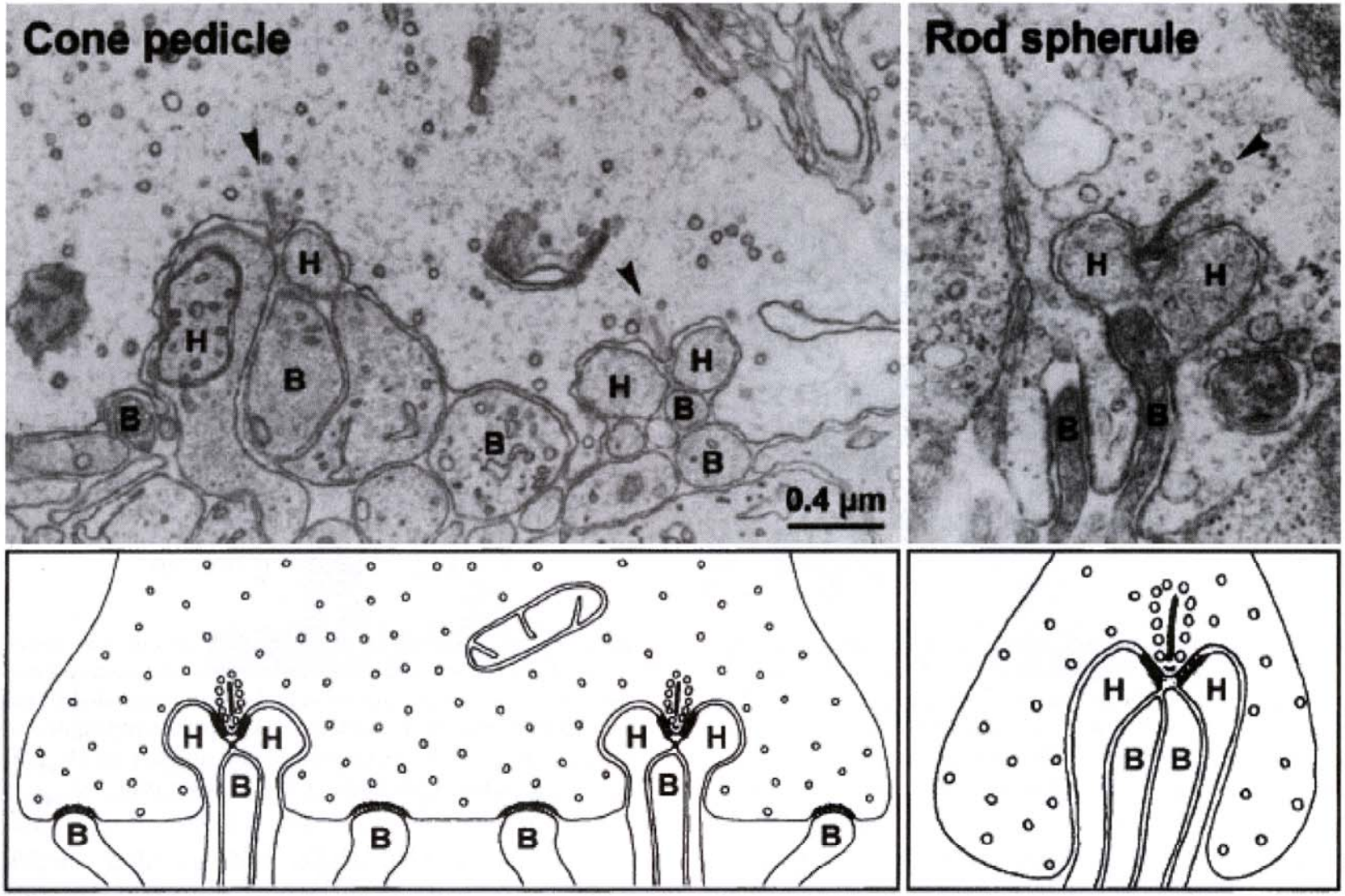


FIGURE 9.3 Below the representative electron micrographs of cone and rod terminals are cartoons of the idealized anatomy. In the cone terminal, two types of synapse are seen. Invaginating synapses are classically described with three postsynaptic elements: two horizontal cell processes (H) and one central bipolar process (B). More conventional synapses, or flat contacts, are seen between bipolar cells (B) and the cone. In the rod terminal, only the invaginating synapse is found, and the postsynaptic elements, consisting of two horizontal cells (H) and the process of two bipolar cells, are seen. The prominent presynaptic ribbon and its cluster of synaptic vesicles are seen in invaginating synapses. Bipolar cells making contact with photoreceptors at invaginating synapses are depolarizing (ON center) whereas those making contact at flat synapses are hyperpolarizing (OFF center). (Source: Figure 2 from Brandstätter JH, Hack I.[22])

The ON and OFF pathways are decided by the types of glutamate receptors of bipolar cells

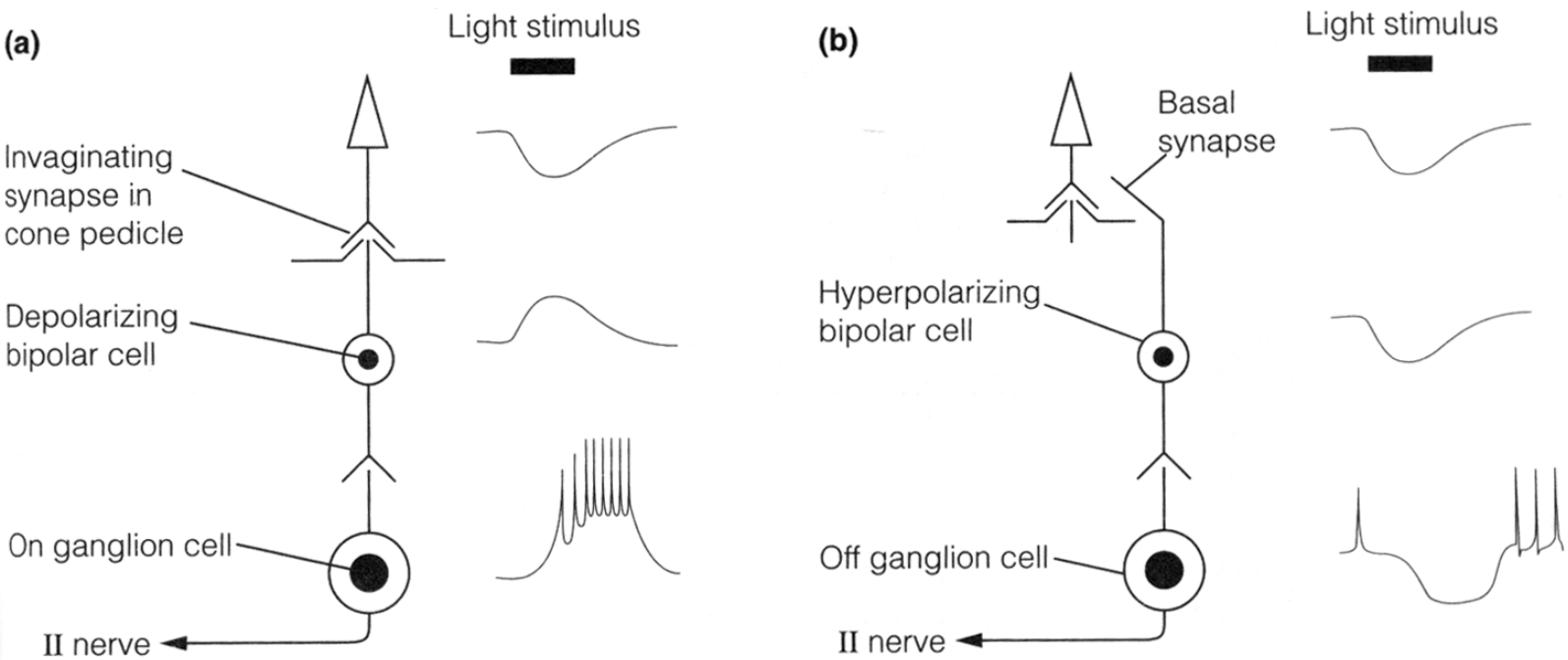


Fig. 2. (a) On channel and (b) off channel in the retina. In each case electrophysiological responses of the cells to light stimulation recorded intracellularly is shown on the right. All cells depicted use glutamate as a neurotransmitter.

Rods => rod ON bipolar cell
Cones => both ON and OFF cone bipolar cells

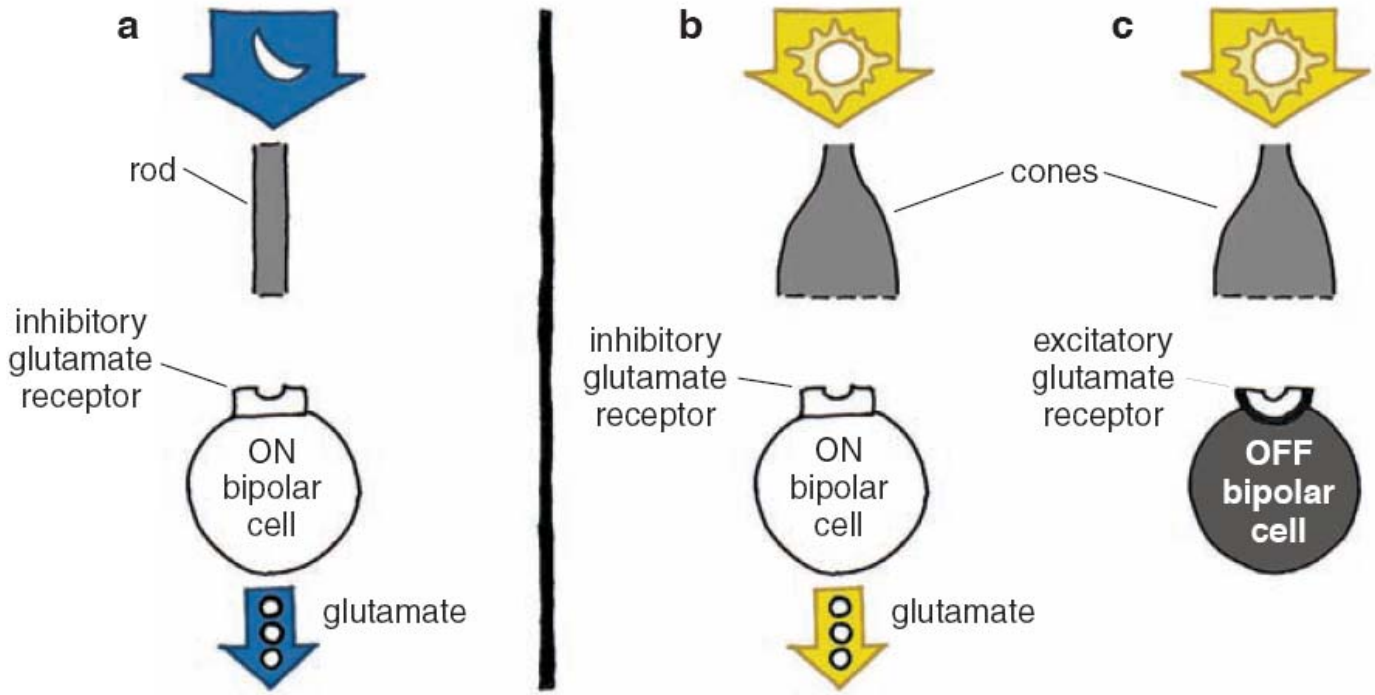


Figure 7. Photoreceptors transmit information to bipolar cells using the molecule glutamate, but different bipolar cells respond differently to the presence of the molecule; some fire in response, whereas others cease firing, depending on the kind of glutamate receptor on their surface. ON bipolar cells have a depolarizing receptive field *(a, b)*; OFF cells have a hyperpolarizing receptive field *(c)*. Contrary to what one might expect, photoreceptors *stop* releasing glutamate when stimulated by light, in turn causing ON bipolar cells to release glutamate.

b-wave <= depolarization of ON bipolar cell
hyperpolarization of OFF bipolar
hyperpolarization of horizontal cells

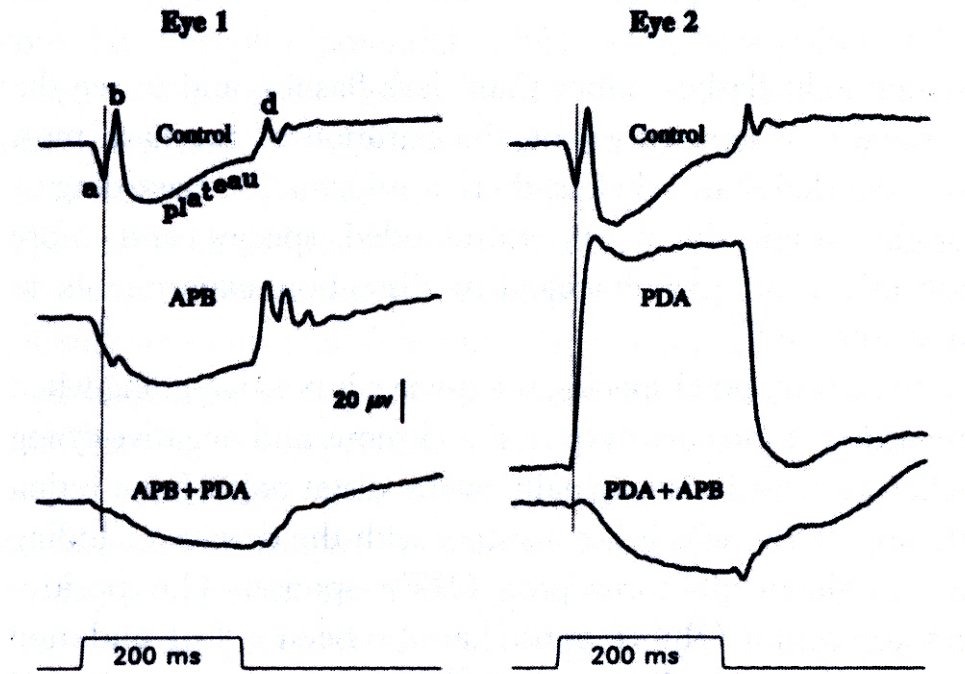


FIGURE 12.24 Effects of APB and PDA on the light-adapted photopic ERG of two monkey eyes. Drugs were given sequentially, APB followed by PDA for eye 1, and PDA followed by APB for eye 2. The vertical line shows the time of the a-wave trough in the control response. The 200-ms stimulus was 3.76 log td (2.01 log cd/m^2) on a steady rod-saturating background of 3.3 log td (1.55 cd/m^2). (Source: From Bush RA, Sieving PA.

APB: blocks mGluR6 receptor
eliminate light-evoked
depolarization of ON bipolar cells

PDA: blocks ionotropic glutamate receptor
eliminate hyperlolarizing of OFF bipolar
cells and inhibitory feedback from horizontal
cells

ON and OFF systems and center-surround organization

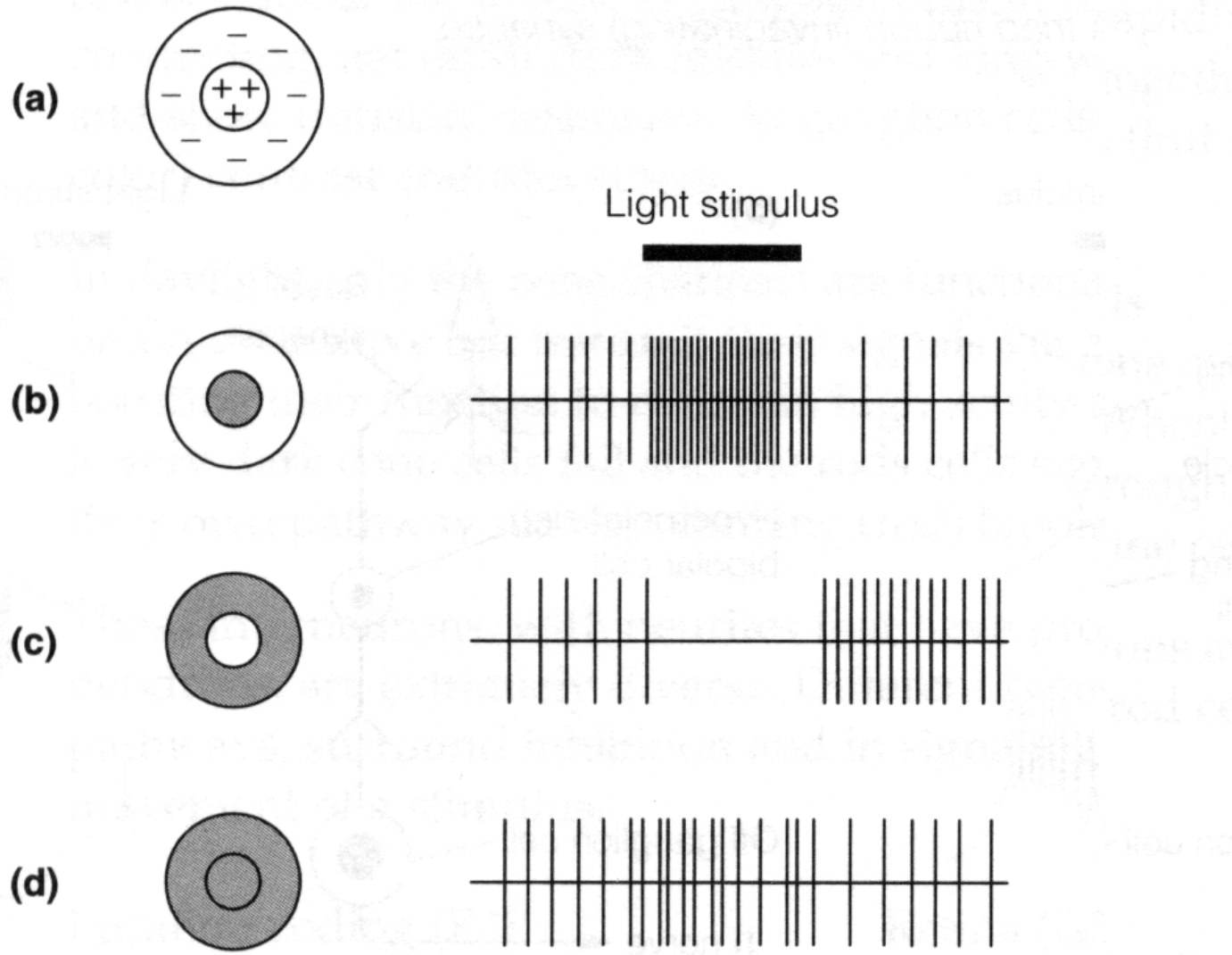


Fig. 3. Extracellular recording from on ganglion cells: (a) receptive field; (b) central illumination; (c) surround illumination; (d) overall illumination.

Lateral inhibition by horizontal cells

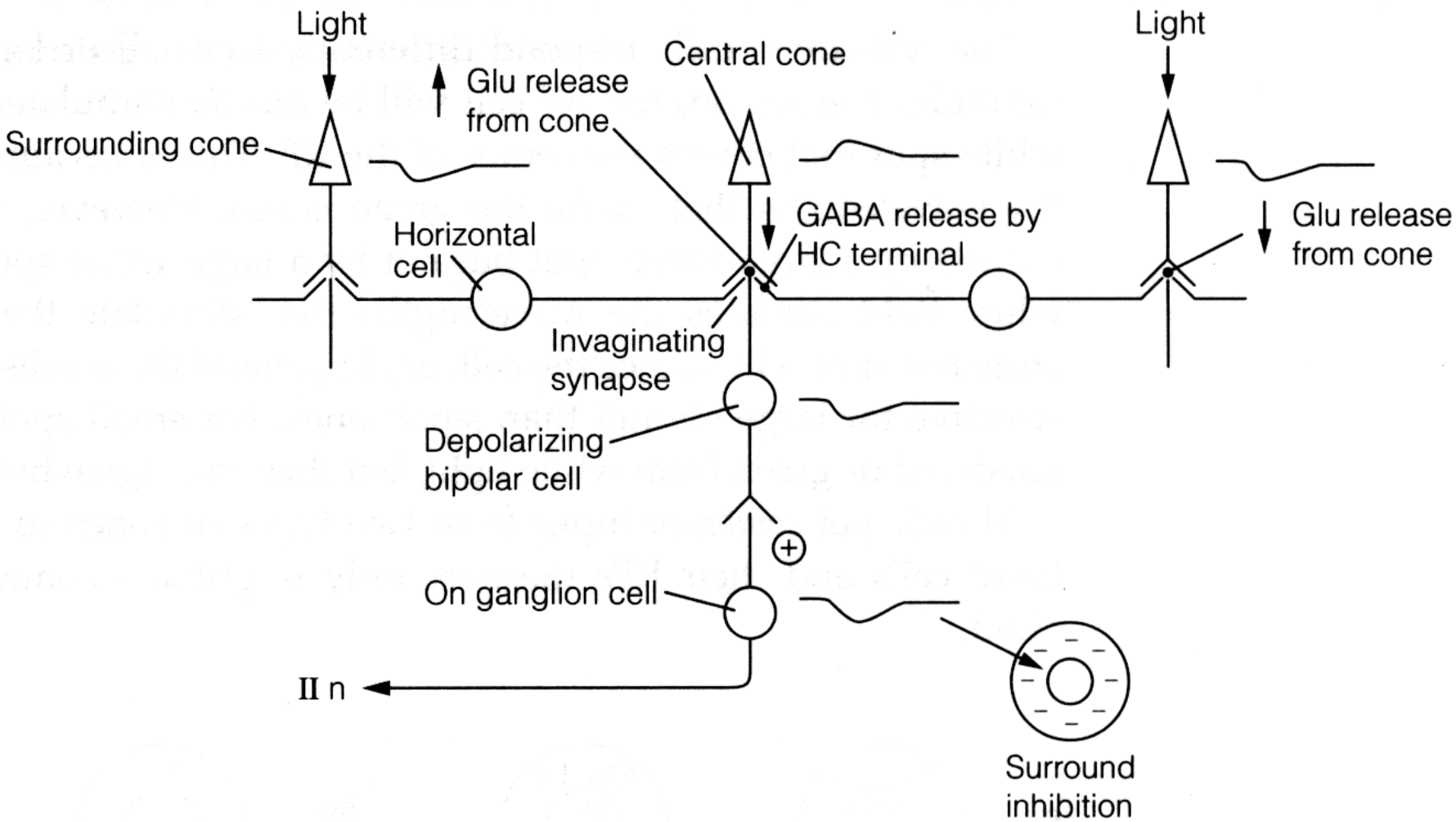
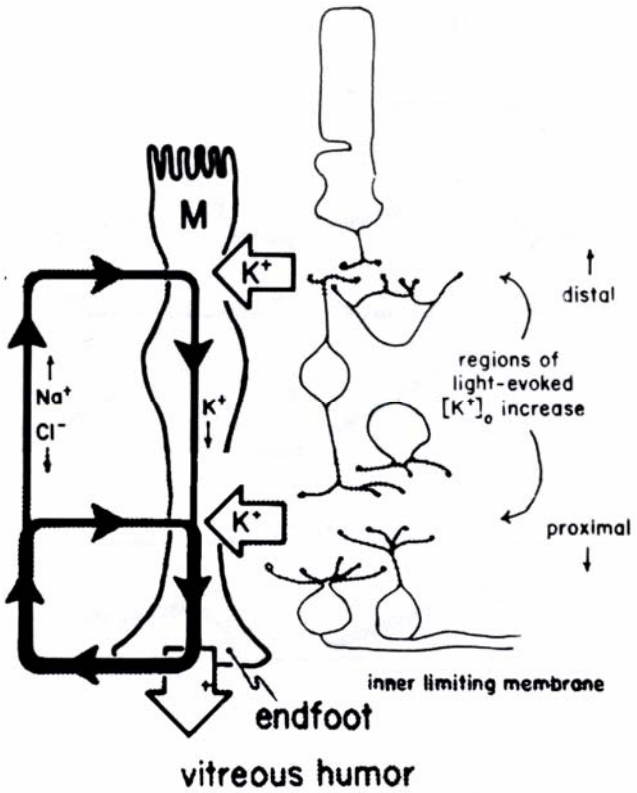


Fig. 4. The mechanism of lateral inhibition by horizontal cells (HC).

C-Wave



Composed of two subcomponents:

a corneal negative by the neural retina

slow PIII, by Muller cells

fast component: the trailing edge of the a-wave

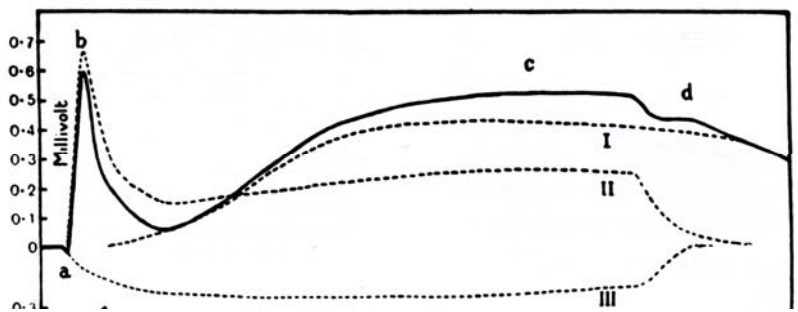
a corneal positive by the RPE

The RPE subcomponent is usually larger => positive c-wave

Both components are caused by the light-evoked increase of [K+]o.

Evidence:

1. intravenous sodium iodate => poison of the RPE =>
   abolish corneal positive component of c-wave
2. isolated retina => only corneal negative c-wave
3. isolated retina with intact RPE attached=>
   regular c-wave
4. Abolish K+ conductance => abolish both components
5. Knockout of K channel in Muller => lose of slow PIII.



d-Wave

-A positive-going defection in the ERG at light offset.

- largely contributed by depolarization of OFF bipolar cells.

- Positive going offset of the late photoreceptor potential

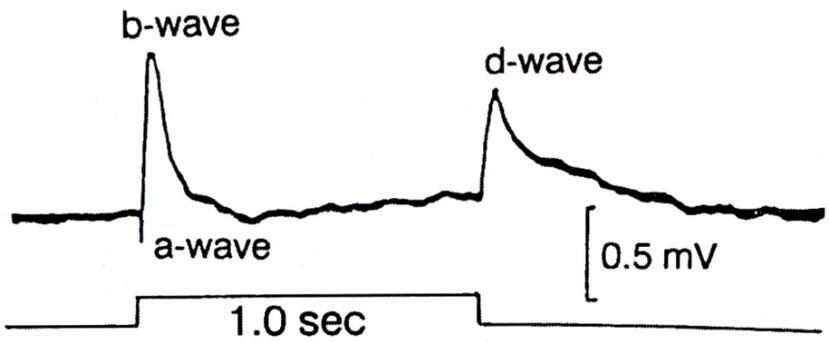


FIGURE 12.27 ERG from the all-cone retina of the squirrel. Recordings were made under light-adapted conditions between a contact lens electrode on the cornea and an electrode on the forehead. (Source: From Arden GB, Tansley K: *J Physiol* 1955; 127:592–602.)

In summary, the corneal d-wave in primates is largely produced by the depolarization of OFF bipolar cells and the positive-going offset of the late receptor potential. It is further shaped by the negative-going offset of PII. In contrast to cold-blooded species, for primates (and other mammals not presented here), there is little evidence for a positive-going d-wave component that originates in the proximal retina, either directly by neurons or indirectly by K^+ spatial buffering.

Questions to think about

1. What are the limitations and advantages of ERG as a diagnostic tool?

2. Is a-wave more helpful than c-wave in pinpointing the pathological sites of the retina?