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**SEMMELWEIS  
UNIVERSITY**



**Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial\* framework\*\***

Consortium leader

**PETER PAZMANY CATHOLIC UNIVERSITY**

Consortium members

**SEMMELWEIS UNIVERSITY, DIALOG CAMPUS PUBLISHER**

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# BEVEZETÉS A FUNKCIONÁLIS NEUROBIOLÓGIÁBA

# INTRODUCTION TO FUNCTIONAL NEUROBIOLOGY

By Imre Kalló

Contributed by: Tamás Freund, Zsolt Liposits, Zoltán Nusser, László Acsády, Szabolcs Káli, József Haller, Zsófia Maglóczky, Nórbert Hájos, Emilia Madarász, György Karmos, Miklós Palkovits, Anita Kamondi, Lóránd Eröss, Róbert Gábrriel, Zoltán Kisvárday, Zoltán Vidnyánszky

# Electrophysiology

**Imre Kalló & Norbert Hájos**

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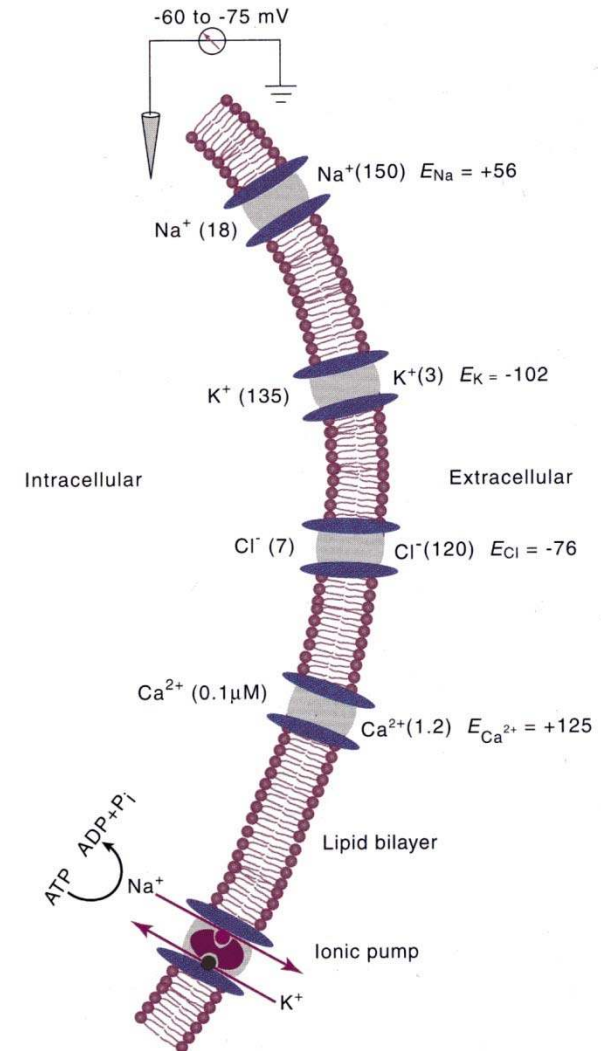
- I. Membrane and action potentials in neurons.
- II. Signal transmission at the synapses.
- III. Synaptic plasticity
- IV. In vitro and in vivo recording techniques
- V. Firing properties of different neuronal phenotypes.

## Ion concentrations and Equilibrium Potentials

Distribution of ions on opposite sides of a cellular membrane is unequal due to the semipermeability of the membrane retaining the negatively charged protein molecules within the cell and the regulated action of ion channels and ion pumps transferring ions from one side of the membrane to the other. The voltage difference generated by the altered ionic concentrations on the opposite sides of the cellular membrane is called the **membrane potential**. The concentration of sodium ( $\text{Na}^+$ ; 145mM vs. 18mM) and chloride ( $\text{Cl}^-$ , 120mM vs 7mM) ions are high in the extracellular region, whereas potassium ( $\text{K}^+$ , 135mM vs. 3mM) ions, along with large protein anions have high concentrations in the intracellular region. Calcium ions are kept intracellularly at nanomolar concentrations (100nM), elevations of which from the extracellular space (1.2 mM) and intracellular stores change the membrane potential, as well as activate calcium-dependent intracellular processes. Each of the ions are characterized by a membrane **potential** at which its flow from one side of the membrane to the other is in **equilibrium**, consequently its net (overall) flow is zero.

## Ion concentrations and Equilibrium Potentials

	Inside (mM)	Outside (mM)	Equilibrium potential (mV)
Squid giant axon			
Na <sup>+</sup>	50	440	+55
K <sup>+</sup>	400	20	-76
Cl <sup>-</sup>	40	560	-66
Ca <sup>++</sup>	0.4 μM	10	+145
Mammalian neuron			
Na <sup>+</sup>	18	145	+56
K <sup>+</sup>	135	3	-102
Cl <sup>-</sup>	7	120	-76
Ca <sup>++</sup>	0.1 μM	1.2	+125



## The Nernst Equation

The equilibrium potential for an ion can be calculated using the Nernst equation:

$$E_{\text{ion}} = 2.303 \frac{RT}{zF} \log \frac{[\text{ion}]_o}{[\text{ion}]_i}$$

where

- $E_{\text{ion}}$  = ionic equilibrium potential
- R = gas constant
- T = absolute temperature
- z = charge of the ion
- F = Faraday's constant
- log = base 10 logarithm
- $[\text{ion}]_o$  = ionic concentration outside the cell
- $[\text{ion}]_i$  = ionic concentration inside the cell

The Nernst equation can be derived from the basic principles of physical chemistry. Let's see if we can make some sense of it.

Remember that equilibrium is the balance of two influences: diffusion, which pushes an ion down its concentration gradient, and electricity, which causes an ion to be attracted to opposite charges and repelled by like charges. Increasing the thermal energy of each particle increases diffusion and will therefore increase the potential difference achieved at equilibrium. Thus,  $E_{\text{ion}}$  is proportional to T. On the other hand, increasing the electrical charge of each

particle will decrease the potential difference needed to balance diffusion. Therefore,  $E_{\text{ion}}$  is inversely proportional to the charge of the ion (z). We need not worry about R and F in the Nernst equation because they are constants.

At body temperature (37°C), the Nernst equation for the important ions—K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, and Ca<sup>2+</sup>—simplifies to:

$$E_K = 61.54 \text{ mV} \log \frac{[\text{K}^+]_o}{[\text{K}^+]_i}$$

$$E_{\text{Na}} = 61.54 \text{ mV} \log \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i}$$

$$E_{\text{Cl}} = -61.54 \text{ mV} \log \frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i}$$

$$E_{\text{Ca}} = 30.77 \text{ mV} \log \frac{[\text{Ca}^{2+}]_o}{[\text{Ca}^{2+}]_i}$$

Therefore, in order to calculate the equilibrium potential for a certain type of ion at body temperature, all we need to know is the ionic concentrations on either side of the membrane. For instance, in the example we used in Figure 3.12, we stipulated that K<sup>+</sup> was twentyfold more concentrated inside the cell:

$$\text{If } \frac{[\text{K}^+]_o}{[\text{K}^+]_i} = \frac{1}{20}$$

$$\text{and } \log \frac{1}{20} = -1.3$$

$$\text{then } E_K = 61.54 \text{ mV} \times -1.3 \\ = -80 \text{ mV.}$$

## The Goldman Equation

If the membrane of a real neuron were permeable only to  $K^+$ , the resting membrane potential would equal  $E_K$ , about  $-80$  mV. But it does not; the measured resting membrane potential of a typical neuron is about  $-65$  mV. This discrepancy is explained because real neurons at rest are not exclusively permeable to  $K^+$ ; there is also some  $Na^+$  permeability. Stated another way, the *relative permeability* of the resting neuronal membrane is quite high to  $K^+$  and low to  $Na^+$ . If the relative permeabilities are known, it is possible to calculate the membrane potential at equilibrium by using the Goldman equation. Thus, for a membrane permeable only to  $Na^+$  and  $K^+$  at  $37^\circ C$ :

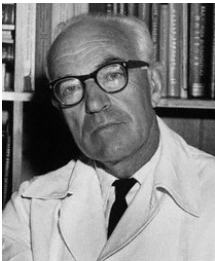
$$V_m = 61.54 \text{ mV} \log \frac{P_K [K^+]_o + P_{Na} [Na^+]_o}{P_K [K^+]_i + P_{Na} [Na^+]_i}$$

where  $V_m$  is the membrane potential,  $P_K$  and  $P_{Na}$  are the relative permeabilities to  $K^+$  and  $Na^+$ , respectively, and the other terms are the same as for the Nernst equation.

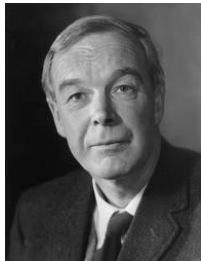
If the resting membrane ion permeability to  $K^+$  is 40 times greater than it is to  $Na^+$ , then solving the Goldman equation using the concentrations in Figure 3.15 yields:

$$\begin{aligned} V_m &= 61.54 \text{ mV} \log \frac{40 (5) + 1 (150)}{40 (100) + 1 (15)} \\ &= 61.54 \text{ mV} \log \frac{350}{4015} \\ &= -65 \text{ mV} \end{aligned}$$

## The Action Potential



Sir J. C.  
Eccles



A. L.  
Hodgkin



A.F.  
Huxley

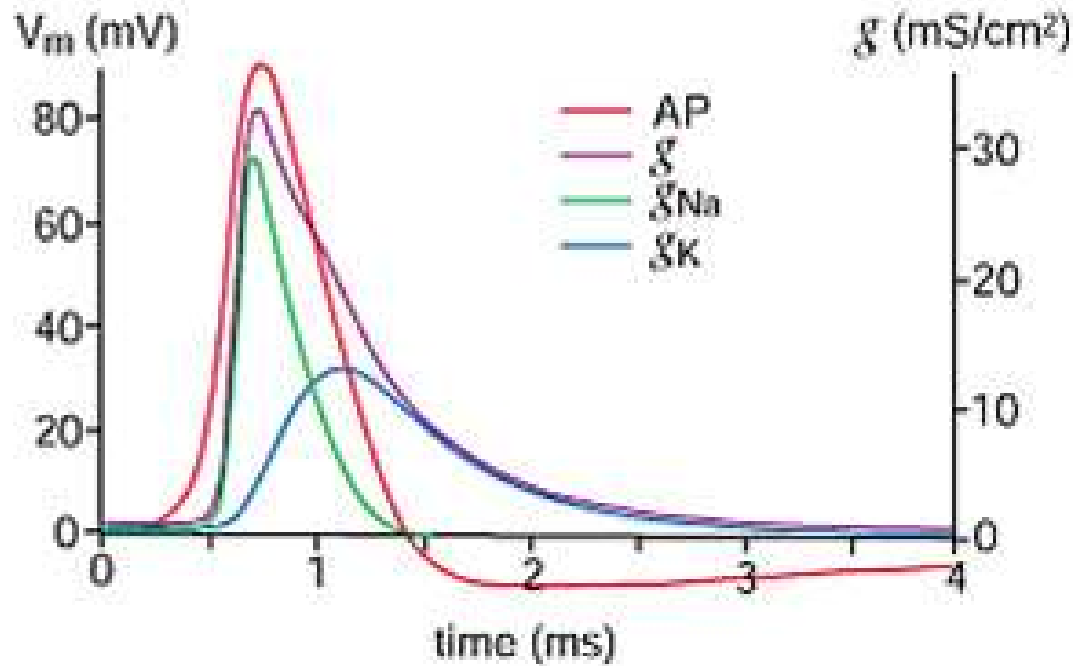
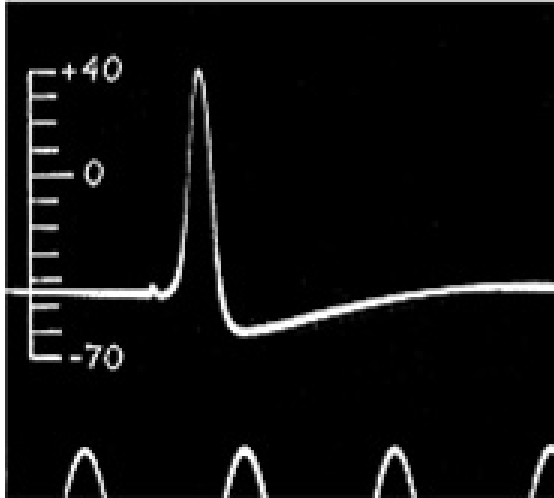
The Nobel Prize in Physiology or  
Medicine 1963

**“for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane”**

Squid



## What is happening during Action Potential?



## The Hodgkin-Huxley equations

Action potential conduction on the squid axon is governed by the partial differential equation

$$-\frac{d}{4R_i} \frac{\partial^2 V}{\partial x^2} + C \frac{\partial V}{\partial t} + G_{\text{leak}}(V - V_{\text{leak}}) + G_{Na} m^3 h (V - V_{Na}) + G_K n^4 (V - V_K) = 0$$

where  $C$  is the capacitance per unit area of the axon (of diameter  $d$  and axoplasmic resistivity  $R_i$ );  $G_{\text{leak}}$ ,  $G_{Na}$  and  $G_K$  are the maximal conductances per unit area of the leak, transient- $\text{Na}^+$  and delayed-rectifier- $\text{K}^+$  currents, respectively, and  $V_{\text{leak}}$ ,  $V_{Na}$  and  $V_K$  are the corresponding reversal potentials. In the space-clamped condition, where an electrode is inserted longitudinally along the axon, the first term (corresponding to the axial current) disappears and the partial differential equation above becomes an ordinary differential equation. An extra term must also be added to the right-hand side to account for the current flowing through the electrode.

The presence of the voltage-dependent variables  $m$  ( $\text{Na}^+$ -current activation),  $h$  ( $\text{Na}^+$ -current inactivation) and  $n$  ( $\text{K}^+$ -current activation) make the partial differential equation above nonlinear. These gating variables satisfy first order kinetic equations of the form

## The Hodgkin-Huxley equations

$$\tau_x(V) \frac{dx}{dt} = x_\infty(V) - x$$

where the sigmoid function  $x_\infty$  gives the level of (in)activation achieved in voltage clamp, and  $\tau_x$  is the relaxation time constant of variable  $x$  ( $x = m, h$  or  $n$ ). These kinetic equations can be rewritten as

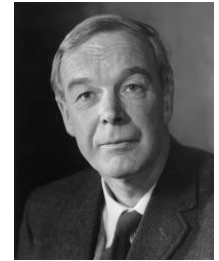
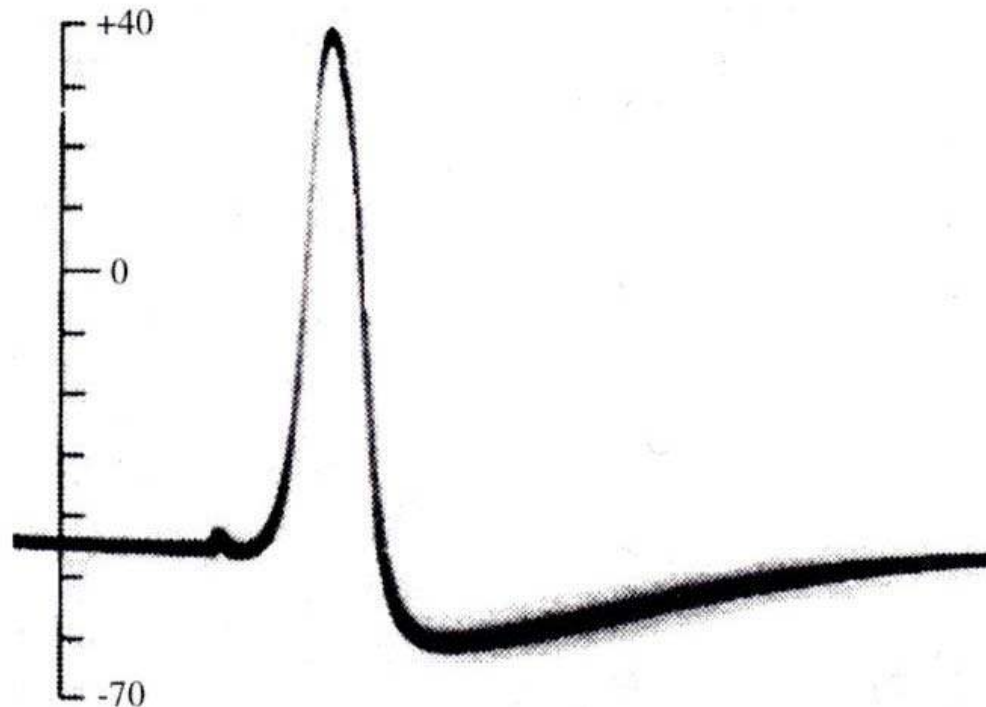
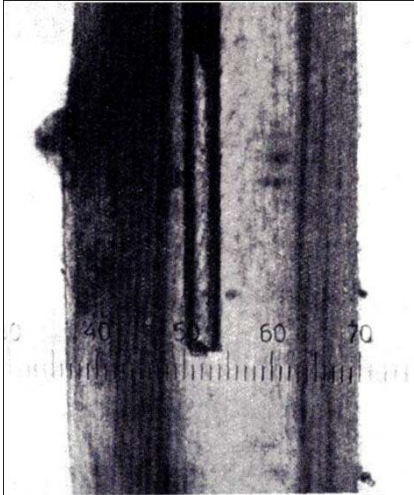
$$\frac{dx}{dt} = \alpha_x(V)(1 - x) - \beta_x(V)x.$$

The rate functions  $\alpha_x$  and  $\beta_x$  are now interpreted as mean transition rates of ionic channels from closed to open state and vice versa. Values of all parameters involved – obtained by fitting voltage-clamp data – can be found in Ref. [a].

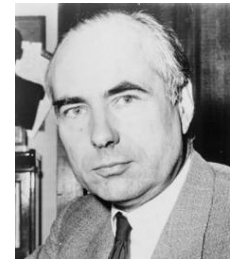
### Reference

- a Hodgkin, A.L. and Huxley, A.F. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* 117, 500–544

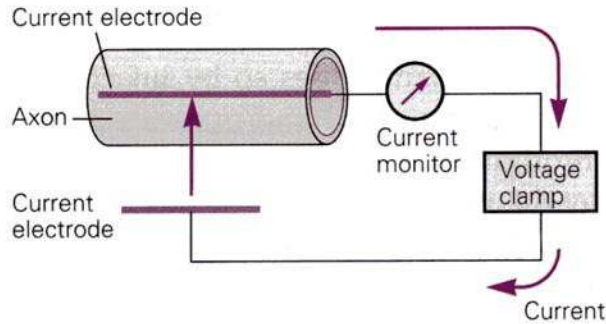
## Potential changes during Action Potential



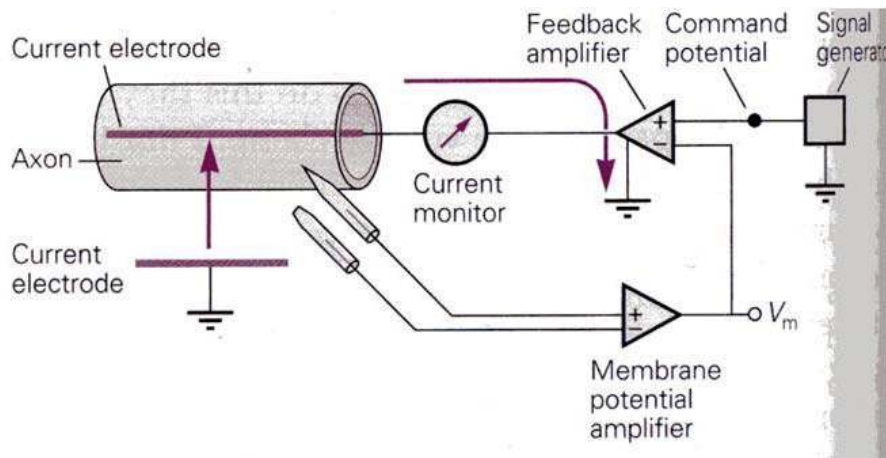
A. L.  
Hodgkin



A.F.  
Huxley

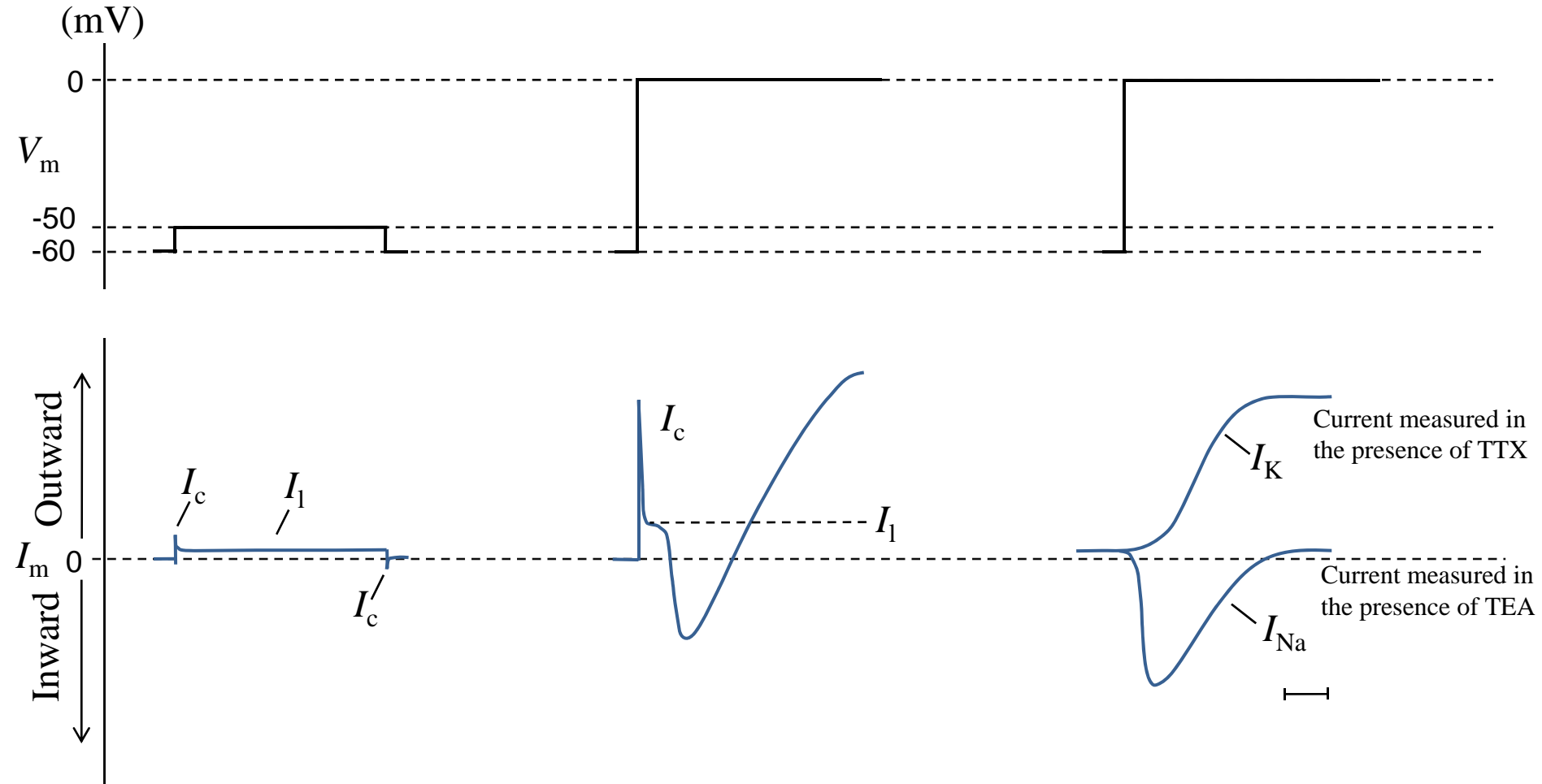


The voltage clamp is a current generator



The voltage clamp operates under a negative feedback mechanism

## Demonstration of ionic movements during Action Potential

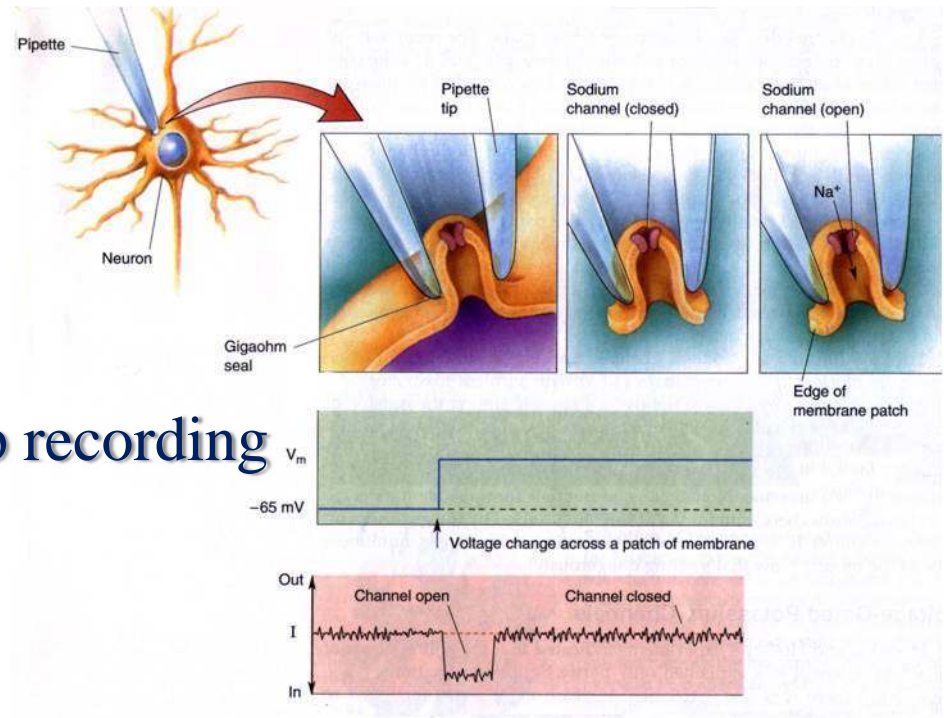
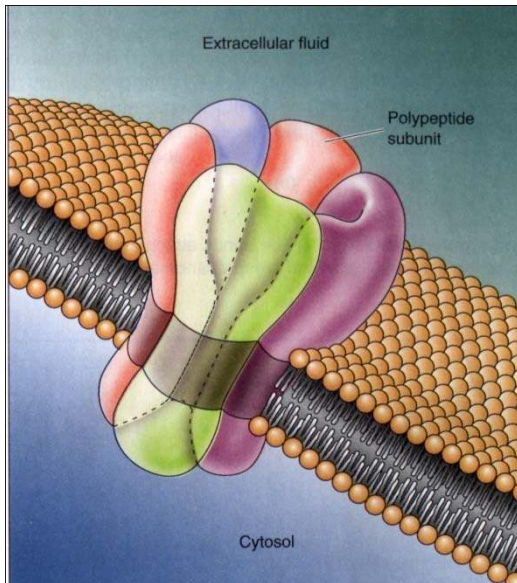




## The Nobel Prize in Physiology or Medicine 1991

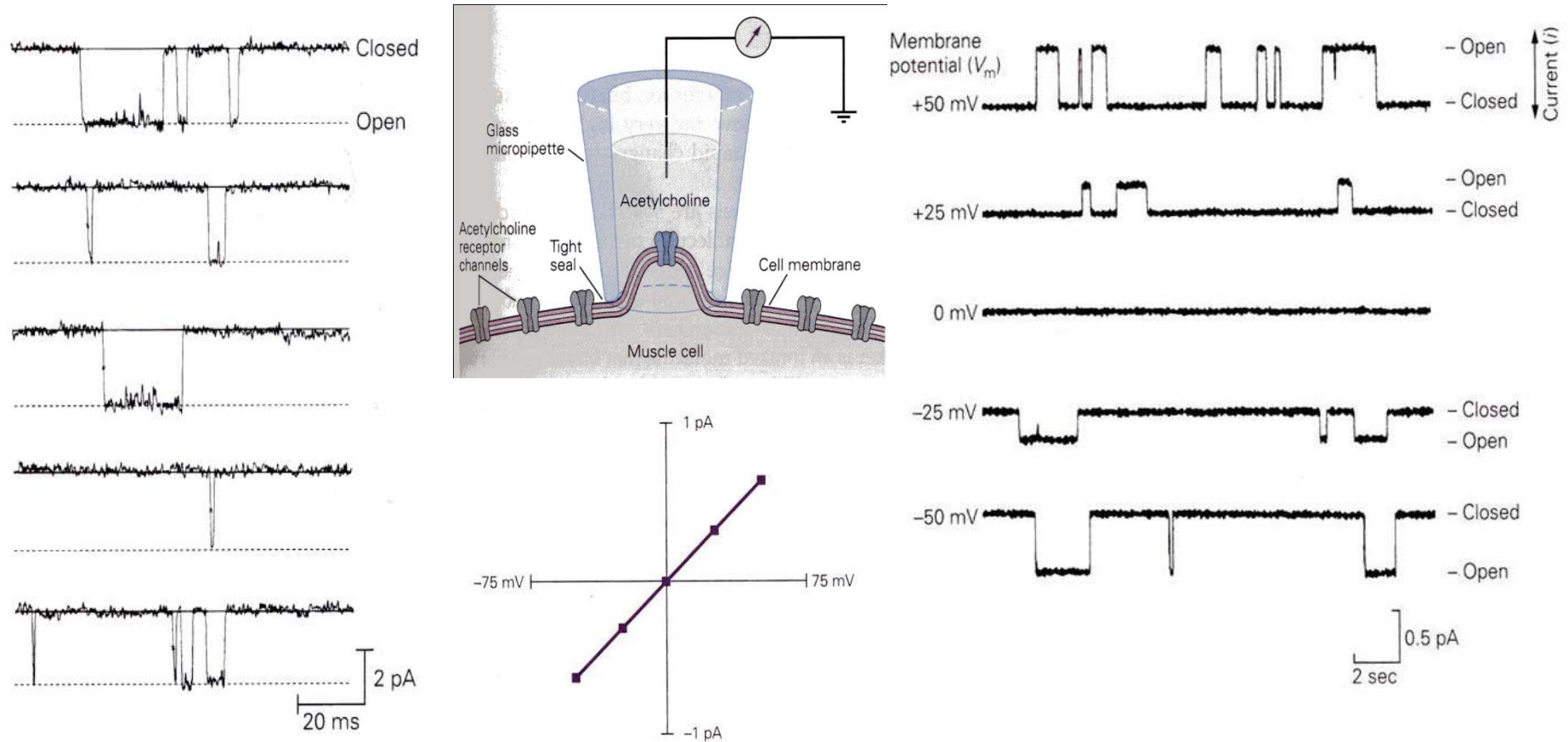
**"for their discoveries concerning the function of single ion channels in cells"**

Erwin Neher Bert Sakmann

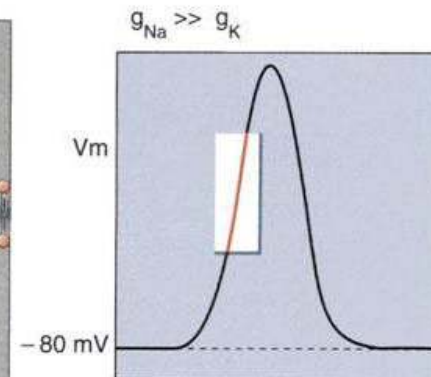
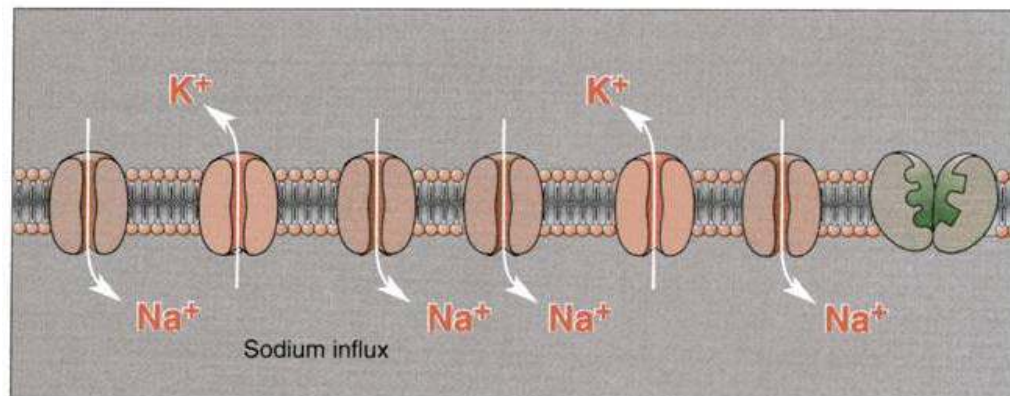
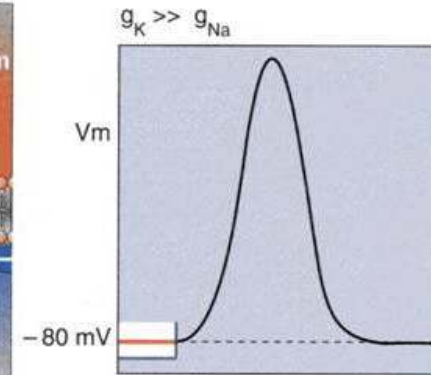
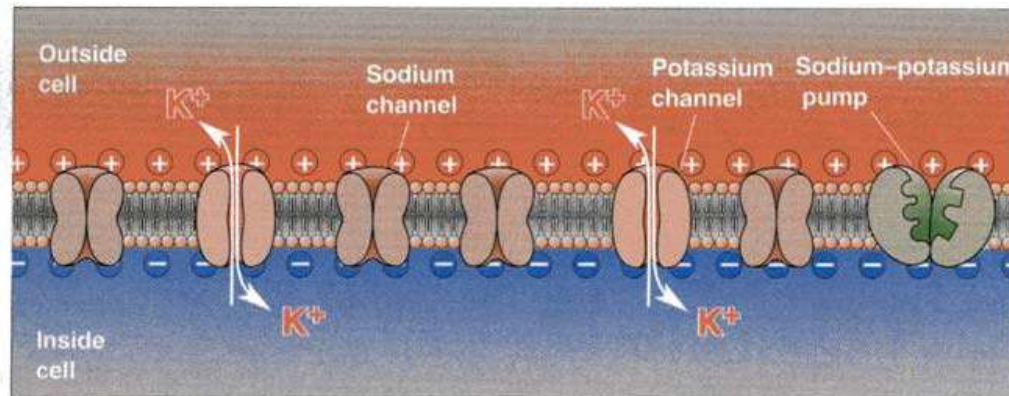


## Patch-clamp recording

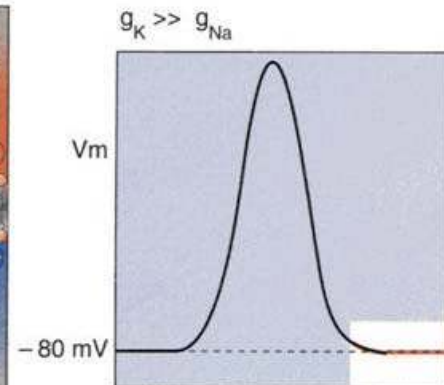
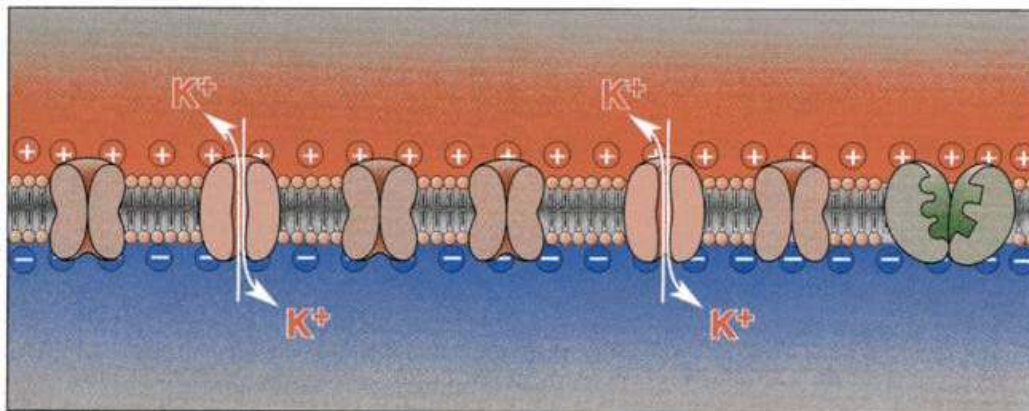
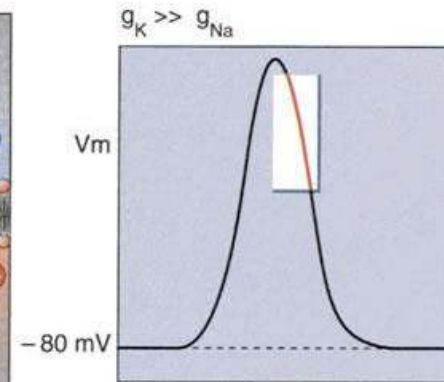
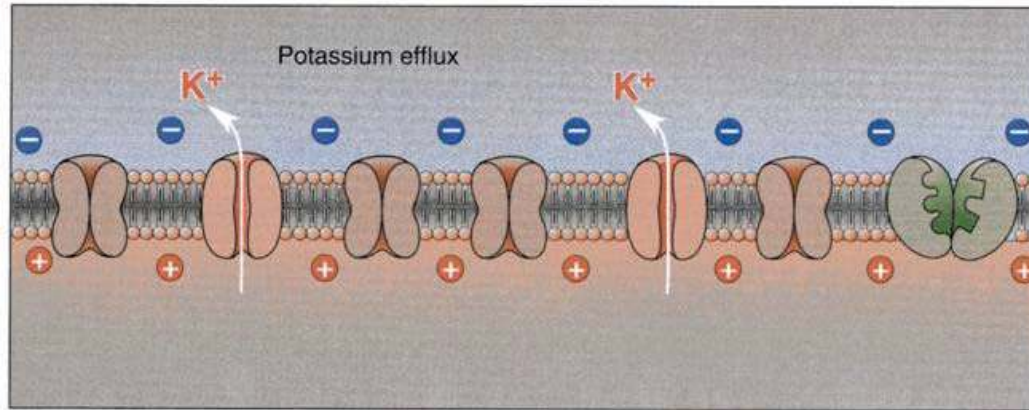
## Direct examination of channel proteins



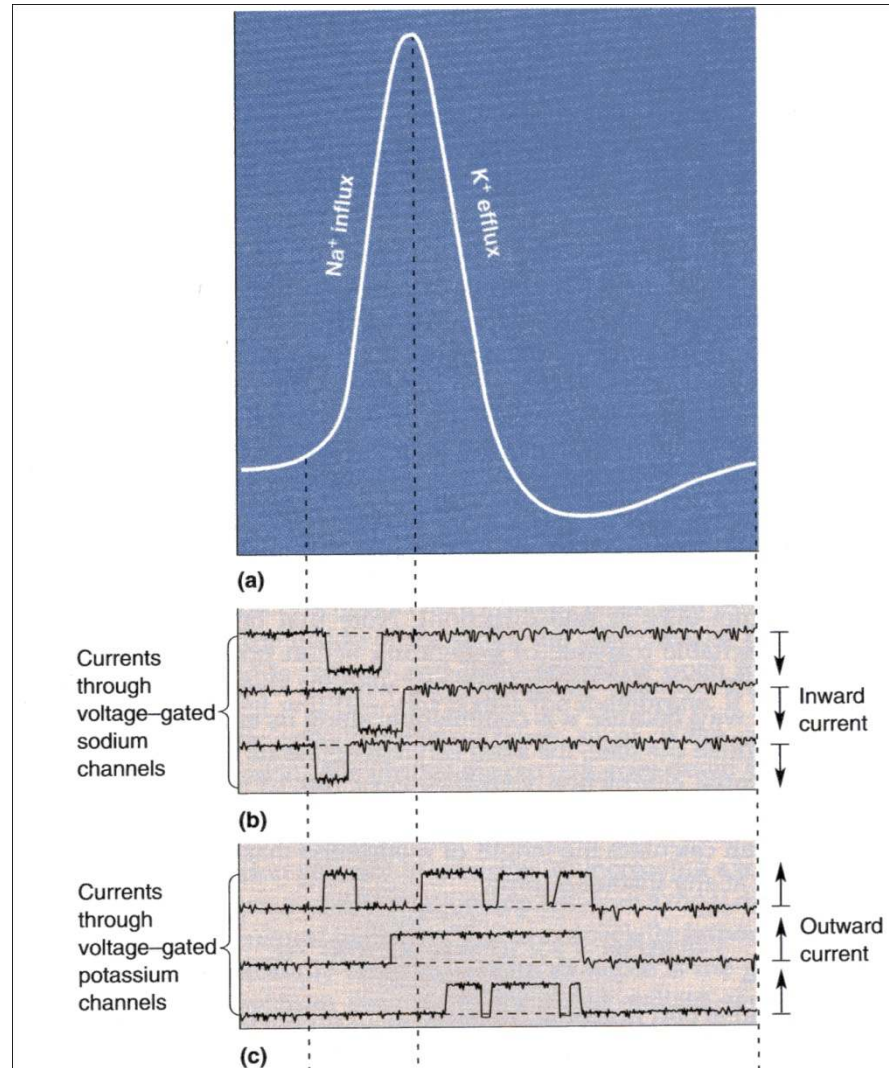
## Movement of ions during Action Potential

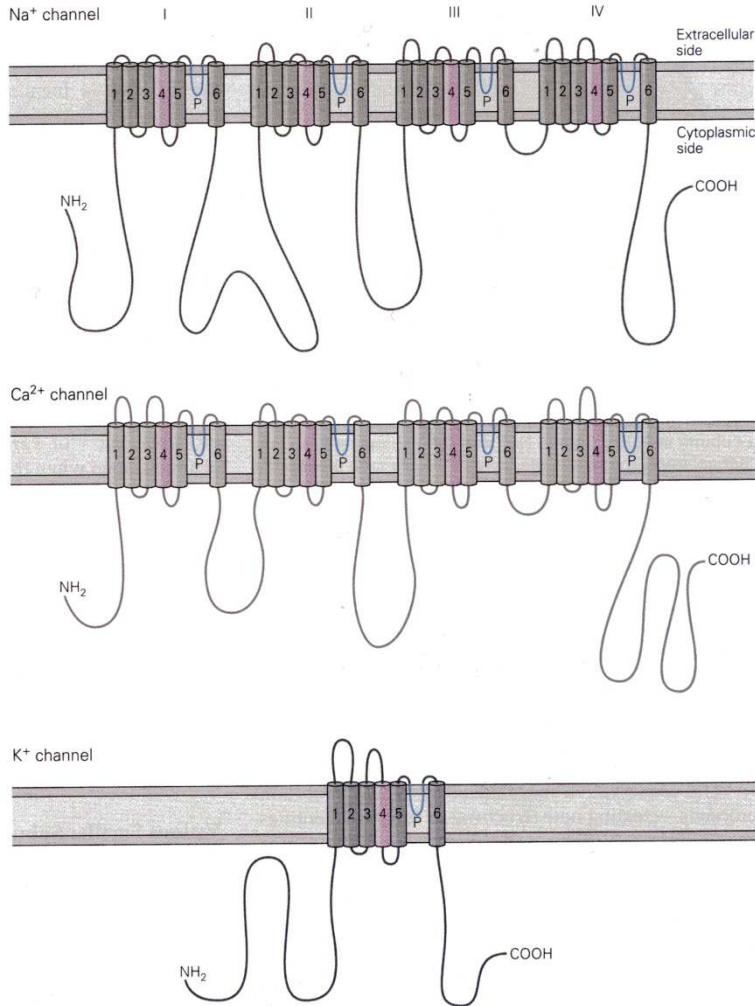


## Movement of ions during Action Potential

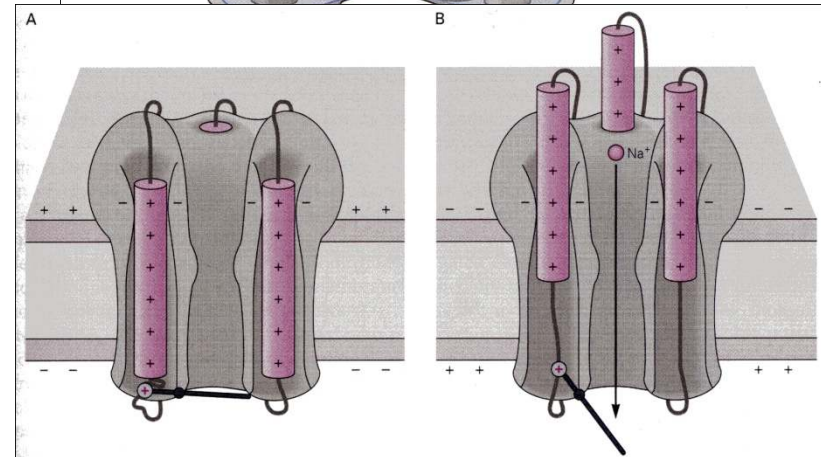
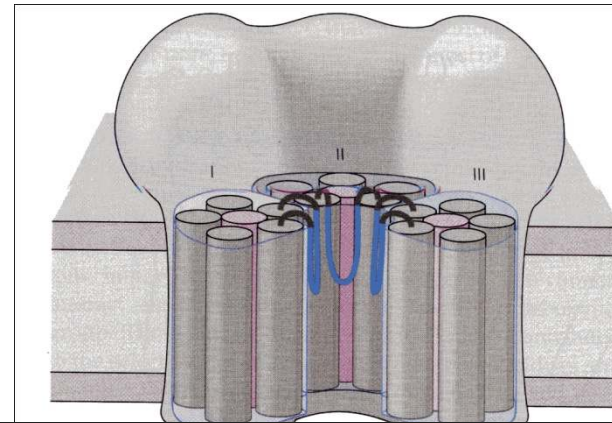


## Opening of ion channels during Action Potential

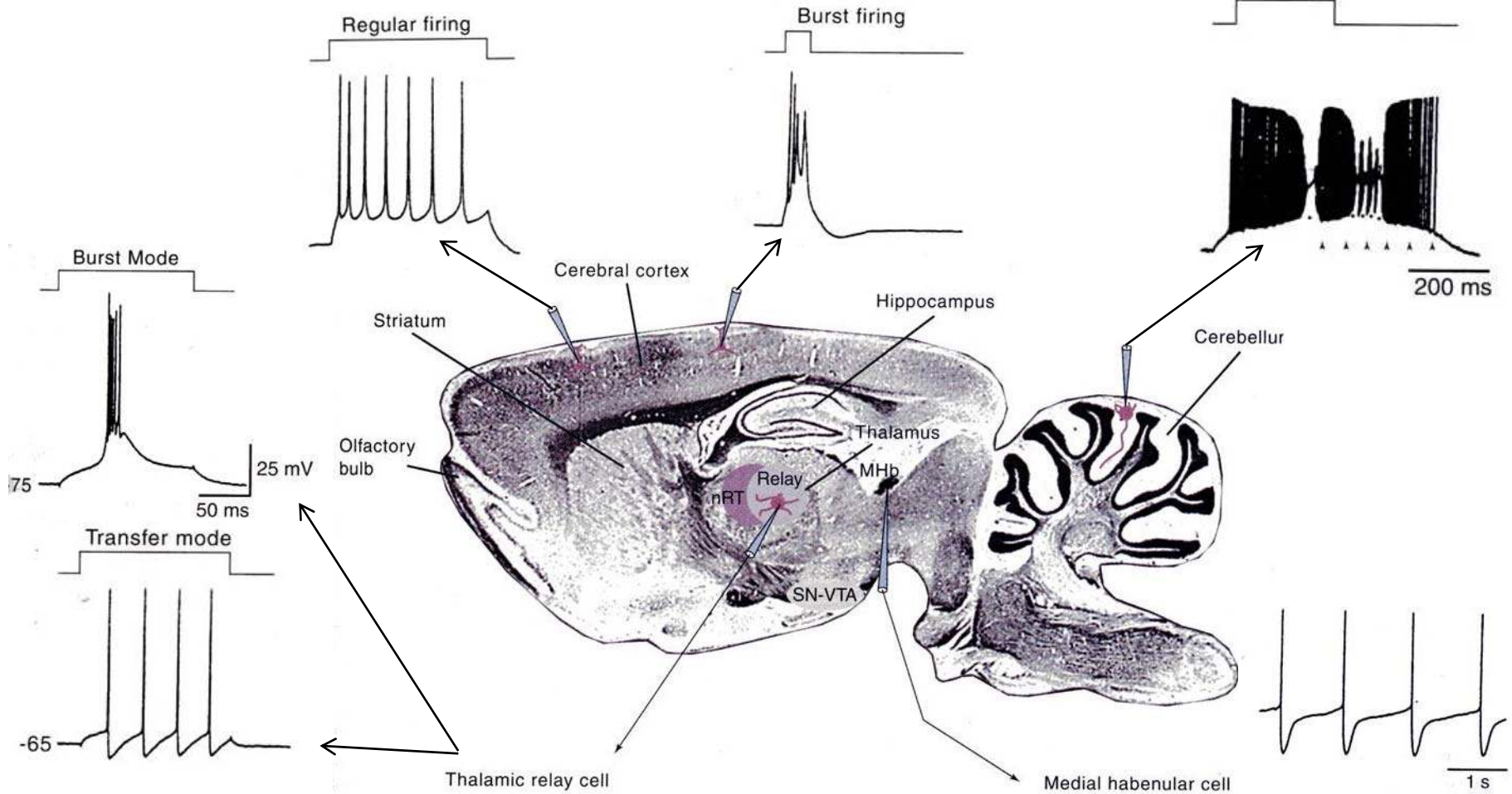




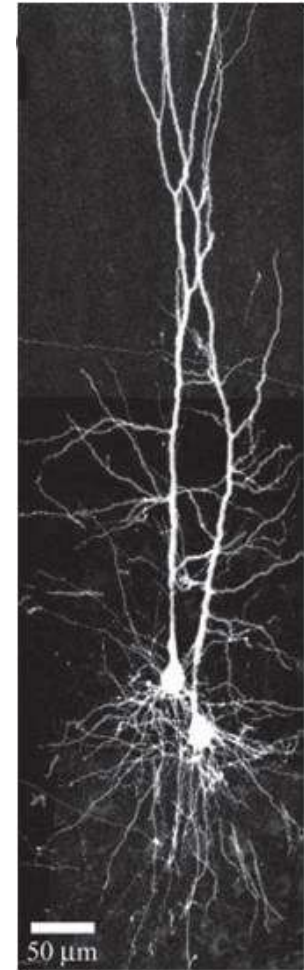
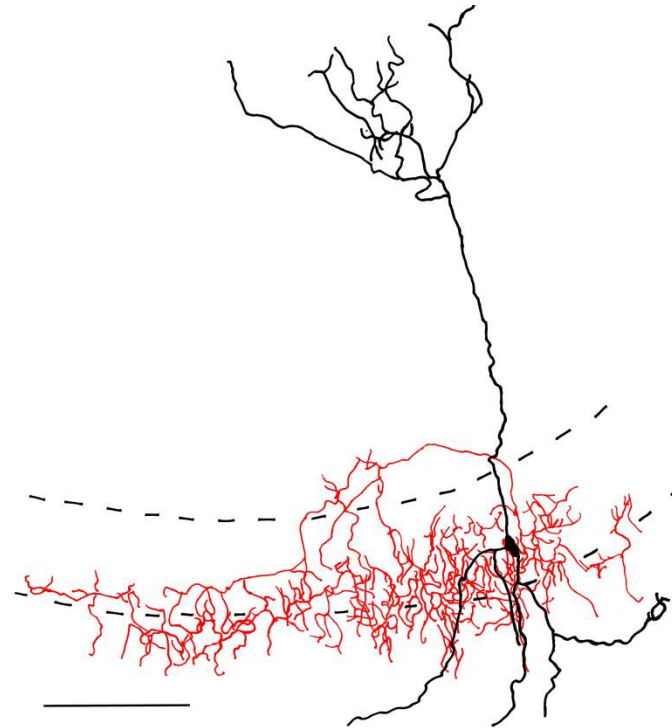
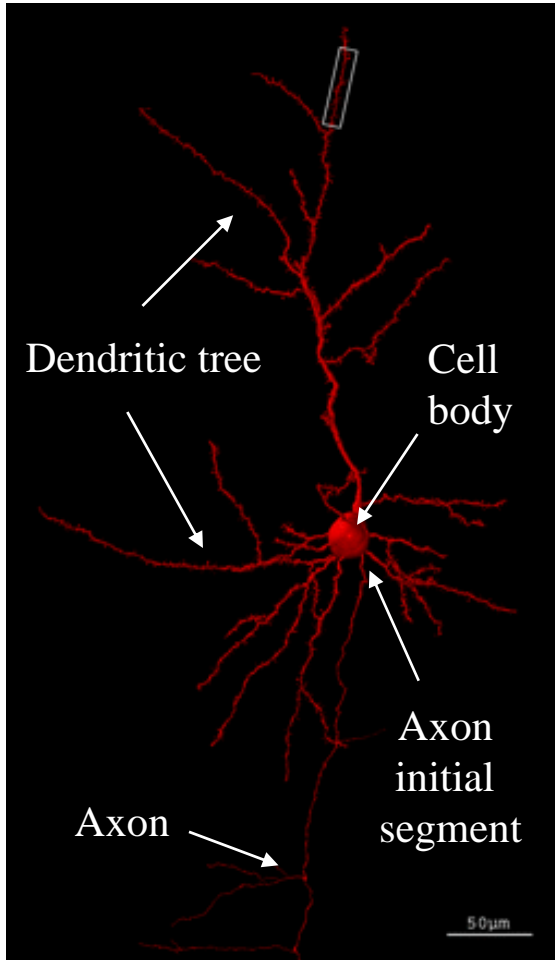
## Structure and function of voltage-gated ion channels



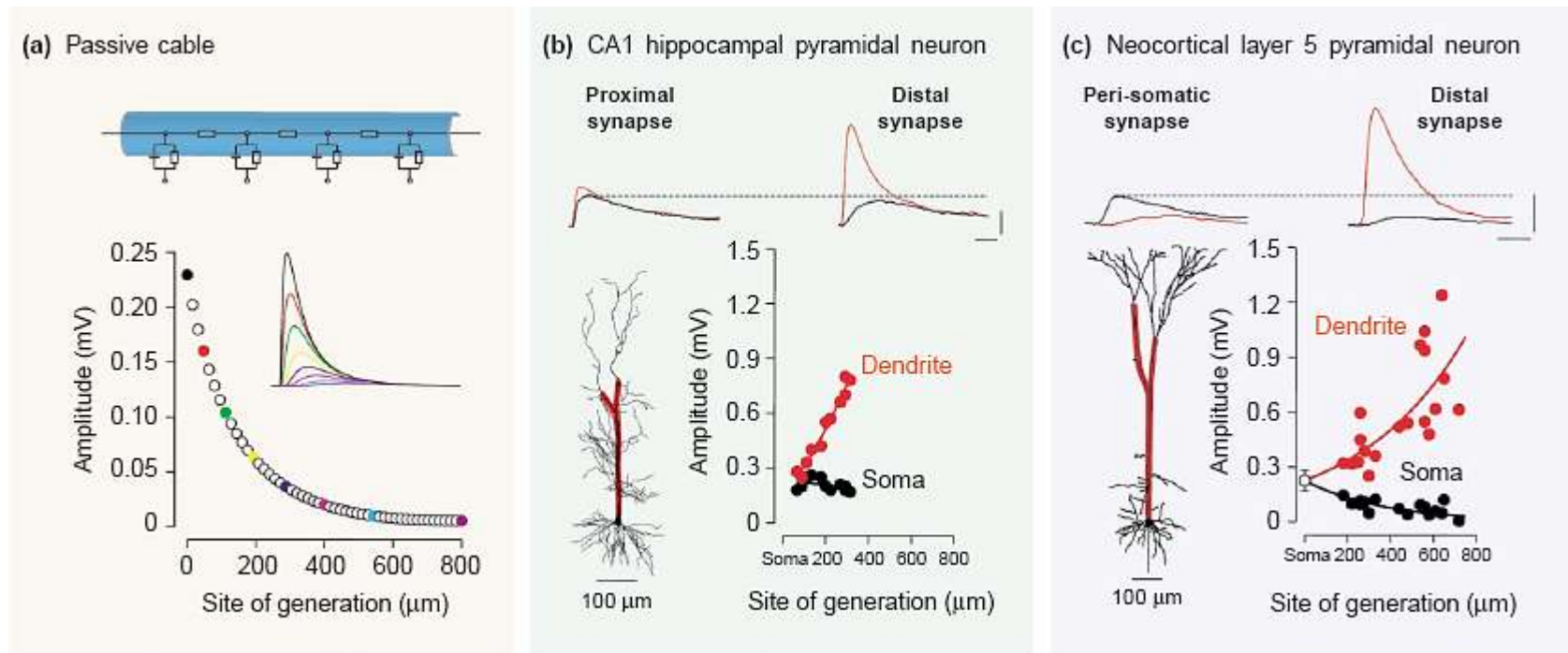
## Great variety of firing activity of neurons



## Morphology of neurons



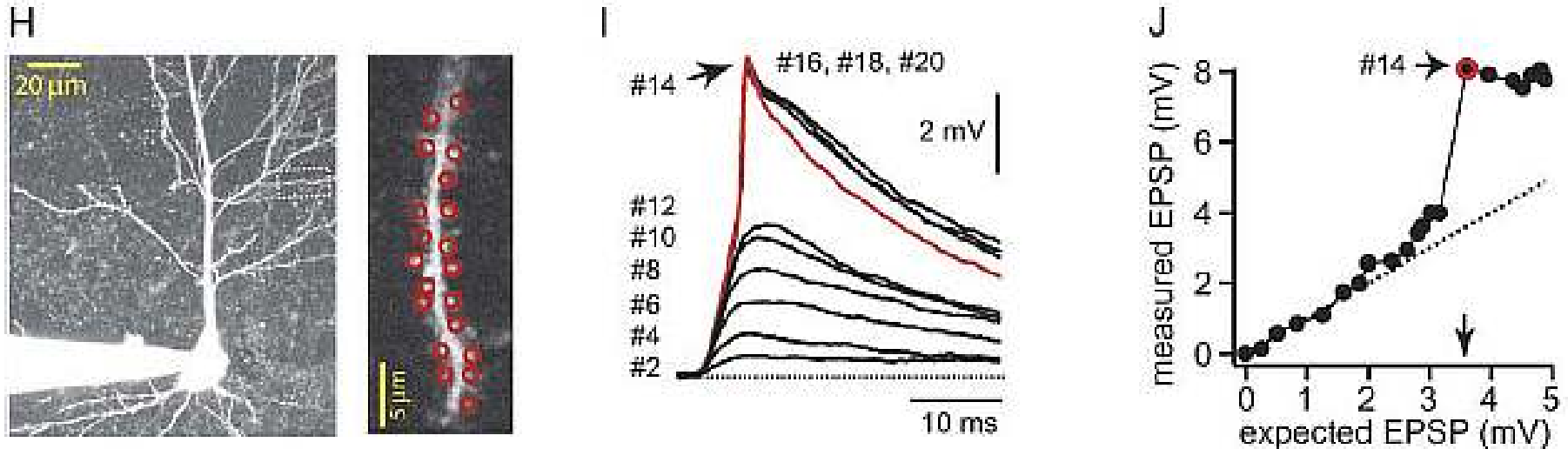
## Synaptic potentials, passive and active dendrites



TRENDS in Neurosciences

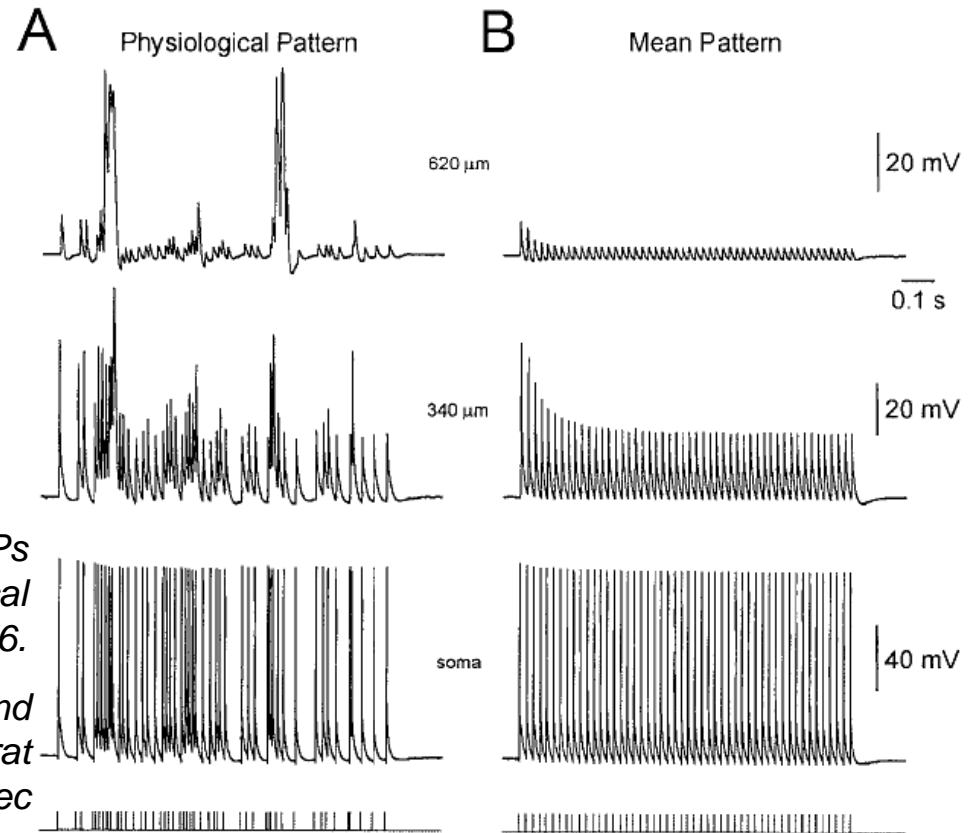
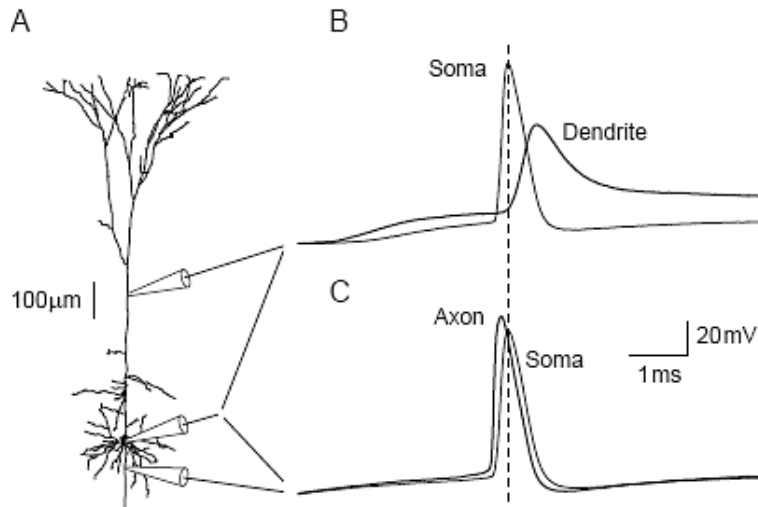
**Ref:** Williams SR, Stuart GJ. Role of dendritic synapse location in the control of action potential output. *Trends Neurosci.* 2003 Mar;26(3):147-54.

The asynchronous excitatory input is summed linearly, whereas the synchronous input supralinearly in the dendrites



**Ref:** Losonczy A, Magee JC. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron*. 2006 Apr 20;50(2):291-307.

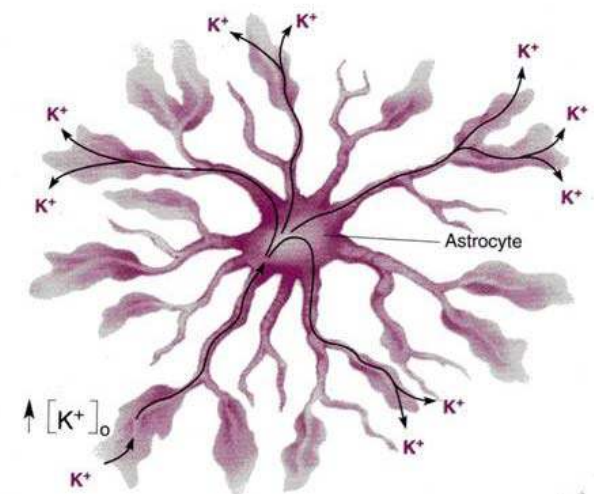
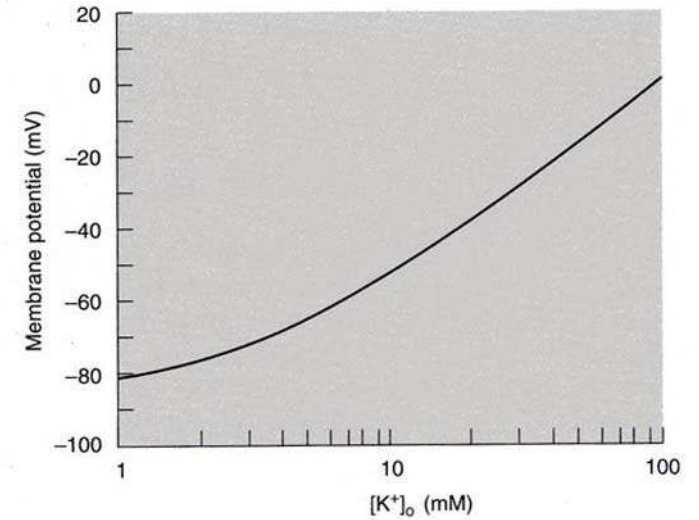
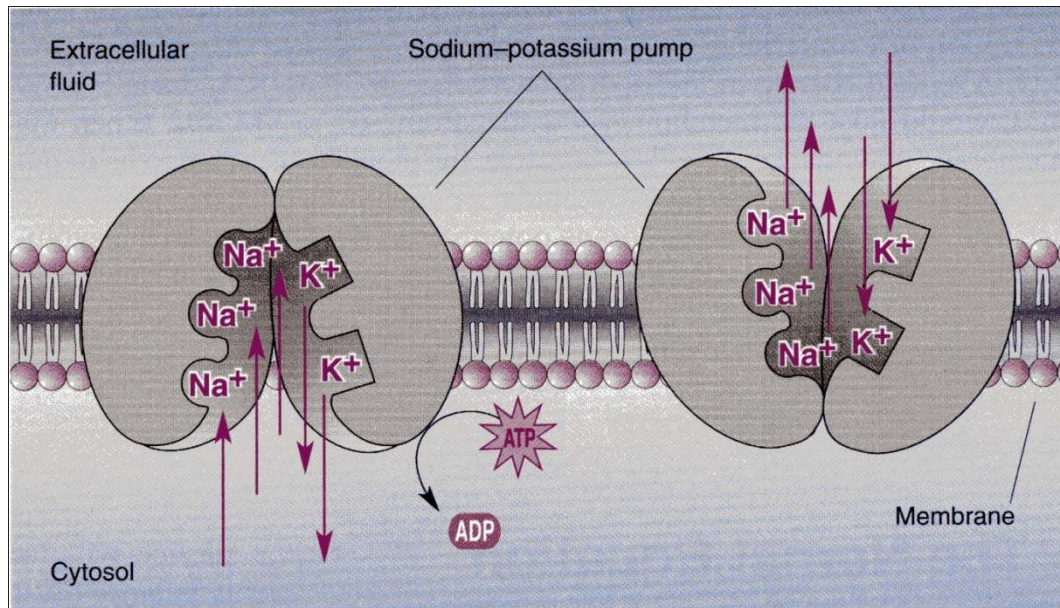
## The amplitude of the retrograde propagating action potential in the dendrite is dependent on the firing pattern



**Ref:** Stuart G, Sakmann B. Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. *Neuron*. 1995 Nov;15(5):1065-76.

**Ref:** Williams SR, Stuart GJ. Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. *J Physiol*. 1999 Dec 1;521 Pt 2:467-82.

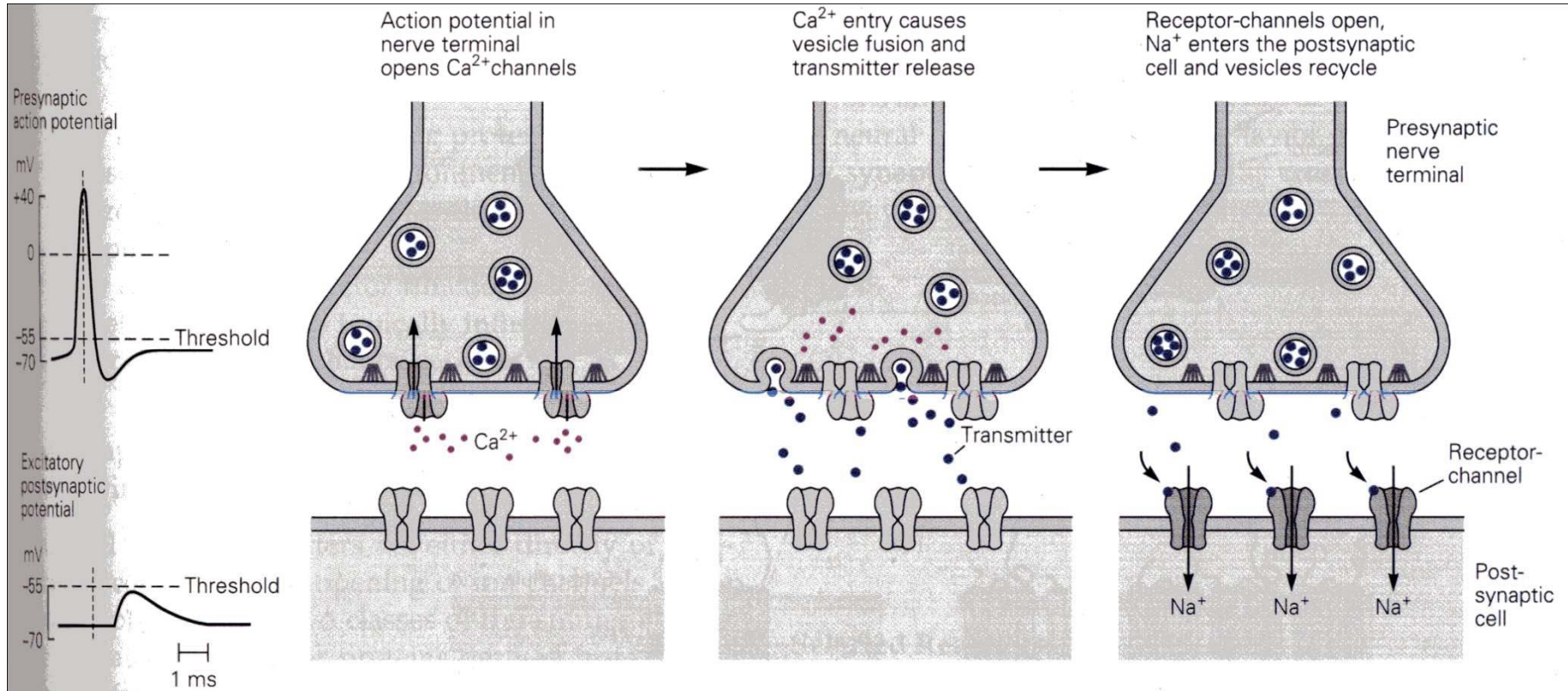
## Removal of $K^+$ ions from the extracellular space



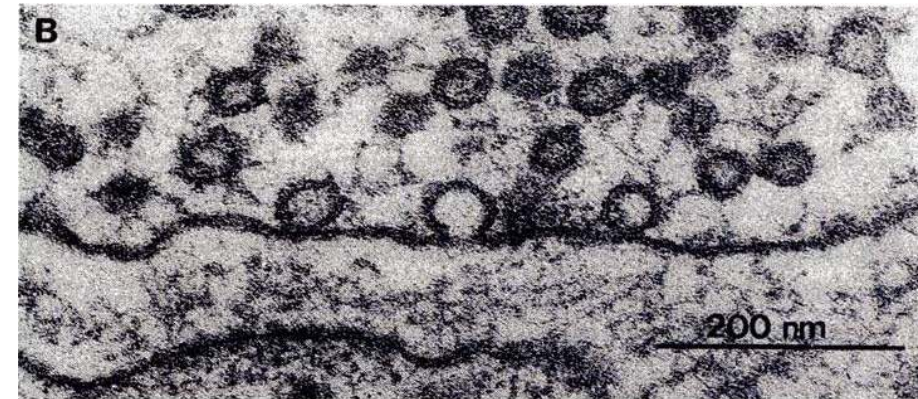
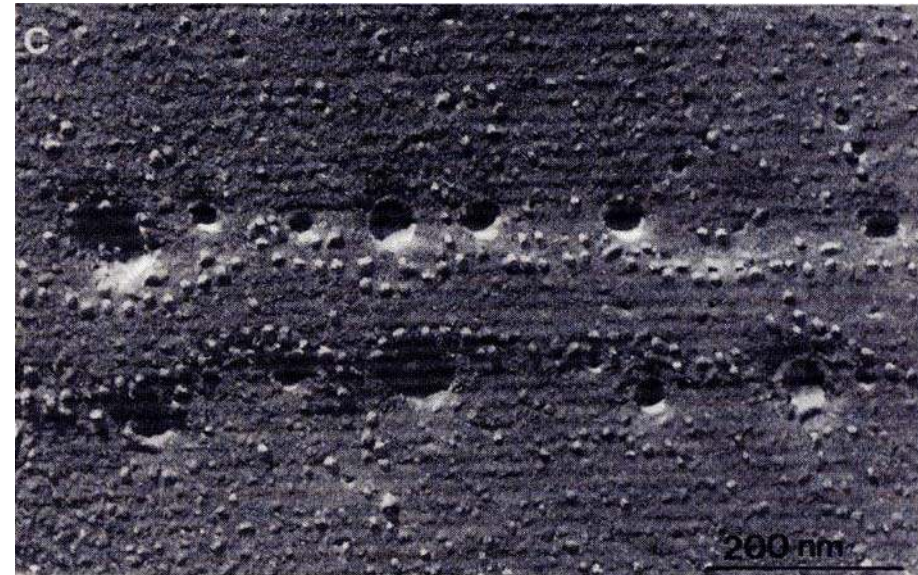
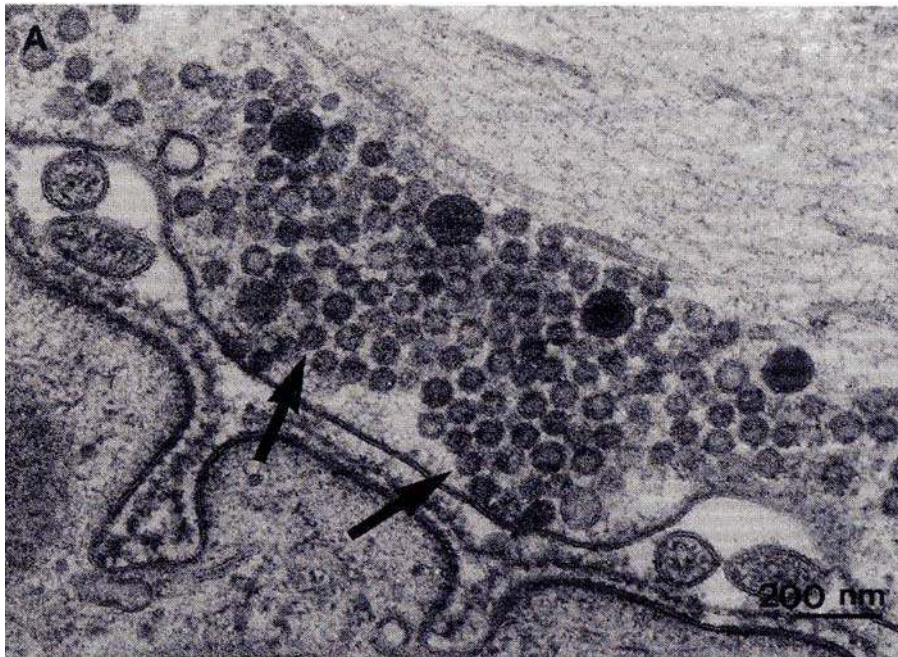


## Synaptic neurotransmission The presynaptic side

## Mechanism of neurotransmission

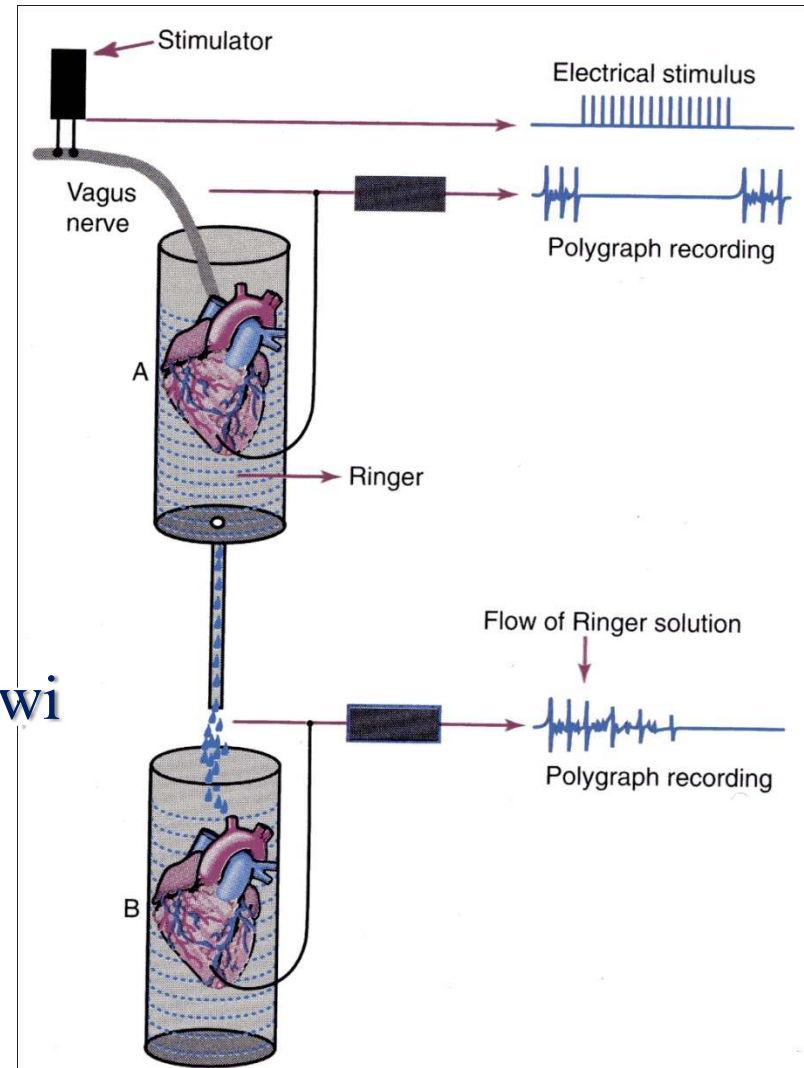


## Ultrastructure of axon terminals

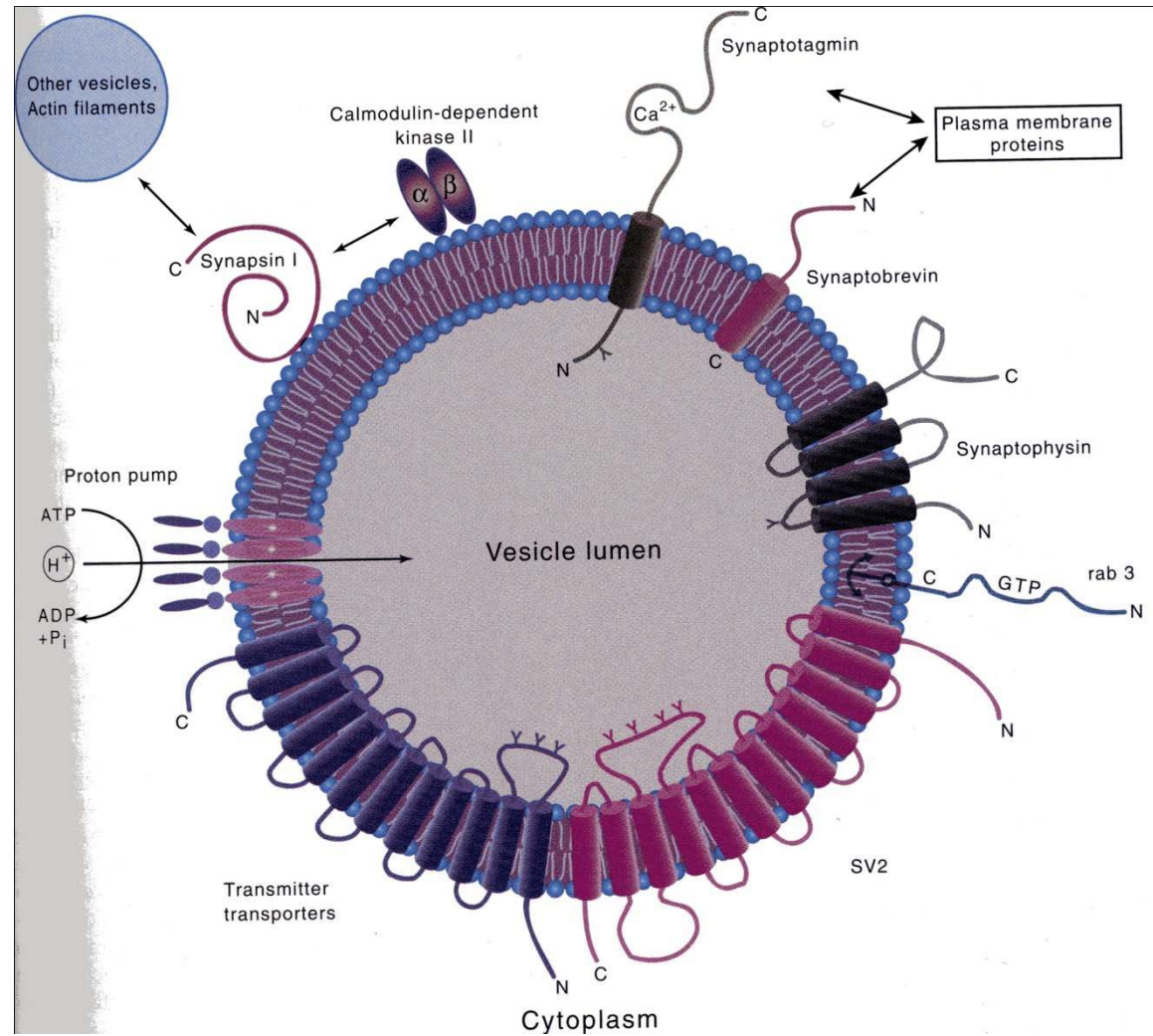


## Proving the chemical nature of neurotransmission

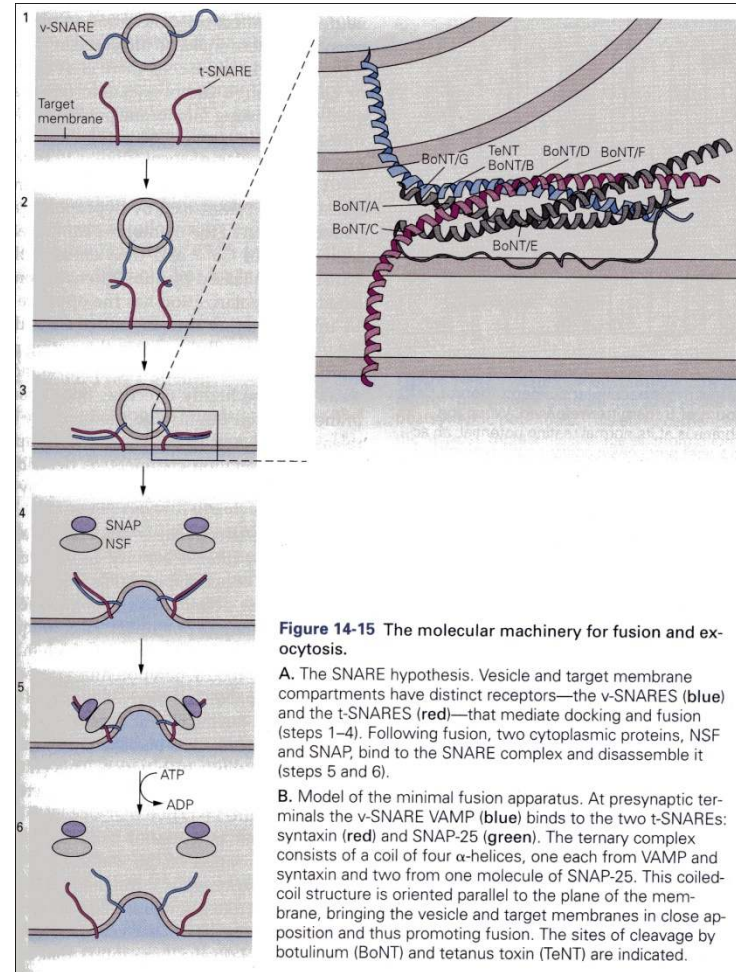
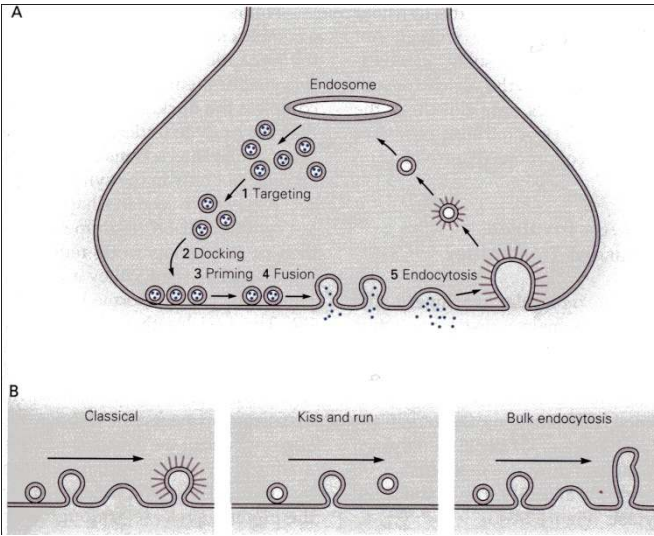
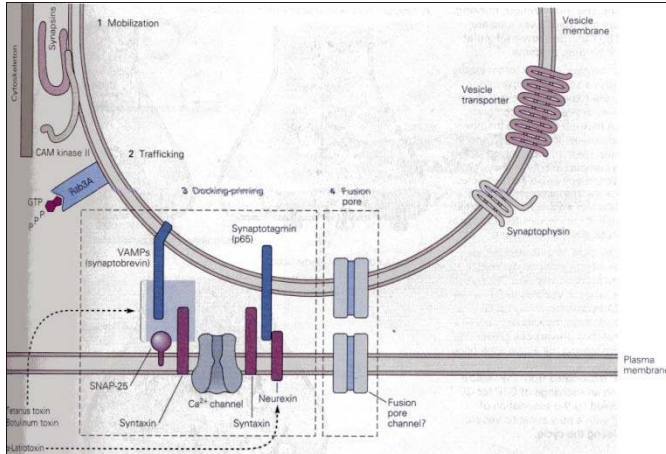
### Experiment of Otto Loewi in 1920



## Synaptic vesicle and its associated proteins



## Release of the content of synaptic vesicle



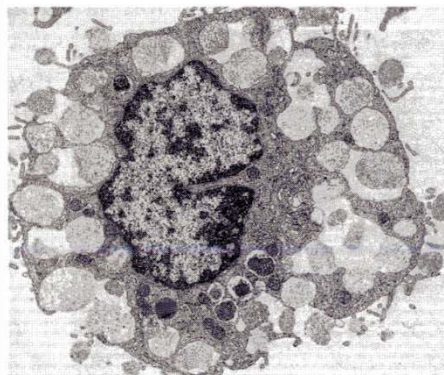
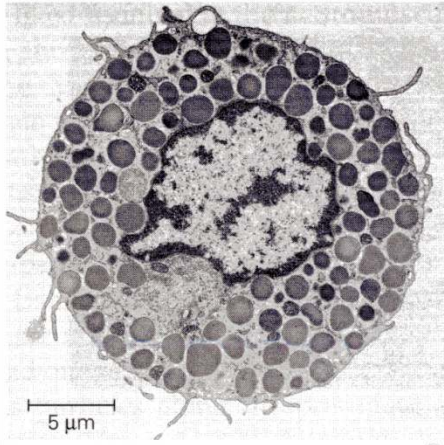
**Figure 14-15** The molecular machinery for fusion and exocytosis.

**A.** The SNARE hypothesis. Vesicle and target membrane compartments have distinct receptors—the v-SNAREs (blue) and the t-SNAREs (red)—that mediate docking and fusion (steps 1–4). Following fusion, two cytoplasmic proteins, NSF and SNAP, bind to the SNARE complex and disassemble it (steps 5 and 6).

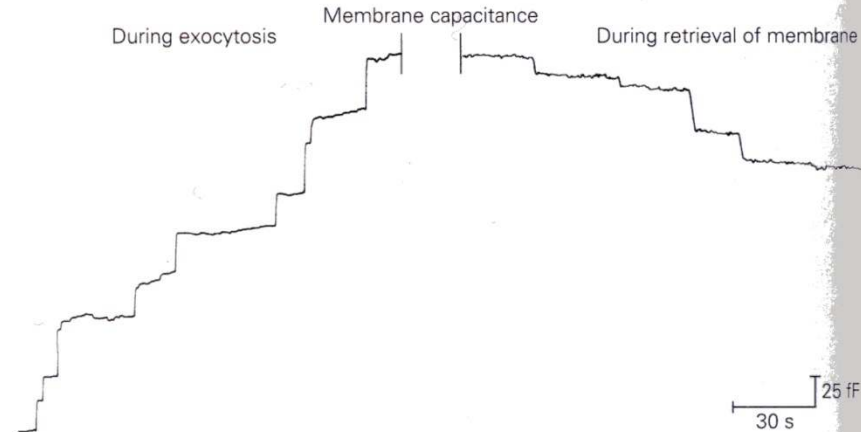
**B.** Model of the minimal fusion apparatus. At presynaptic terminals the v-SNARE VAMP (blue) binds to the two t-SNAREs: syntaxin (red) and SNAP-25 (green). The ternary complex consists of a coil of four  $\alpha$ -helices, one each from VAMP and syntaxin and two from one molecule of SNAP-25. This coiled-coil structure is oriented parallel to the plane of the membrane, bringing the vesicle and target membranes in close apposition and thus promoting fusion. The sites of cleavage by botulinum (BoNT) and tetanus toxin (TeNT) are indicated.

## Exocytosis increase the capacitance of the cell

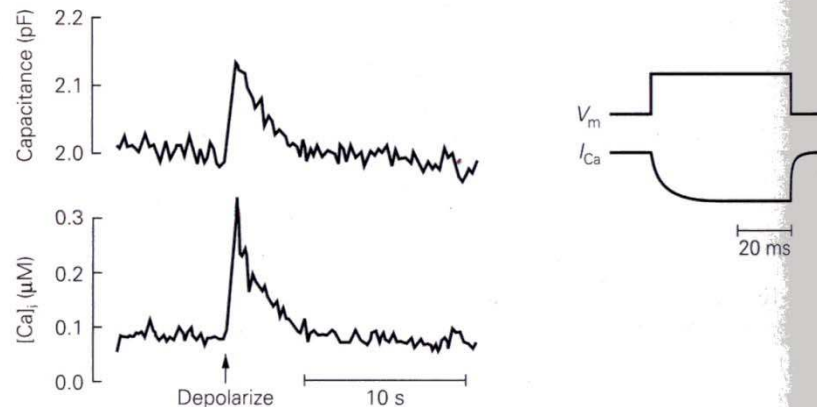
A Mast cell before and after exocytosis



B Membrane capacitance during and after exocytosis of mast cell vesicles



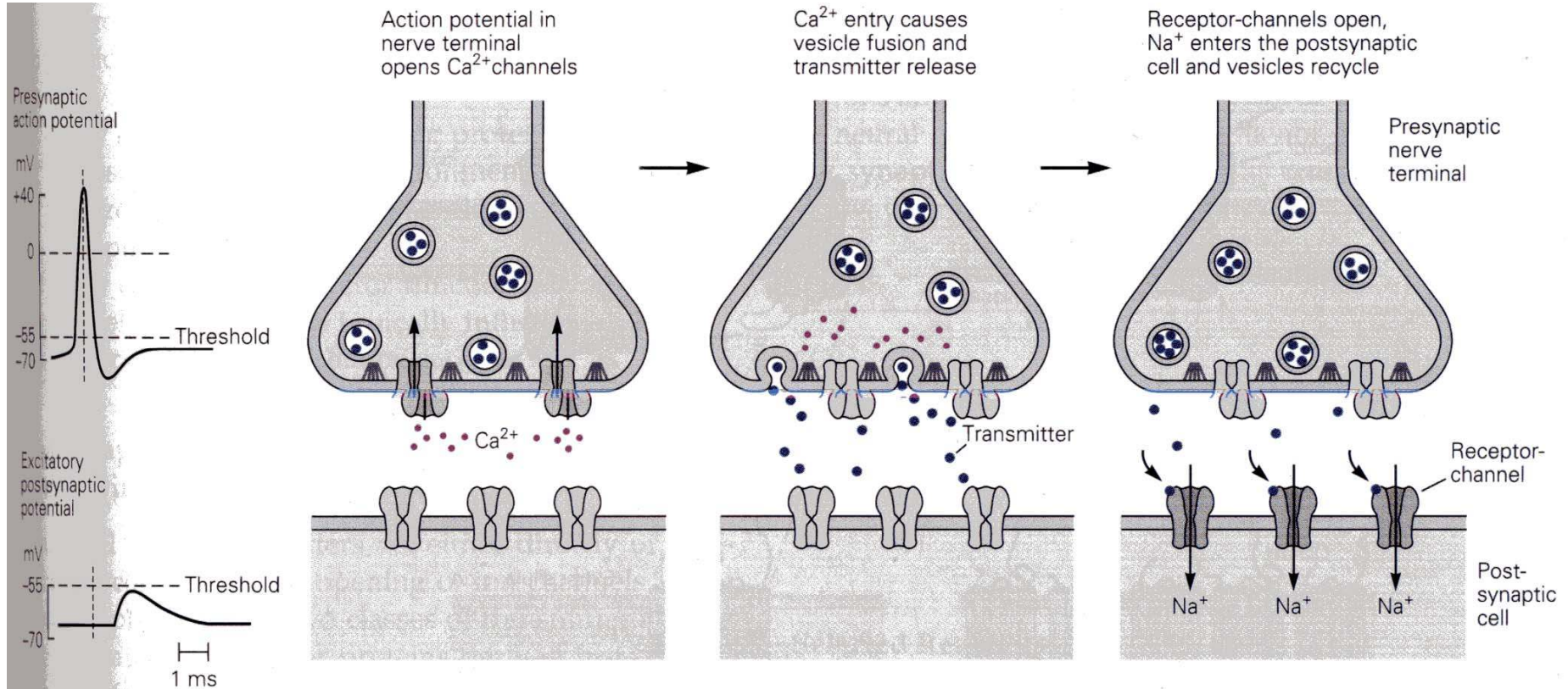
C Calcium-dependent exocytosis of synaptic vesicles





## Synaptic neurotransmission The postsynaptic side

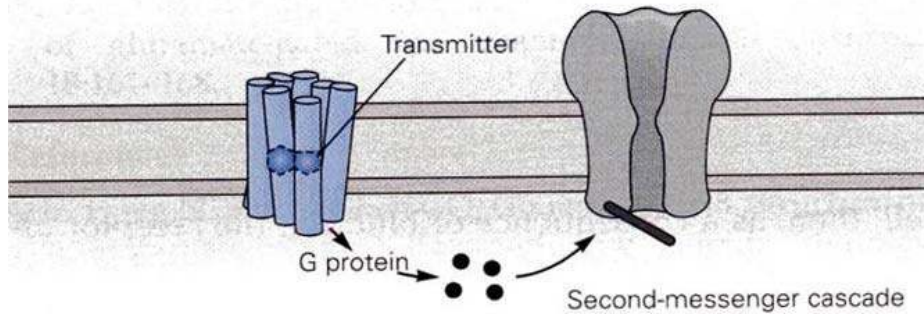
## Mechanism of neurotransmission



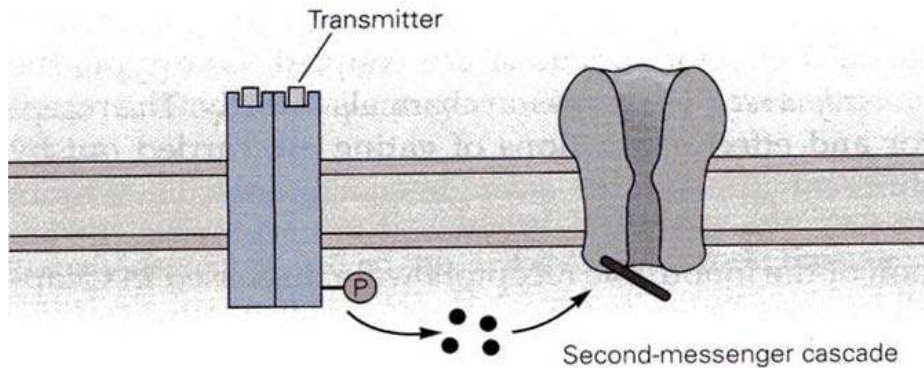
## Structure of postsynaptic ion channels and receptor proteins

### Indirect gating

#### *G protein-coupled receptor*

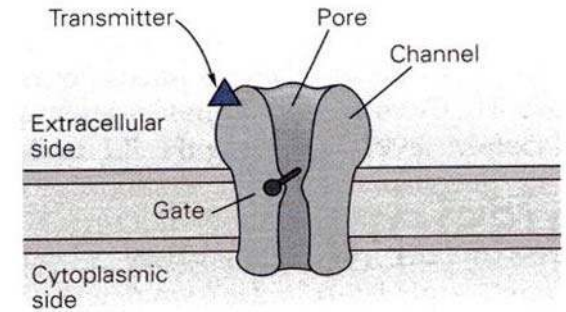


#### *Receptor tyrosine kinase*

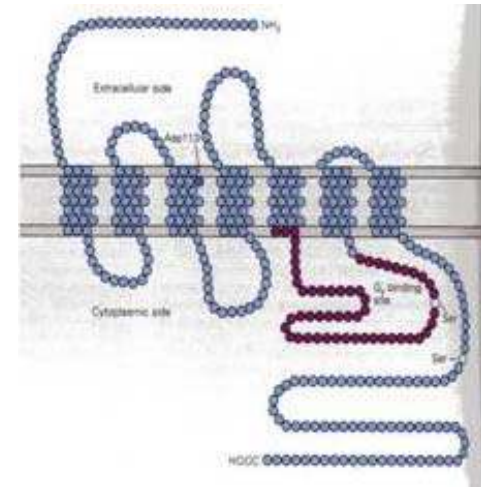


### Direct gating

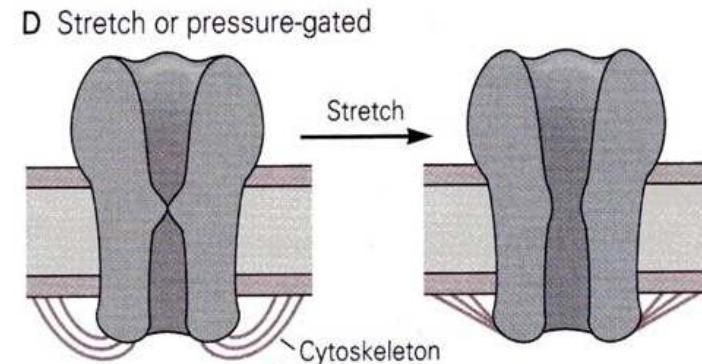
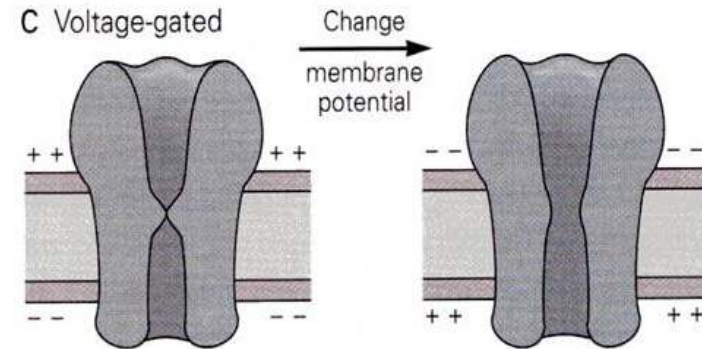
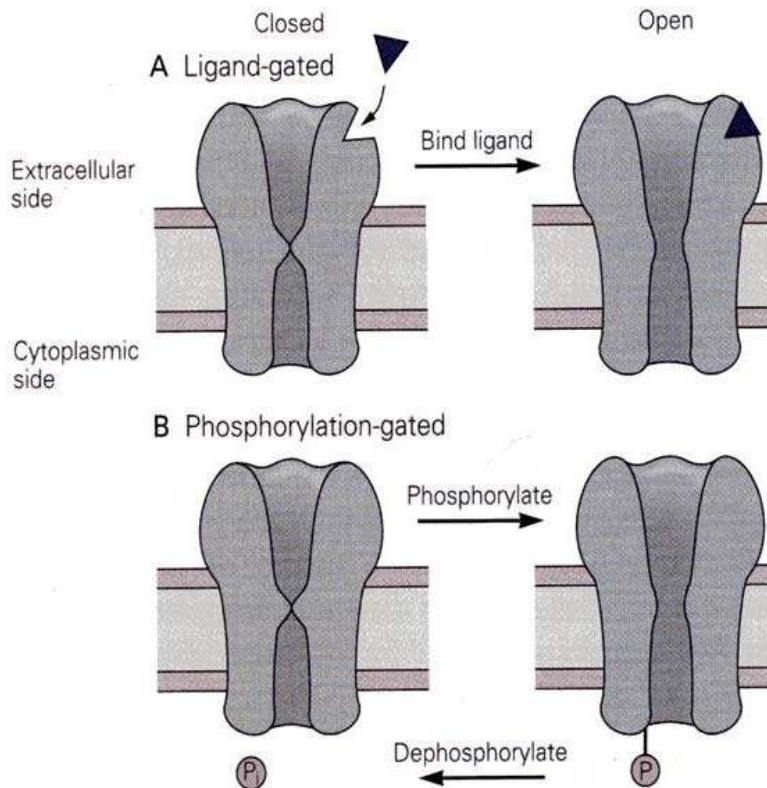
#### *Ionotropic receptor*



### Structure of metabotropic receptors



## Function of channel proteins



## Fast (10ms) – ionotropic - neurotransmission

### Excitatory:

- glutamate receptors - AMPA r. ( $\text{Na}^+$  and  $\text{K}^+$ )  
kainate r. ( $\text{Na}^+$  and  $\text{K}^+$ )  
NMDA r. ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ )
- acetylcholine receptors - nicotine r. ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ )
- serotonin receptors - 5HT3 r. ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ )

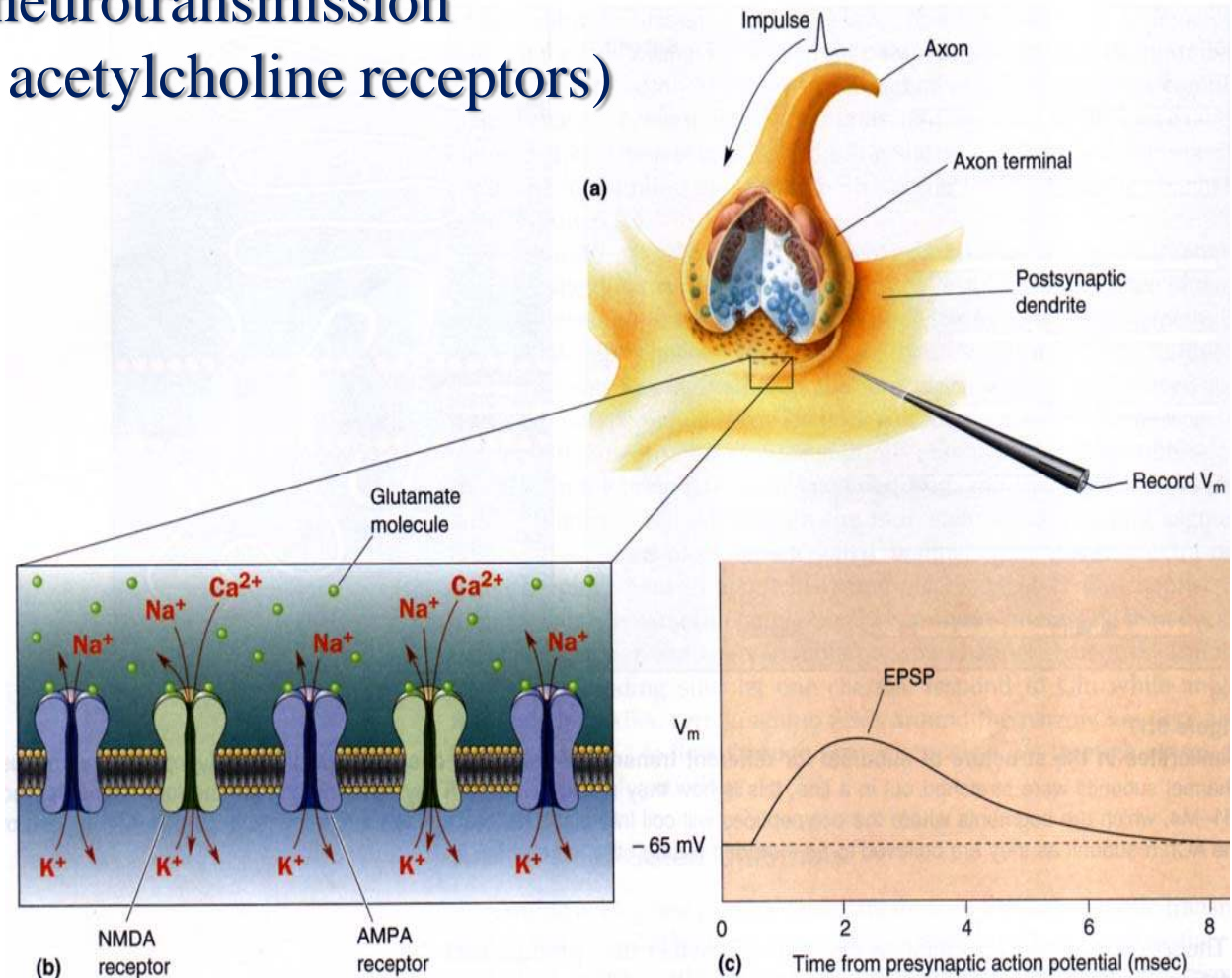
### Inhibitory:

- GABA receptors - GABA<sub>A</sub> r. ( $\text{Cl}^-$ )
- glycine receptors ( $\text{Cl}^-$ )

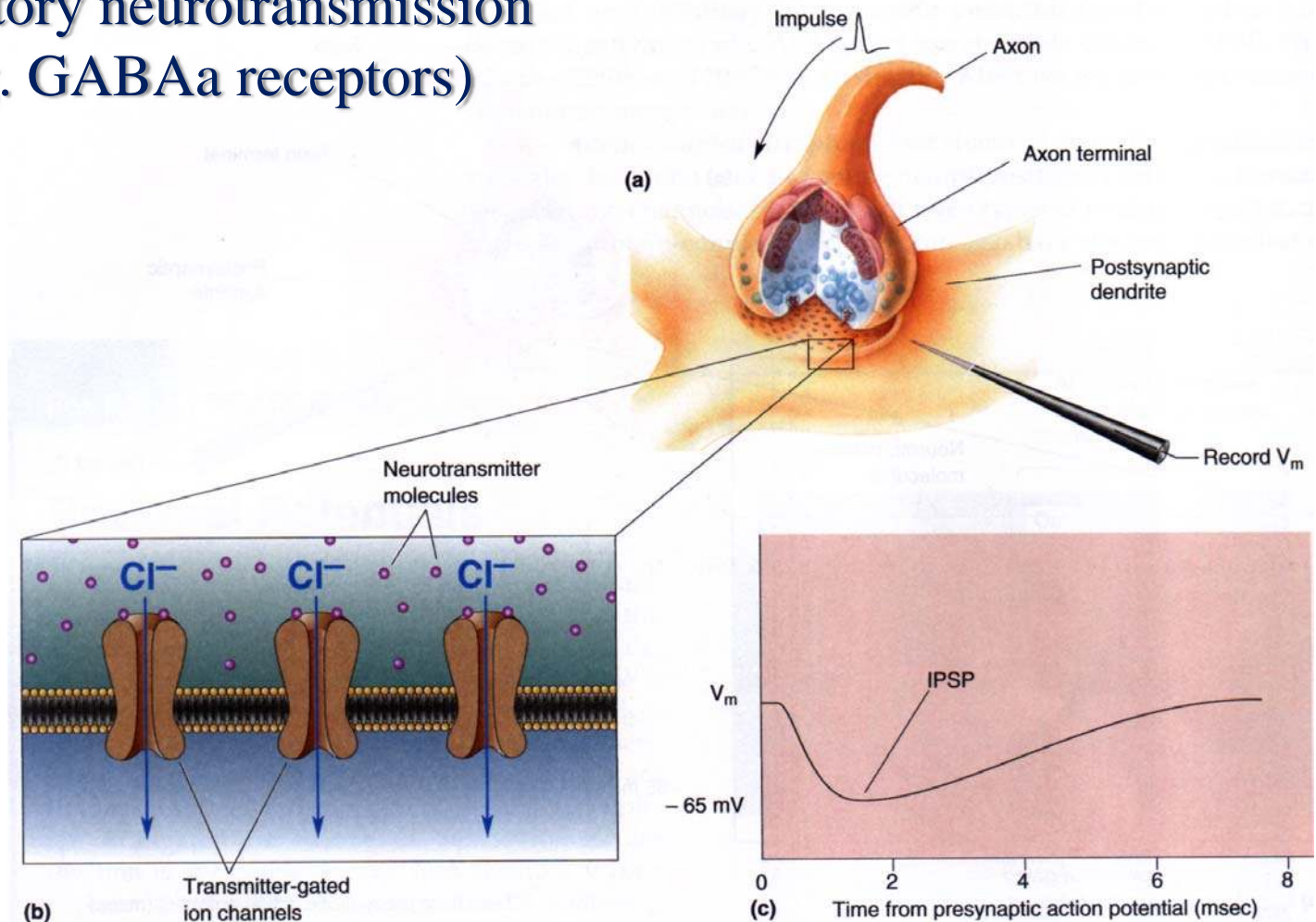
## Slow (100ms) – metabotropic - neurotransmission

- glutamate receptors - mGluR r. (mGluR1-8)
- GABA receptors - GABA<sub>B</sub> r.
- acetylcholine receptors - muscarinic r. (M1-5)
- serotonin receptors - 5HT1-8 r.
- dopamine receptors - D1-D6 r.
- adrenergic receptors - alpha 1,2 r.; beta1,2 r.
- histamine - H1-3 r.

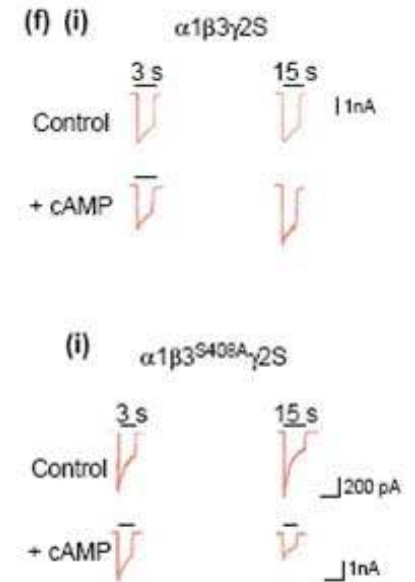
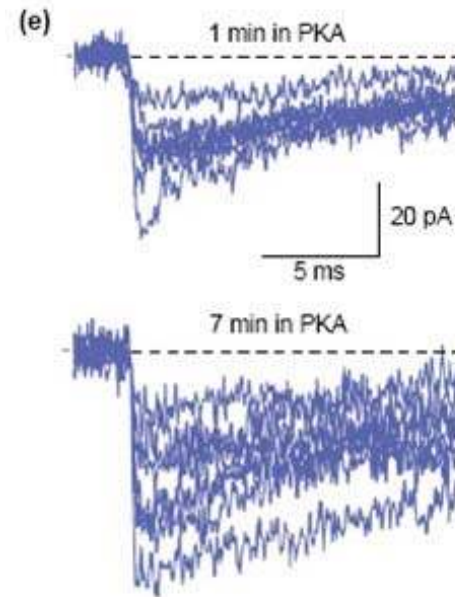
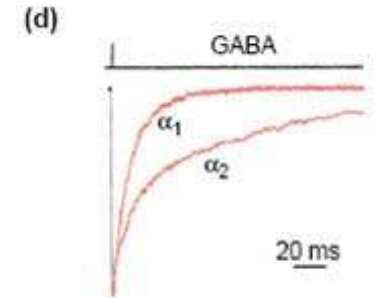
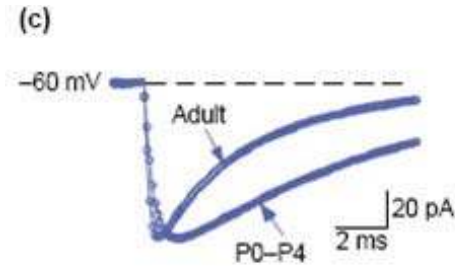
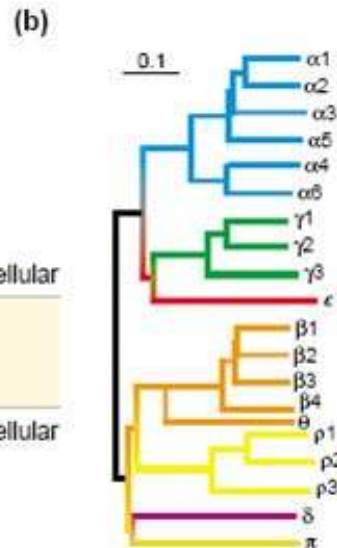
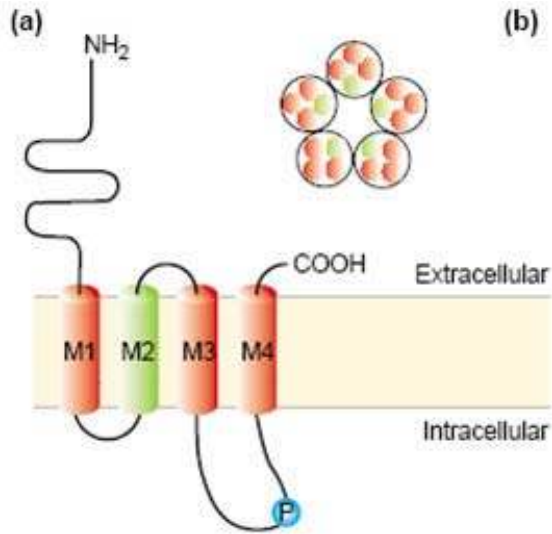
## Excitatory neurotransmission (e.g. glutamate or acetylcholine receptors)



## Inhibitory neurotransmission (e.g. GABA<sub>A</sub> receptors)

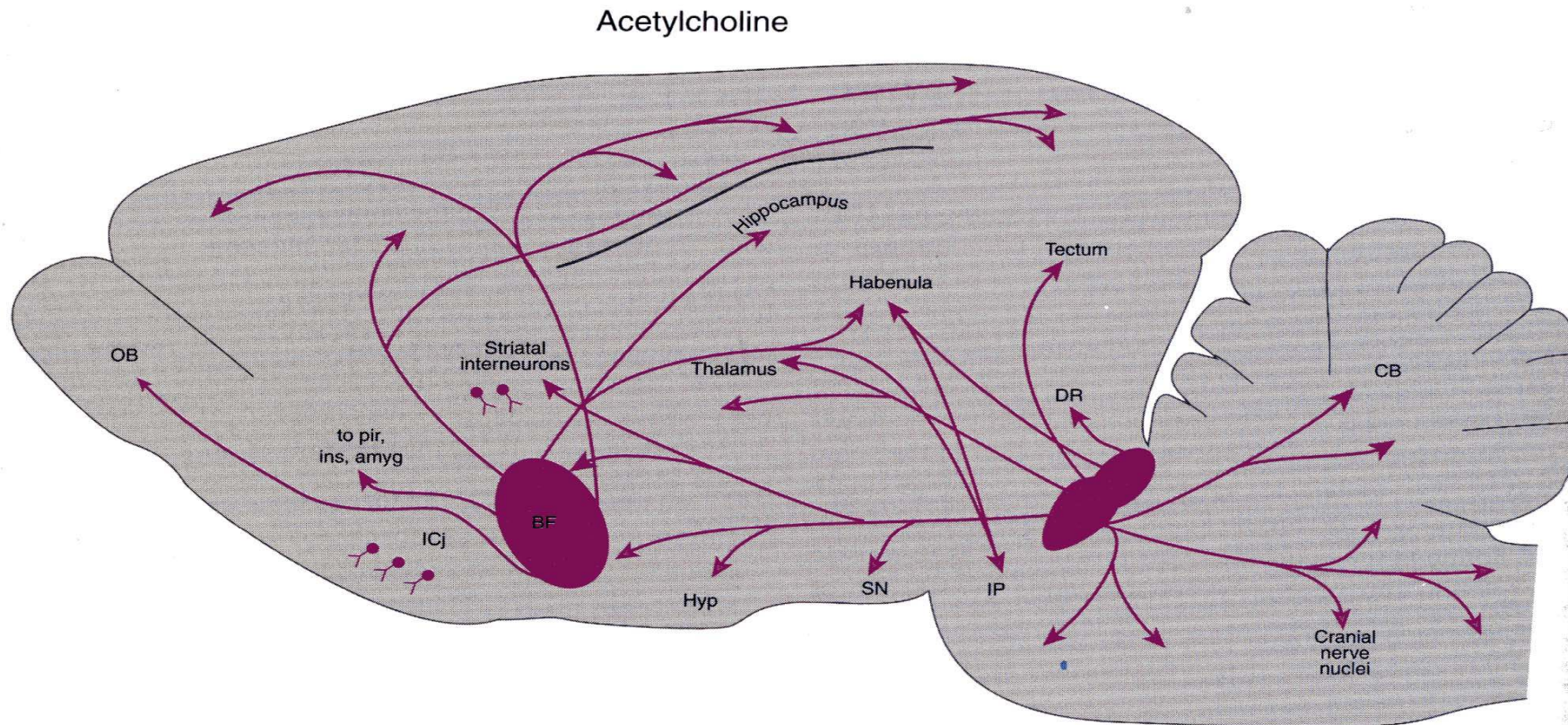


## Structure and modifiability of a large variety of postsynaptic receptors.



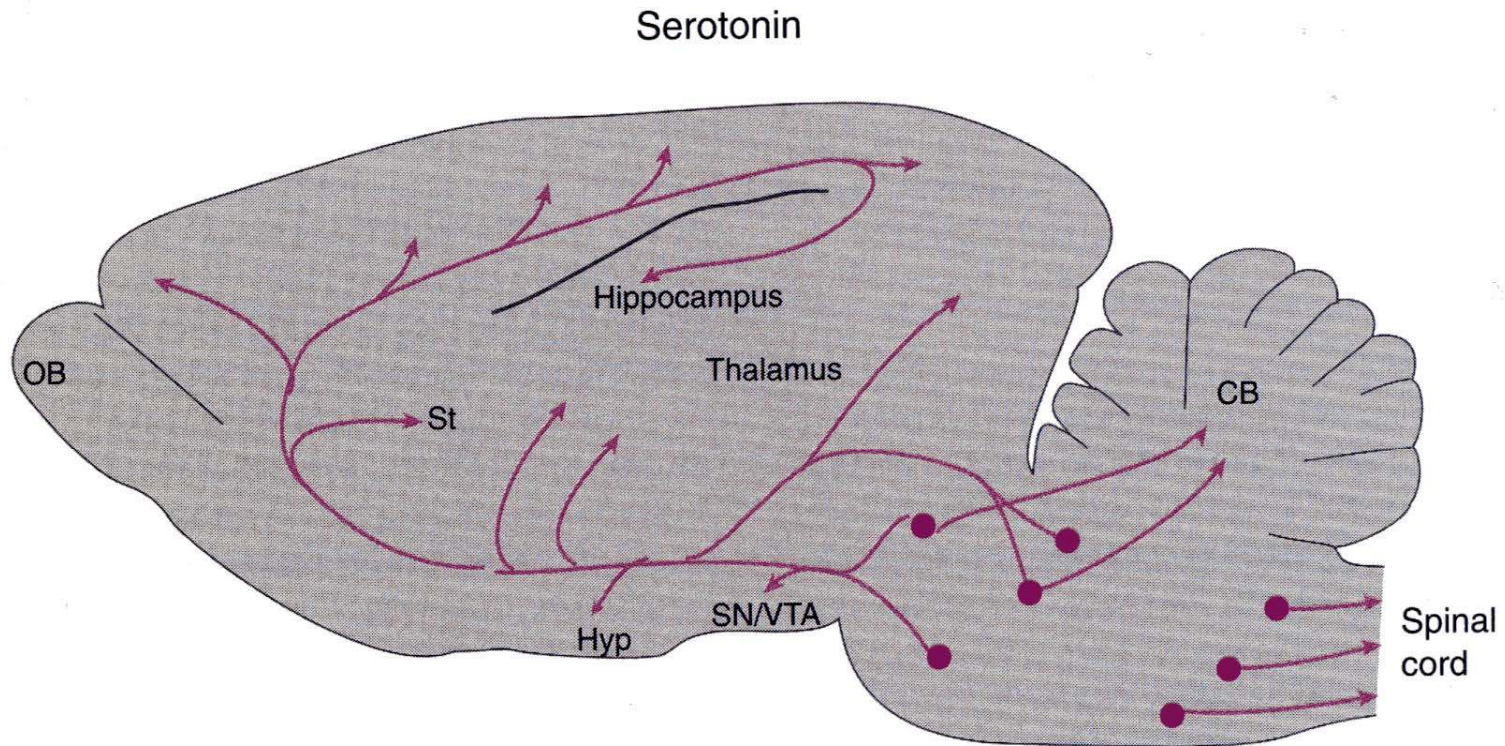
TRENDS in Neurosciences

## Cholinergic pathways in the rat brain



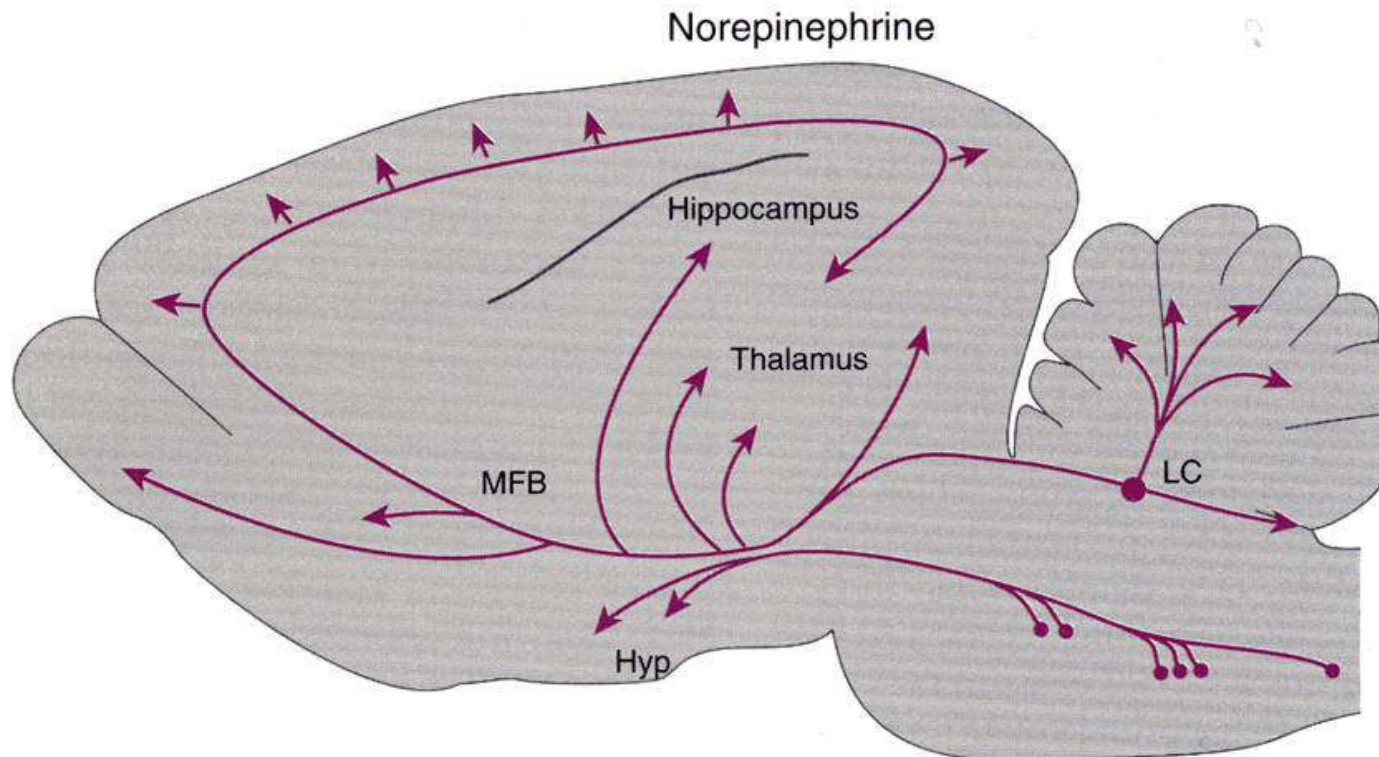
**FIGURE 8.12** Schematic of acetylcholine cell bodies and projections in a sagittal section of the rat. Abbreviations: as in Figure 8.6; BF, basal forebrain.

## Serotonergic pathways in the rat brain

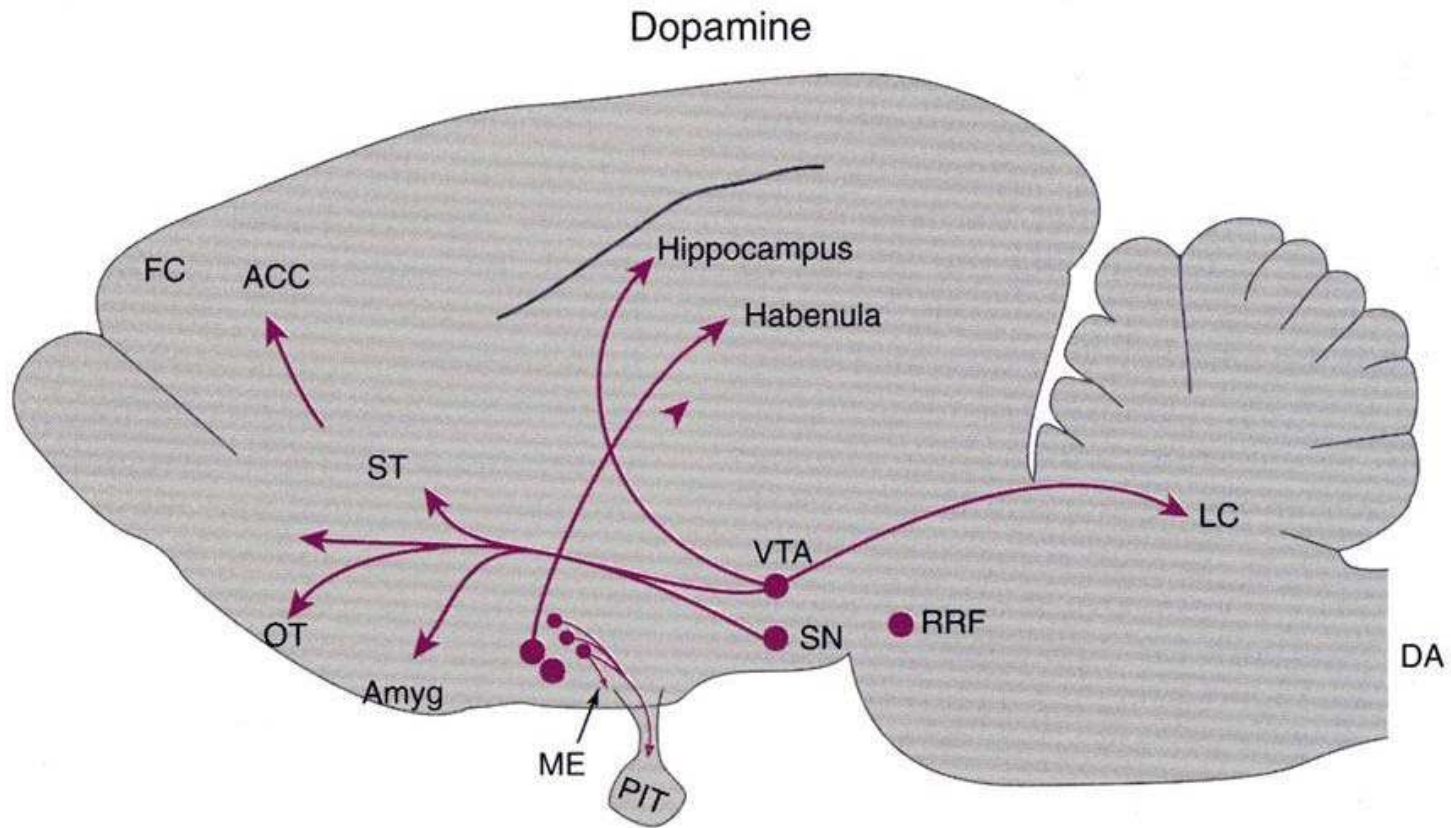


**FIGURE 8.8** Schematic illustrating some of the major collection of serotonergic cell bodies (red circles) and projection areas. Abbreviations: as in Figure 8.6.

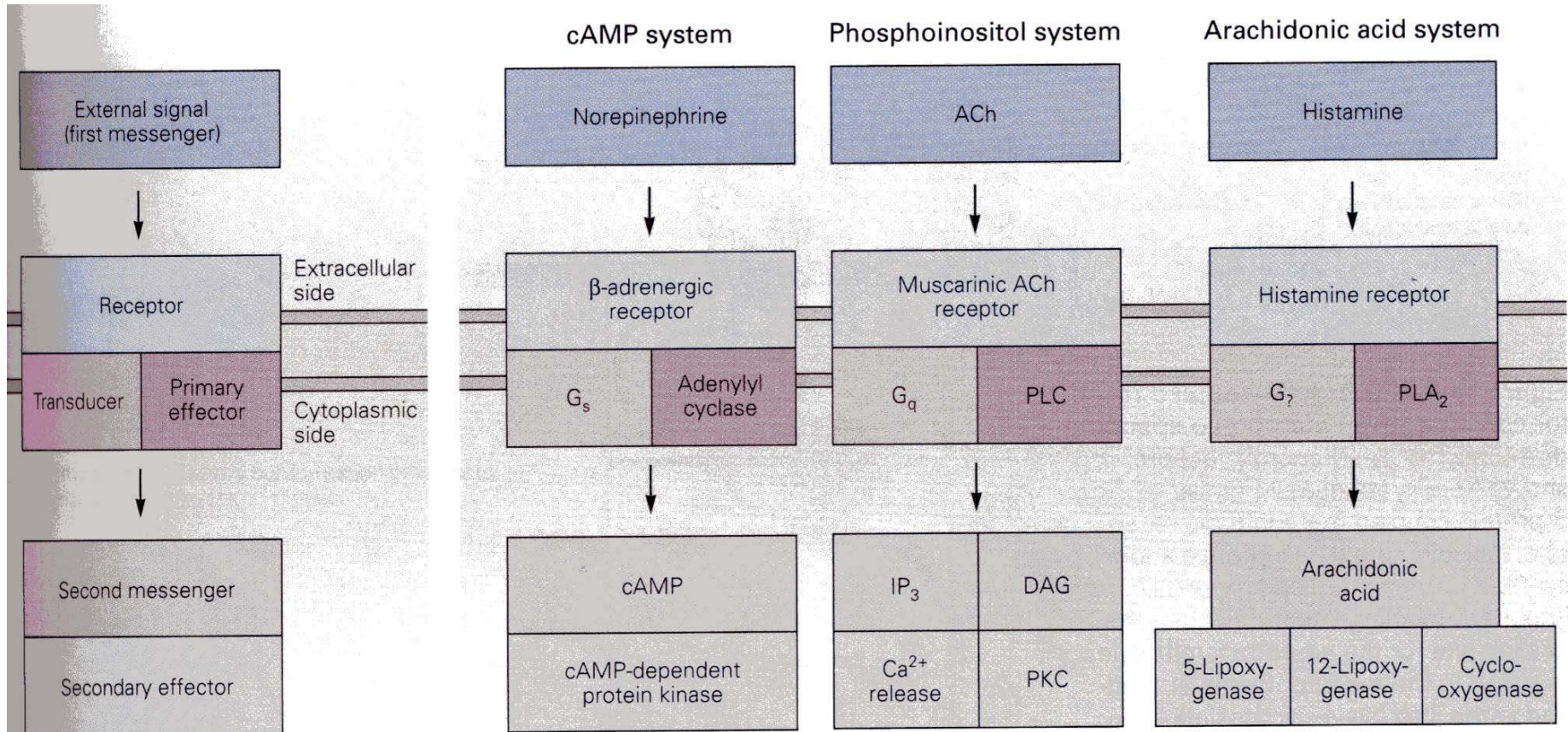
## Noradrenergic pathways in the rat brain



## Dopaminergic pathways in the rat brain



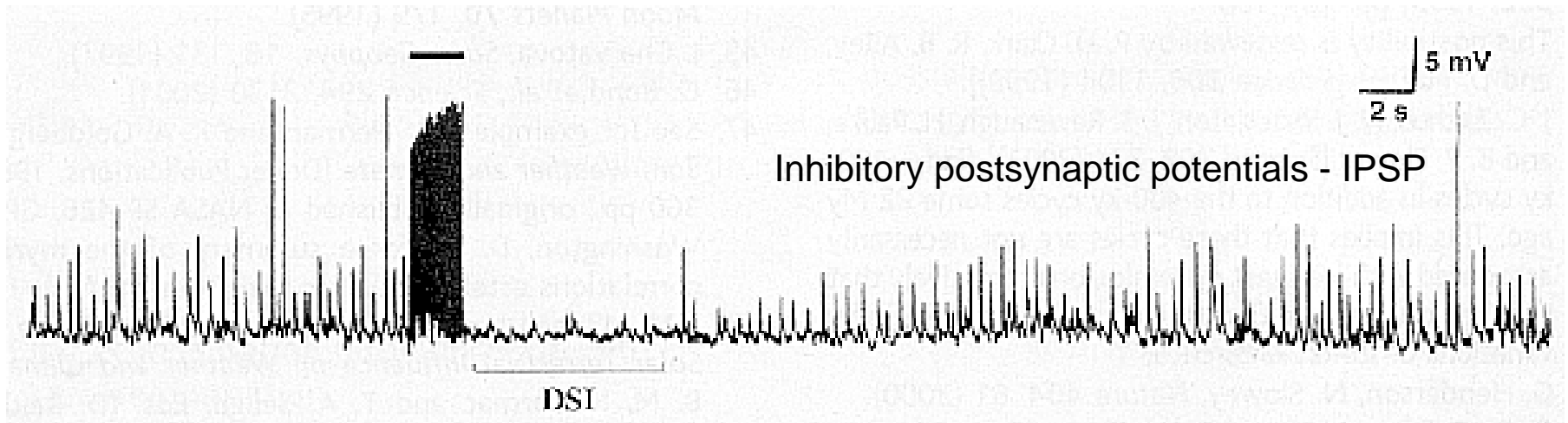
## Activation of second messengers I.



## Retrograde signalization

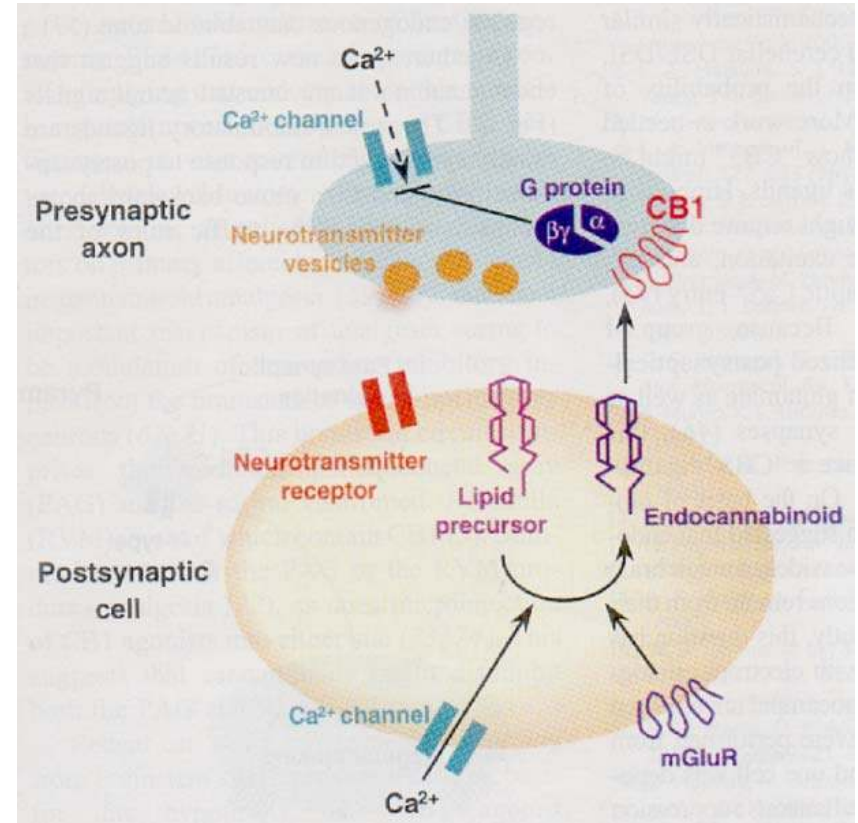
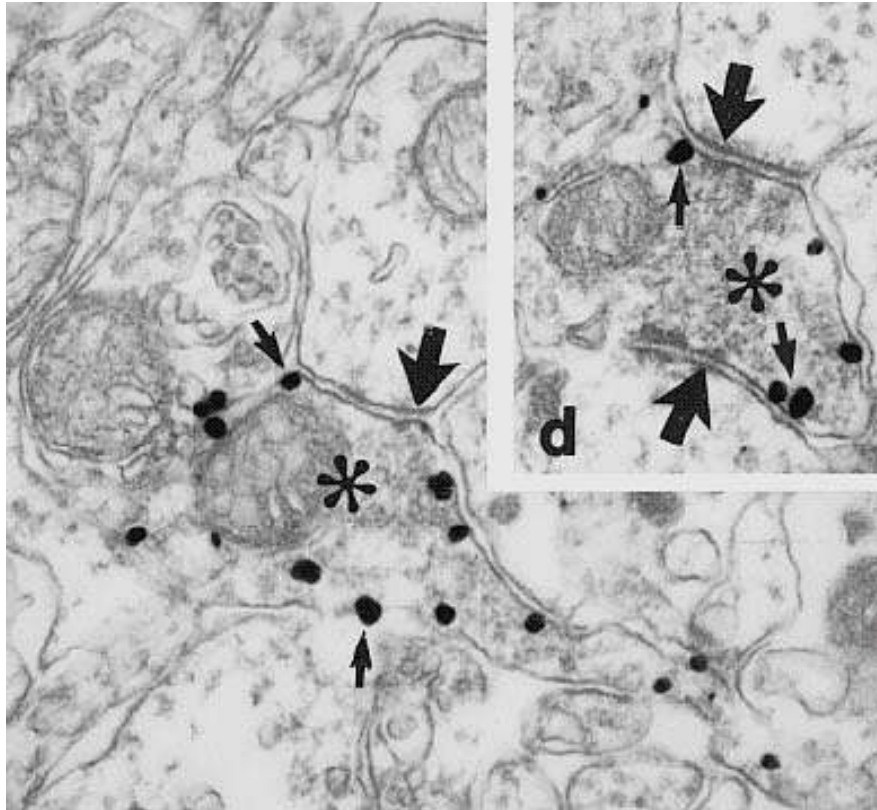
- i) Gaseous: nitrogen monoxide (NO), carbon monoxide (CO)
- ii) Peptides: BDNF, dynorphin
- iii) Lipids: endocannabinoids, arachidonyl acid
- iv) Classical neurotransmitters: GABA, glutamát

## Retrograde synaptic signalization



**Ref:** Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science*. 2002 Apr 26;296(5568):678-82. Review.

## Retrograde synaptic signaling



## Short term plasticity of synapses

It is dependent on the quality of target elements

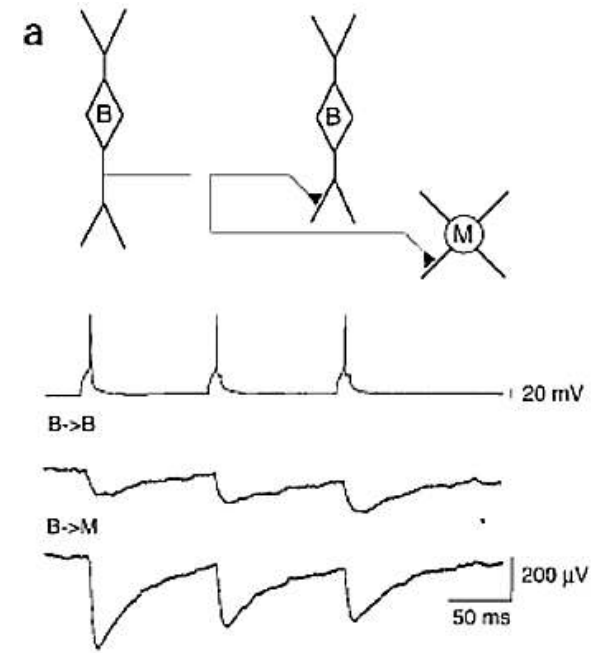
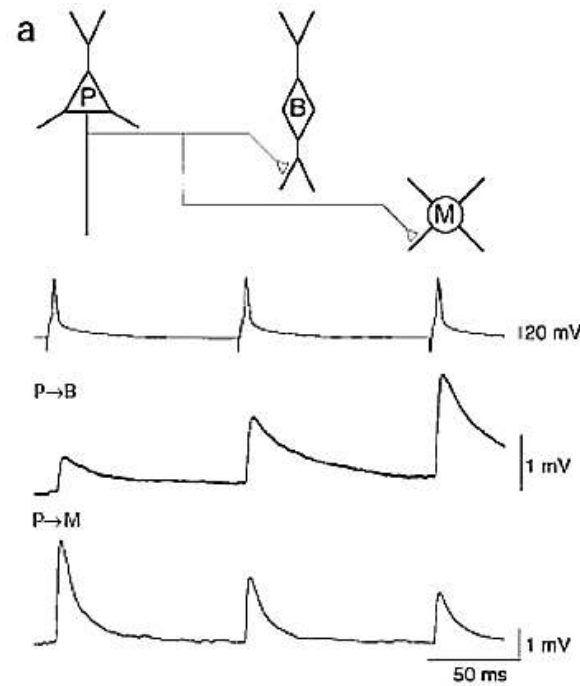
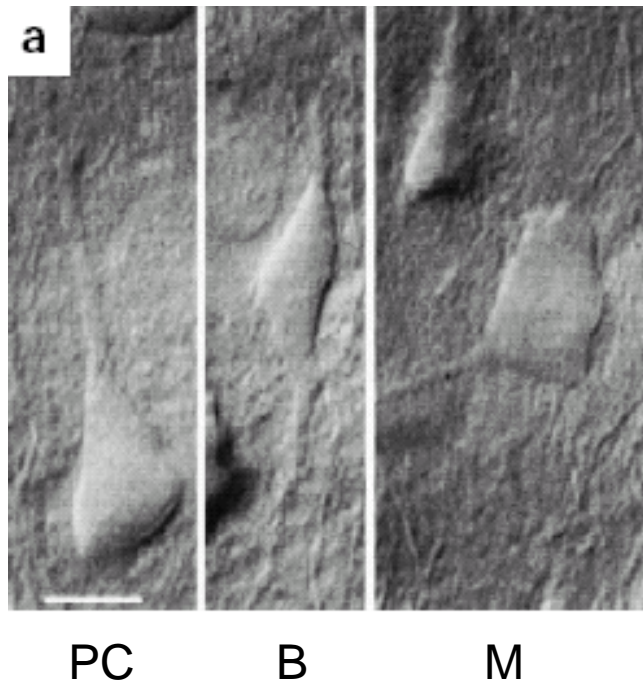
It can be depressive, facilitative and temporally stable

Causes of depression: i) probability of transmitter release is high  
ii) desensitization of receptors  
iii) intracellular factors (e.g. spermin)

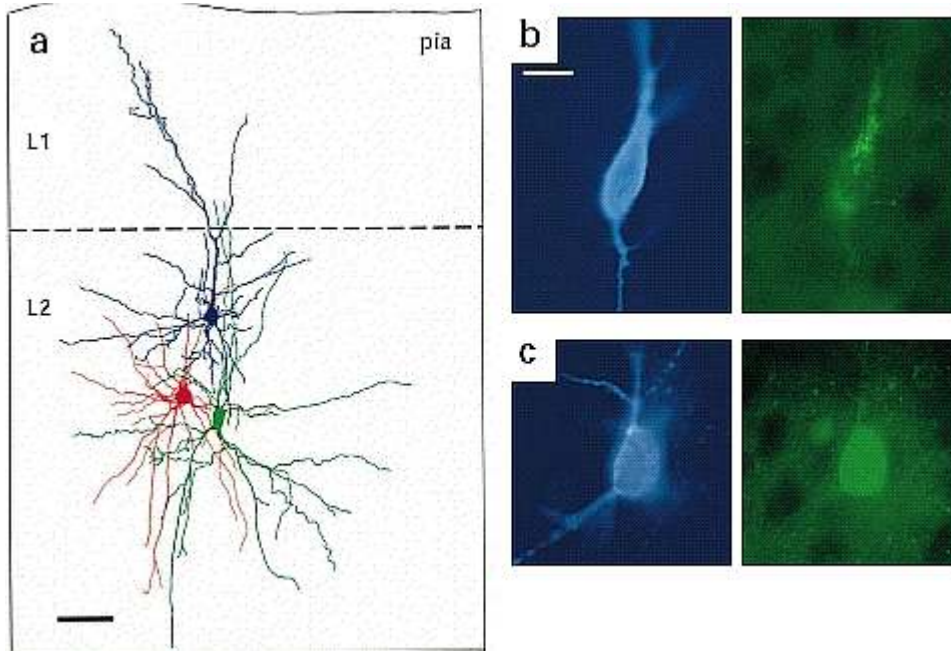
Causes of facilitation: accumulation of  $\text{Ca}^{2+}$  in the presynaptic terminal

It can be influenced by e.g. activation of presynaptic receptors

## Elements of the neocortical network



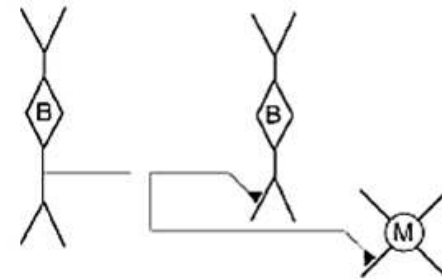
## Elements of the neocortical network



**The somatostatin-containing cells** show bitufted (B) morphology, which innervate the dendritic tree of other neurons.

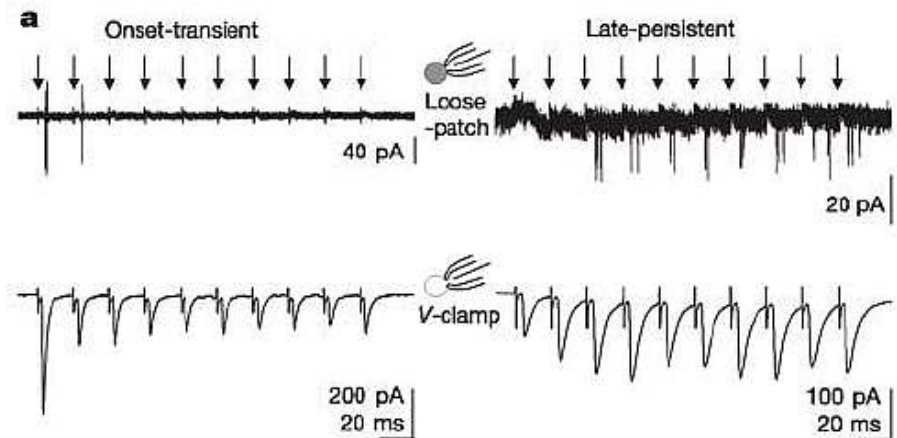
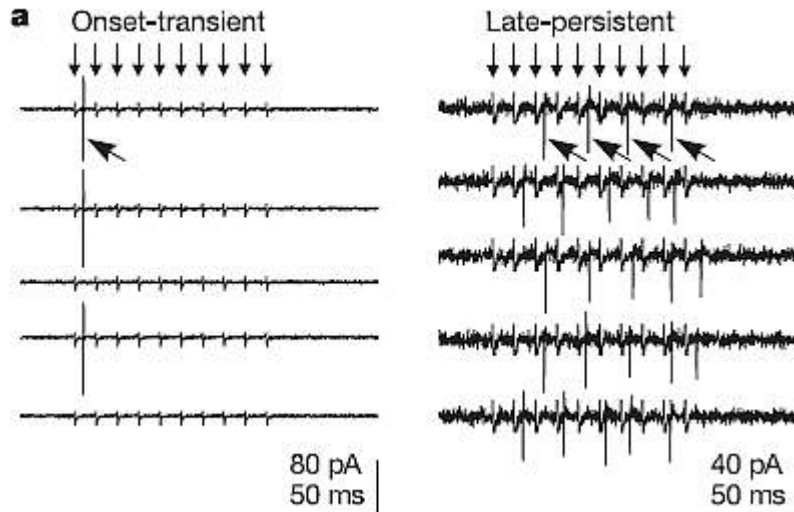
**The parvalbumin-containing cells** show multipolar (M) morphology, which innervate the perisomatic region of other neurons (basket or axo-axonic cells)

**Ref:** Reyes A, Lujan R, Rozov A, Burnashev N, Somogyi P, Sakmann B. Target-cell-specific facilitation and depression in neocortical circuits. *Nat Neurosci.* 1998 Aug;1(4):279-85.



## Spatially and temporally distinguished role of multipolar perisomatic and bitufted dendritic inhibitory cells:

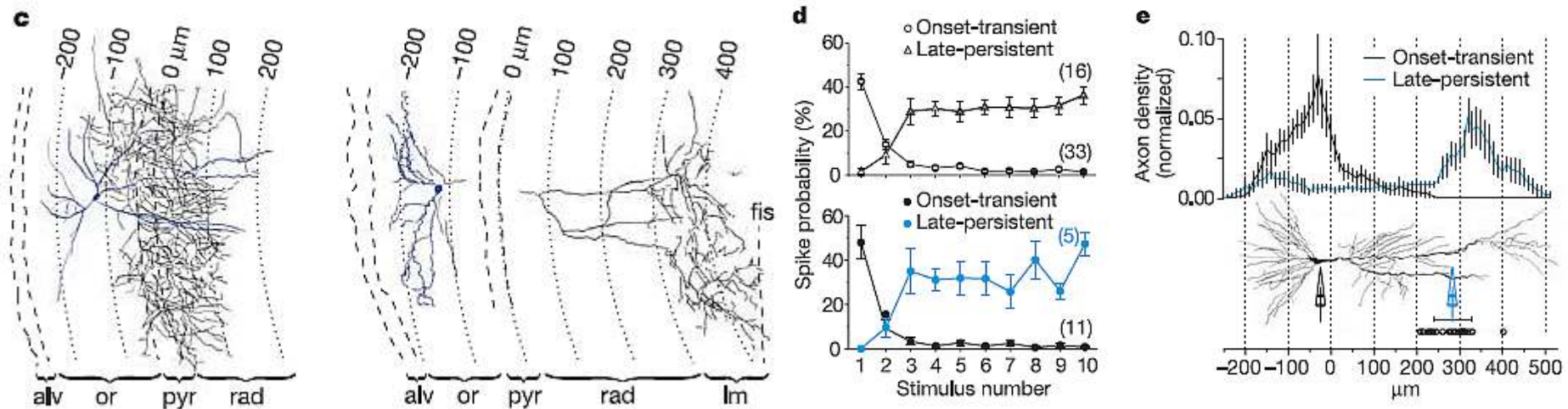
The functional difference originate from the different plasticity of excitatory synapses



**Ref:** Pouille F, Scanziani M. Routing of spike series by dynamic circuits in the hippocampus. *Nature*. 2004 Jun 17;429(6993):717-23.

## Spatially and temporally distinguished role of multipolar perisomatic and bitufted dendritic inhibitory cells:

The functional difference originate from the different plasticity of excitatory synapses



**Ref:** Pouille F, Scanziani M. Routing of spike series by dynamic circuits in the hippocampus. *Nature*. 2004 Jun 17;429(6993):717-23.

## Long-term plasticity of synapses

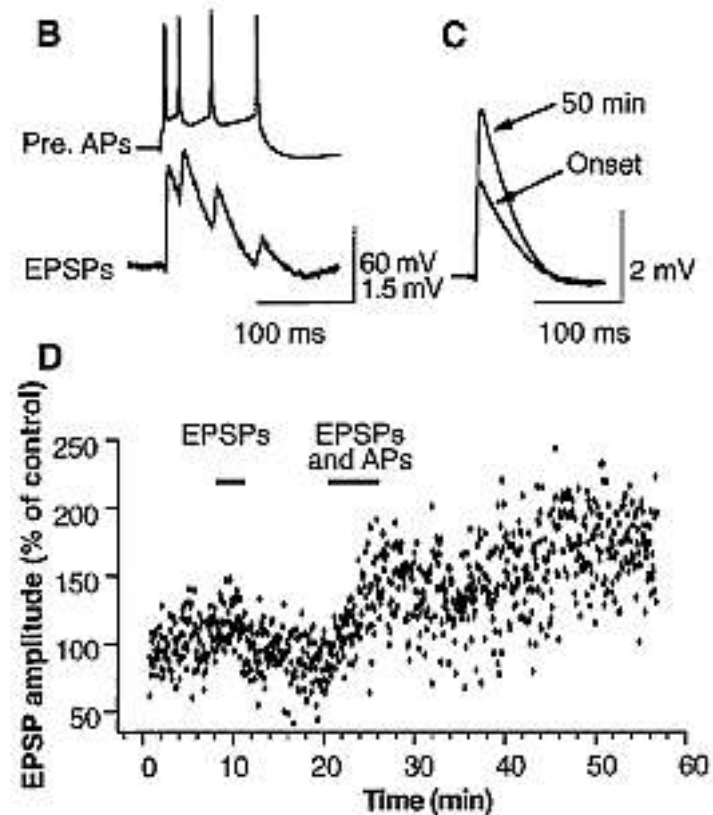
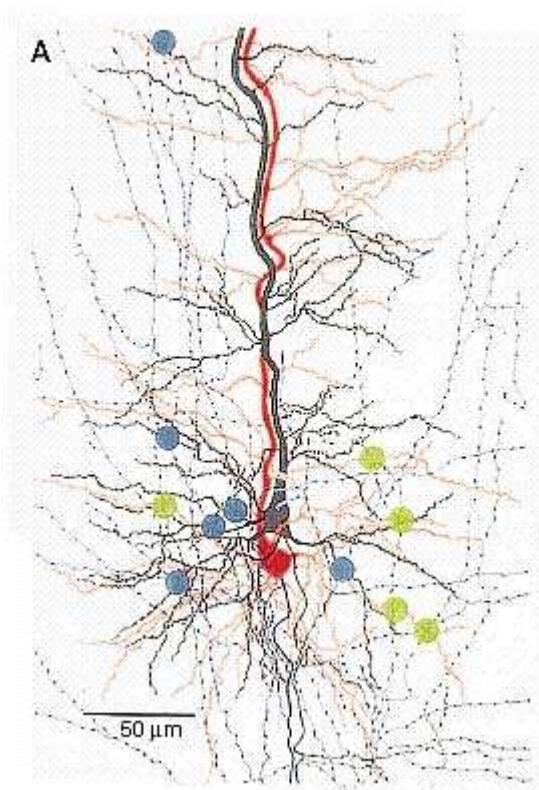
It is dependent on the quality of target elements

It can be LTP – Long Term Potentialiation  
or LTD – Long Term Depression

Methods which can induce: i) high frequency stimulus of the fibers (**Bliss & Lomo, 1973**)  
ii) synchronous co-activation of pre- and postsynaptic cells  
(plasticity window: presynaptic and postsynaptic spikes less than 15 ms apart)  
iii) temporal difference between the activation of pre- and postsynaptic cells

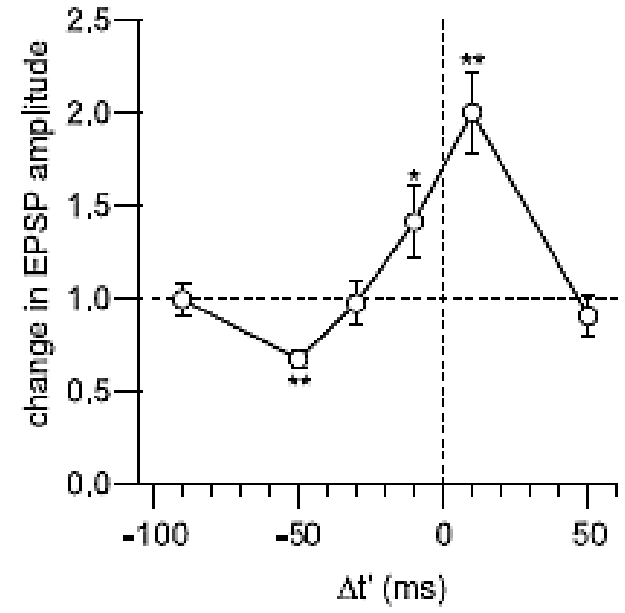
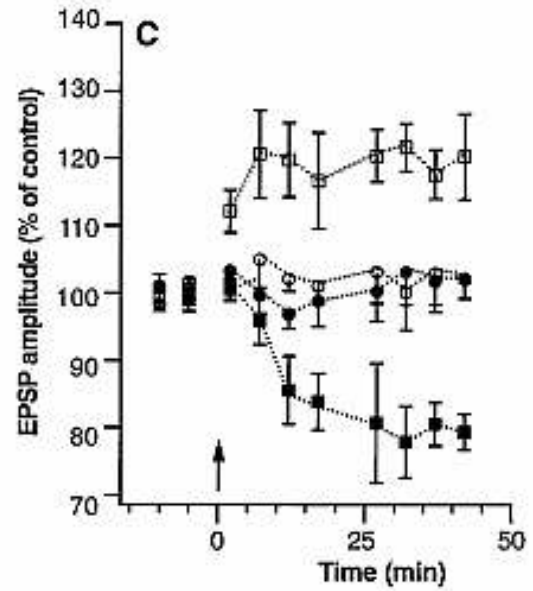
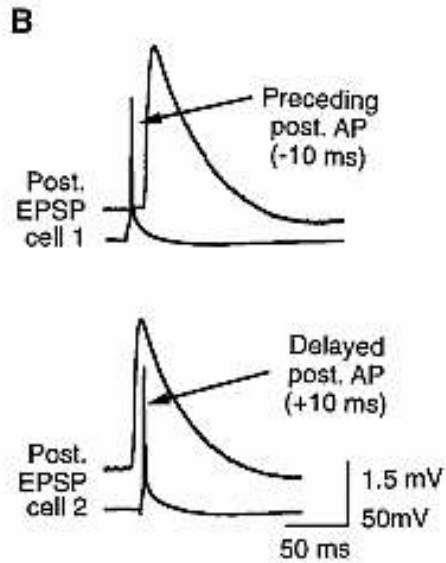
Mechanisms: NMDA-dependent, non-NMDA dependent, receptor insertion, receptor subunit exchange, retrograde messengers etc.

## Spike-time dependent plasticity - STDP



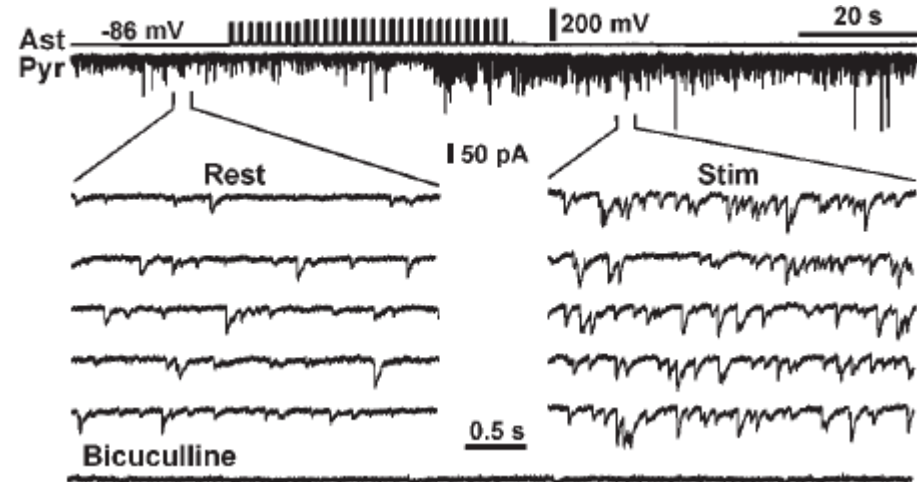
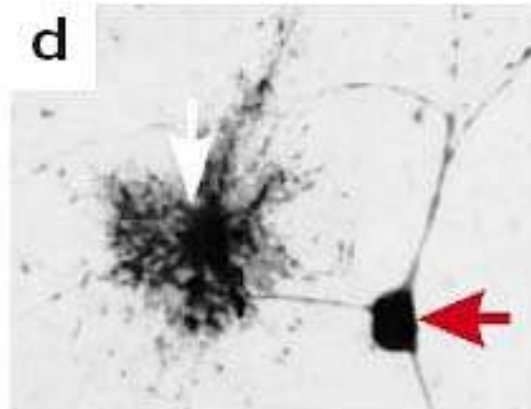
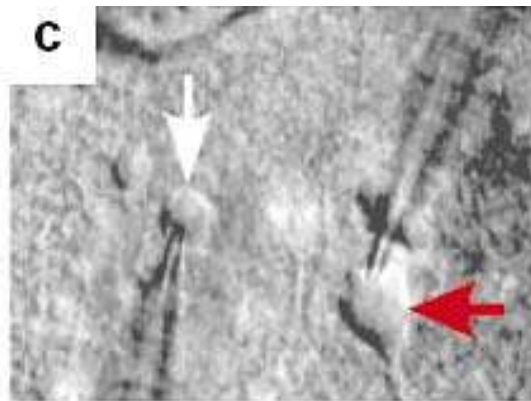
**Ref:** Markram H, Lübke J, Frotscher M, Sakmann B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science*. 1997 Jan 10;275(5297):213-5.

## Spike-time dependent plasticity - STDP



**Ref:** Markram H, Lübke J, Frotscher M, Sakmann B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science*. 1997 Jan 10;275(5297):213-5.

## Direct activation of astroglial cells through depolarization or $Ca^{2+}$ uncaging can enhance GABA release via kainate receptors



**Ref:** Kang J, Jiang L, Goldman SA, Nedergaard M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat Neurosci.* 1998 Dec;1(8):683-92.