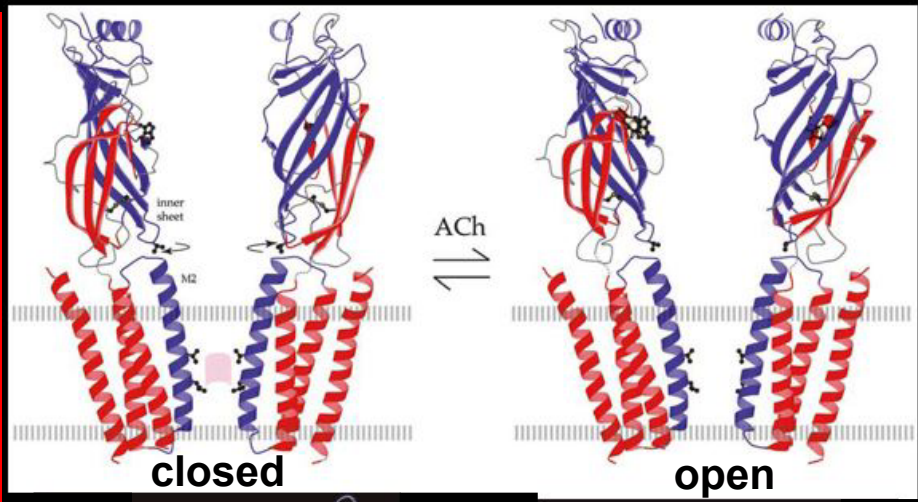
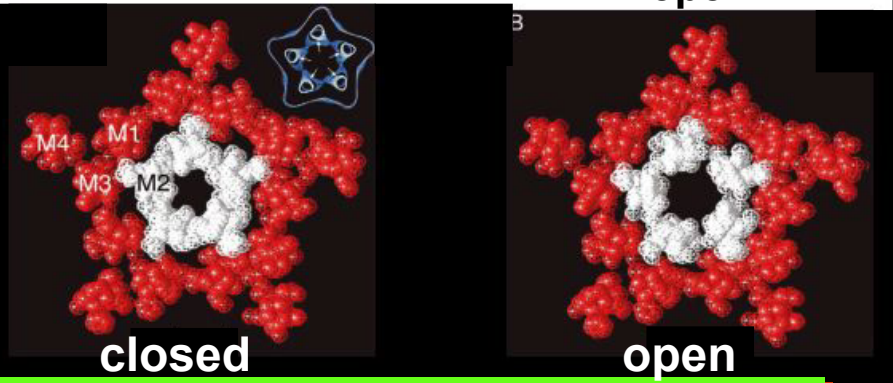
Ионные каналы и синапсы: ключевые молекулярные блоки биологической жизни

26 октября, 2009

Междисциплинарный курс лекций "Молекулярная физиология"

МГУ Москва





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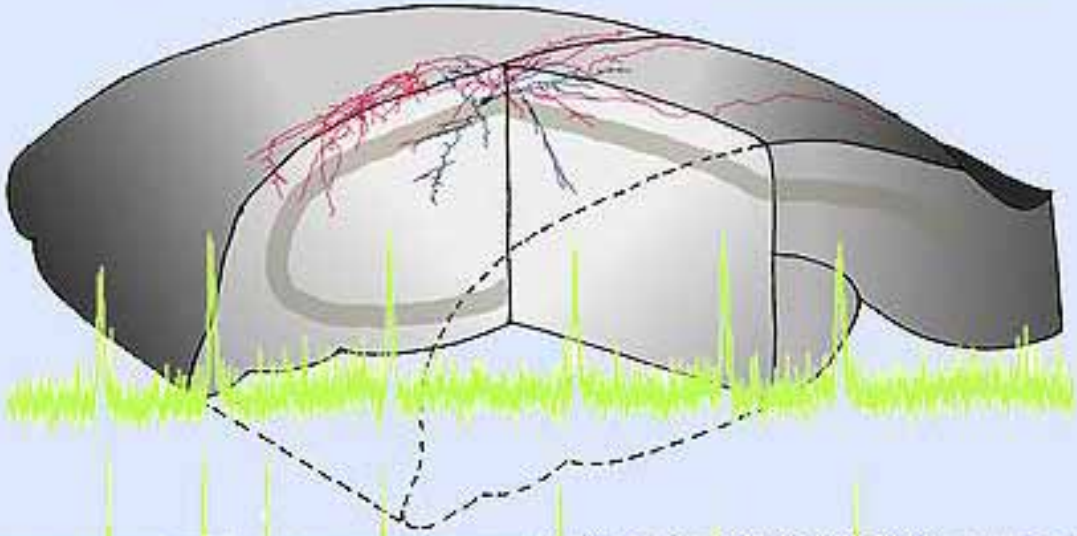

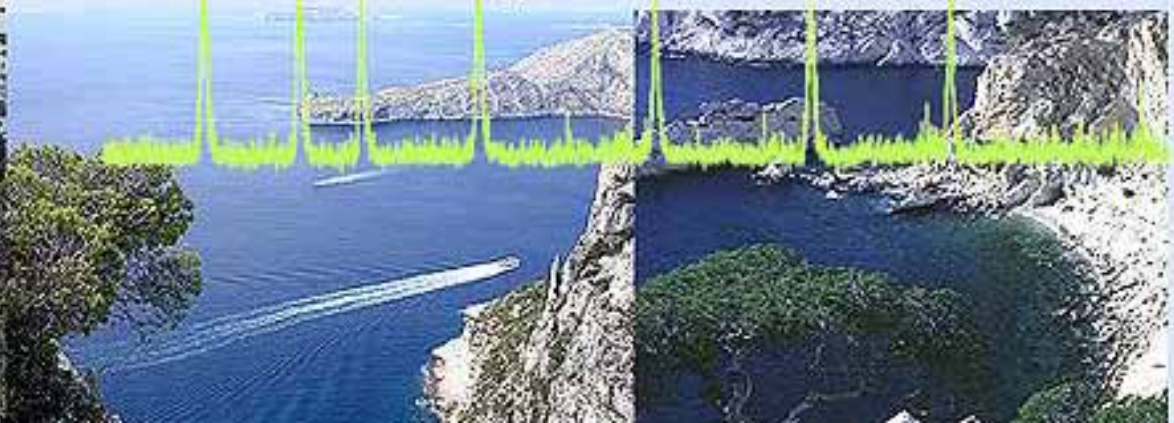

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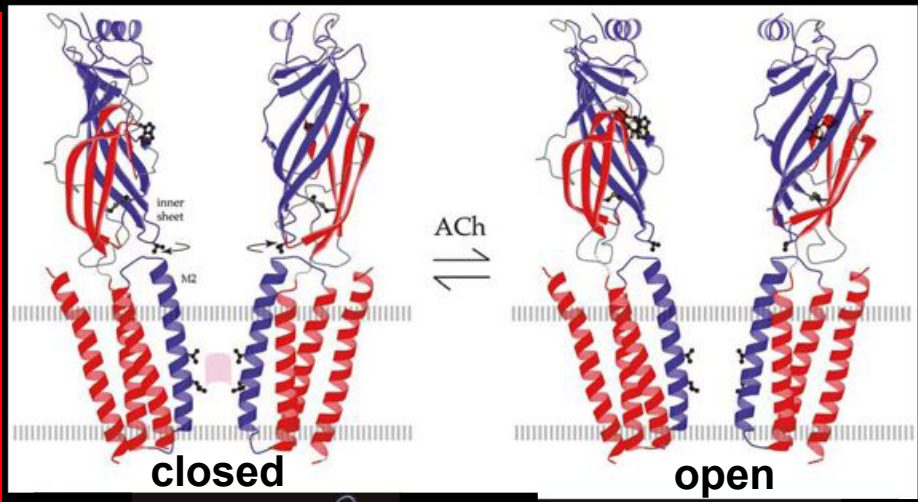

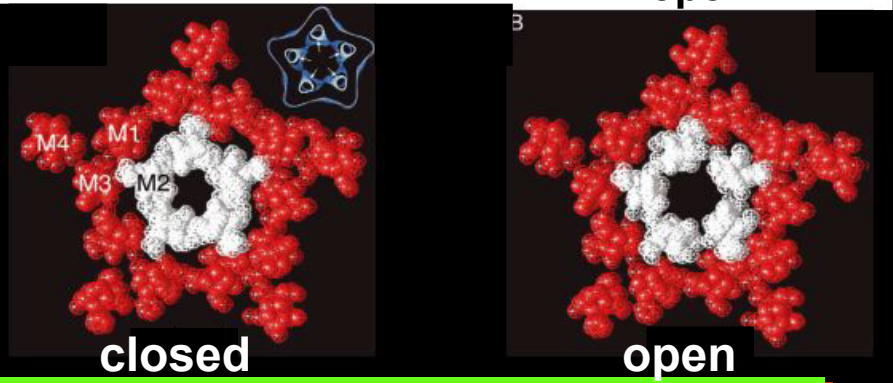

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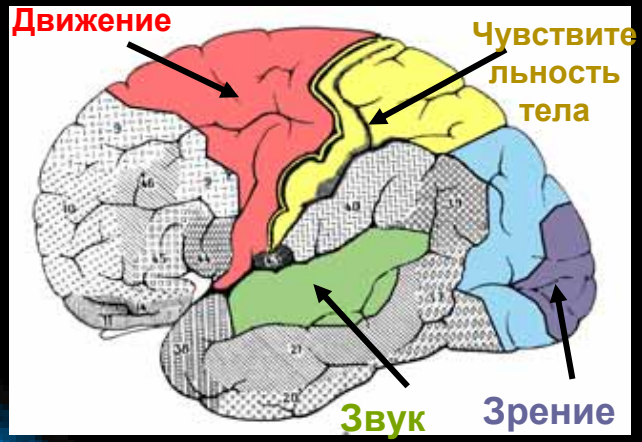
Визуализация мозга: Позитронная эмиссионная томография



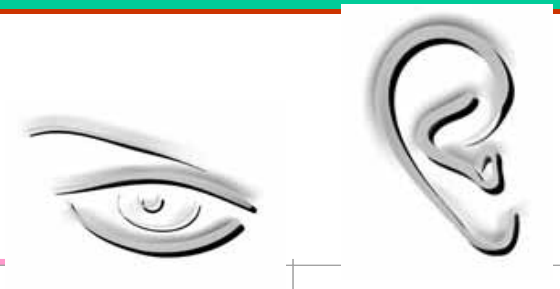
Найти слово

Произнести слово

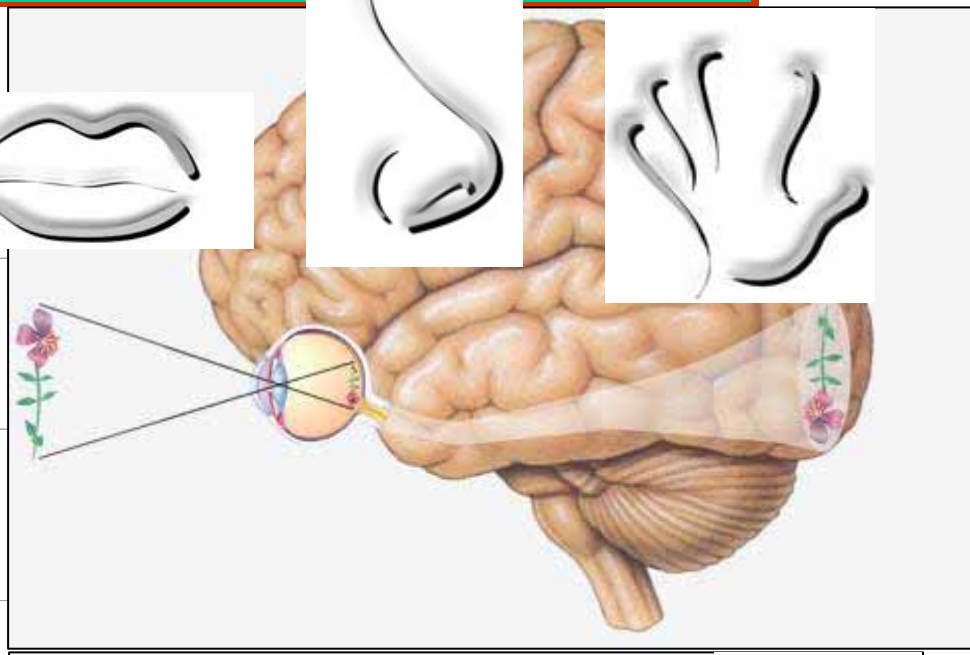
Слушать слово

Читать слово

ОРГАНЫ ЧУВСТВ: Общие правила



чувства	зрение	слух
органы чувств	глаза	уши



Сигнал:
Свет
Звук
Запах...

→

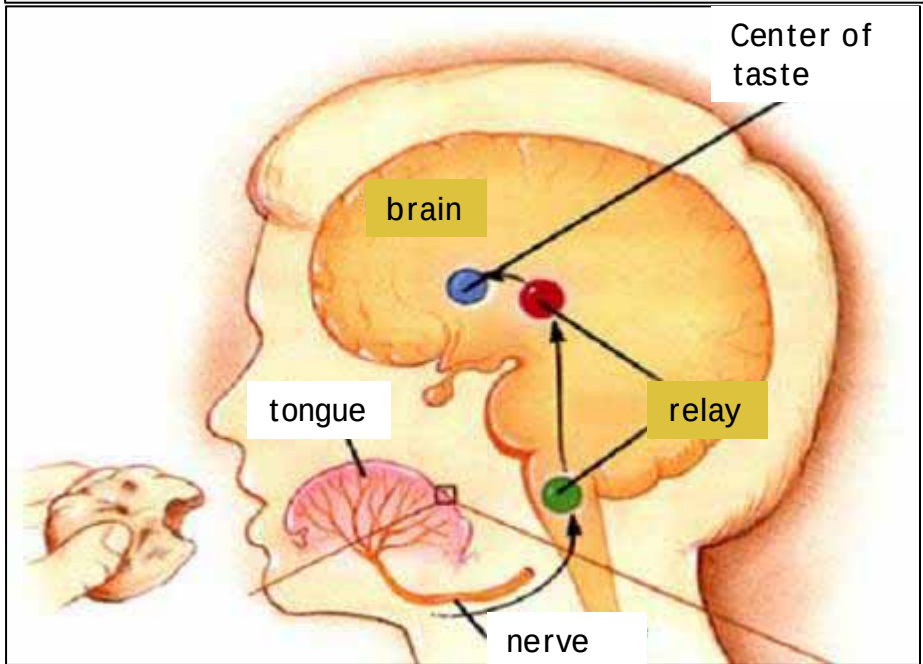
Орган:
Глаз
Ухо
Нос…

↓

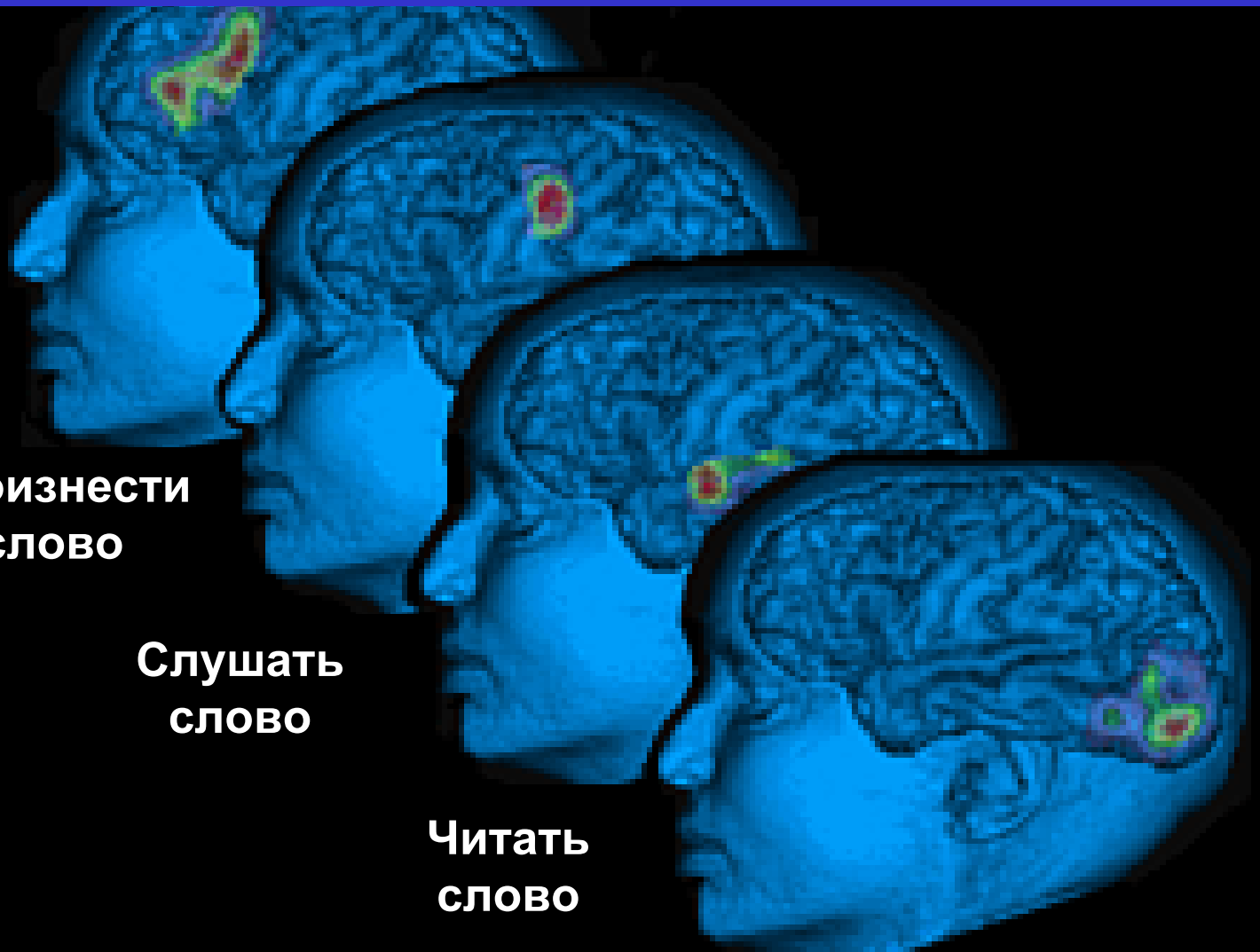
Трансформация
в электрические сигналы

↓

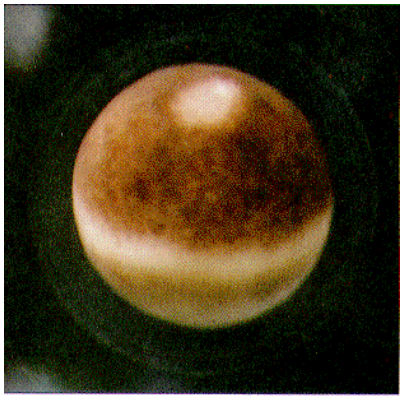
Интеграция и обработка информации



Биологические организмы - "молекулярные машины", способные превращать световые, звуковые, химические, механические и др. внешние сигналы в электрические.

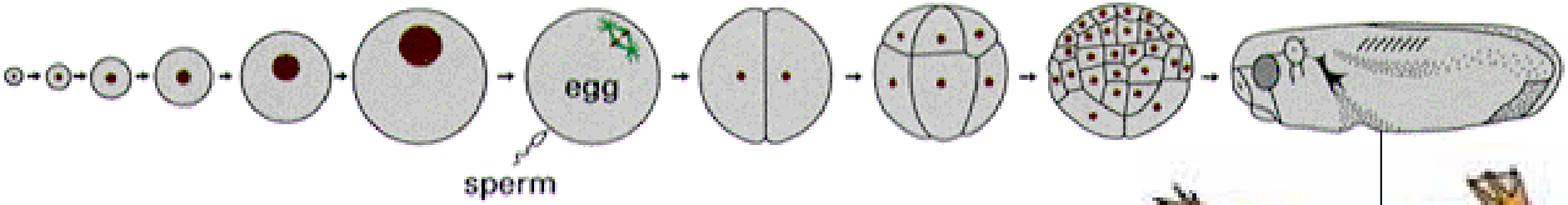


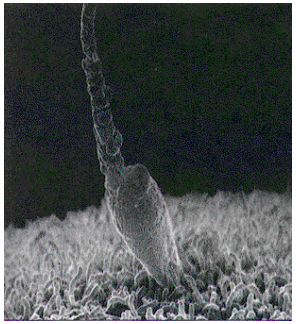
Основные стадии развития

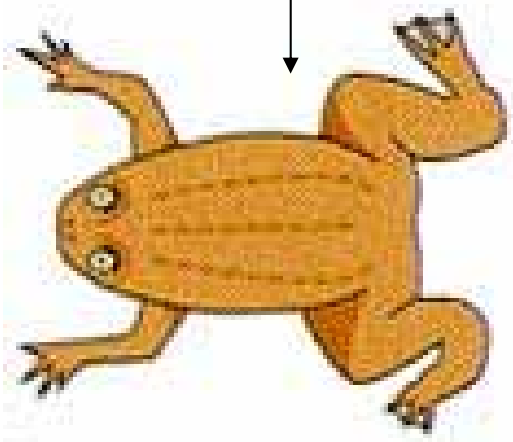


oocyte grows without dividing
(months)

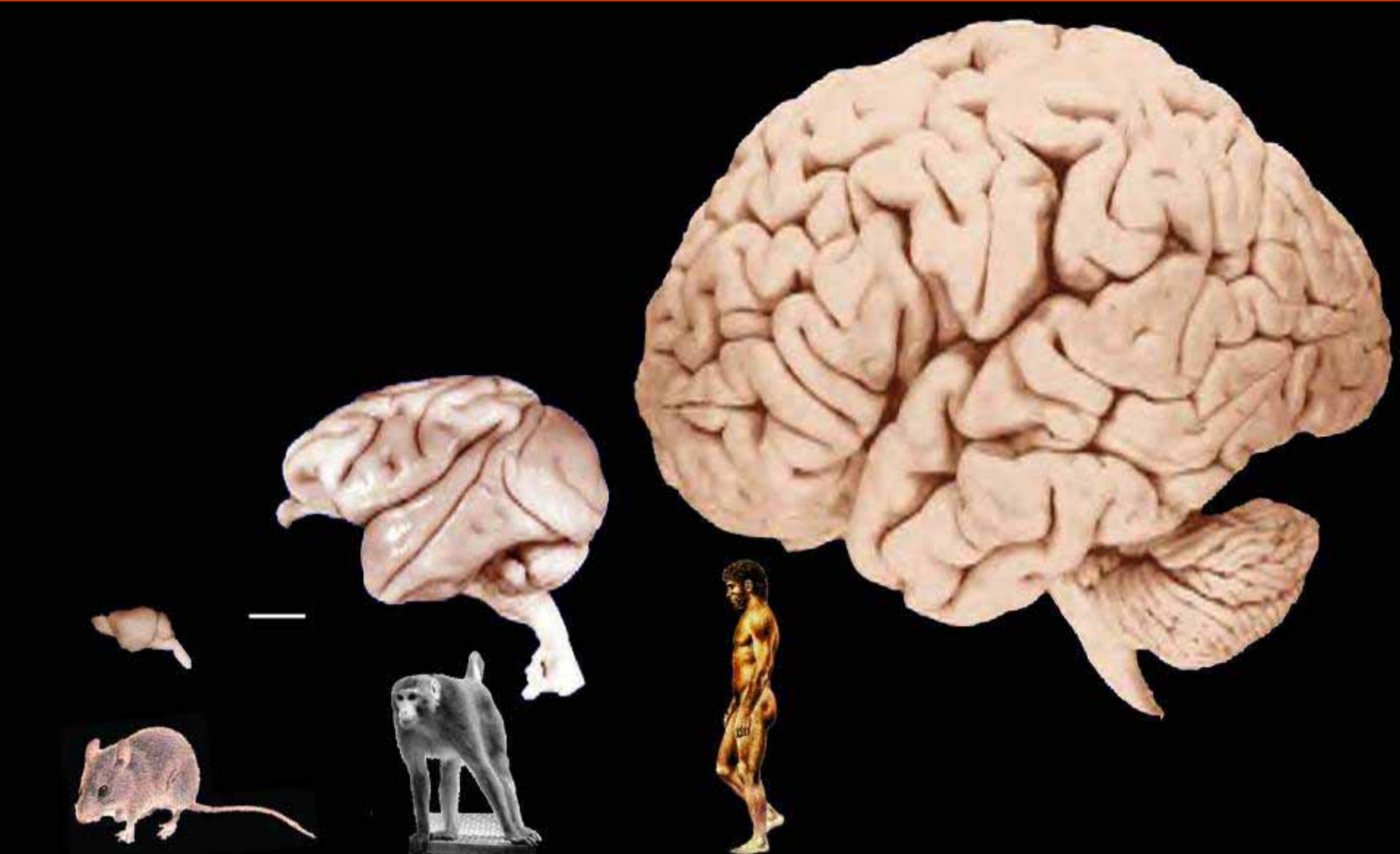
fertilized egg divides without growing
(hours)



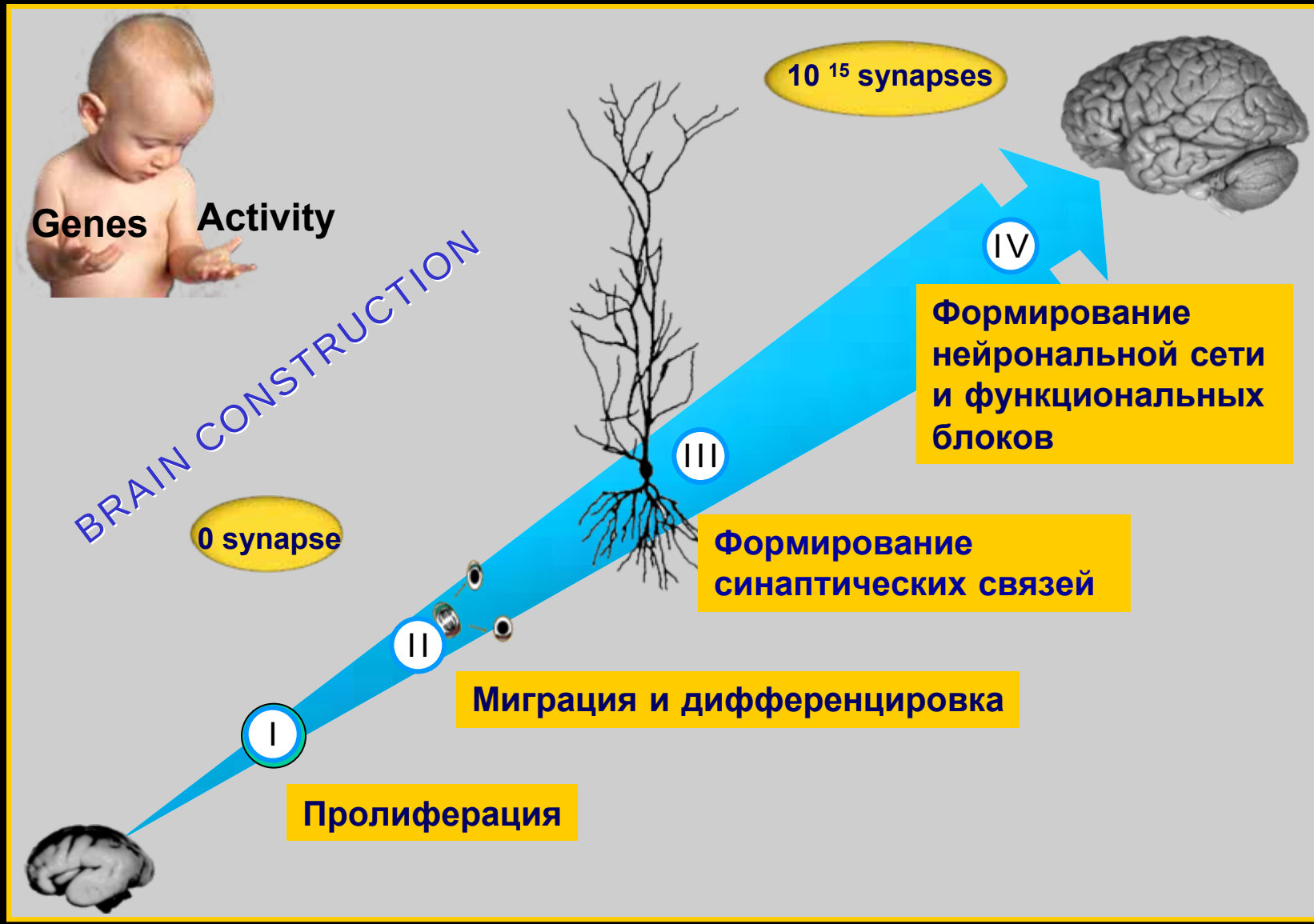




Относительные размеры мозга млекопитающих



Основные этапы развития мозга

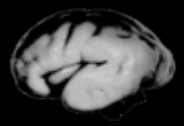


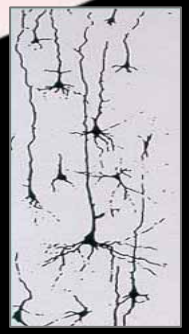
Основные этапы развития мозга и формирование нейрональной сети

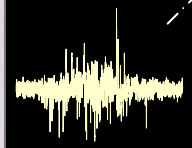
10 15 synapses

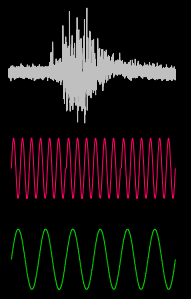
Микросети: локальные взамиодействия

0 synapse





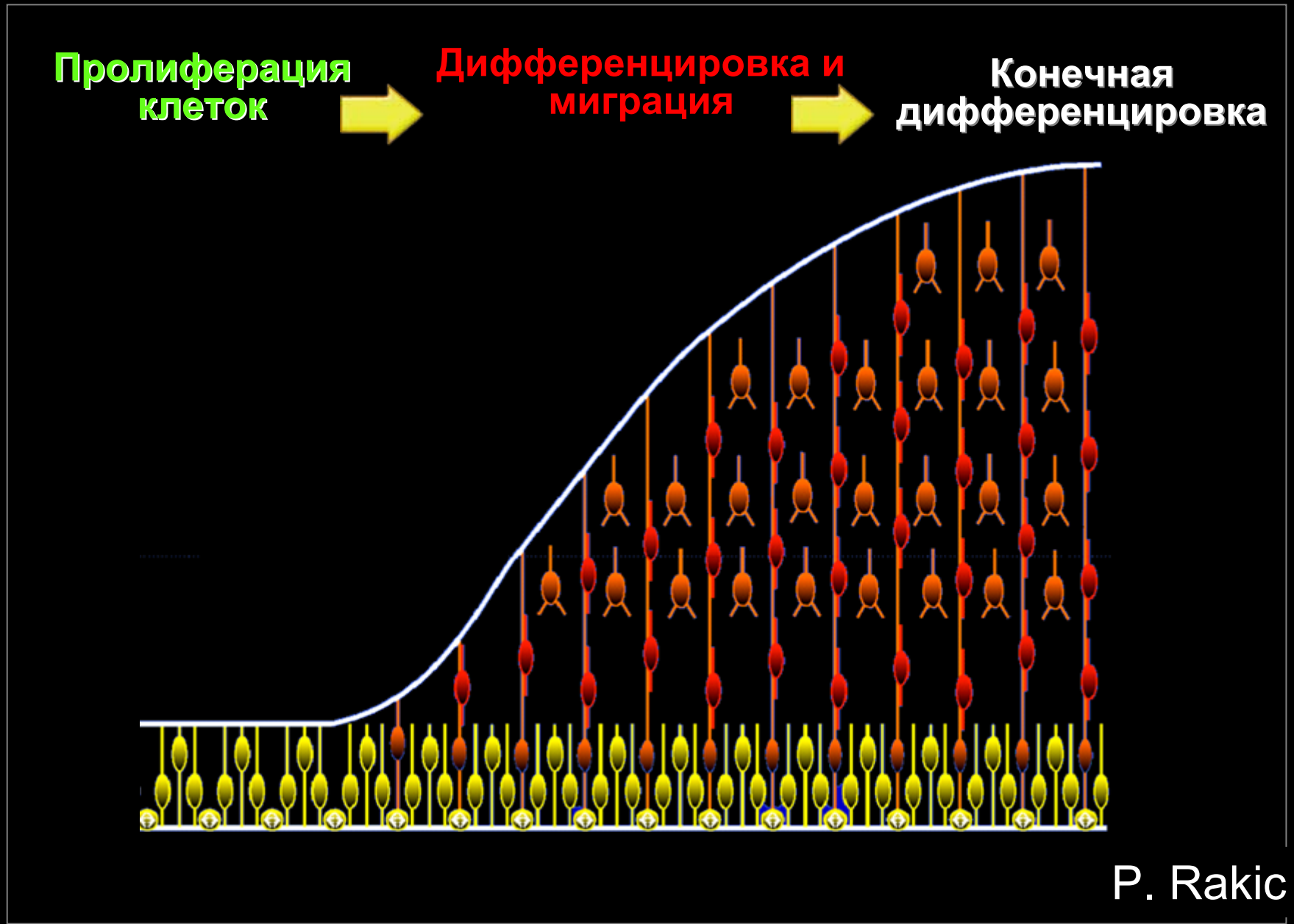




Сложные осцилляции нейрональных сетей

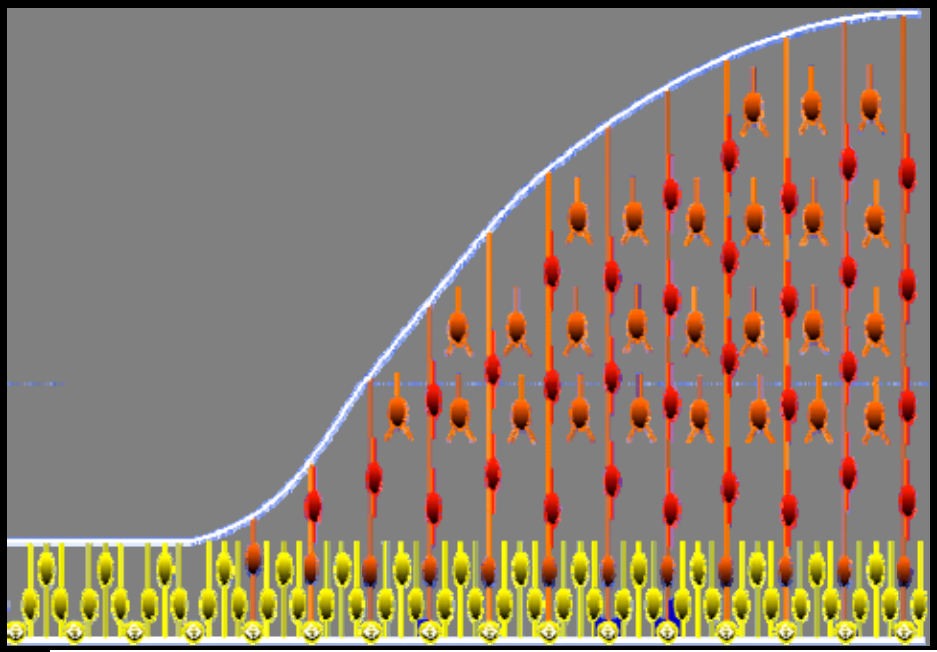
Ранние осцилляции нейрональных сетей

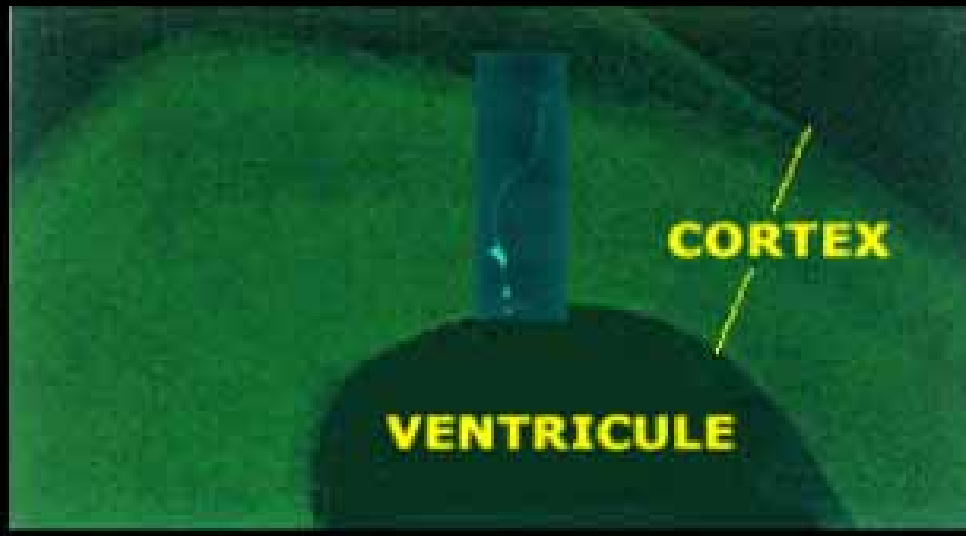
Этапы развития коры

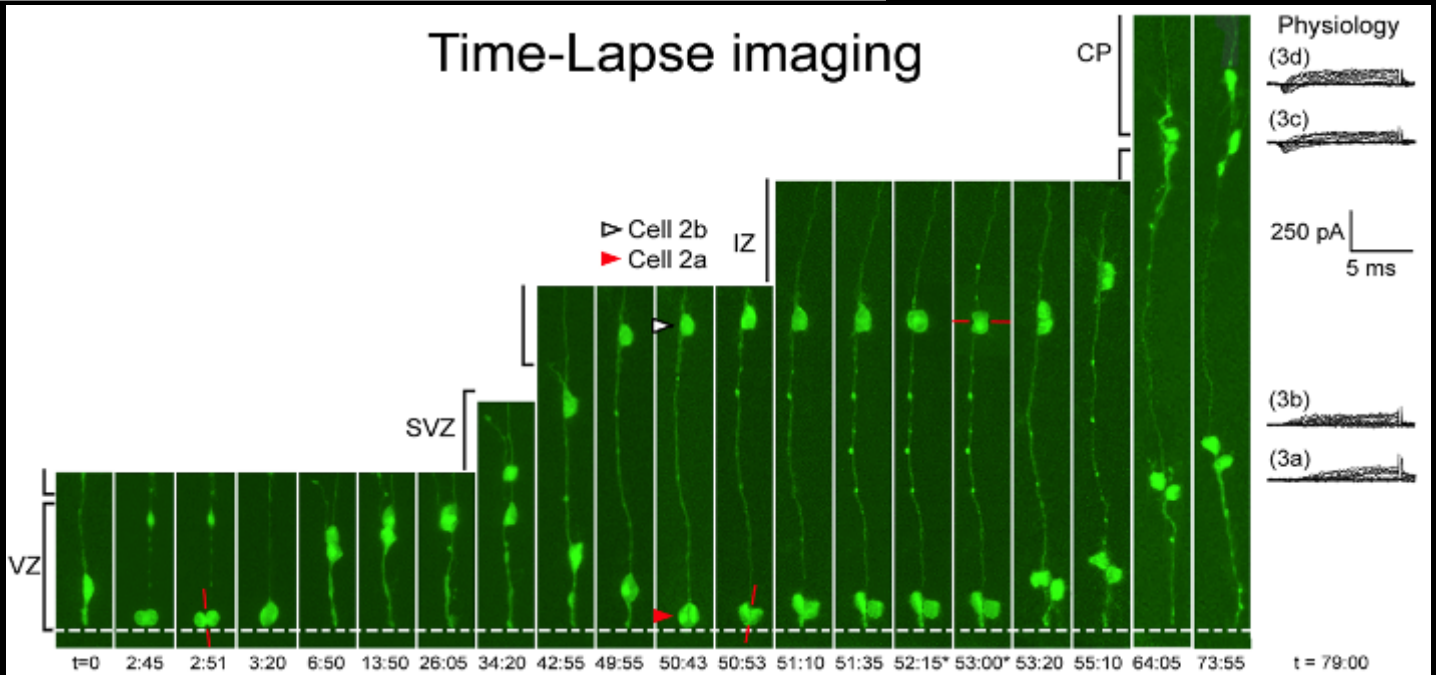


Этапы развития коры

Kriegstein et al.







Гены, нейроны и синапсы

	Species	Nb genes	Nb neurones	Nb synapses
	*C. elegans*	20 000	302	
	*Drosophila*	12 000	205 000	
	*Mus musculus*	33 000	100 000 000	1.2×10^{11}
	*Macacus mulatus*	30000	10 000 000 000	1.2×10^{13}
	*Homo sapiens*	30 000	100 000 000 000	1.2×10^{15}

To calculate 10^{11} (100 billion) seconds: more then 3000 years (3168 years).
10^{13} - > 3 million years; 10^{15} - > 300 million years

Физика

Химия

Биология

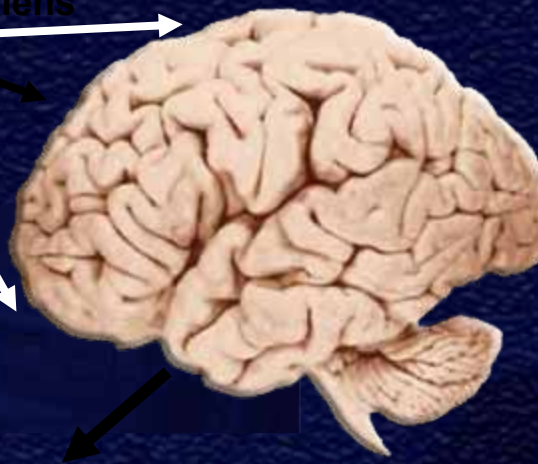
"Мозг - последняя и грандиознейшая биологическая вершина, наиболее глубокая тайна, которую нам еще предстоит разгадать "

James D. Watson "Discovering the Brain", 1992

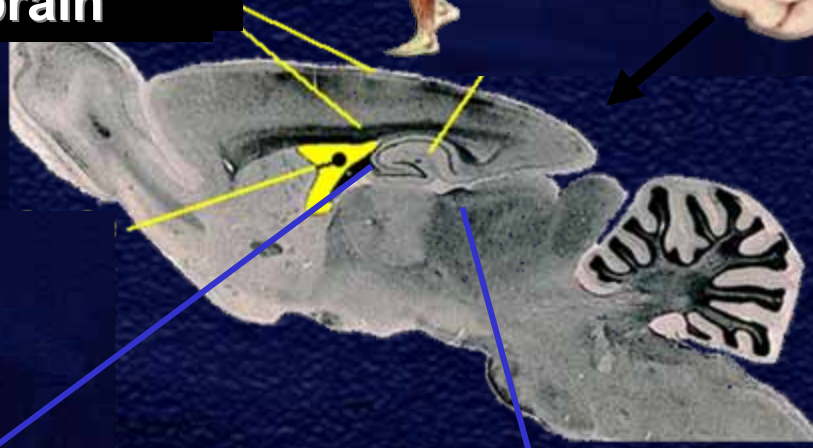
Ion channel

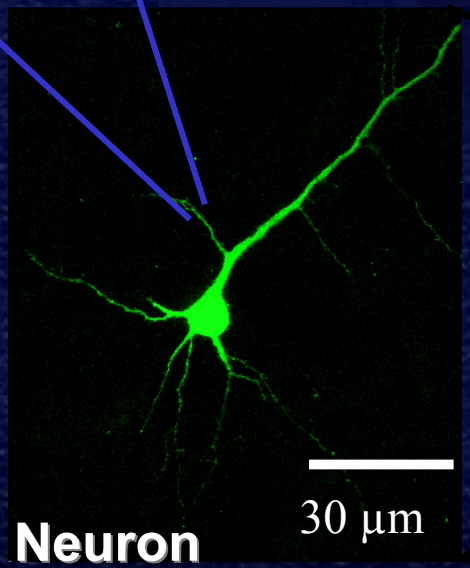
Dendrite
Synapses

Homo sapiens sapiens

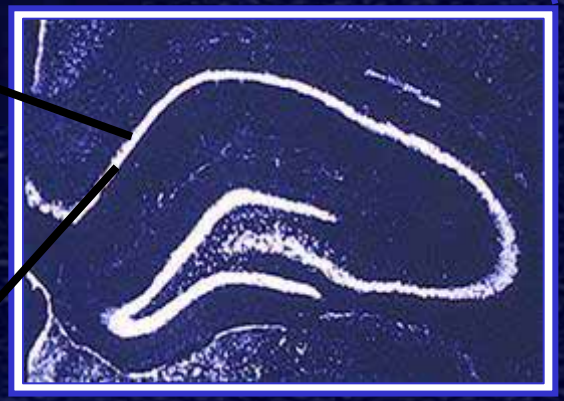


Slice of the brain





Neuron



Slice of the hippocampus

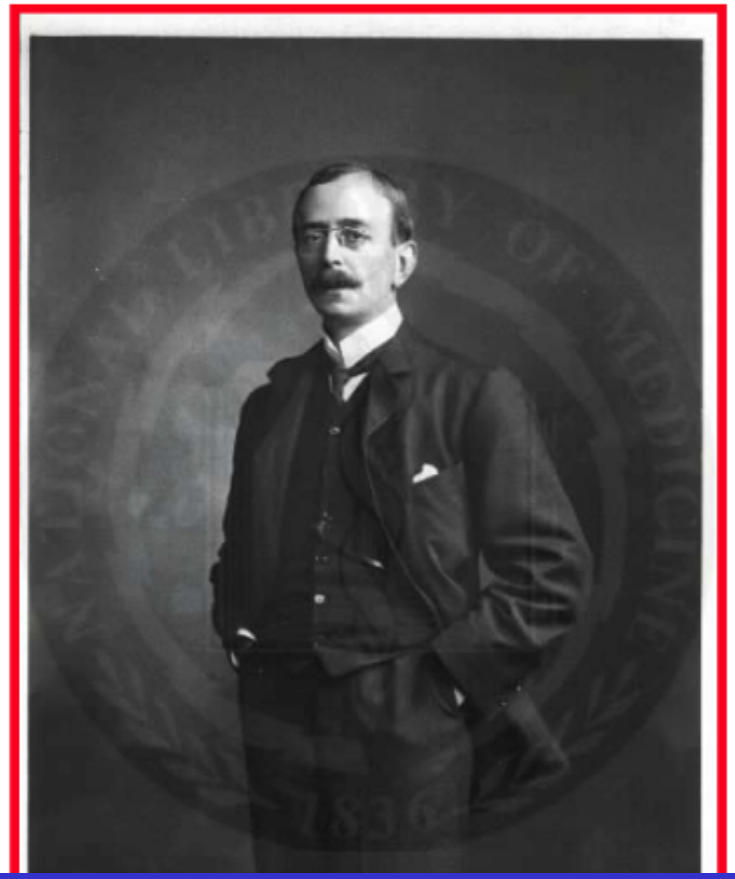
Мы сегодня обсудим:

- Синапс: основные принципы организации
- Ионные каналы - ключевые белки трансформации електро-химических сигналов:
  - основные модули ионных каналов
  - принциапы функционирования ионных каналов
  - что мы знаем о структуре ионных каналов
- Возбуждающие и тормозные синапсы
- Формирование и пластичность синапсов

Синапс

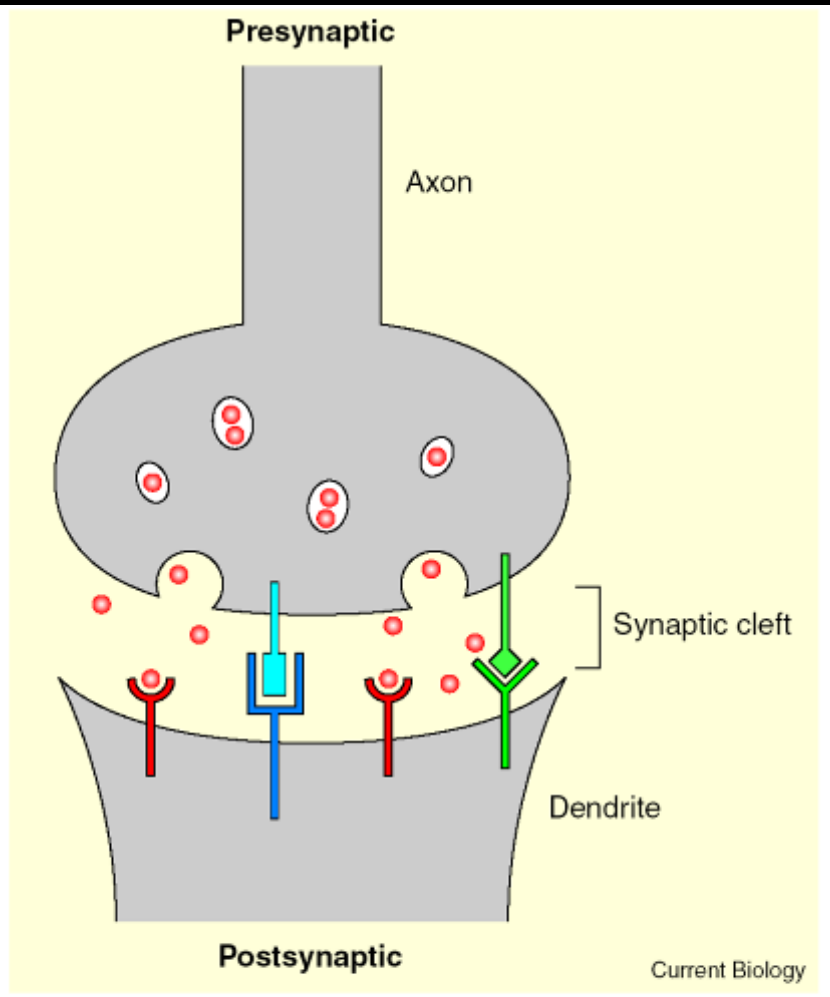
Шеррингтон - 1897

Lord C.S. Sherrington 1857-1952;
Étude sur les réflexes (réflexe myotatique)



Synapse – synaptein (Greek)

syn - together (вместе)
haptein-to hold (держать)

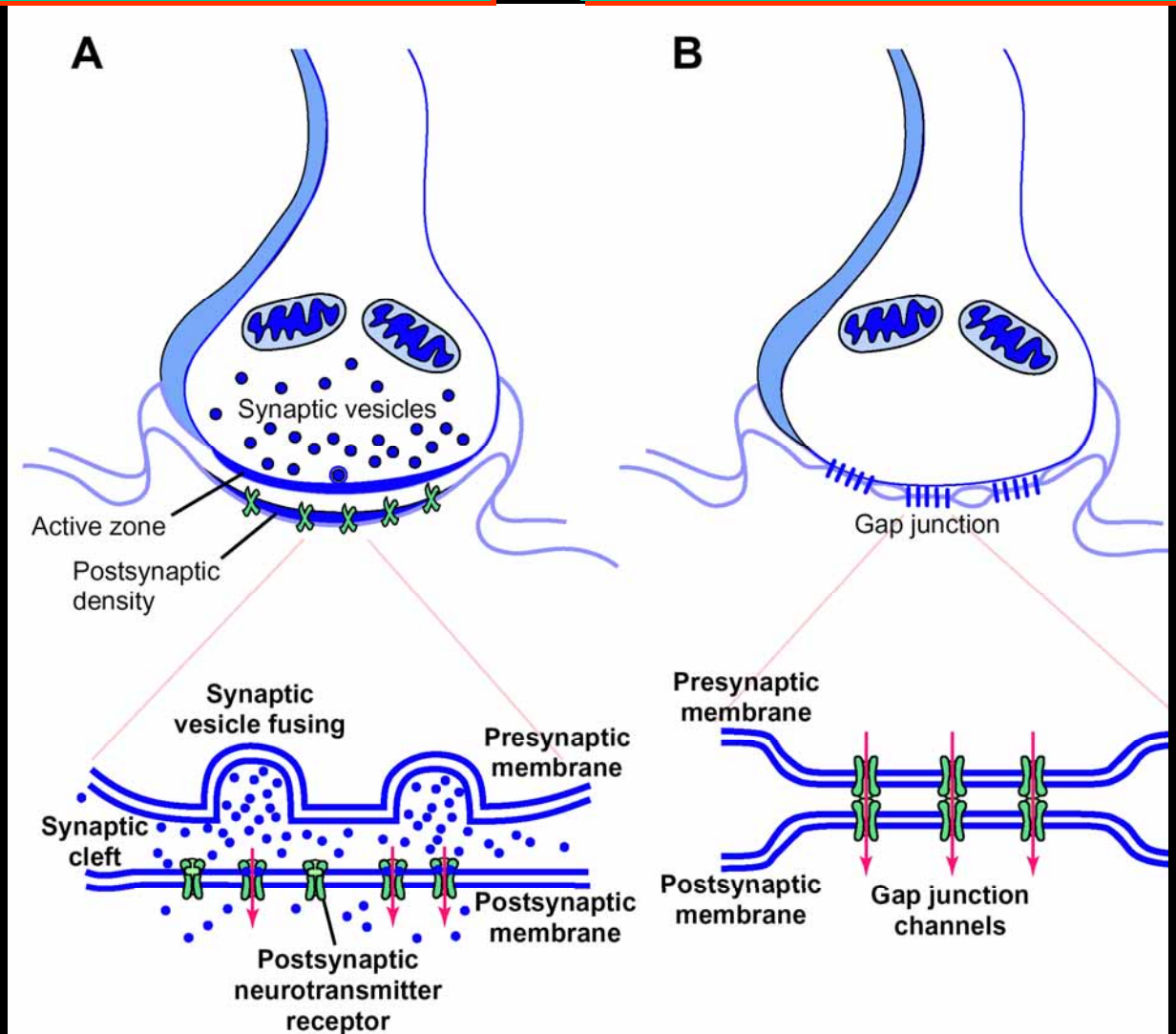


Synapse - region (site) of the contact of neuron with the other cell.

Синапс - место (участок) контакта нейрона с другой клеткой.

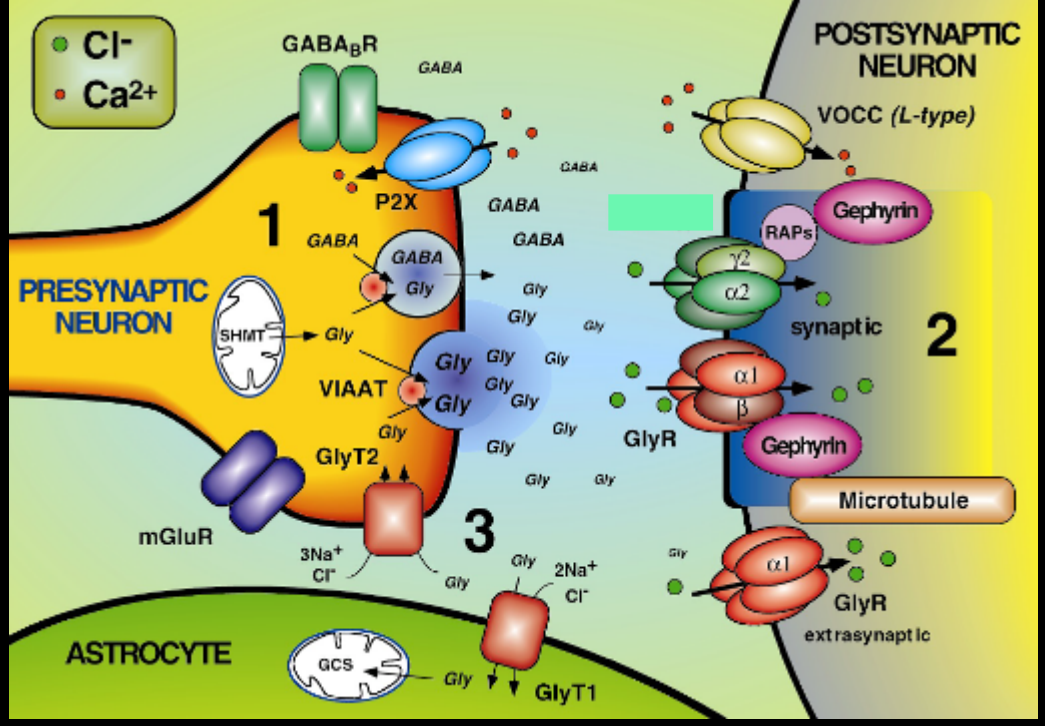
Основные типы синапсов

Химические **Электрические**

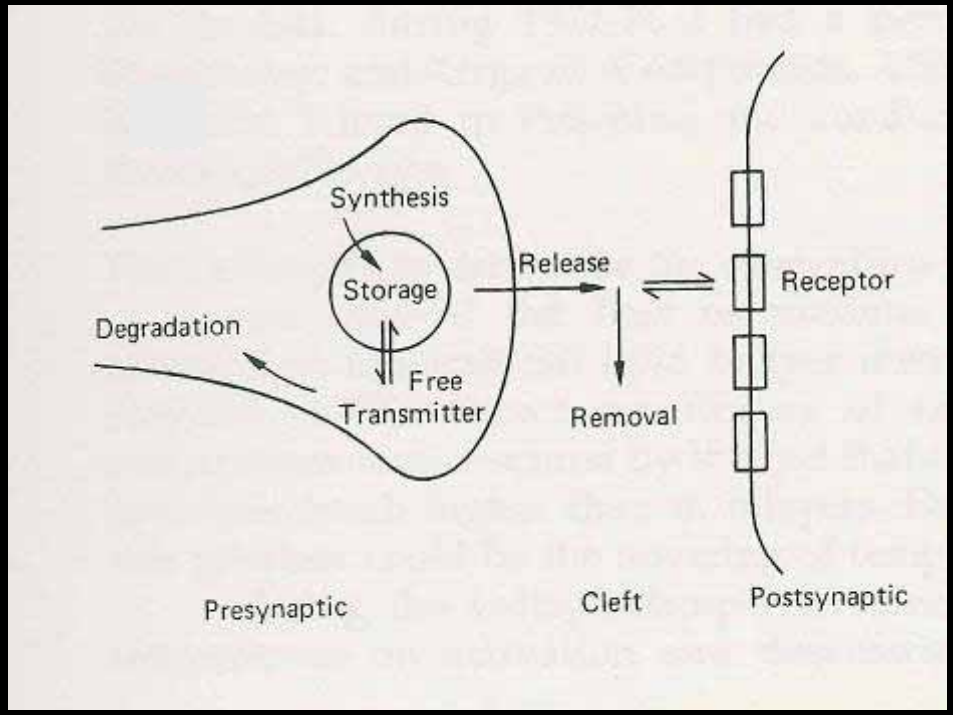


Synapse

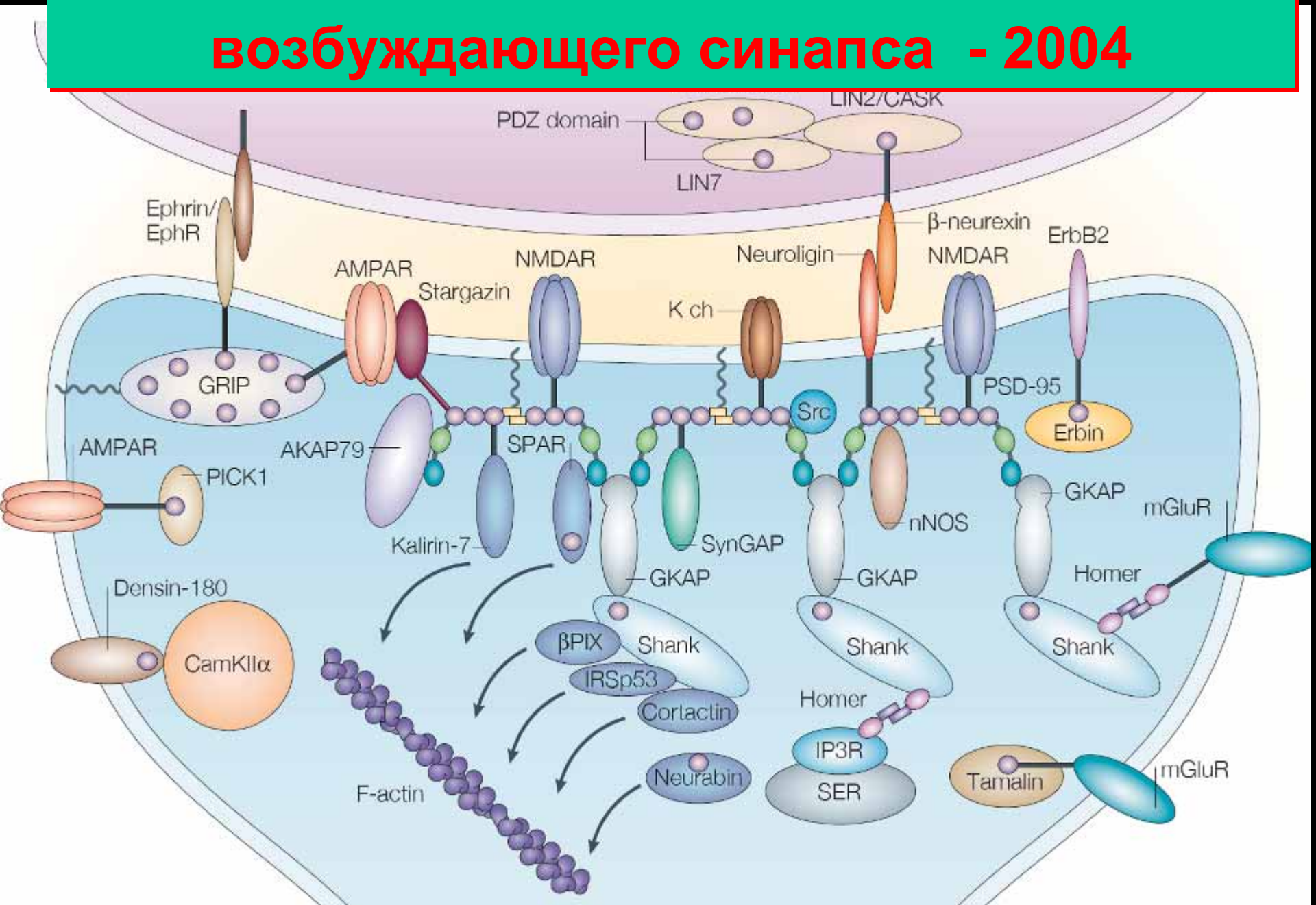
2000-e



1970-e

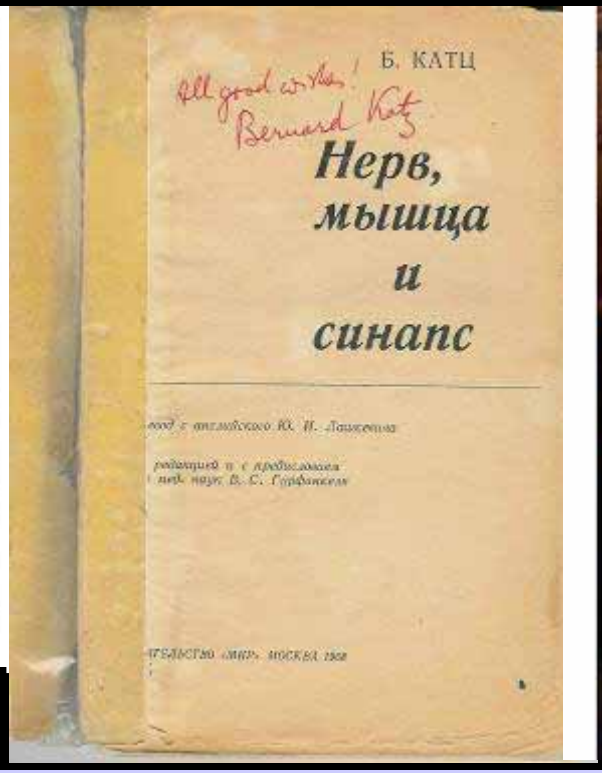


Компоненты постсинаптической области возбуждающего синапса - 2004



Bernard KATZ 1911-2003

B. Katz-1978

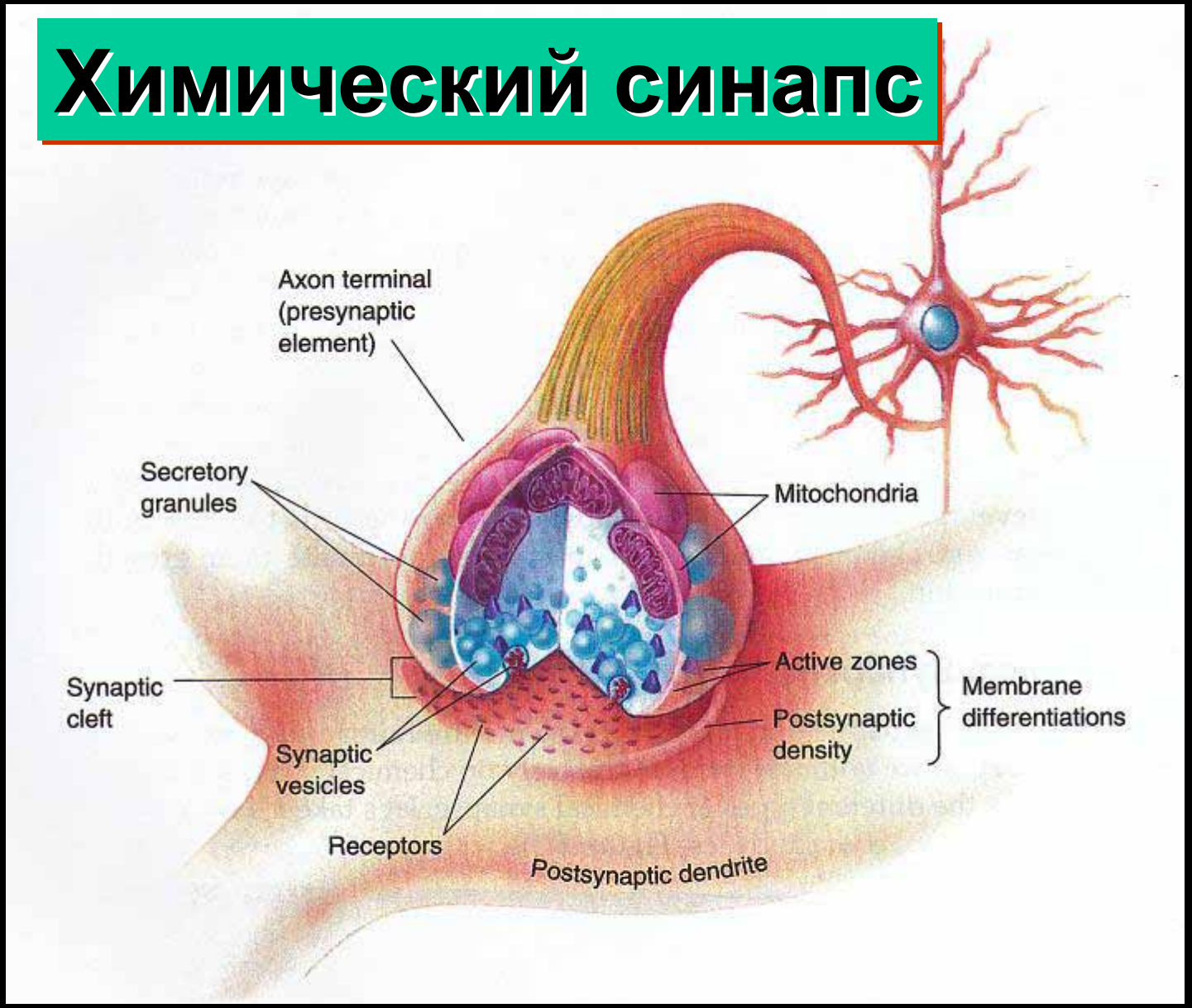


B. Katz-2002

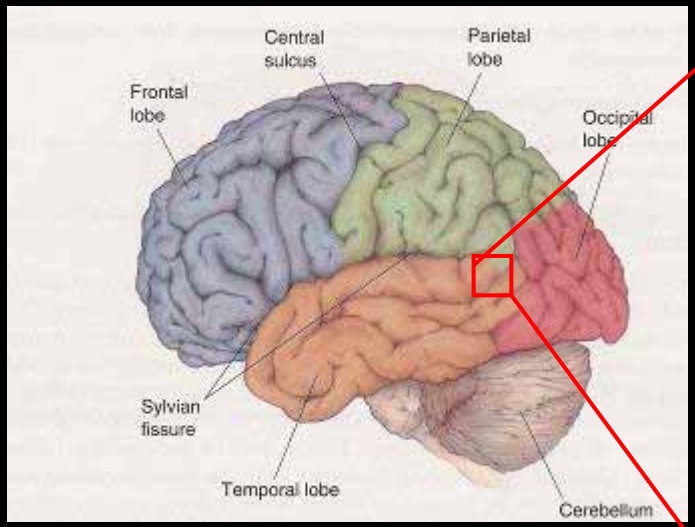
- квантовый выброс нейромедиатора
- ключевая роль кальция в выбросе нейромедиатора
- оценка параметров одиночных каналов

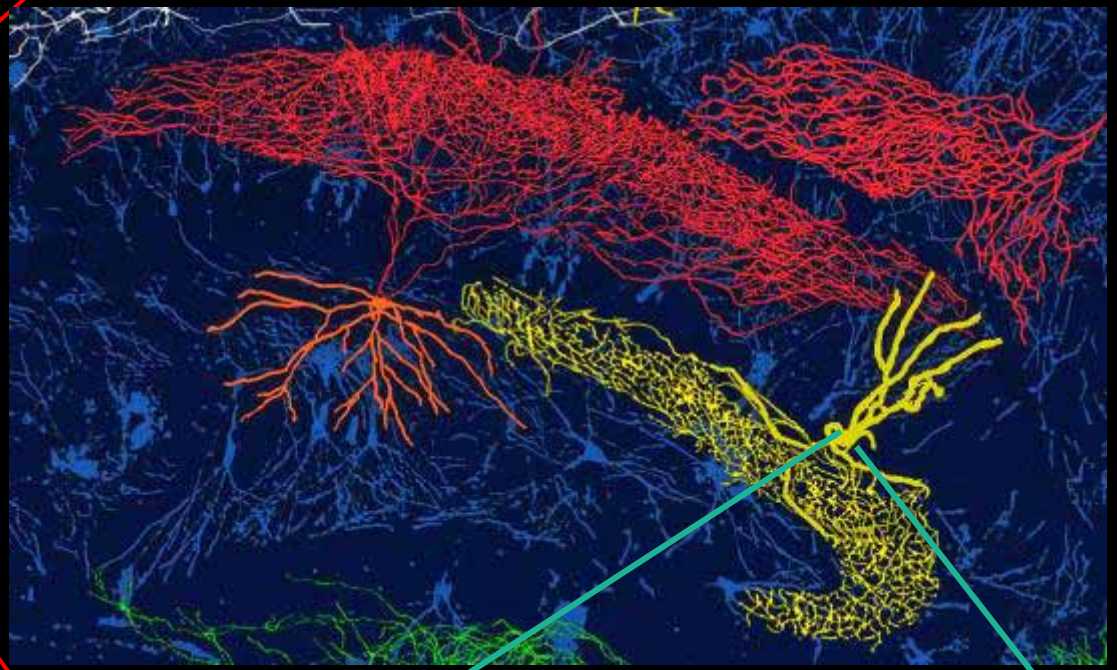
Nobel Price - 1970

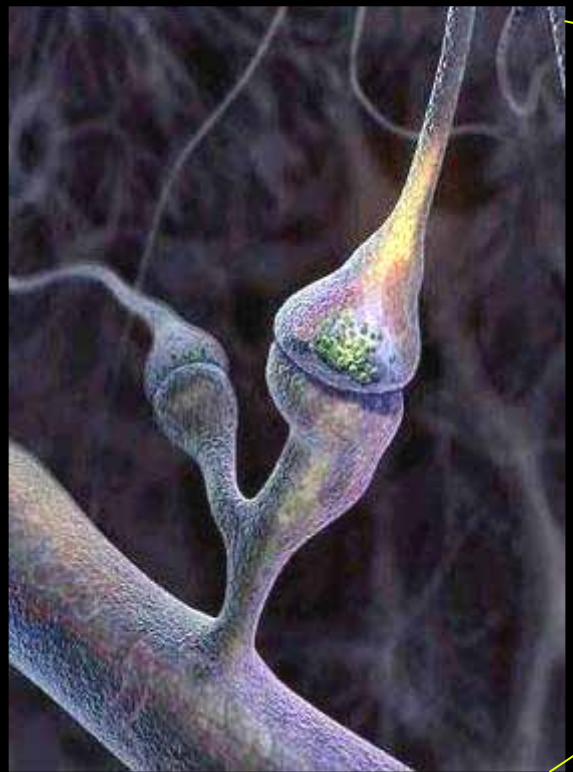
Химический синапс

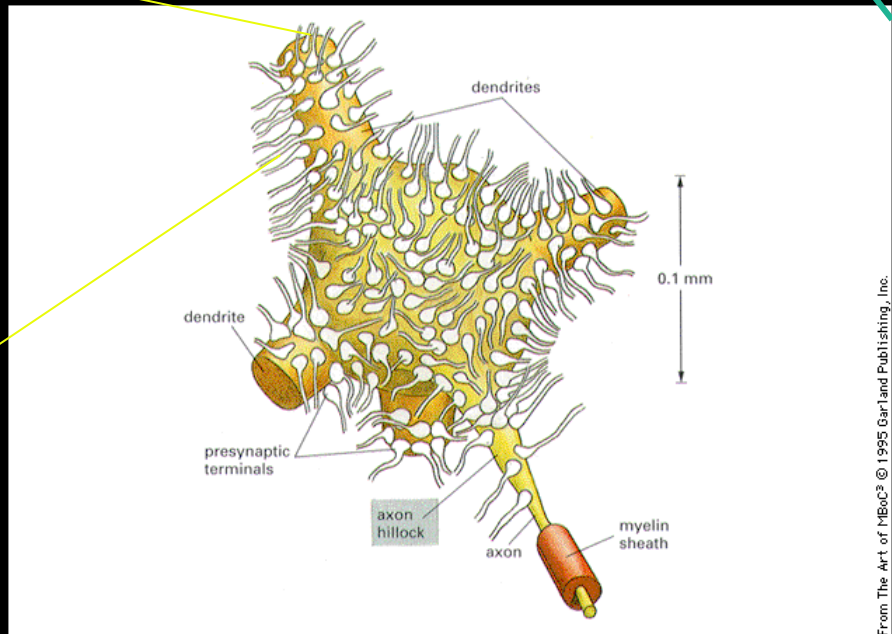


В химических синапсах передача сигнала осуществляется химическими соединениями (нейромедиаторами)

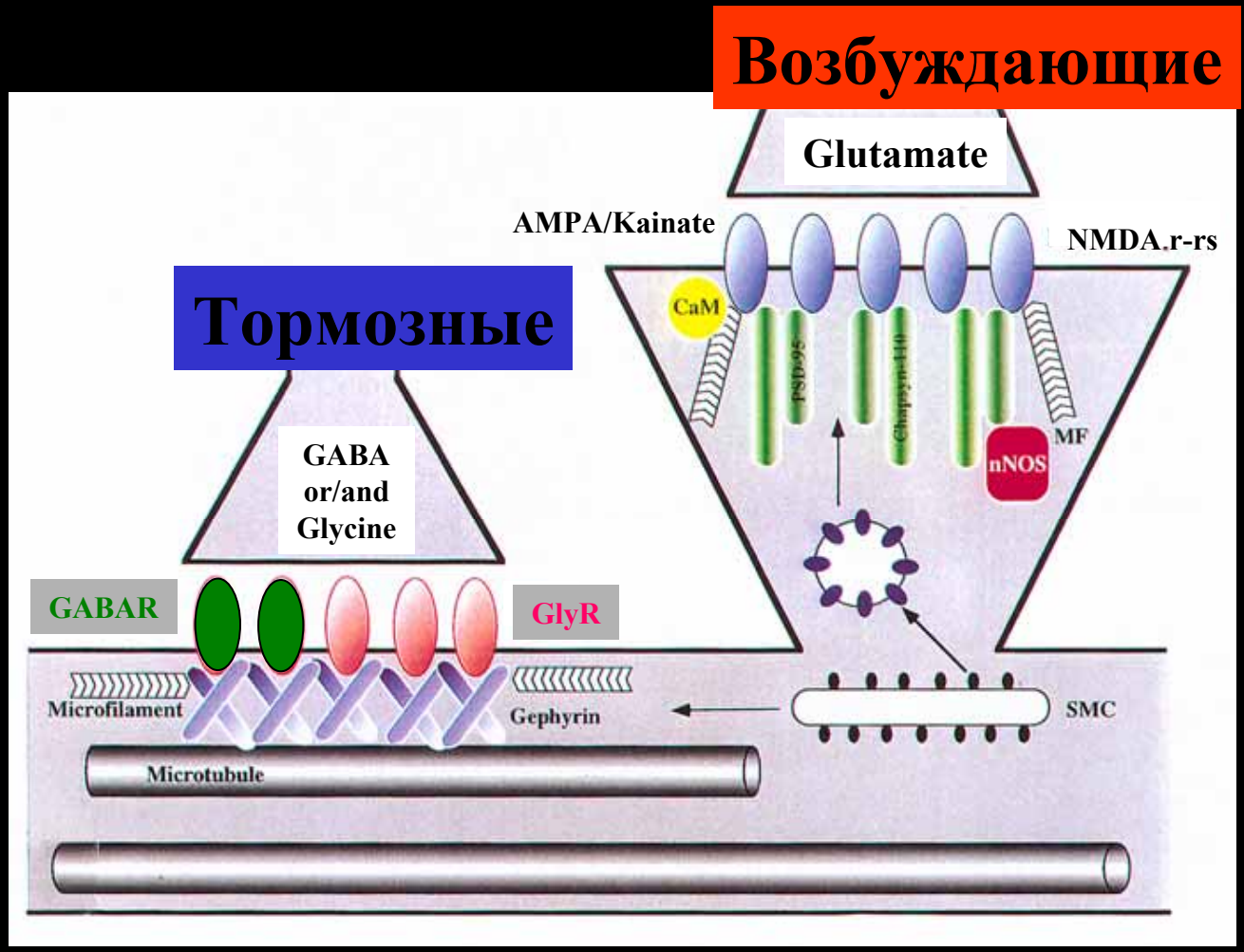






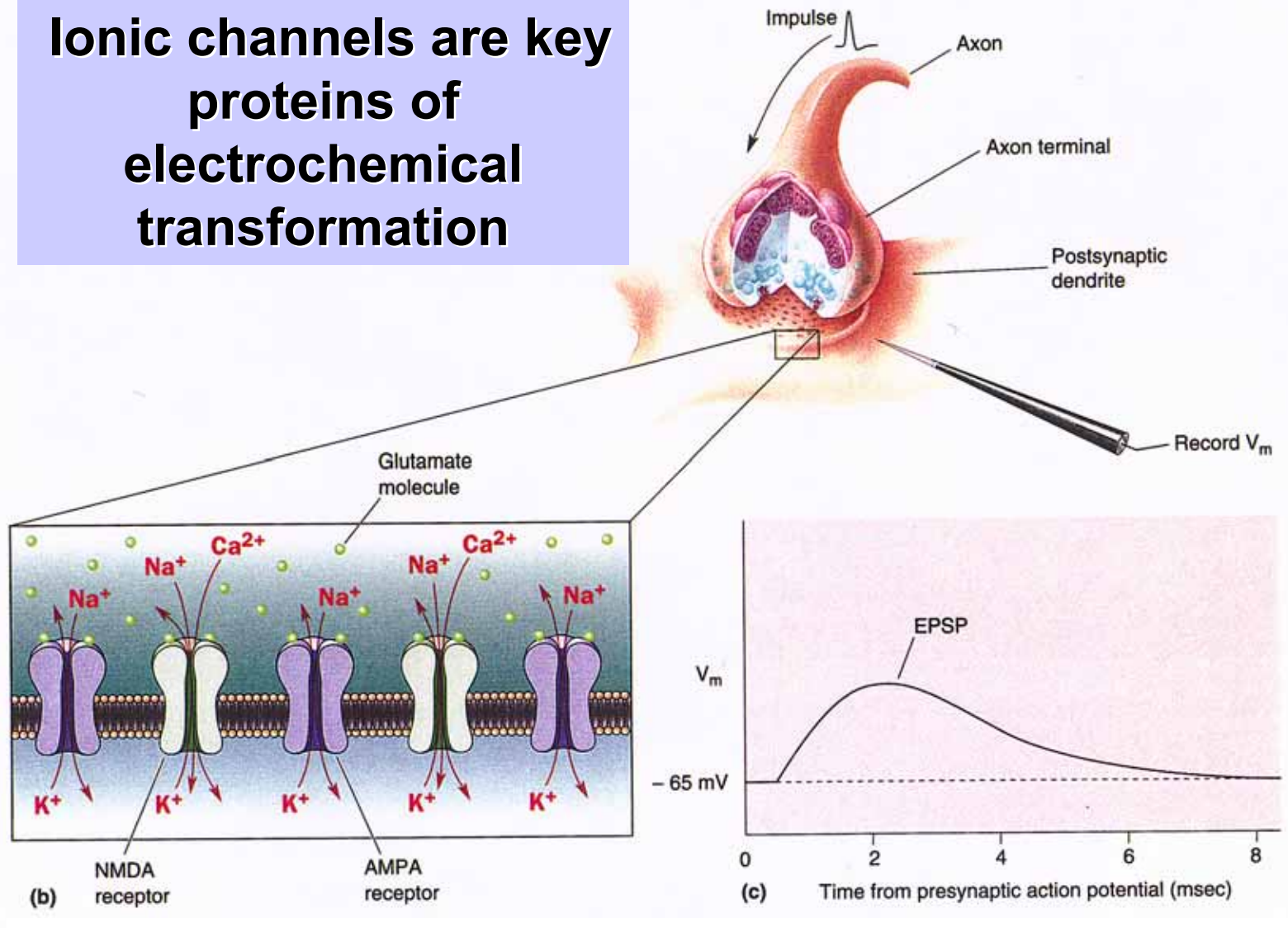


Синапсы в мозге позвоночных

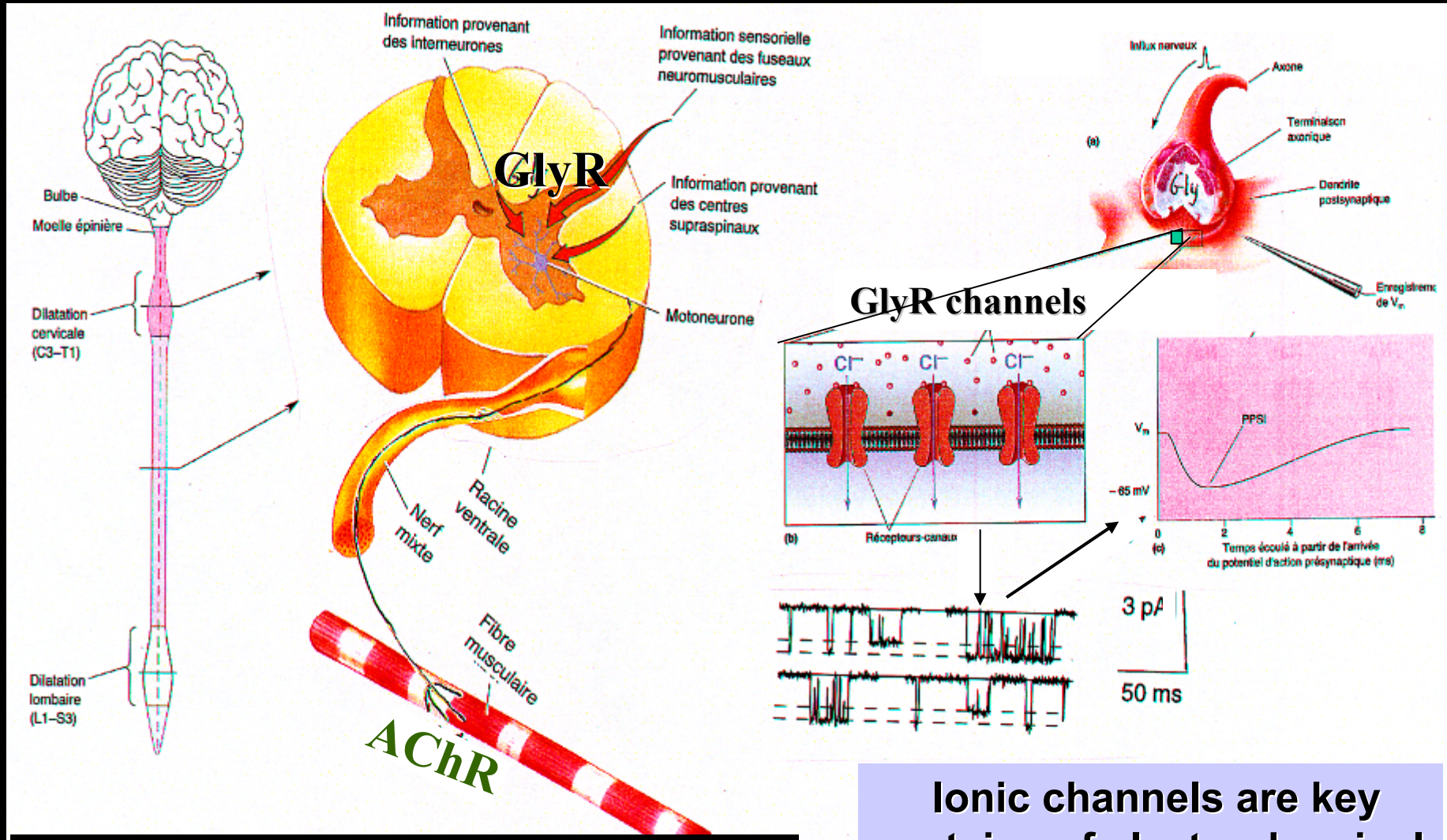


Возбуждающие синапсы: глутаматергические

Ionic channels are key proteins of electrochemical transformation



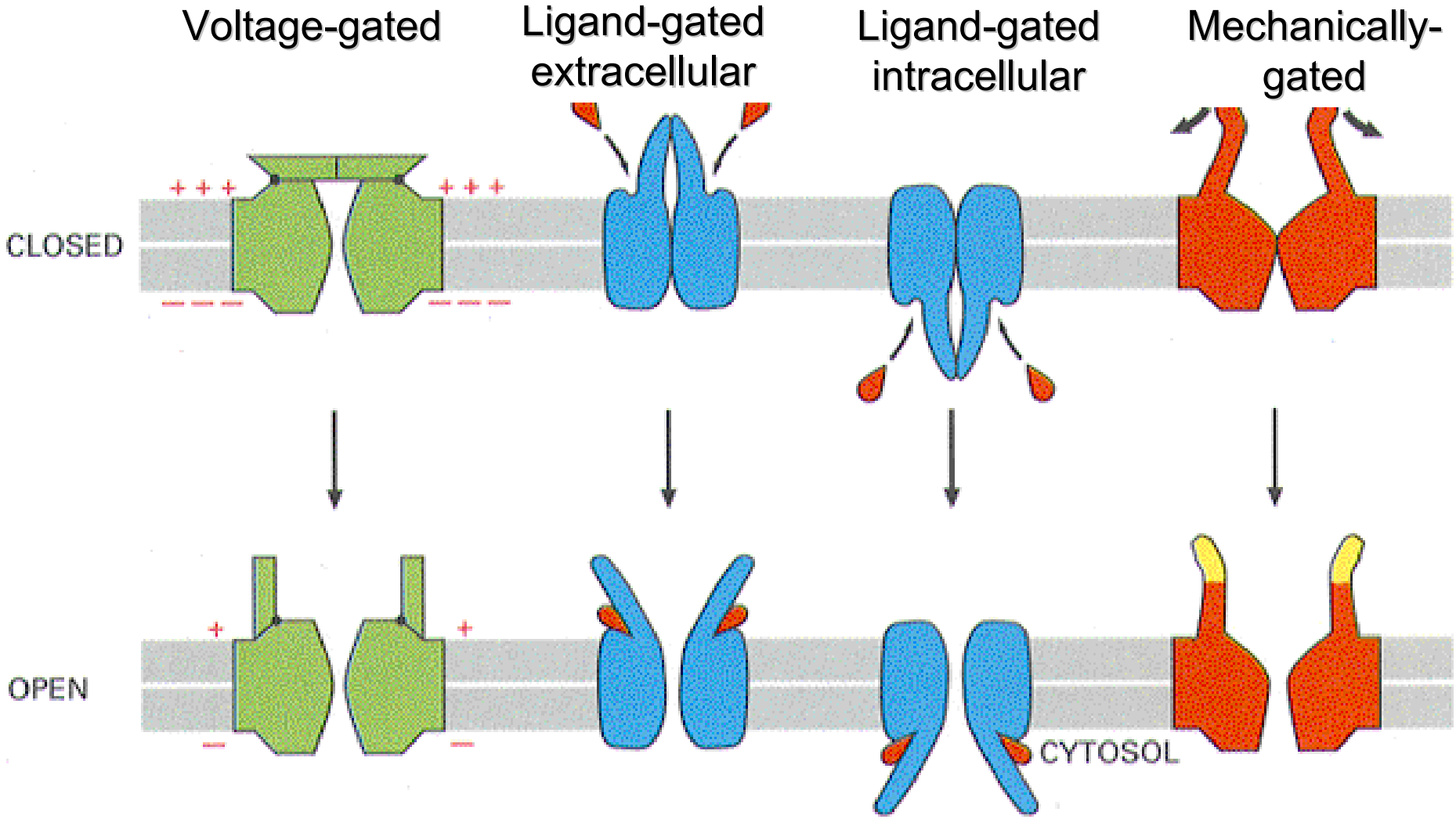
Глицин и ГАБА - тормозные нейромедиаторы в синапсах ЦНС



Ионные каналы - ключевые белки биологической жизни

Ionic channels are key proteins of electrochemical transformation

Основные типы ионных каналов



Medina & Bregestovski, Proc.Roy Soc, 1991

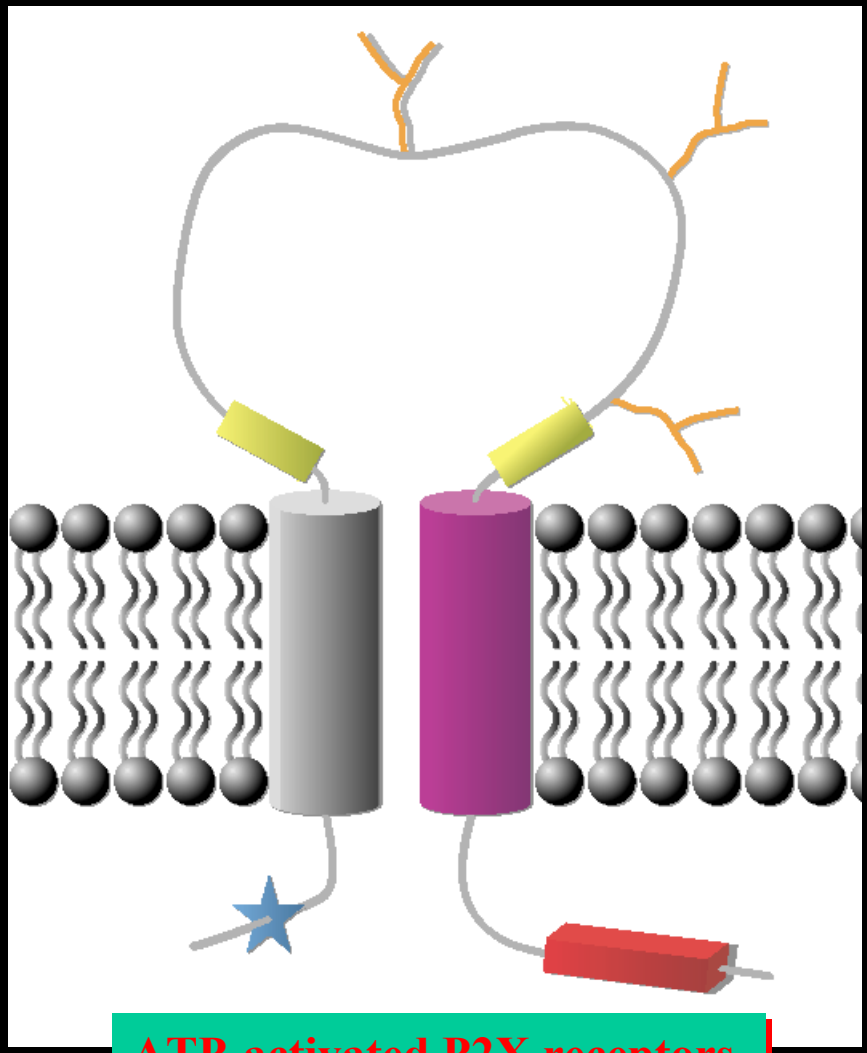
Ионные каналы - молекулярные комплексы, превращающие потенциал, химические или механические сигналы в потоки ионов

Основные функциональные блоки ионных каналов

Extracellullar domain:
-receptor site;
-extrasynaptic targeting

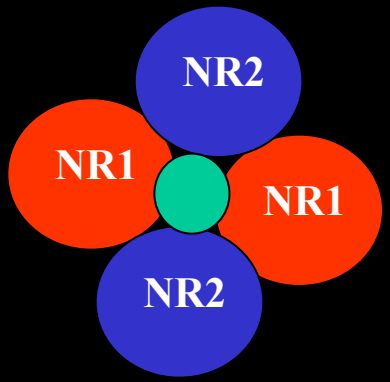
Transmembrane domain:
-ion selectivity;
-gating

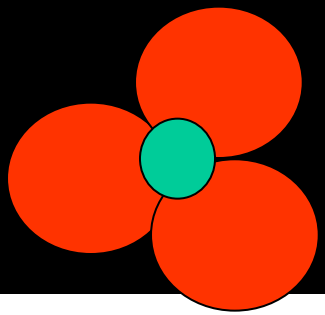
Intracellullar domain:
-phosphorylation;
-protein-protein interaction

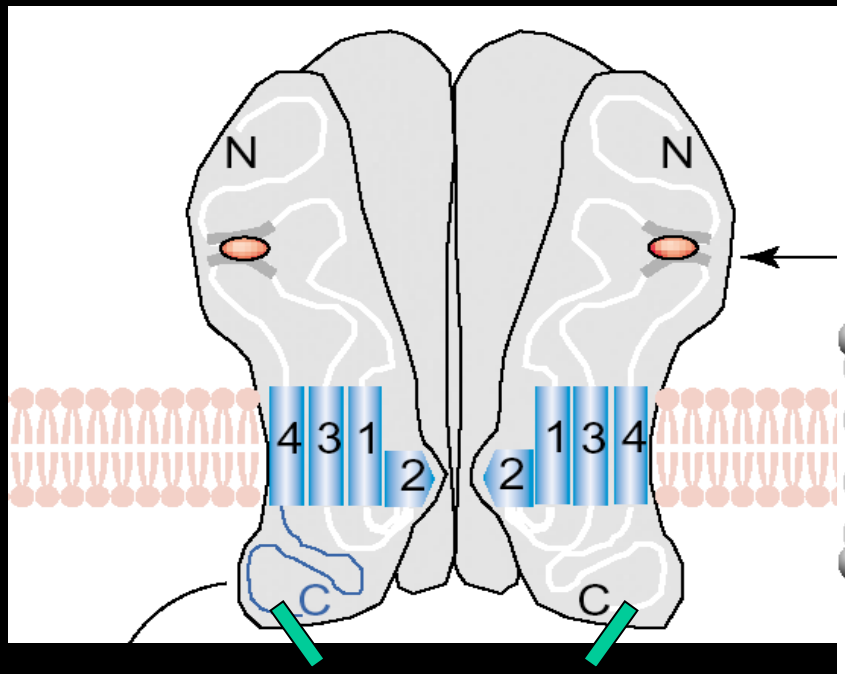


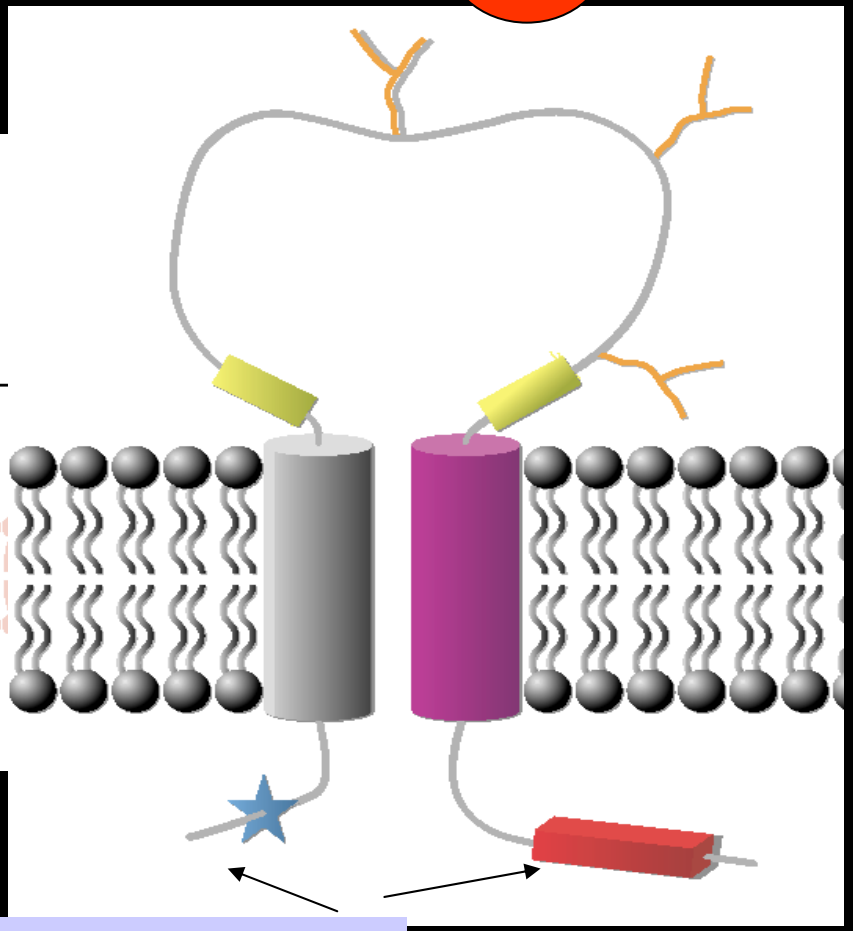
ATP-activated P2X receptors

Субъединичная организация каналов









Domains for interaction with cytoplasmic poteins

Субъединичная организация каналов

Cation-selective

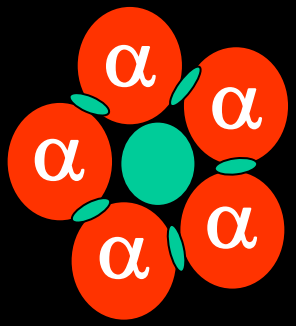
Acetylcholine

Serotonine

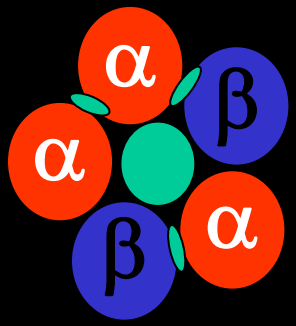
Anion-selective

GABA

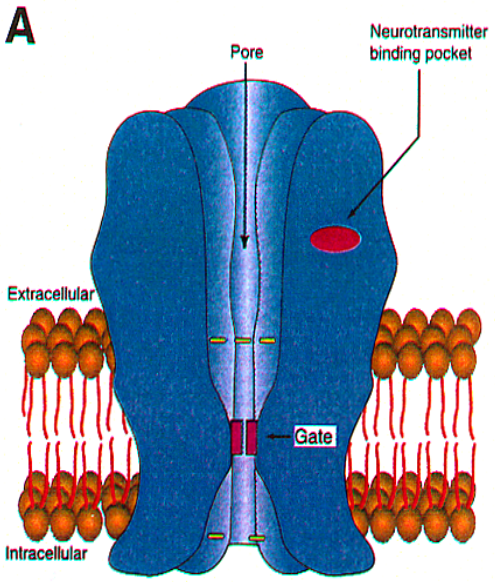
Glycine

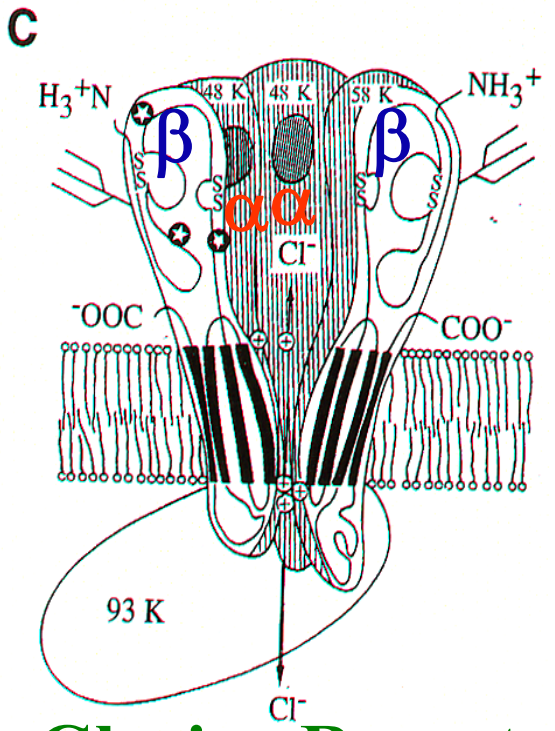


homo-



hetero-oligomeric

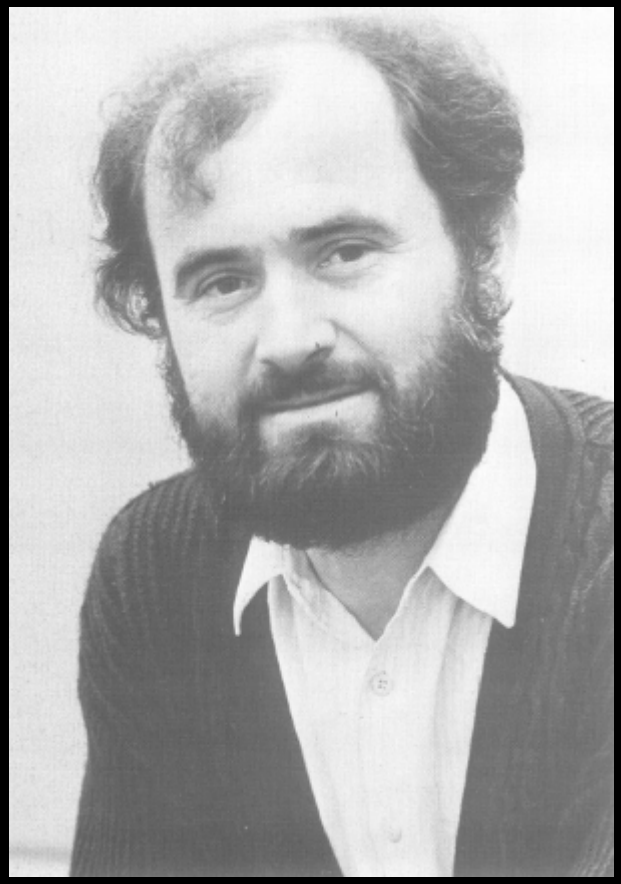




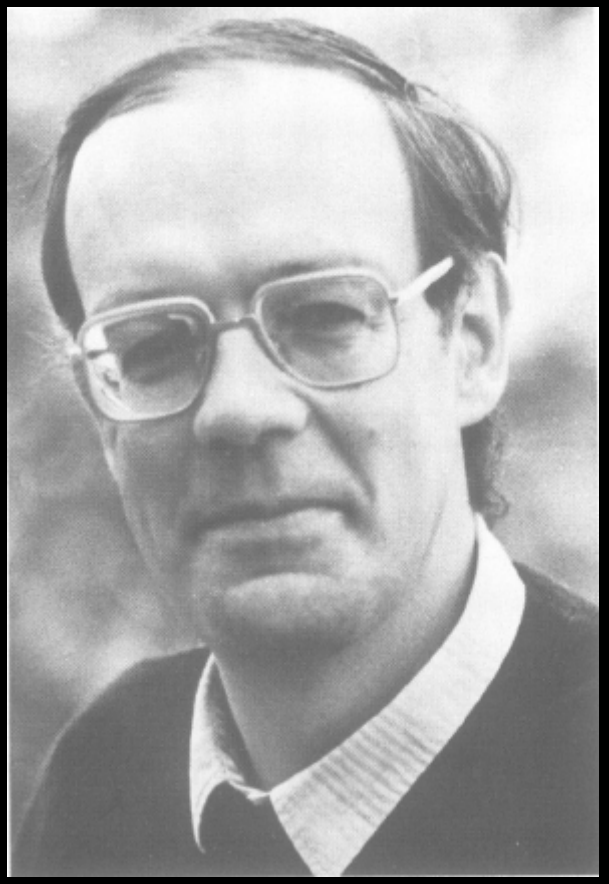
Glycine Recepto

Basic principles of ionic channels functioning

Как увидеть активность одиночных ионных каналов?

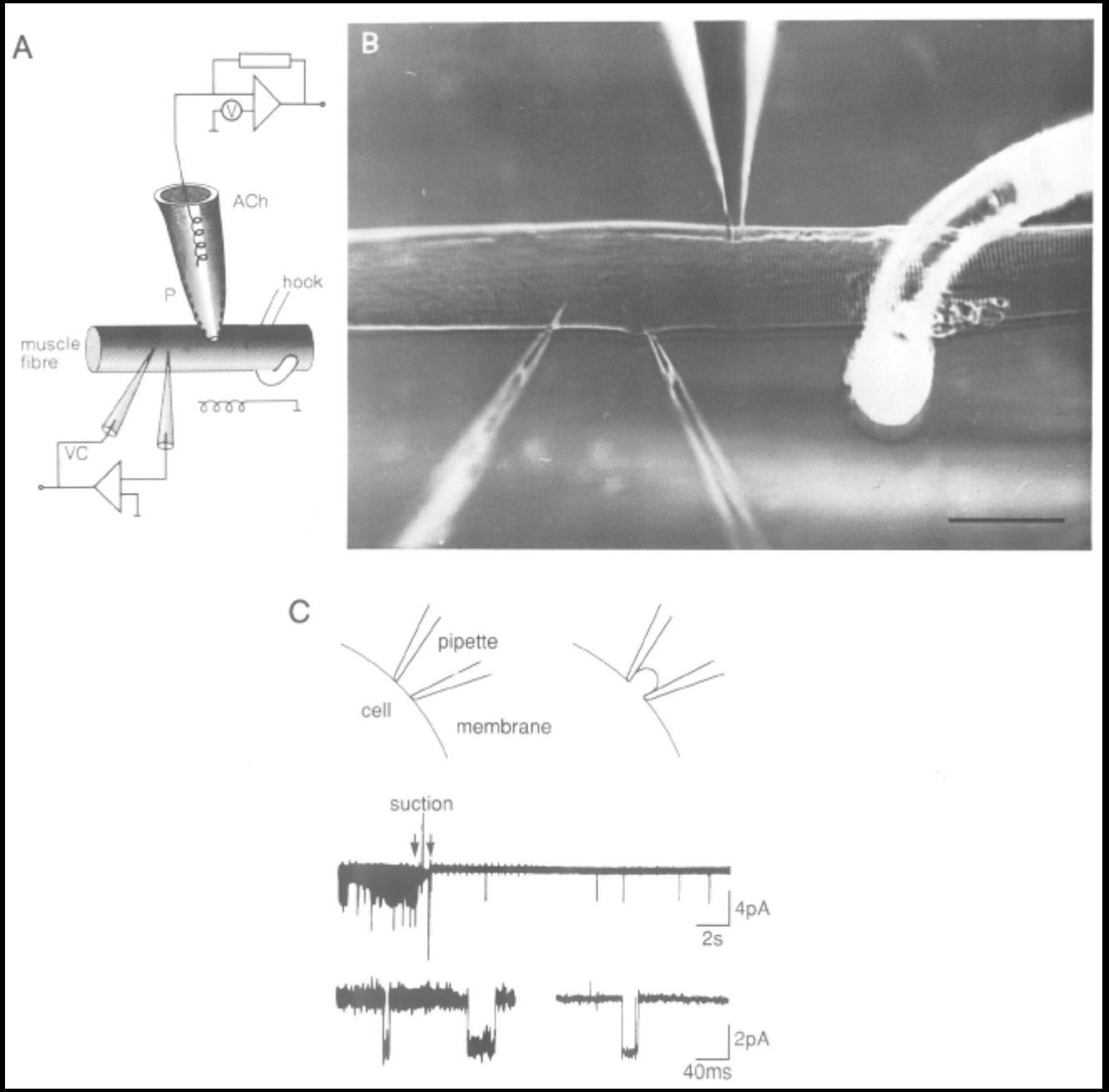


E. Neher



B. Sakmann

Nobel Price-1991



Neher & Sakmann, Nature, 1976

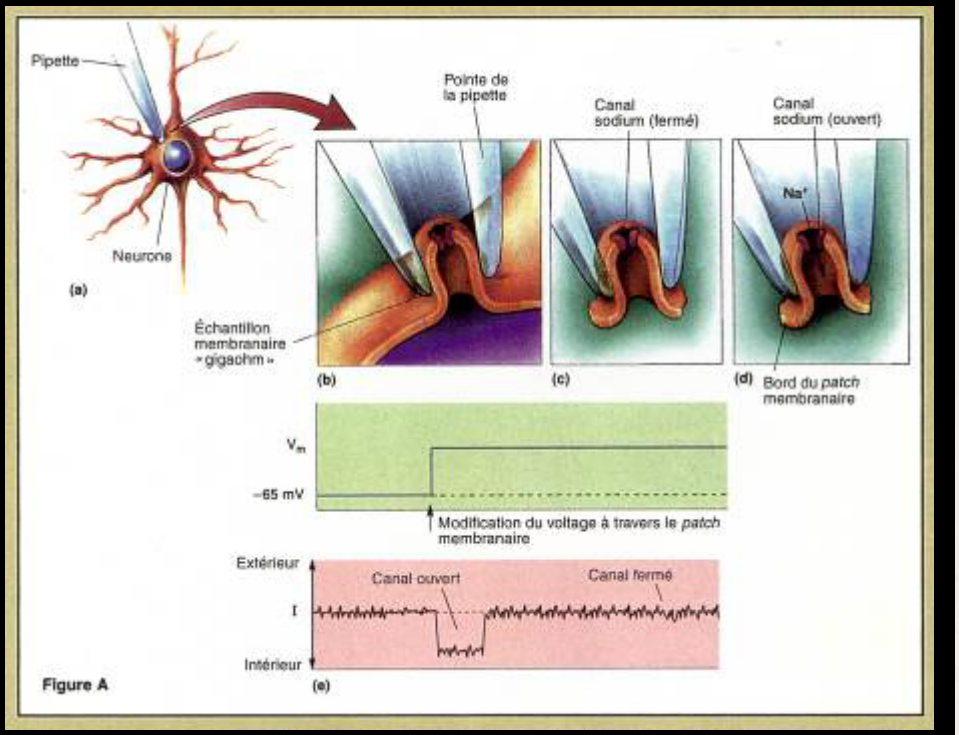


Figure A

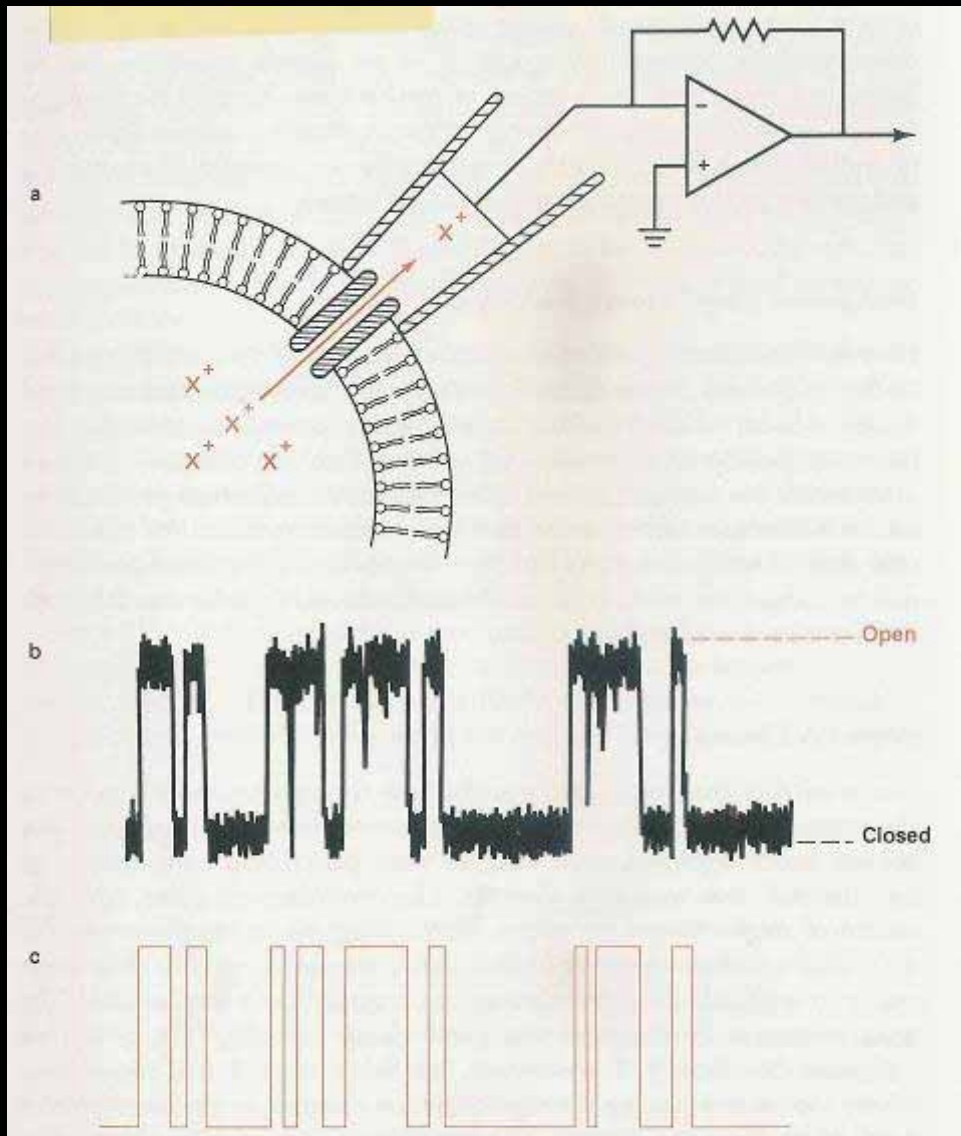
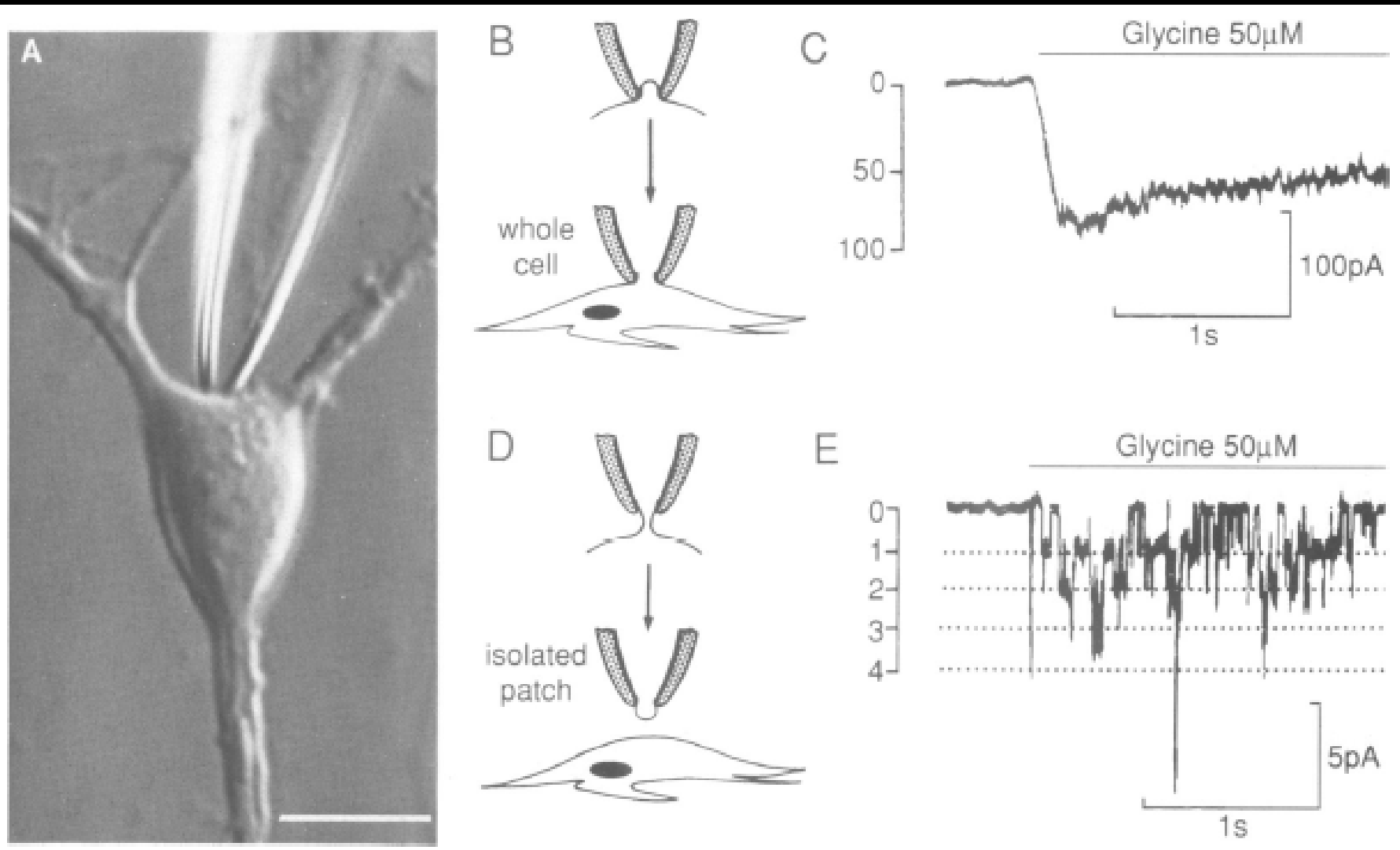
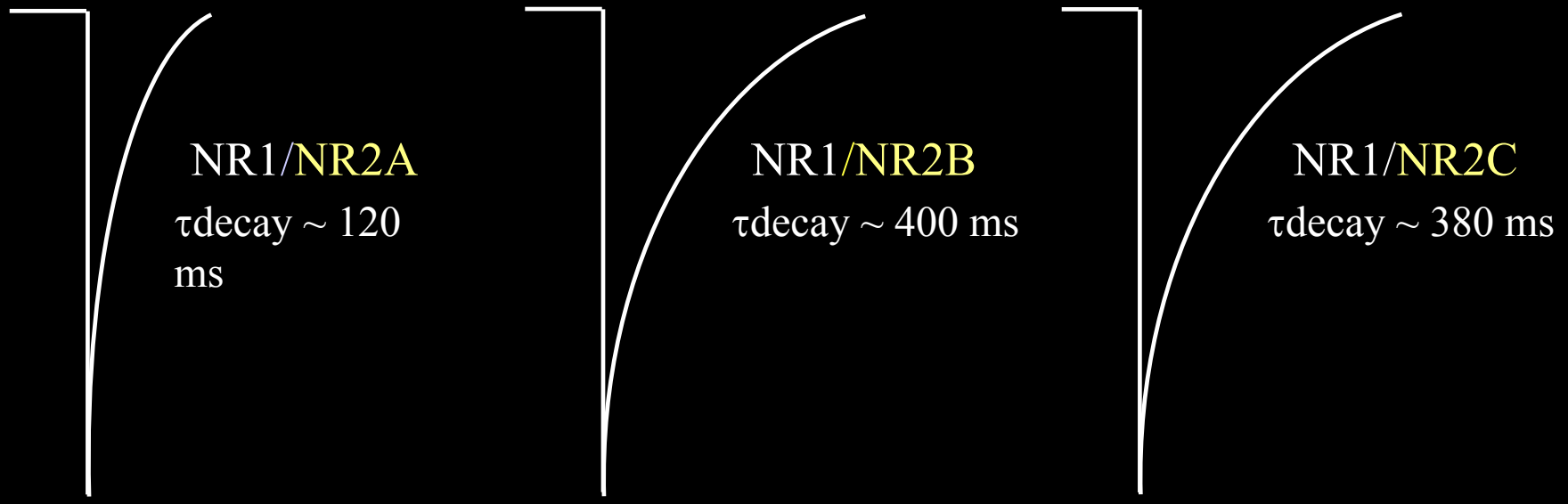


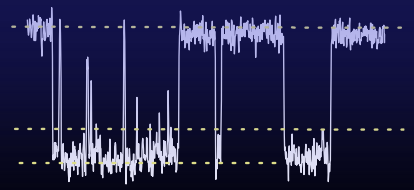
FIGURE 3-4. Patch clamp recording of single ion channel activity. *a*: Illustration of the cell-attached mode of patch clamp recording, with a current-to-voltage converter that is connected to the electrode (Hamill et al., 1981). *b*: An example of recordings of single channel activity obtained with this method. *c*: Simple computer programs can be used to produce idealized single channel records, which reproduce faithfully the openings and closings seen in the real record.


A
B
whole
cell
C
Glycine 50μM
0
50
100
100pA
1s
D
isolated
patch
E
Glycine 50μM
0
1
2
3
4
5pA
1s

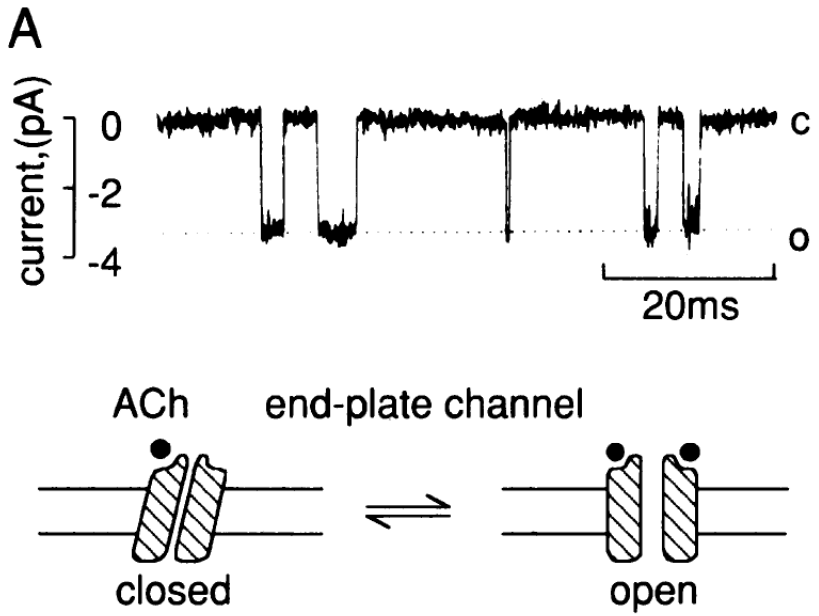
Кинетика деактивации интегральных ионных токов

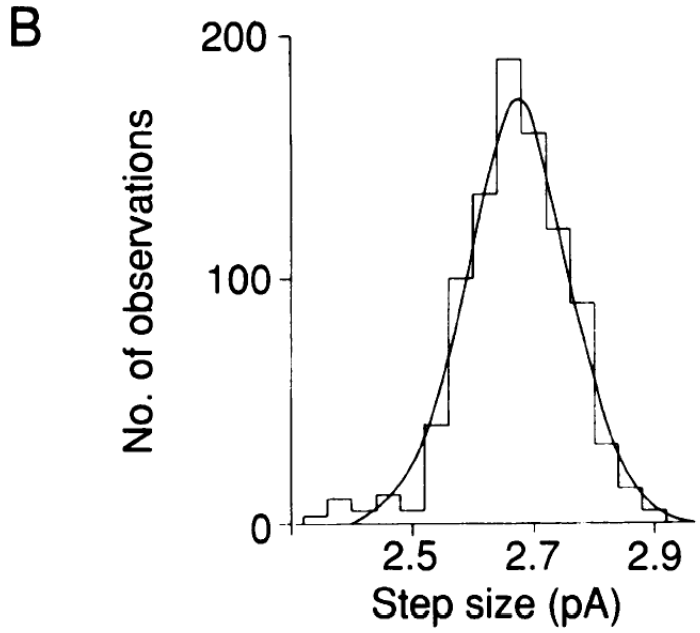


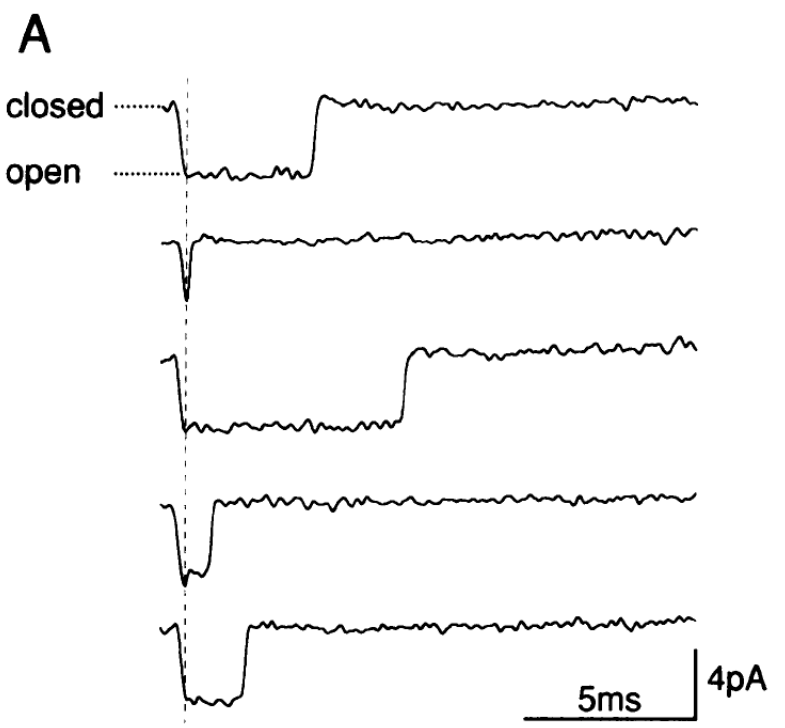
Интегральные токи

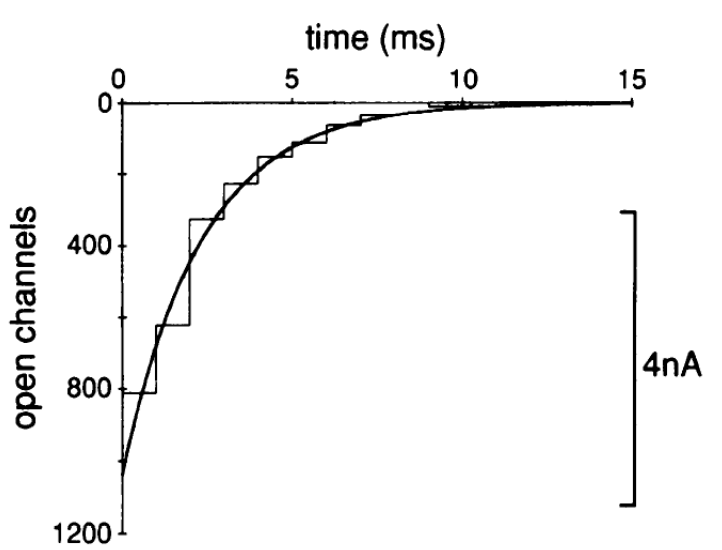


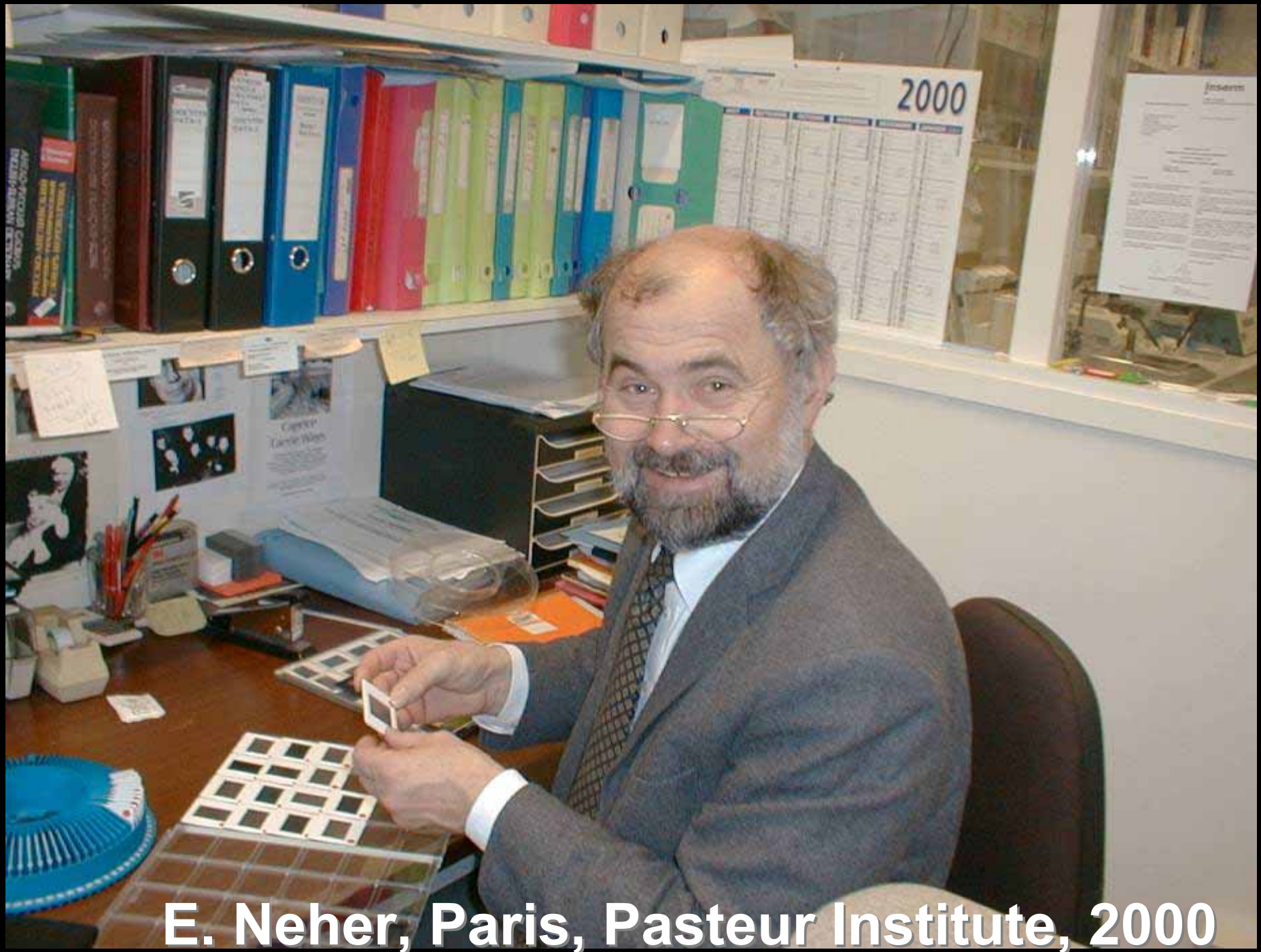
Токи одиночных каналов


A
current,(pA)
0
-2
-4
c
o
20ms
ACh
end-plate channel
closed
open


B
200
100
0
No. of observations
2.5
2.7
2.9
Step size (pA)


A
closed
open
5ms
4pA


B
time (ms)
0
5
10
15
0
400
800
1200
open channels
4nA



E. Neher, Paris, Pasteur Institute, 2000

Paris, Pasteur Institute, 2000

B. Sakmann & N. Spitzer, Rome 2007

Некоторые работы

Доказательство конформационных изменений рецептора ацетилхолина **Bregestovski et al., Nature, 1977**

Доказательство кальциевой проницаемости рецептора ацетилхолина **Bregestovski et al.,Nature, 1979**

Потенциал-зависимый блок магнием рецепторов NMDA **Nowak, Bregestovski, Ascher.,Nature, 1984**

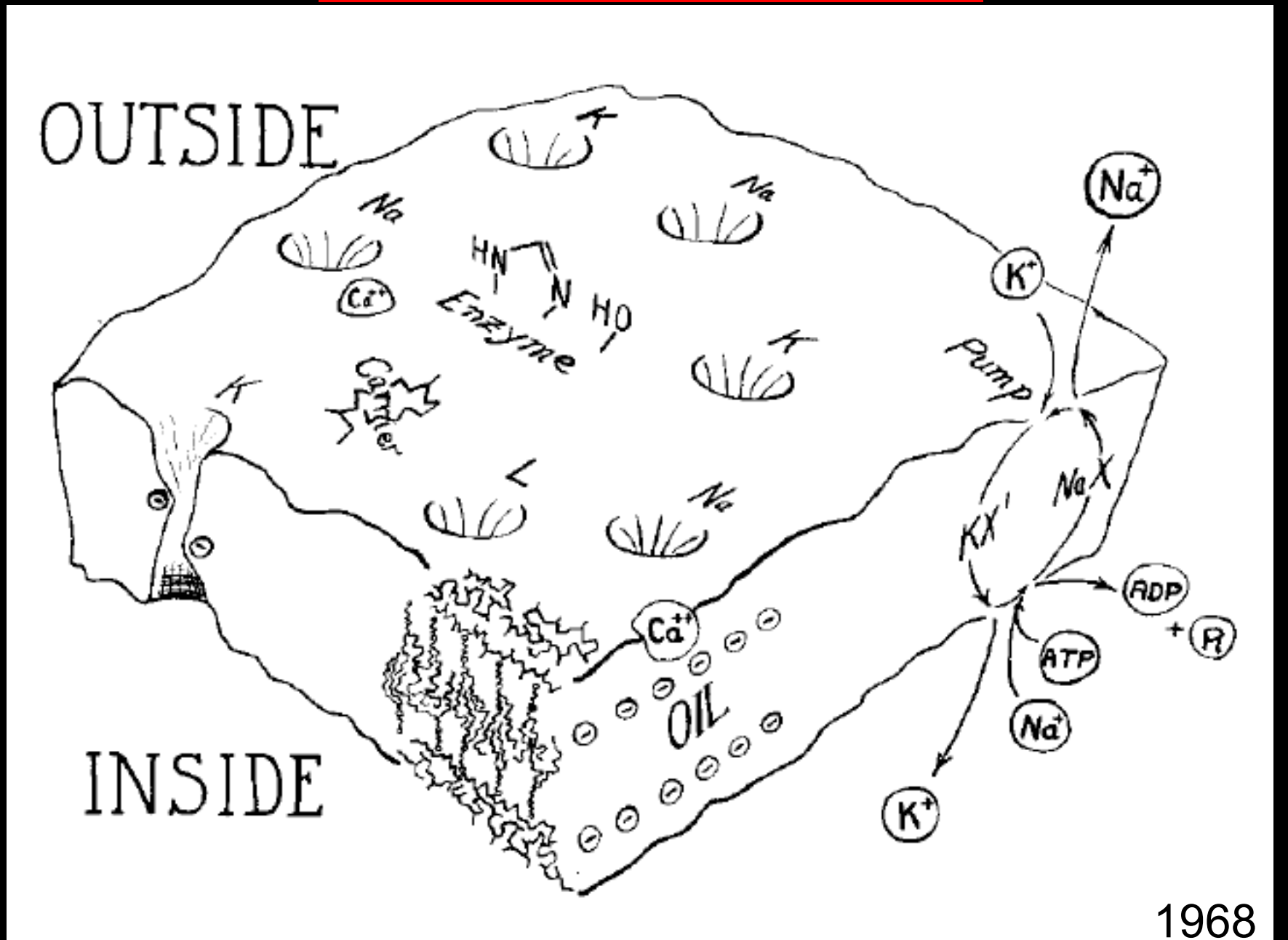
Кальций-зависимая модуляция K^+ каналов лимфоцитов **Bregestovski, Redkozubov, Alexeev,Nature, 1986**

Открытие нового типа механо-чувствительных каналов **Medina, Bregestovski, Proc.Roy.Soc, 1991**

- what we know about structure of ion channels

Как увидеть молекулярную организацию ионных каналов?

Ion Channels -1968



1968

Albert Lasker
Basic Medical Research Award

Clay Armstrong, Bertil Hille and Roderick MacKinnon

For elucidating the functional and structural architecture of ion channel proteins, which govern the electrical potential of membranes throughout nature, thereby generating nerve impulses and controlling muscle contraction, cardiac rhythm, and hormone secretion. (More >)

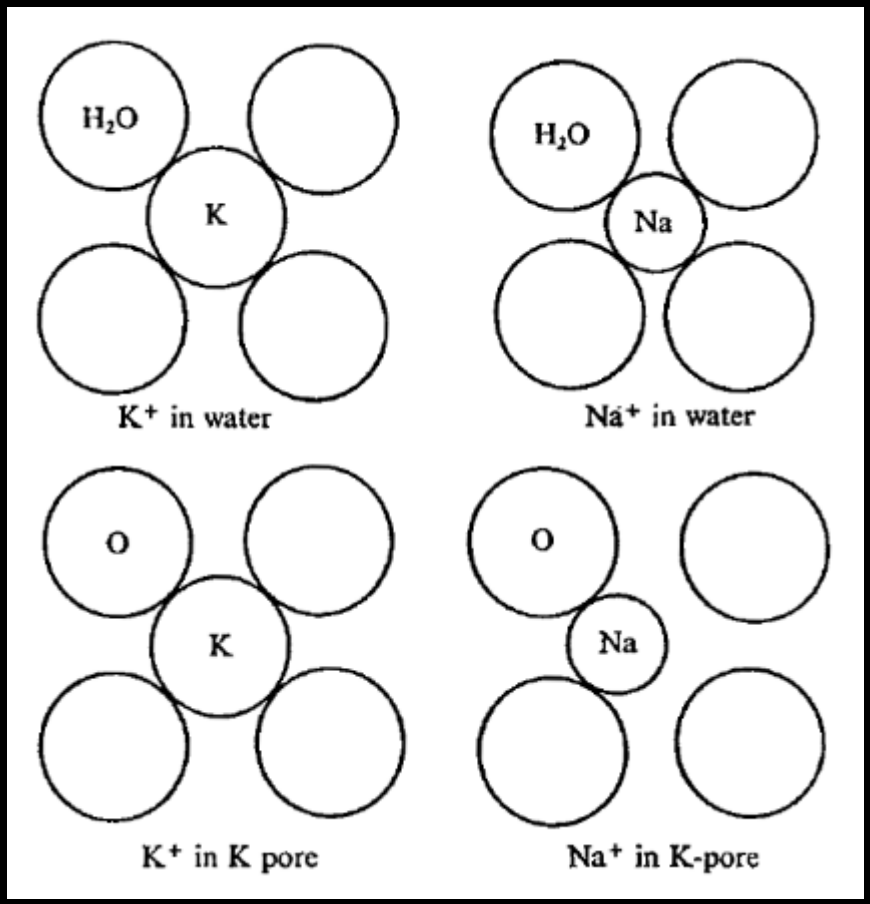
Clay Armstrong
University of Pennsylvania
School of Medicine

Bertil Hille
University of Washington
School of Medicine

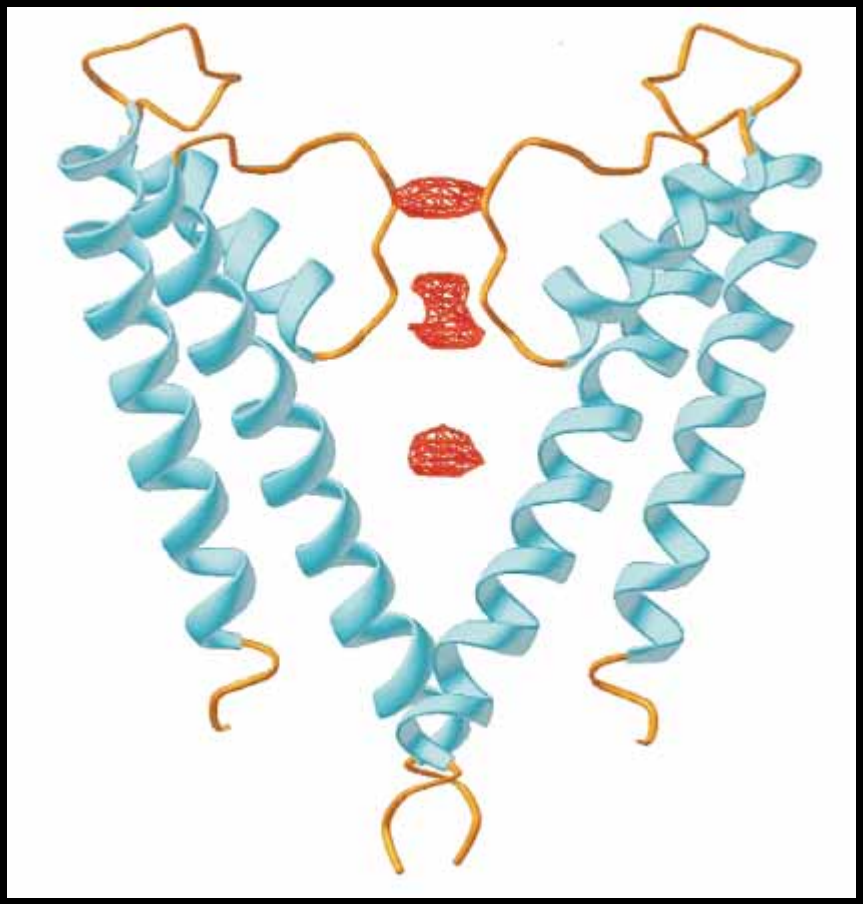
Roderick MacKinnon
The Rockefeller University
Howard Hughes Medical Institute

Idea and reality for K channels selectivity

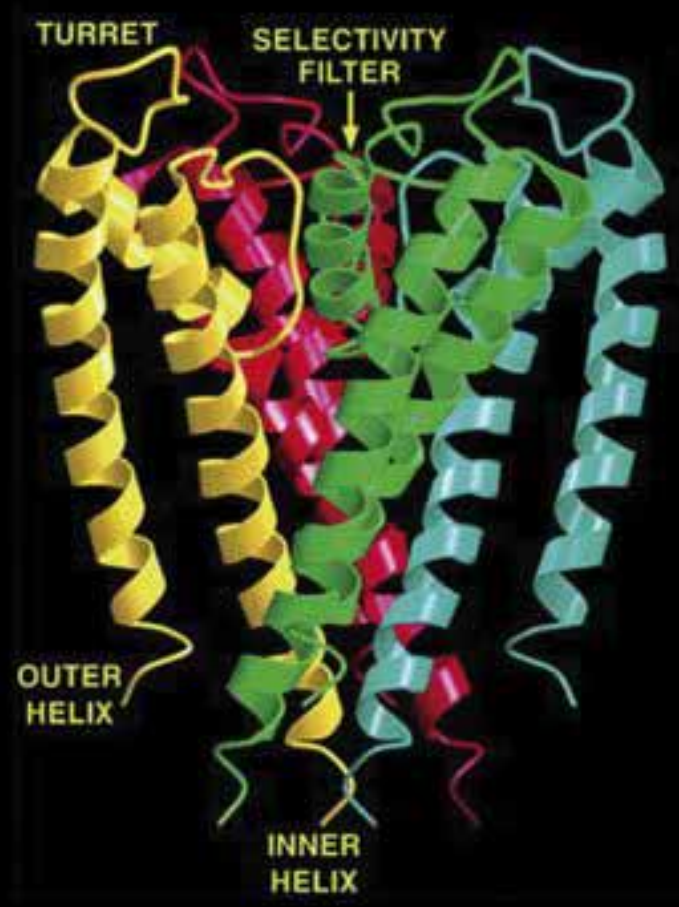
Idea - 1968

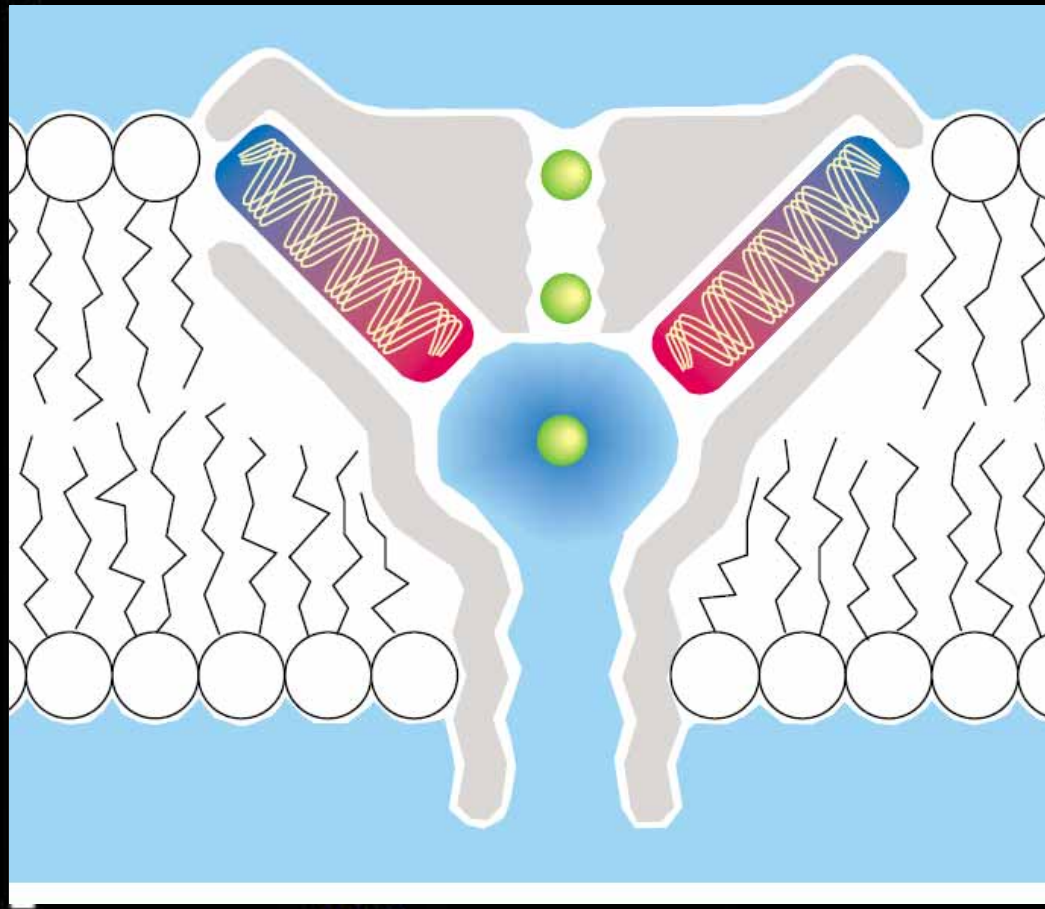


Reality-1998



The crystal structure of K^+ channel





MacKinnon R. Nobel price - 2003

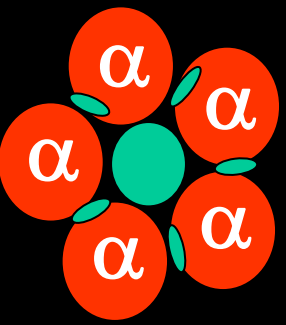
Cys-Loop ligand-gated channels

Cation-selective

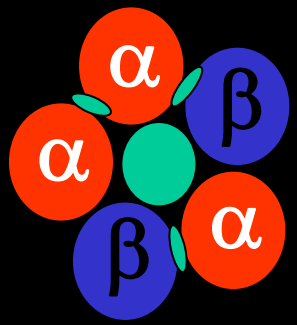
- Acetylcholine
- Serotonine (5-HT)

Anion-selective

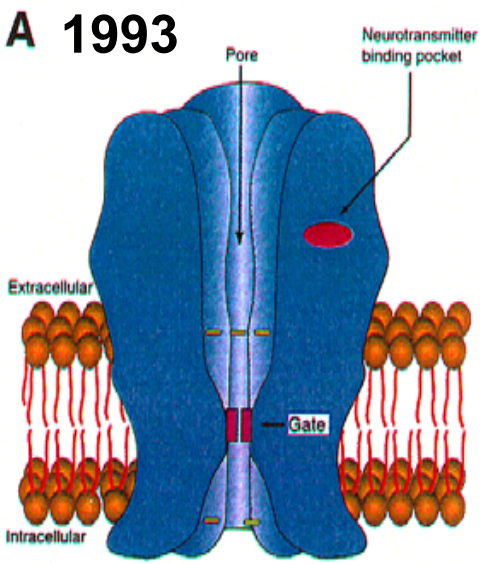
- GABA
- Glycine



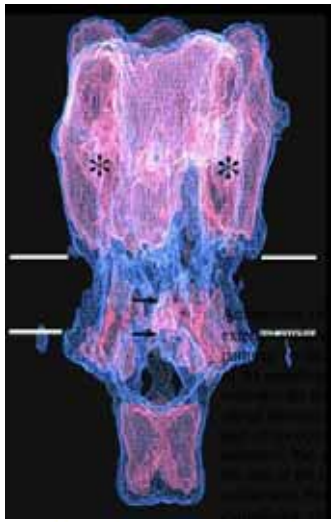
homo-

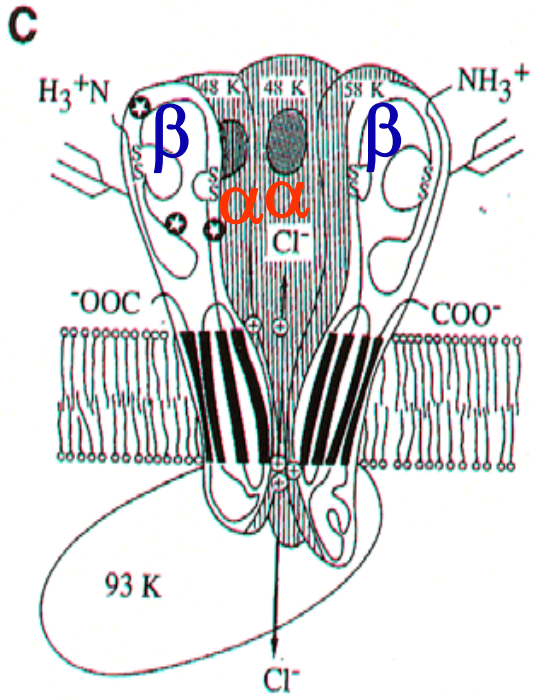


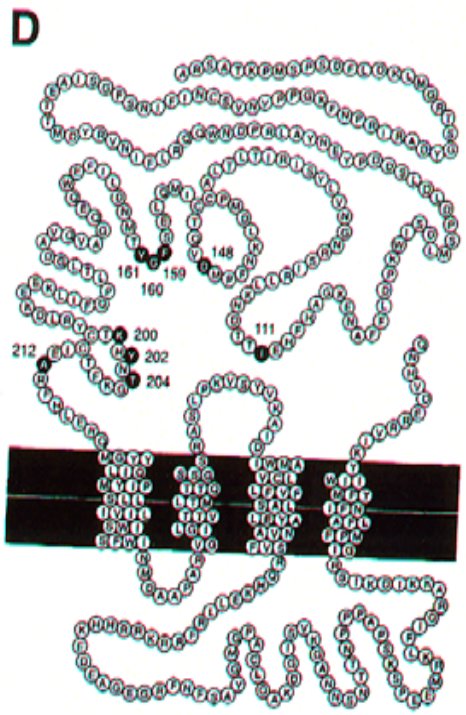
hetero-oligomeric



B 1999



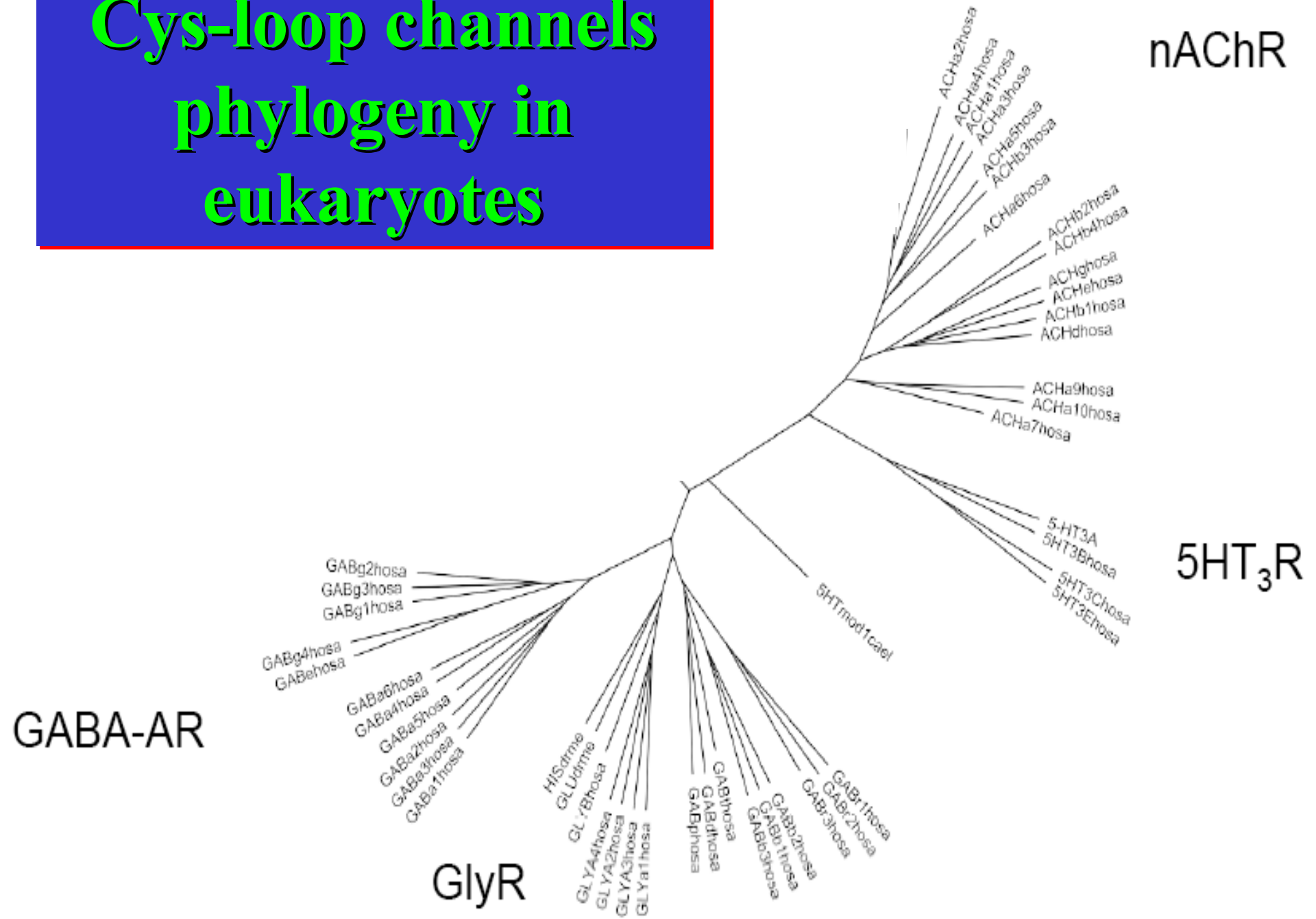


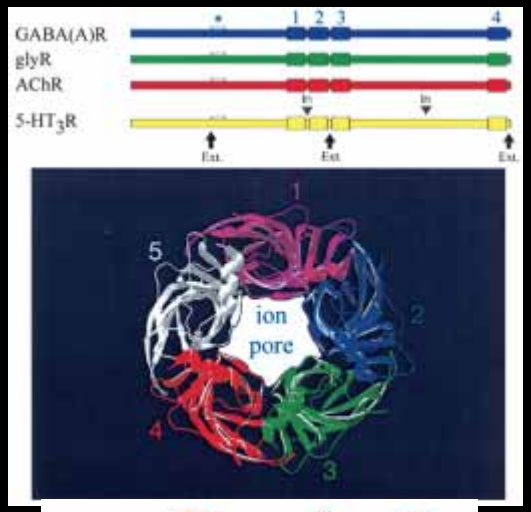


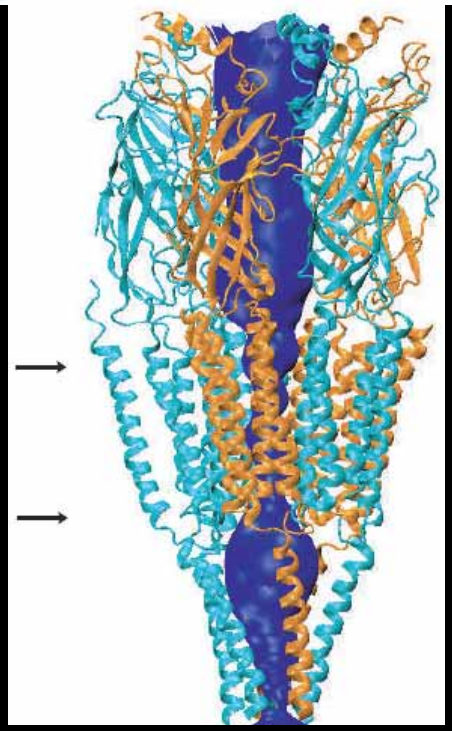
Glycine Receptor

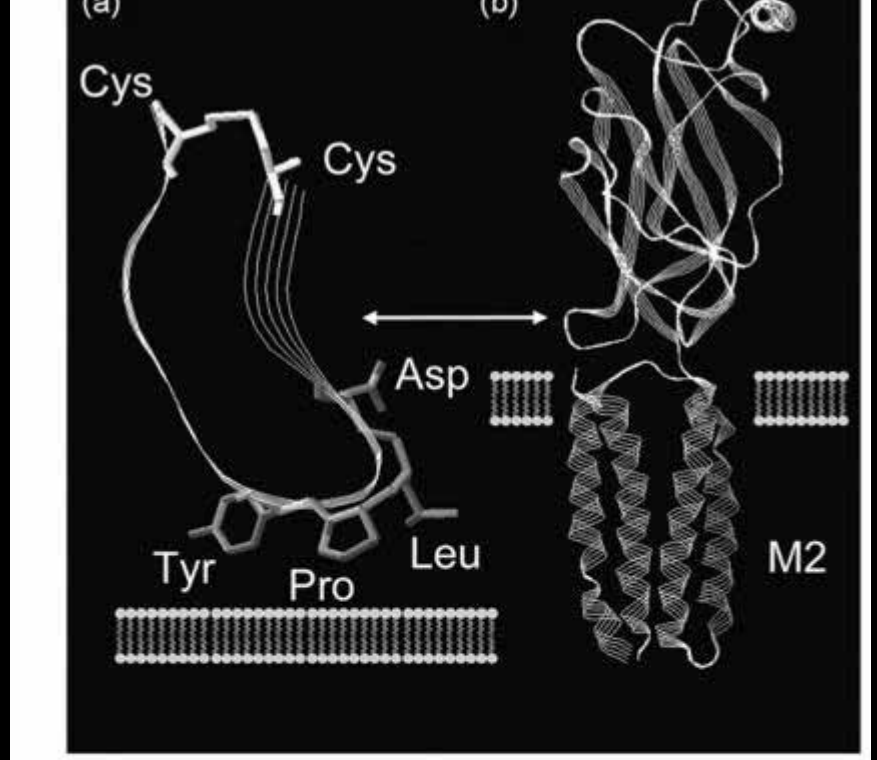
GlyR α subunit

Cys-loop channels phylogeny in eukaryotes









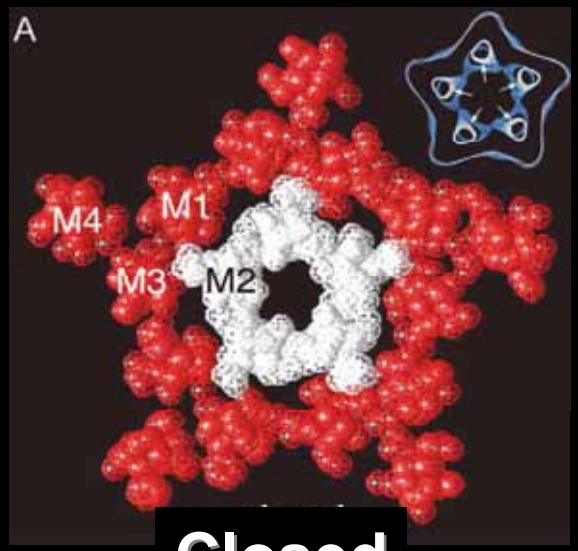
(B)

	1*	5*	9*	15*
AChBP	C	DVSG	VDTES	.GATC
H_GABA$_A$_α1	C	PMHL	ED**FPM**	DAHAC
H_GABA$_A$_β2	C	MMDL	RR**YPL**	DEQNC
H_GABA$_A$_γ2	C	QLQL	HN**FPM**	DEHSC
T.ma_α1	C	EIIV	THFPF	DQQNC
T.ma_δ	C	PINV	LYFPF	DWQNC
H_α7	C	YIDV	RWFPF	DVQHC
H_Gly_α1	C	PMDL	KNFPM	DVQTC

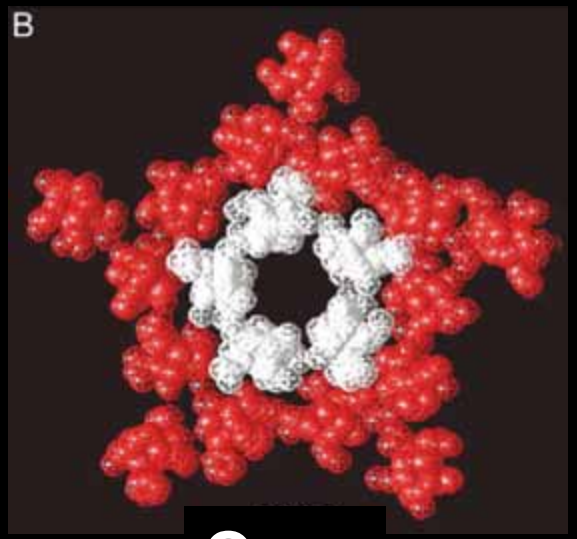
Signature sequence of 13 residues flanked by cysteines, form a closed loop situated between binding and channel domains

Cys-loop

Ключевые вопросы:



Closed

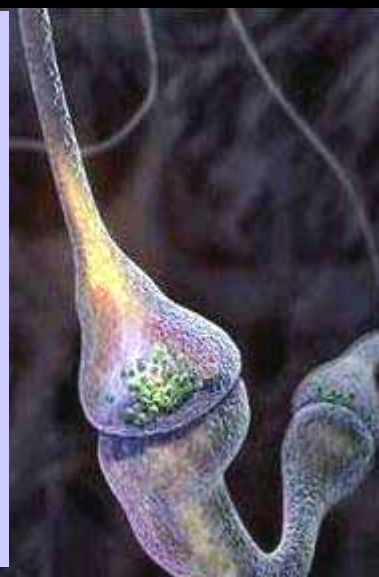


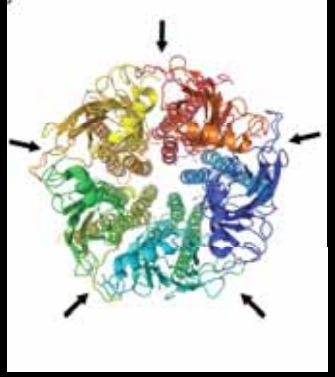
Open

-Как связывание лиганда с рецептором приводит к открыванию ионного канала?

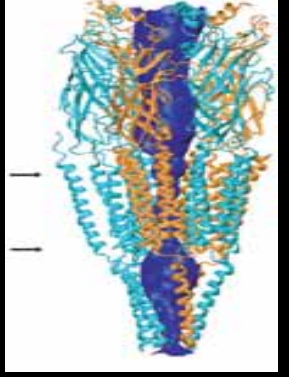
-Какие конформационные изменения претерпевает ионный канал при активации?

- Как регулируются функции ионных каналов?





Some key events in cys-loop receptors life

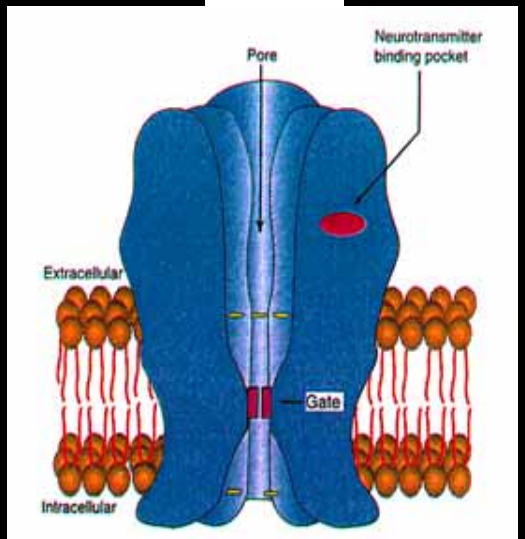


1993 - **structure of AChR from *T.Californica*** (9A) S. Unwin

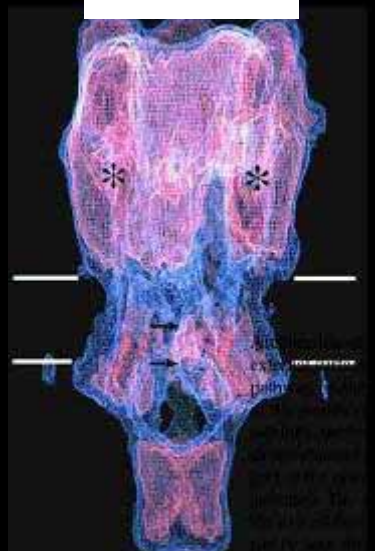
1999 - **structure of AChR from *T.Californica*** (4.6A) S. Unwin & co

2001 - **crystal structure of ACh-binding protein from *Lymnaea Stagnalis*** (2.7A) G. Smit, T. Sixma & co-authors

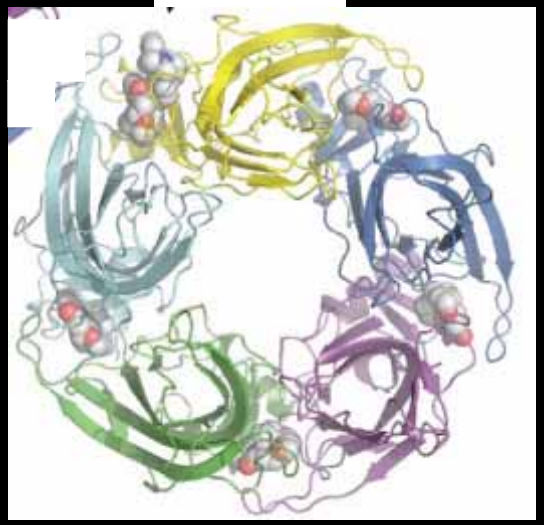
9 A



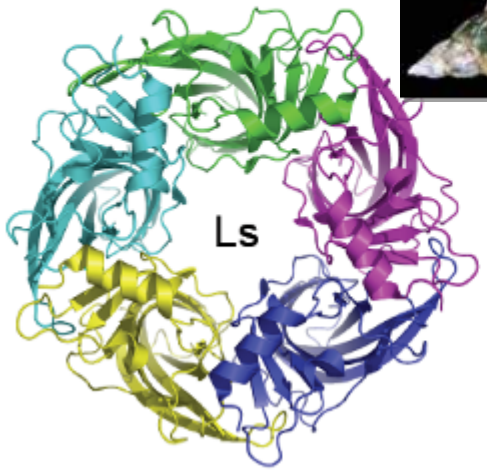
4.6 A



2.7 A



A conserved architectural fold among different AChBPs



(Brejc et al., *Nature*, 2001)
(Celie et al., *Neuron*, 2003)

AChBPs

nAChR

5HT$_3$R

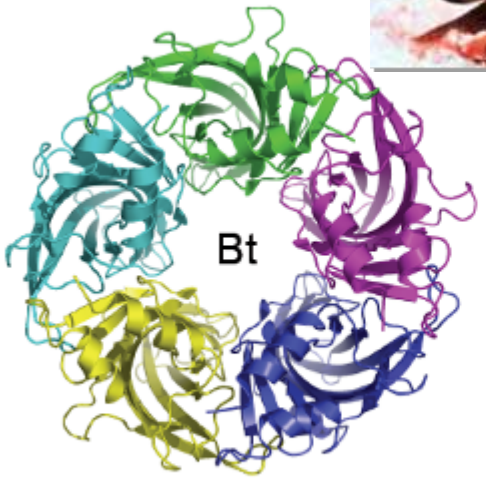
GABA-AR

GlyR

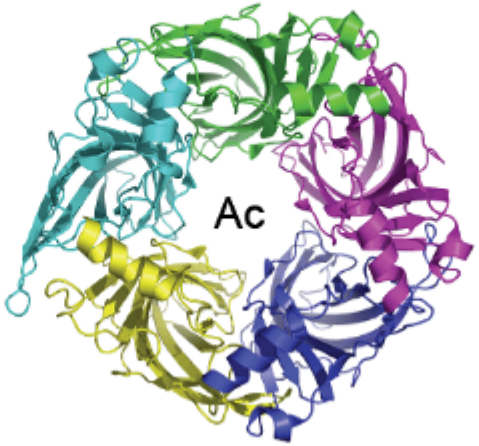
Lymnaea stagnalis

Bulinus truncatus

Aplysia californica



(Celie et al., *J. Biol. Chem.*, 2005)



(Celie et al., *Nat. Struct. Mol. Biol.*, 2005)

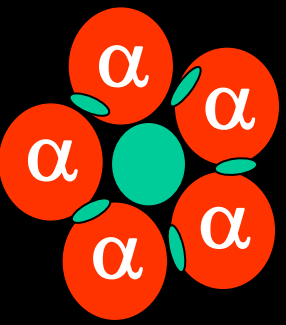
Cys-Loop ligand-gated channels

Cation-selective

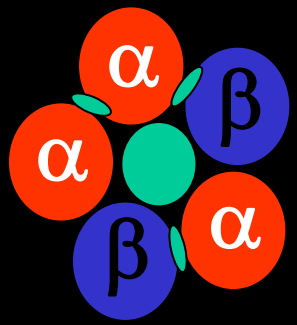
- Acetylcholine
- Serotonine (5-HT)

Anion-selective

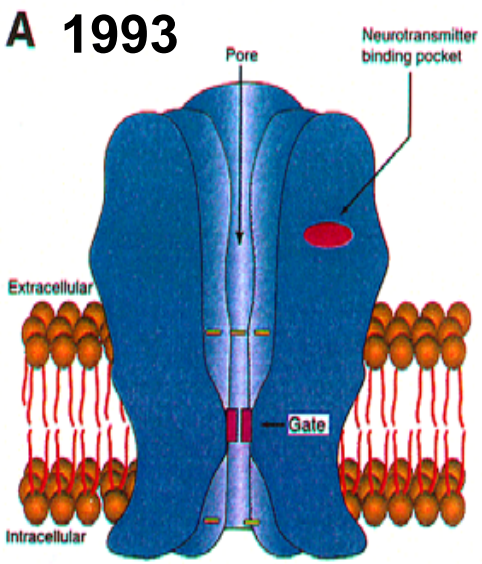
- GABA
- Glycine



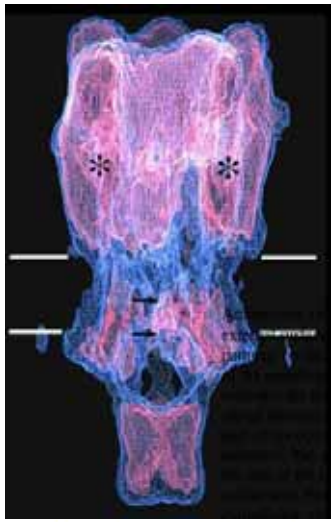
homo-

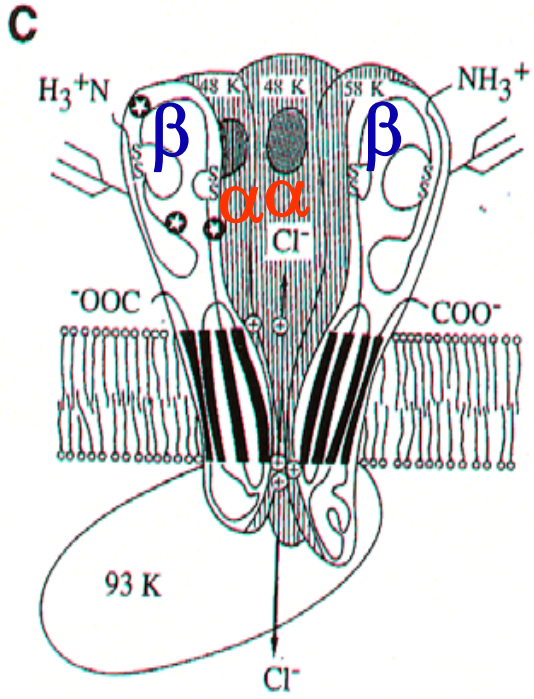


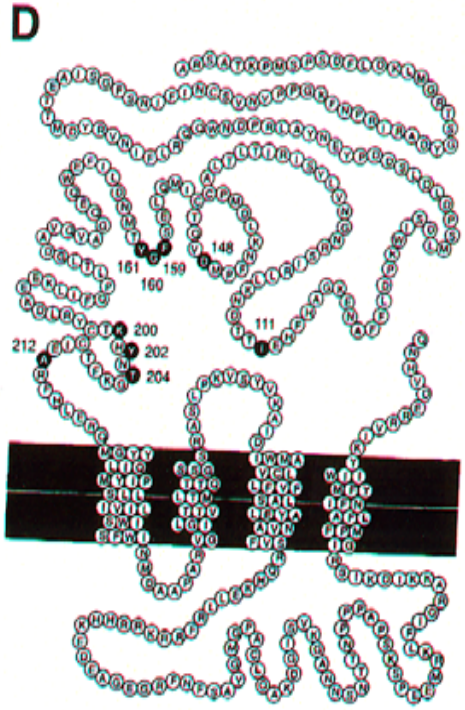
hetero-oligomeric



B 1999



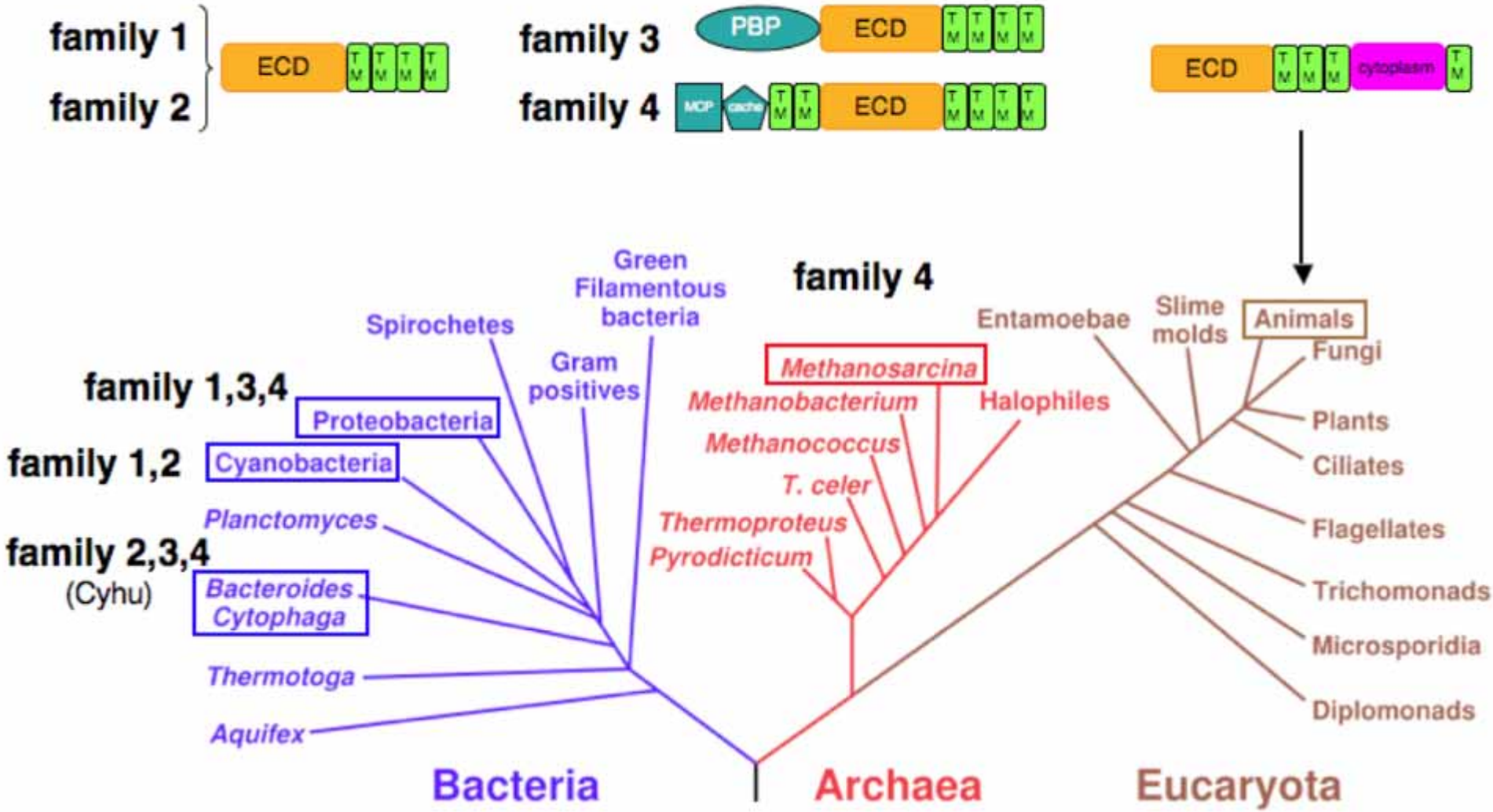




Glycine Receptor

GlyR α subunit

4-TM каналы обнаружены во всех биологических царствах



Tasneem, Aravind et al., 2005

Bacterial CLR history

2005 **Genomic identification**

Tasneem et al. *Genome Biol* 6 (1) pp. R4

2007 **Functional identification**

Bocquet et al. *Nature* 445 pp. 116-9

2008 **X-ray structure closed-pore state**

Hilf & Dutzter, Nature, 2008 (ELIC)

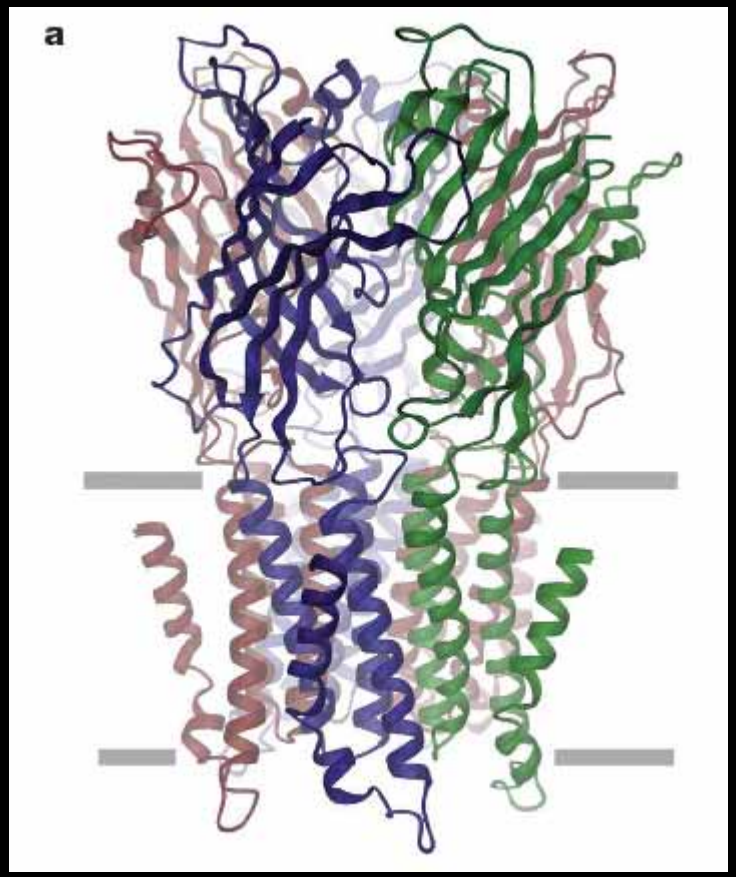
2009 **X-ray structure open-pore state**

Bocquet et al.*Nature* 457 pp. 111-4 **(GLIC)**

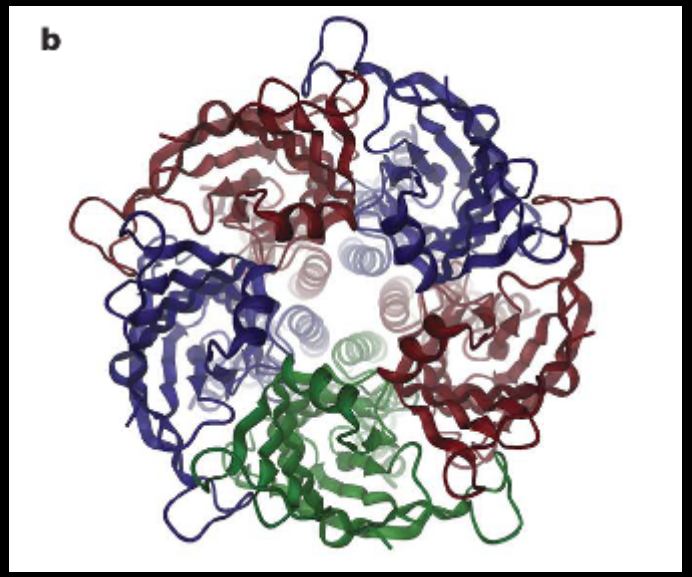
Hilf & Dutzter, Nature, 2009

Pentameric channel from bacterium *Erwinia chrysanthemi* (ELIC) at 3.3 A resolution

16 A



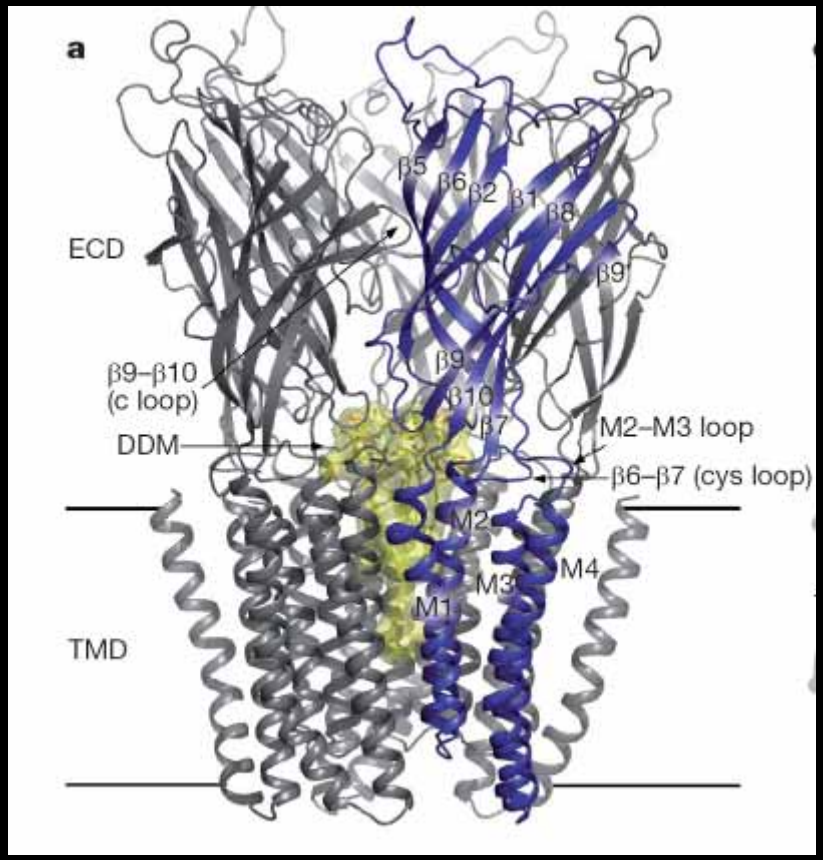
7 A

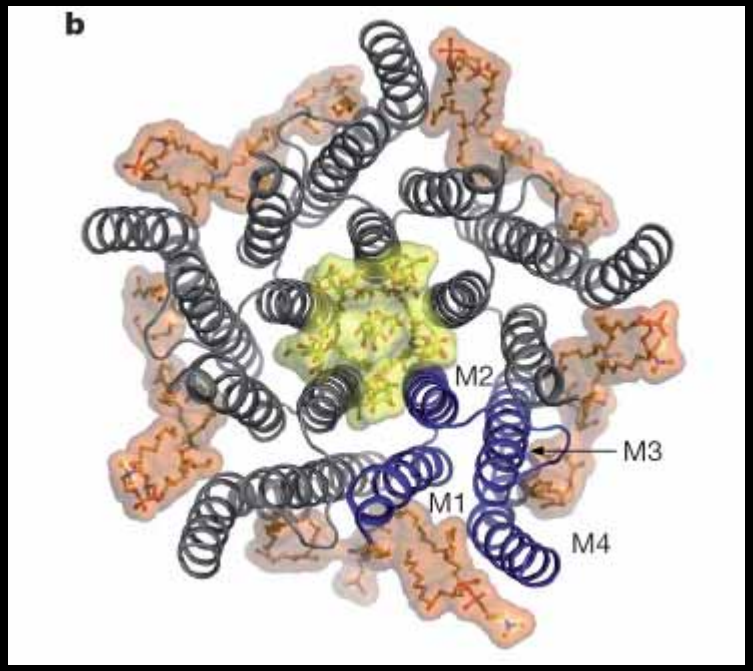


Hilf & Dutzler, Nature, 2008

ELIC - crystal in closed state

Proton channel from cyanobacterium *Gloeobacter violaceus*
Crystal structure in the open conformation

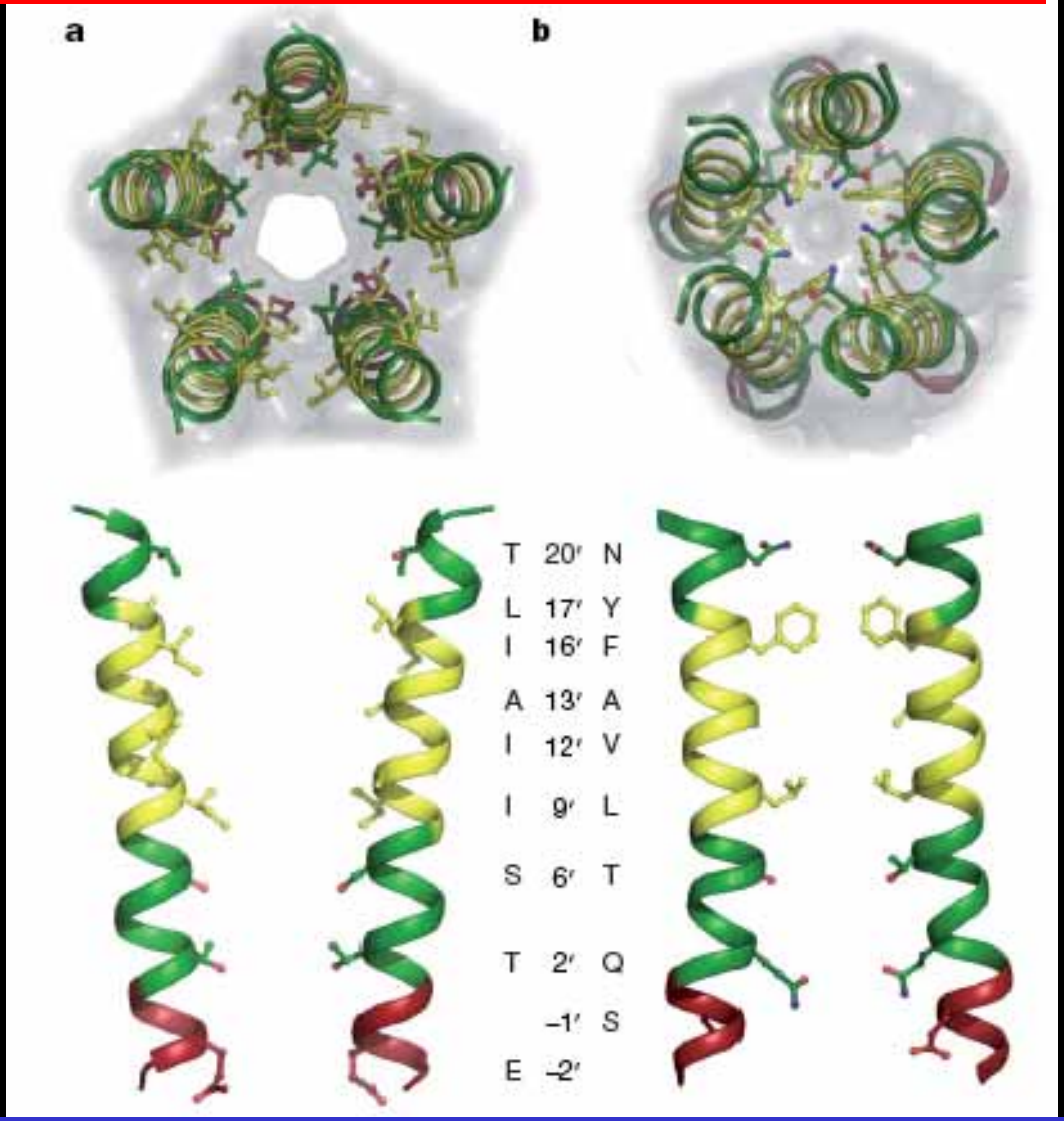




Bocquet et al., Nature, 2009

GLIC - crystal in open state

Proton channel from cyanobacterium *Gloeobacter violaceus*
Crystal structure in the open conformation

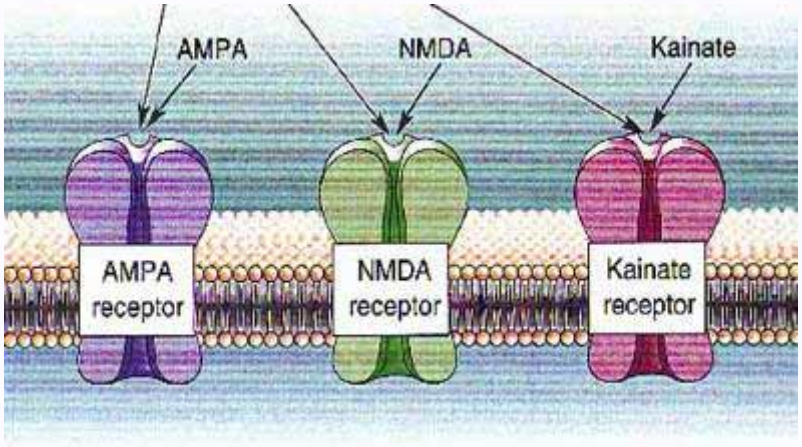


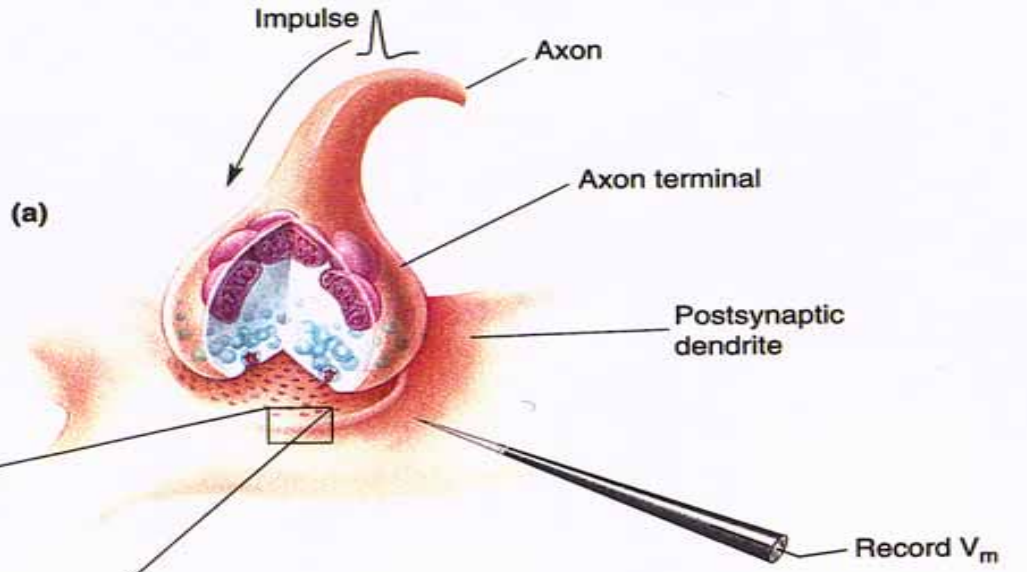
Bocquet et al., Nature, 2009

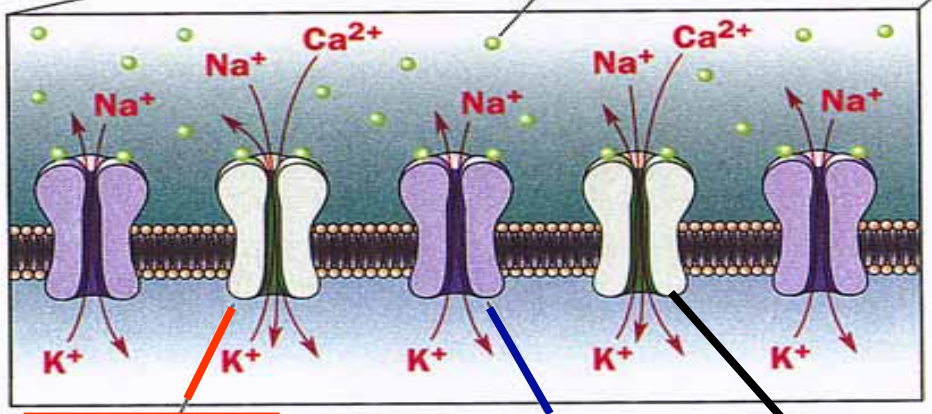
GLIC - crystal in open state

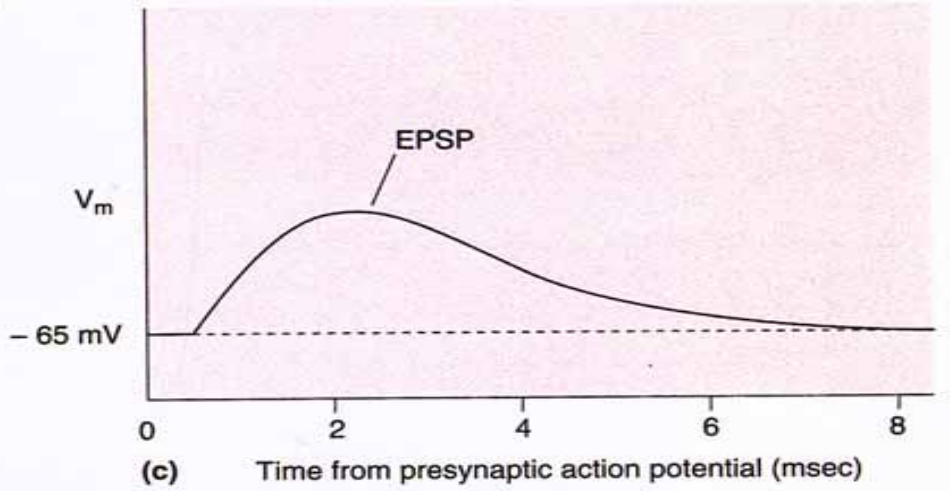
Самосборка ионных каналов в синапсах

Как эти рецепторы ко-локализуются ?









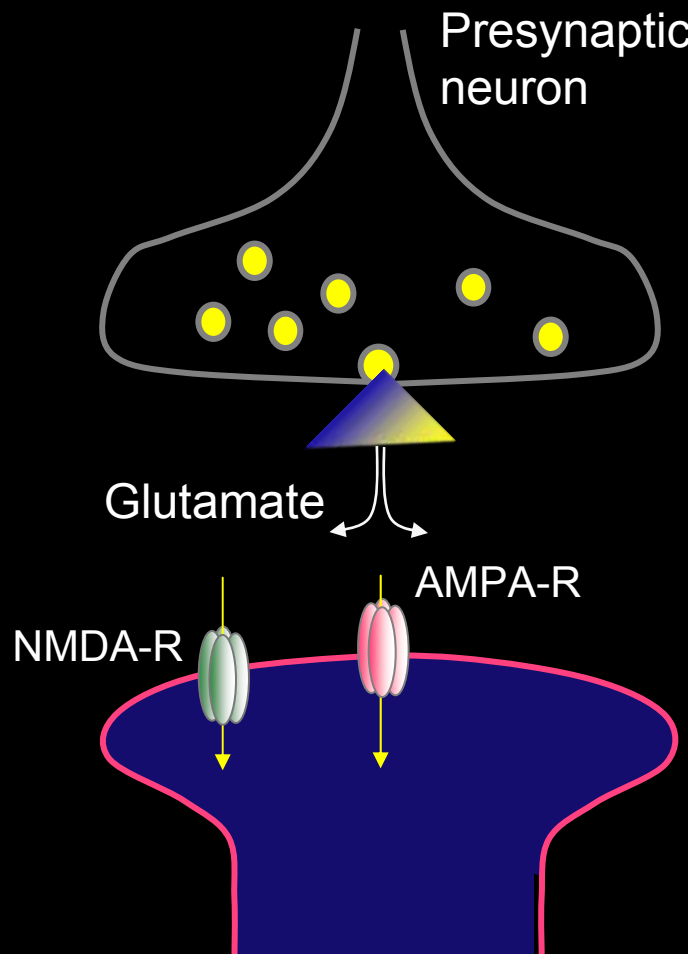
NMDA receptor

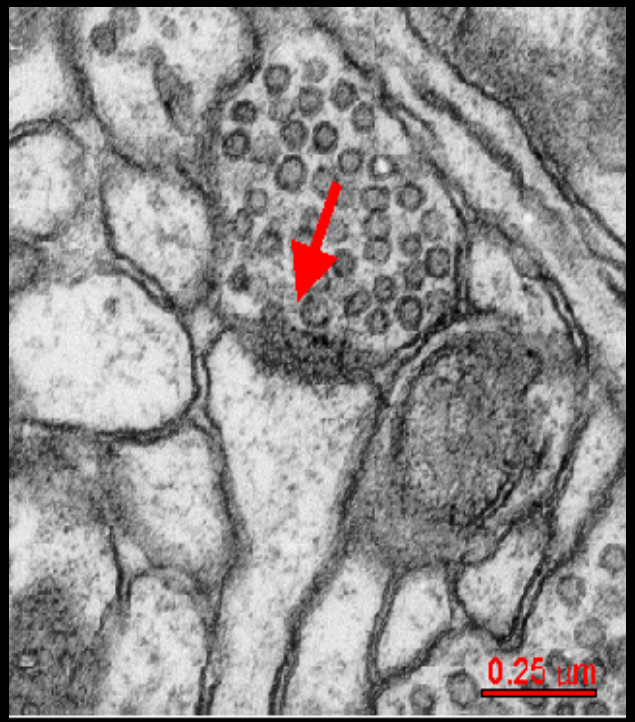
AMPA receptor

Kainate receptor

Постсинаптическая плотность глутаматергических синапсов

Glutamate synapse in the CNS

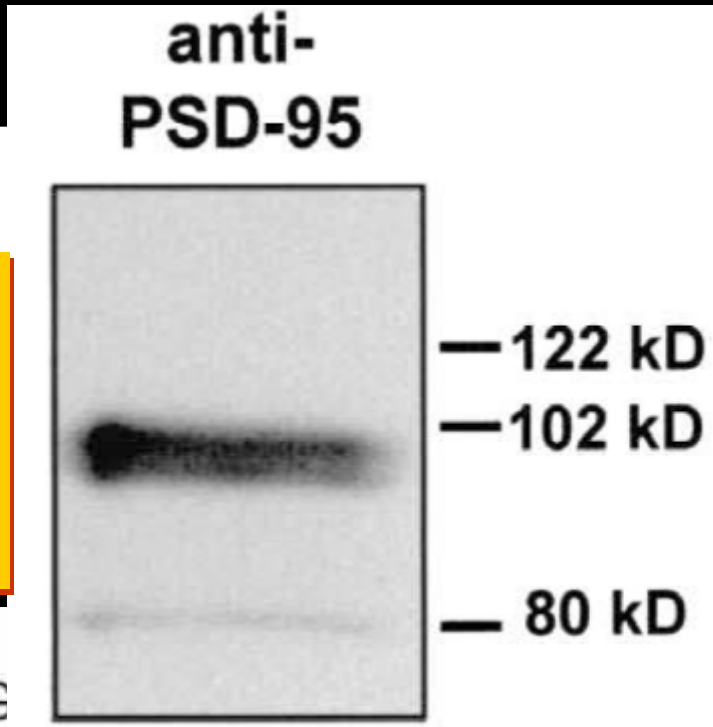


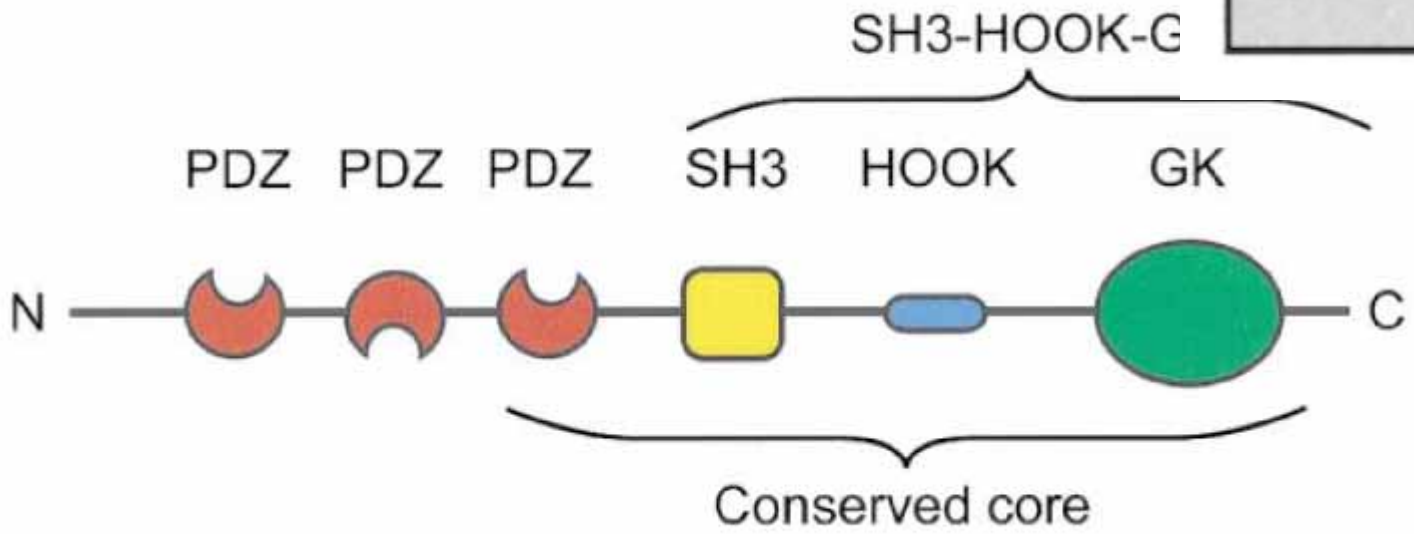


PSD-95-domain organization

PDZ domains- modular protein-interaction domains that are specified for binding a short peptide motifs at the extreme caboxy (C) termini of other proteins.

PDZ domains-are located at about 12 nm below postsynaptic membrane.





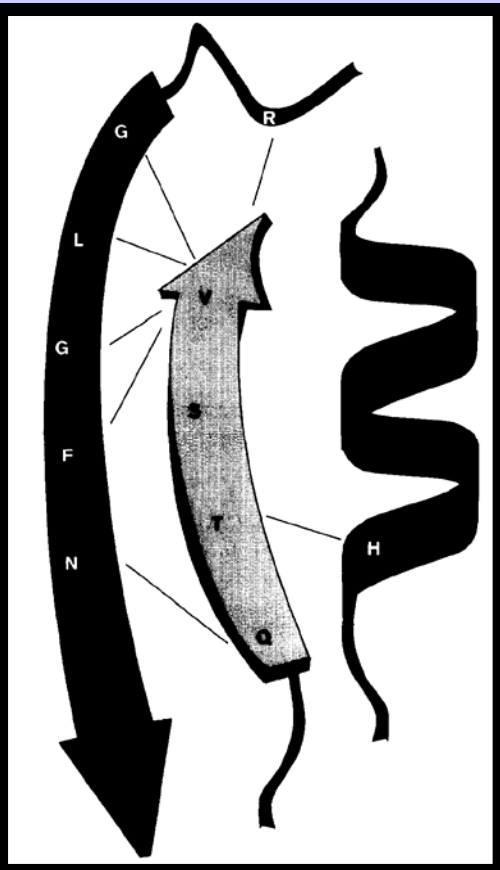
P. Seeburg et al, 1995 : PSD-95 stimulate clustering of NMDAR channels

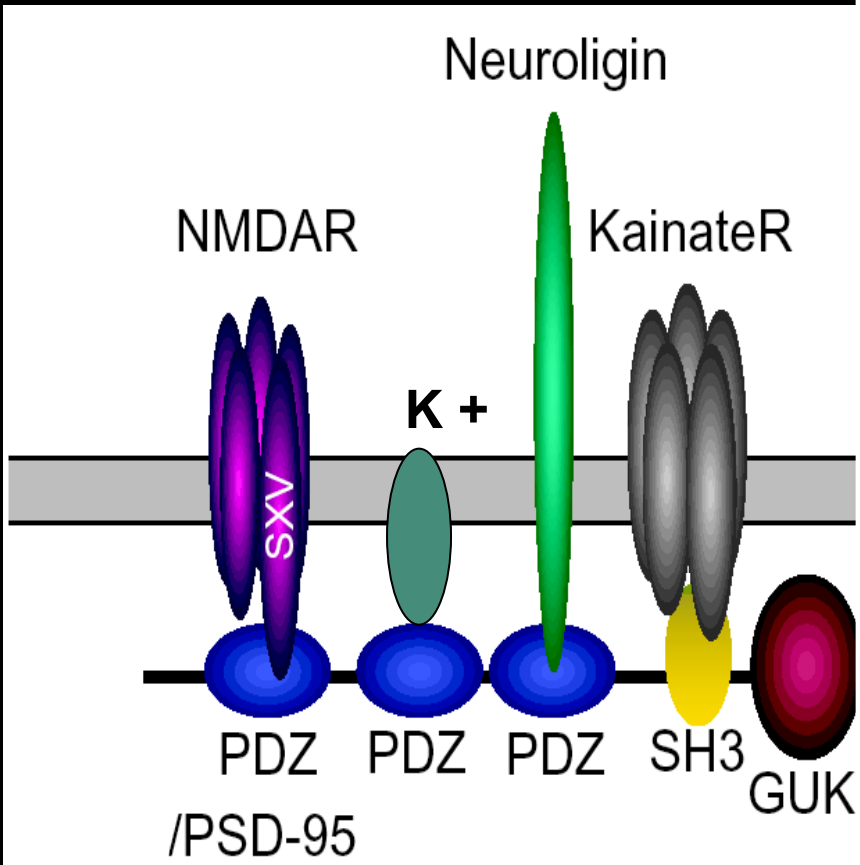
M. Sheng et al, 1995 : PSD-95 stimulate clustering of potassium channels

ETXV
ESSV

protein–protein interactions between synaptic components of the postsynaptic density (PSD)

- 90 amino acids
-Six β-strands and two α-helices





-ETDV - K + channel
-ESDV - NMDA receptor (NR2A/B subunits)

Schematic diagram of the protein–protein interactions between synaptic protein components of the postsynaptic density (PSD)

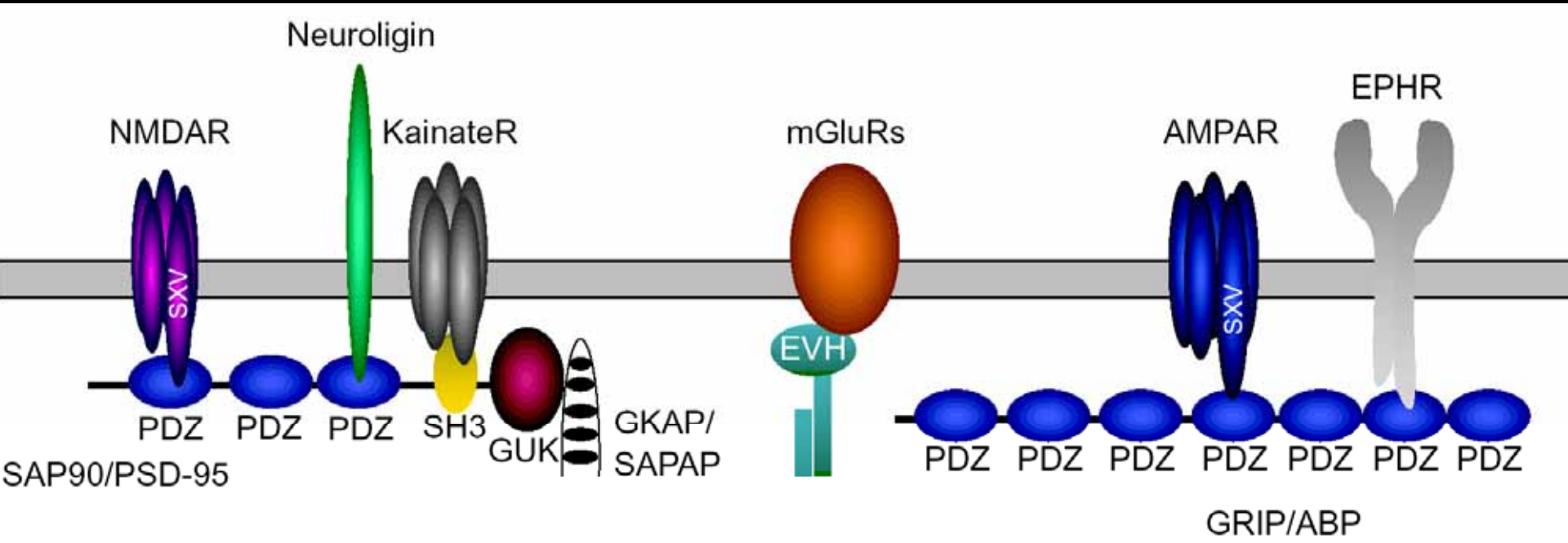
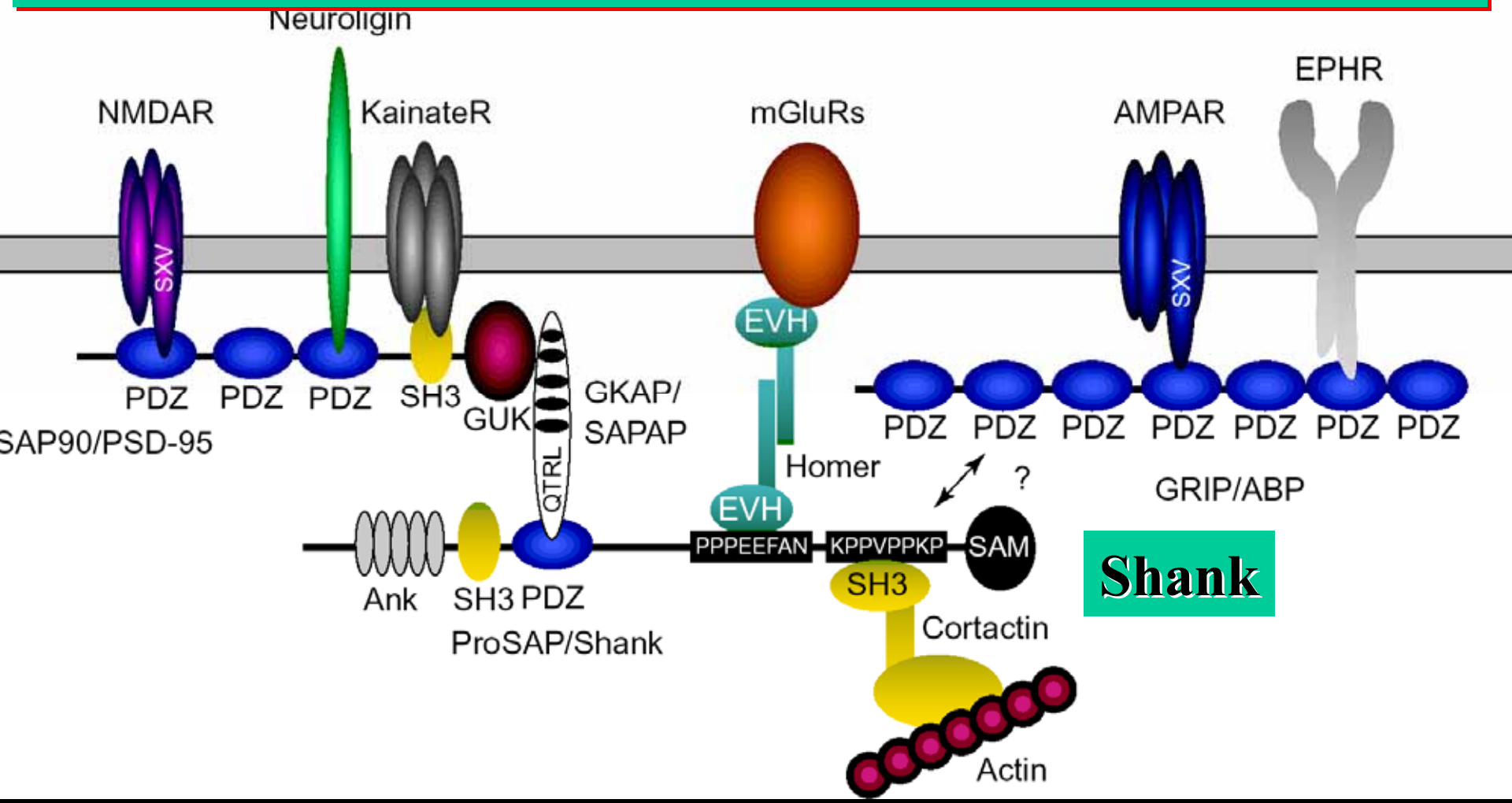
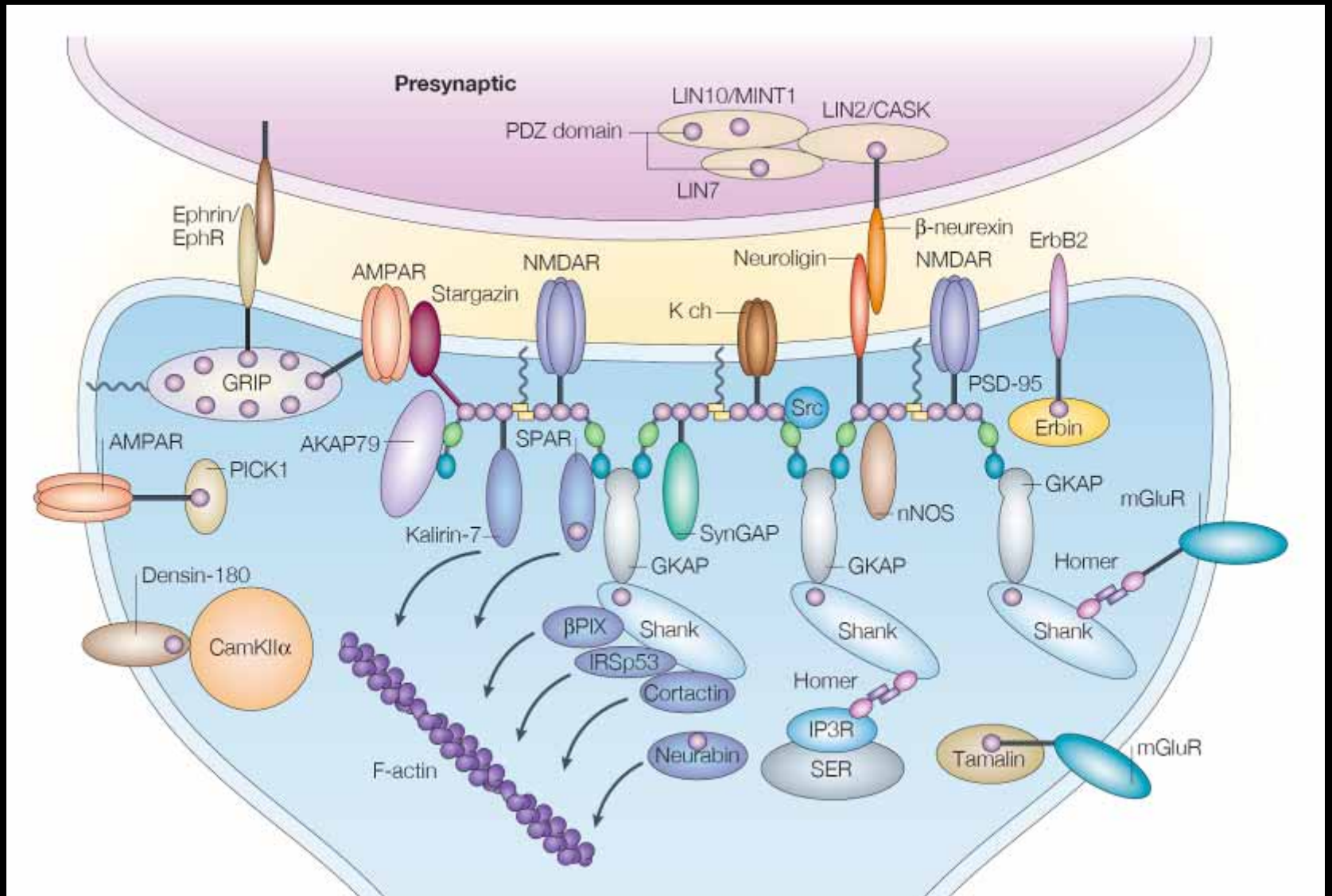


Diagram of the protein–protein interactions between components of the postsynaptic density (PSD)

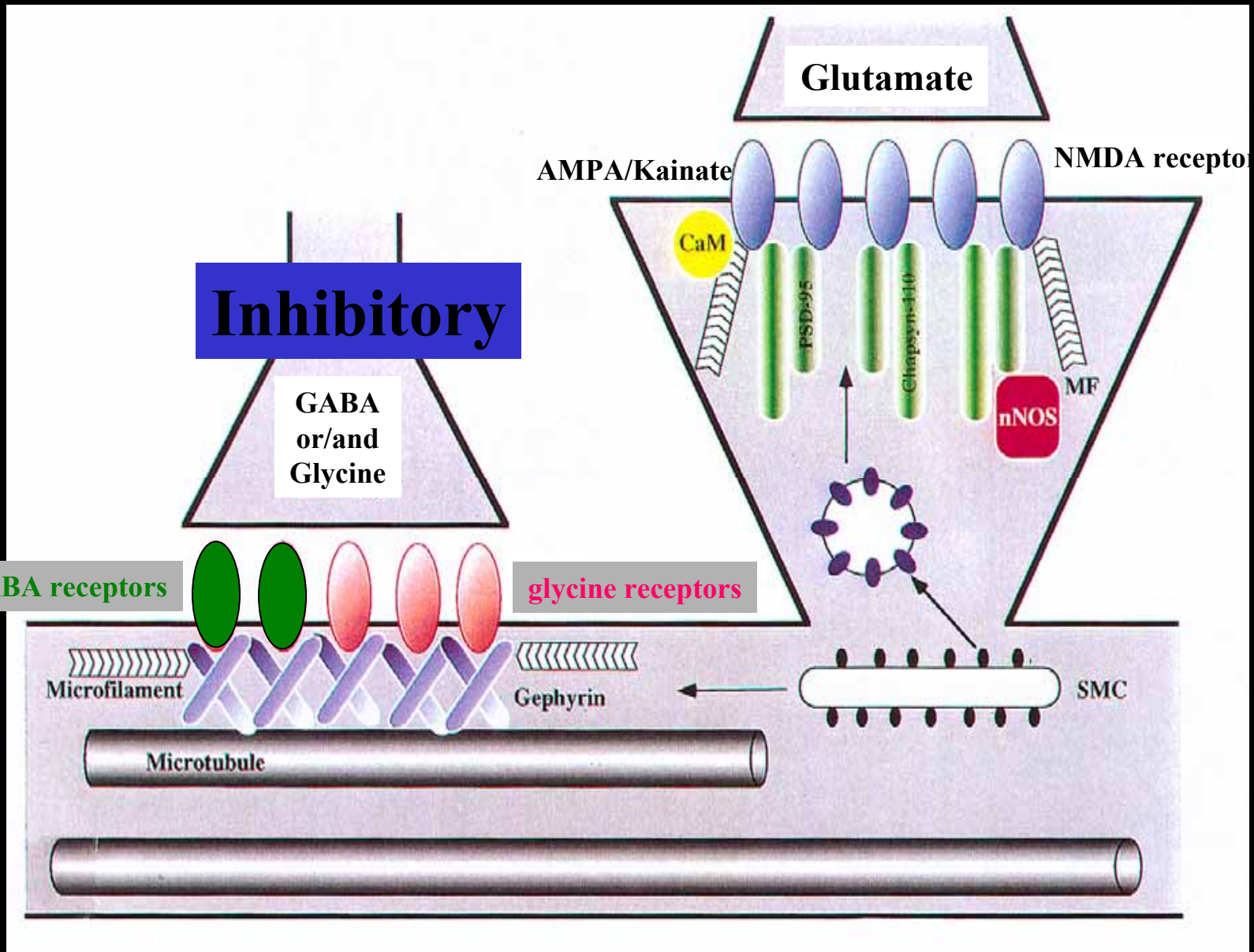


Архитектура постсинапса

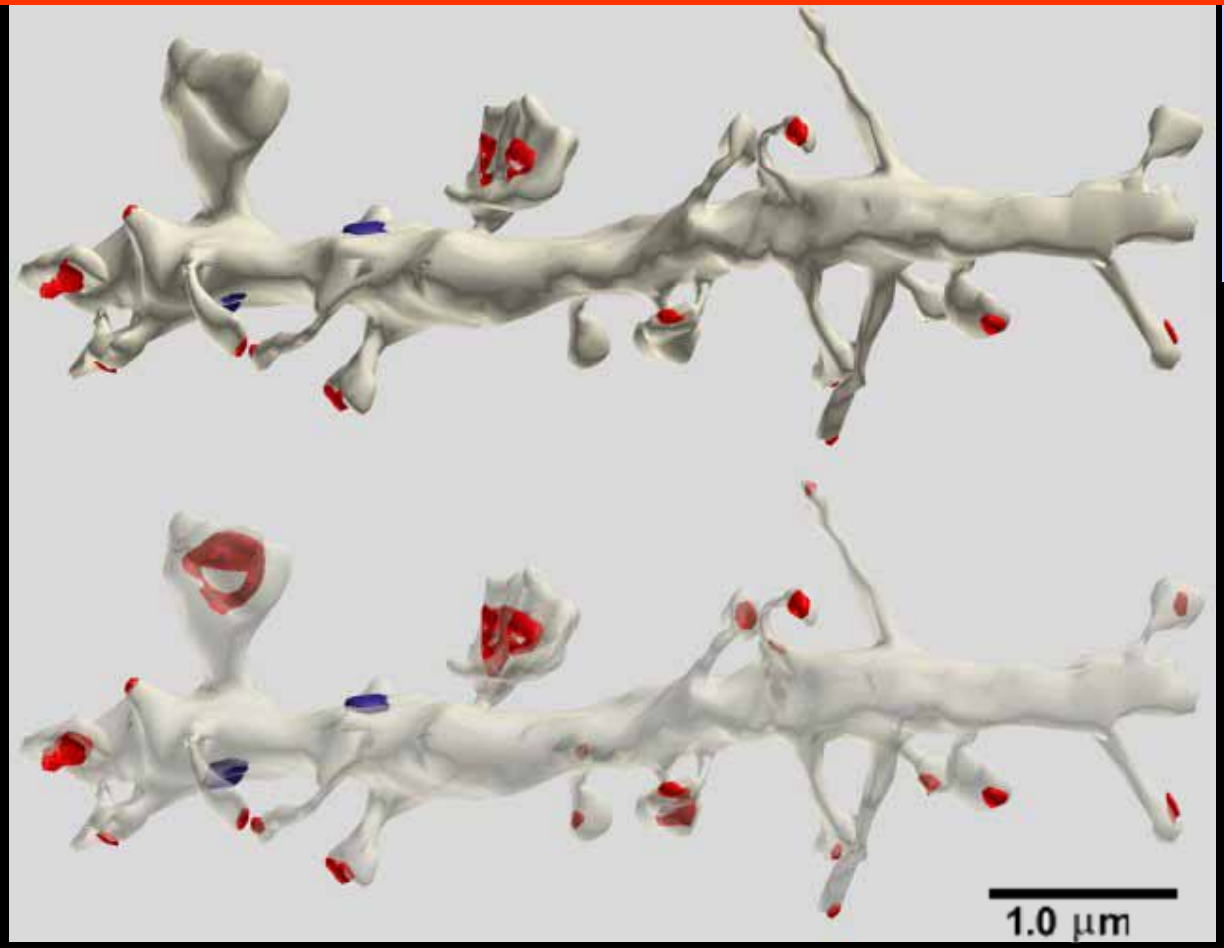


Формирование и подвижность синапсов

Model of synapses



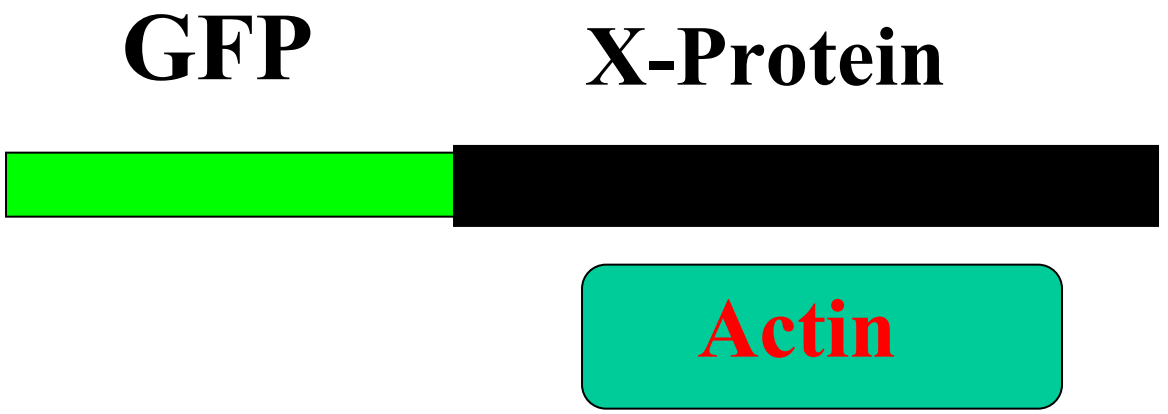
Дендриты и постсинаптические шипики содержат актин



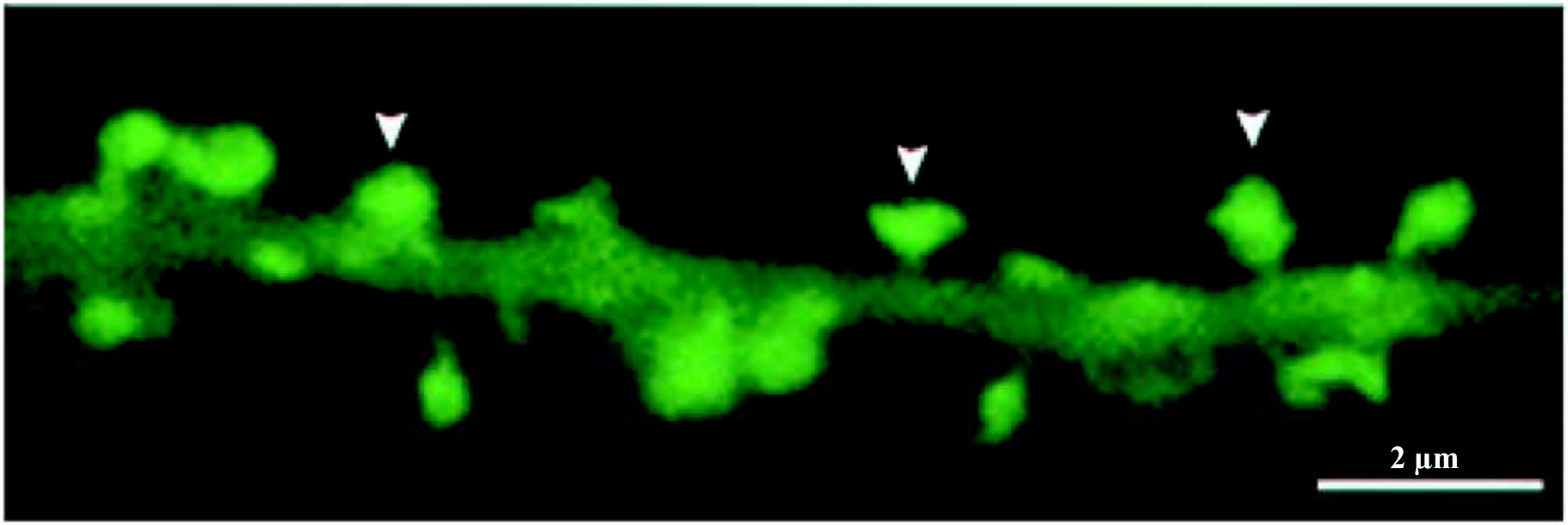
Fifkova, E., and Delay, R.J. (1982.) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. J. Cell Biol. 95, 345–350.

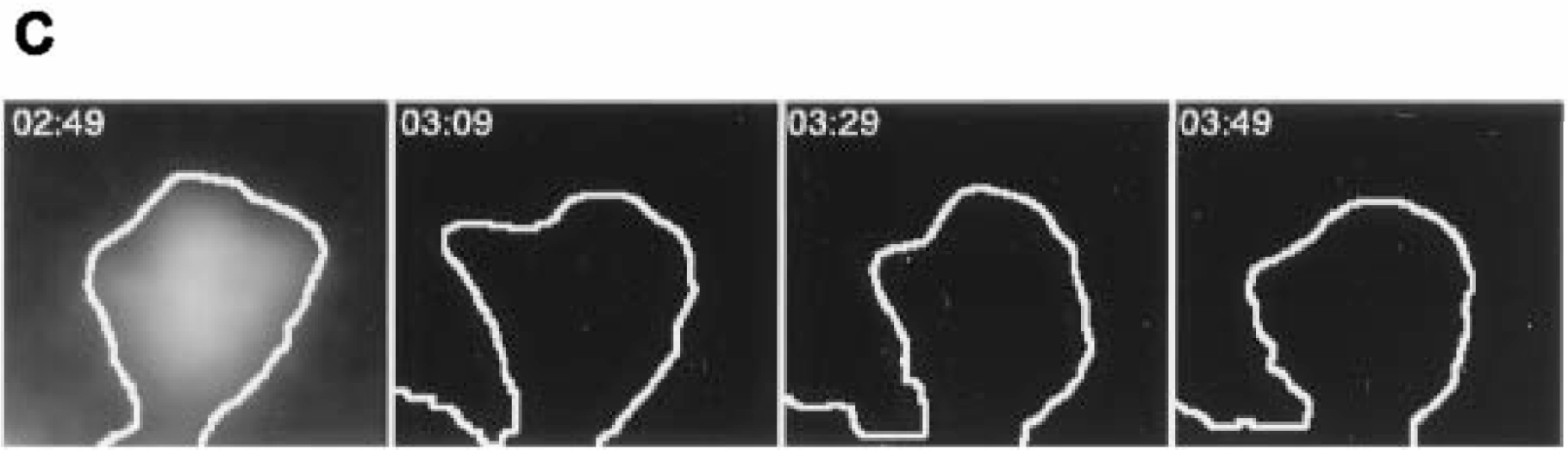
Matus, A., Ackermann, M., Pehling, G., Byers, H.R., and Fujiwara, K. (1982.) High actin concentrations in brain dendritic spines and postsynaptic densities. Proc. Natl. Acad. Sci. USA 79, 7590–7594.

Excitatory synapses are shown in red. Inhibitory synapses in blue.


GFP
X-Protein
Actin

Подвижность дендритных шипиков



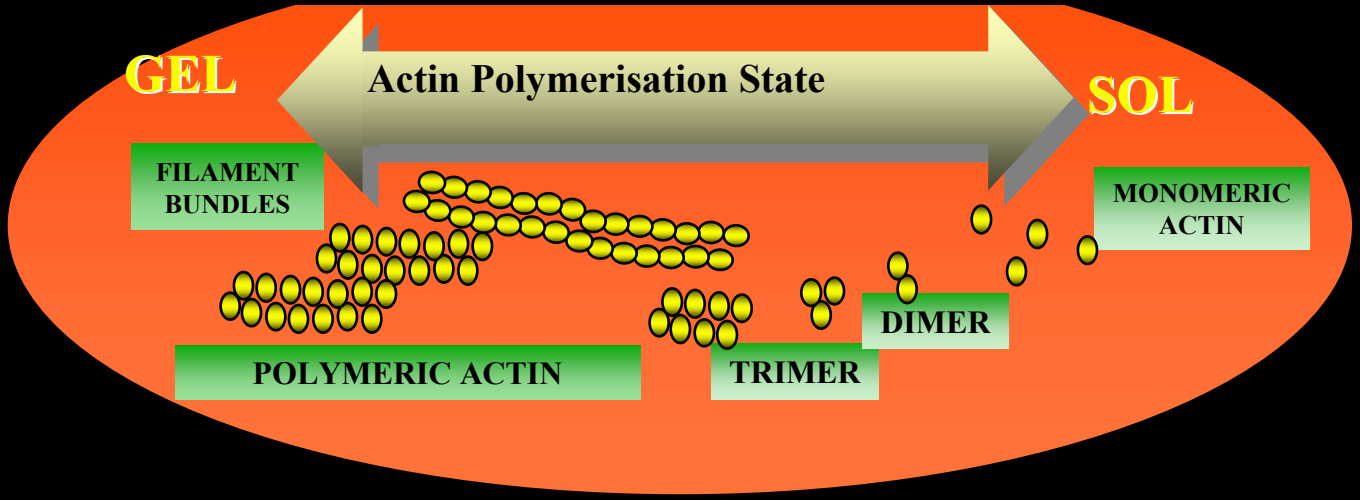


Matus et al. 1998

Actin in spines of hippocampal neurons

Matus

F/G-actin modulators



CYTOCHALASIN Depolymerization: cap to the fast-growing end

LANTRUCULIN Depolymerization: binds to monomeric actin (marine sponge)

PHALLOIDIN Polymerization: prevents depolymerization (mushroom)

JASPLAKINOLIDE Polymerization: prevents depolymerization (marine sponge)

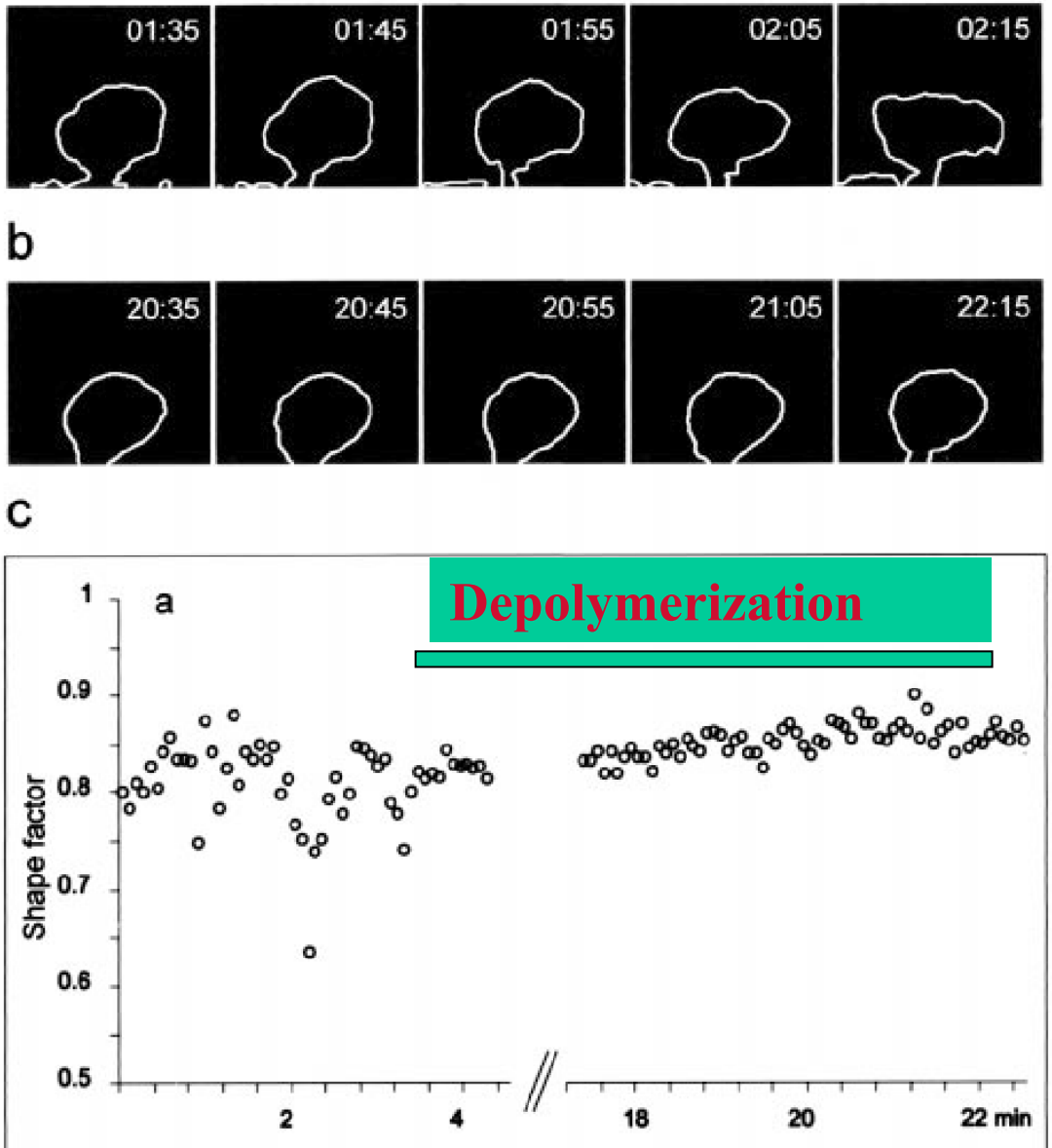
Proteins

GELSOLIN Depolymerization: (i) Ca-dependent F-actin severing; (ii) displace phalloidin from actin filaments;

COFILIN Depolymerization: pH-dependent;

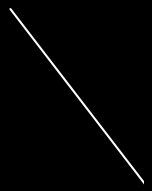
PROFILIN Polymerization

Деполимеризация актина блокирует подвижность шипиков

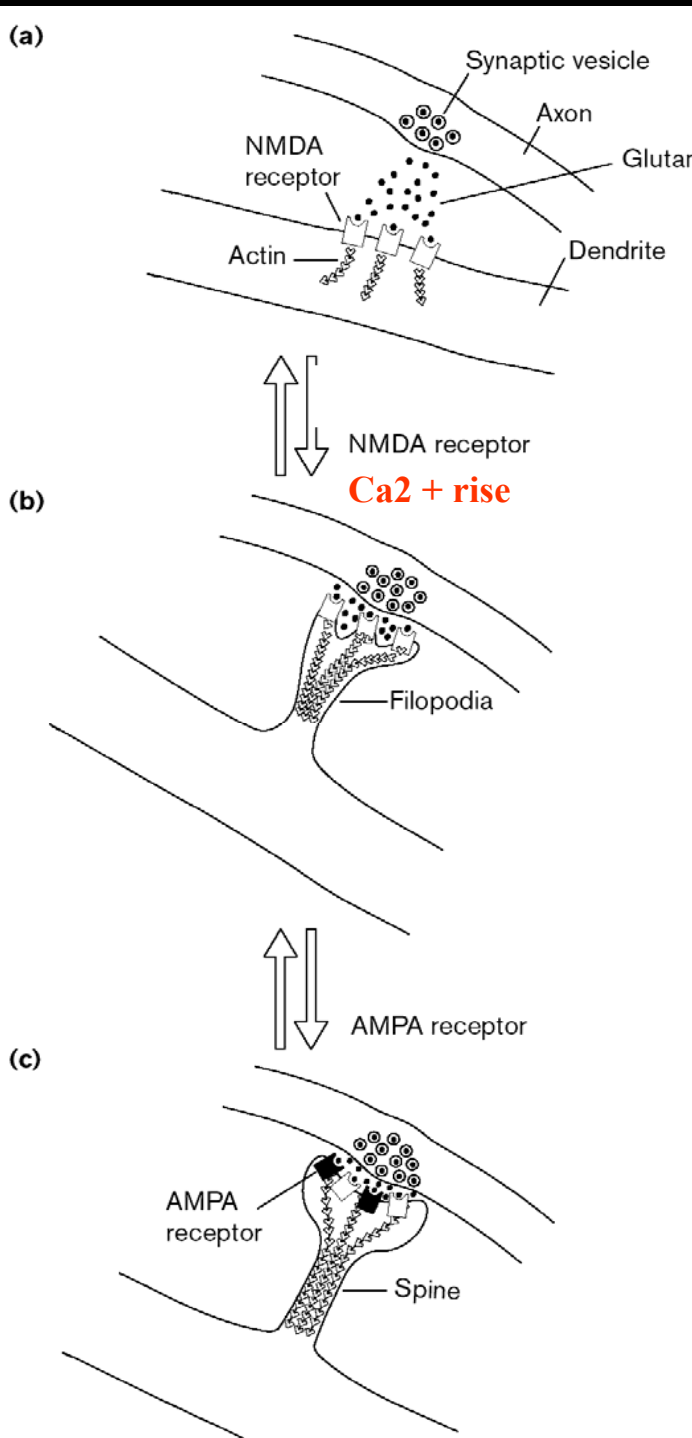


Actin in spines + CytD

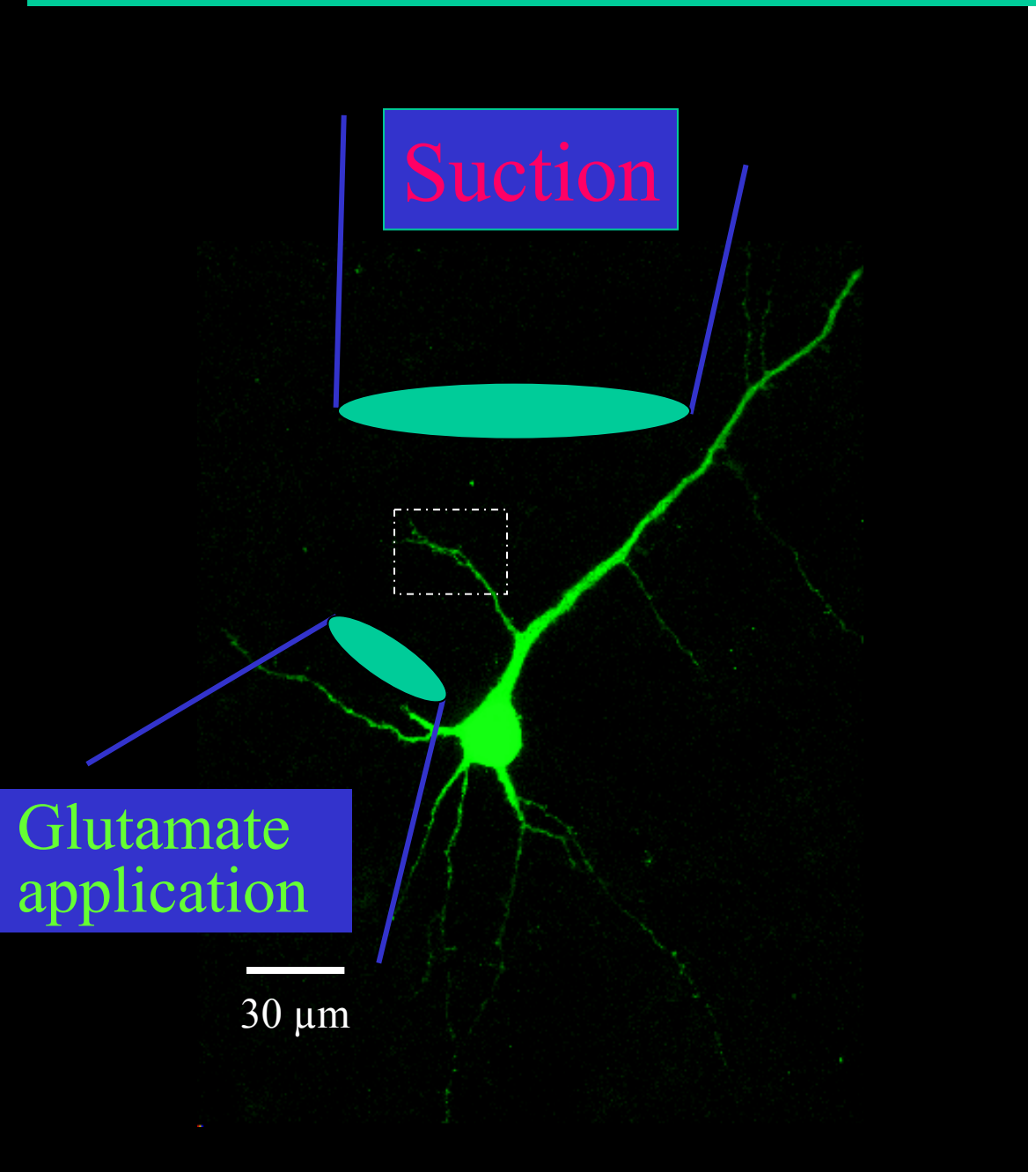
Matus

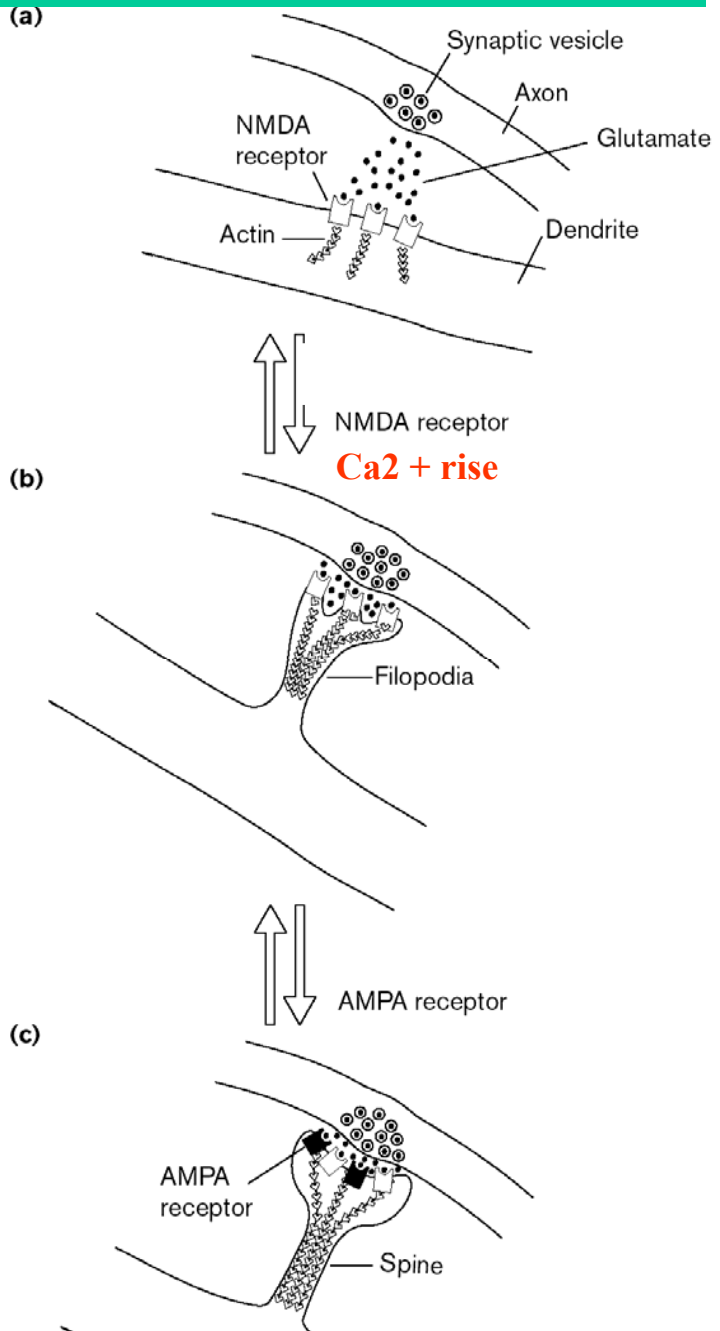


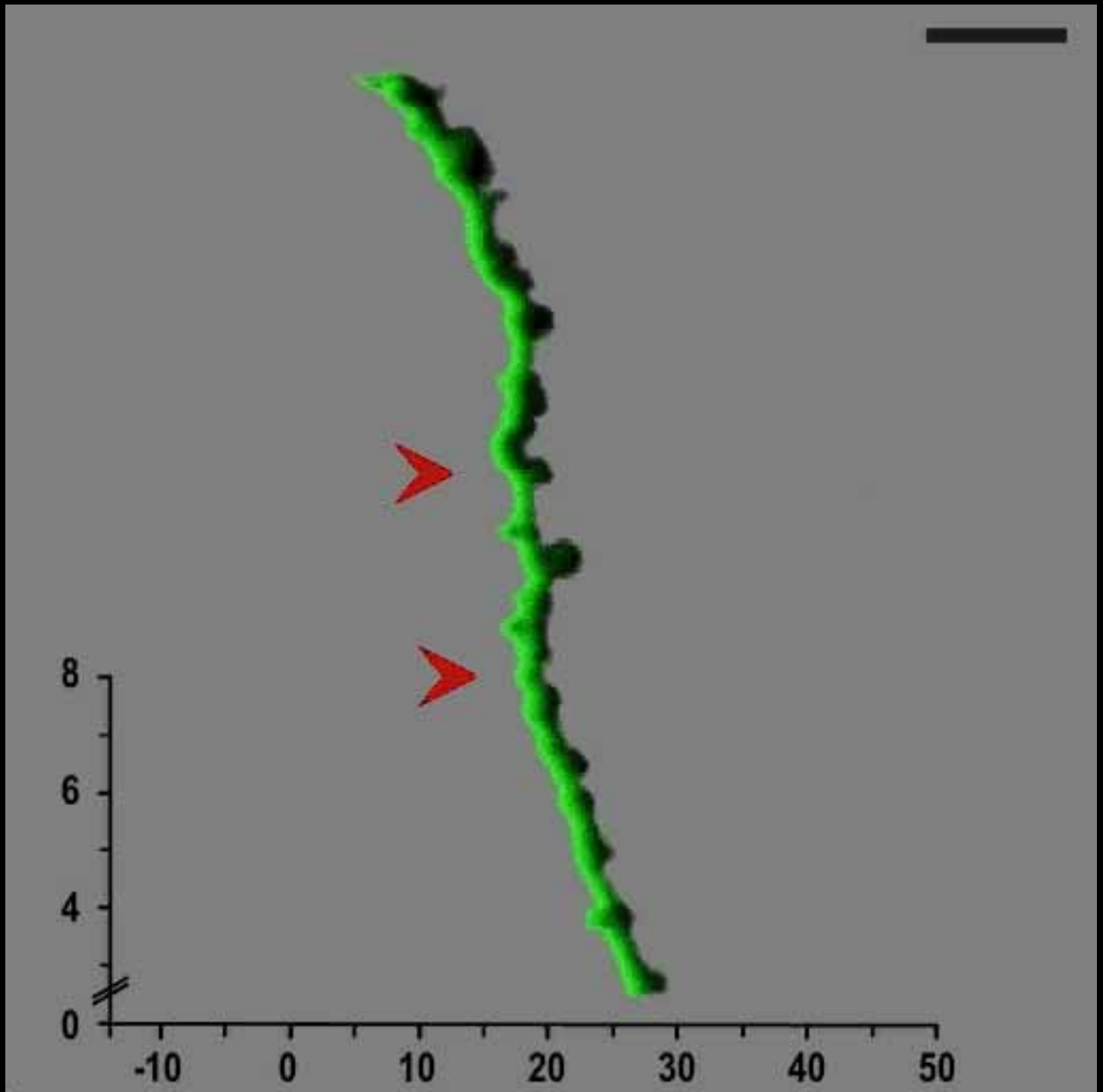
Ca^{2+}-стимулируемое формирование дендритных шипиков



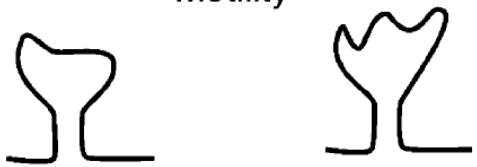
Локальная активация рецепторов в дендритах



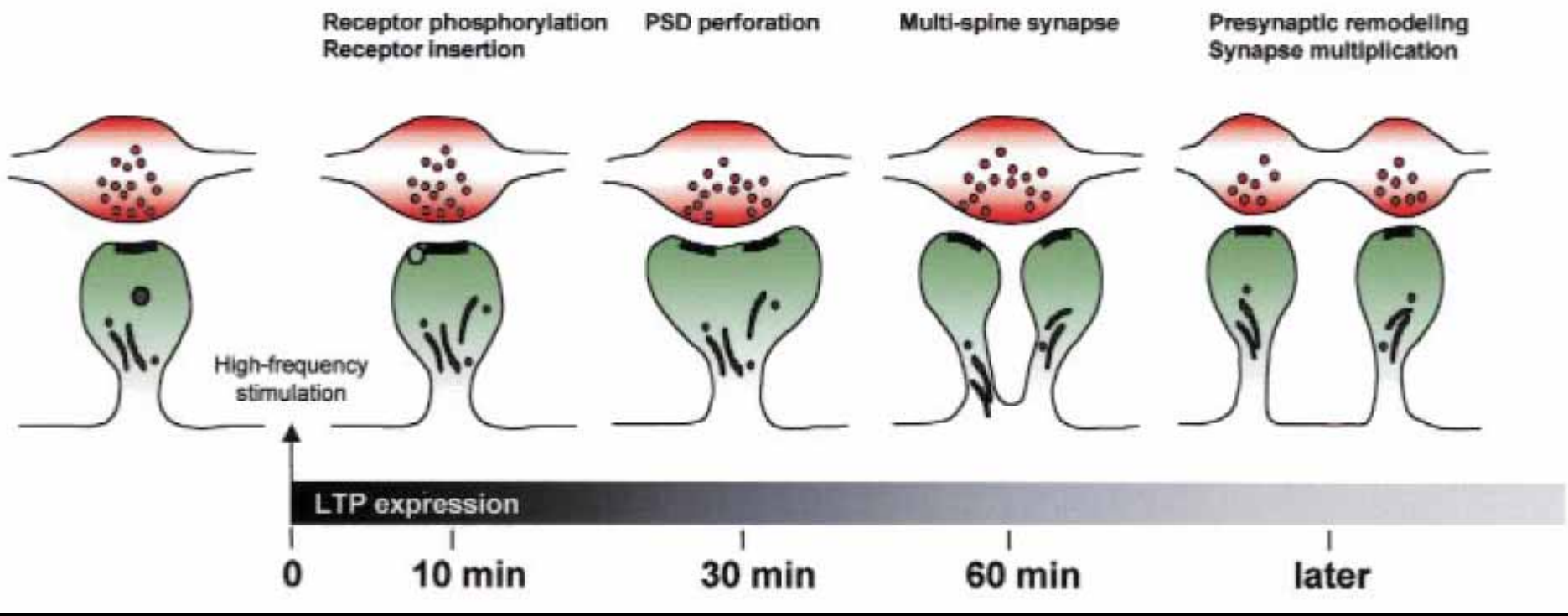


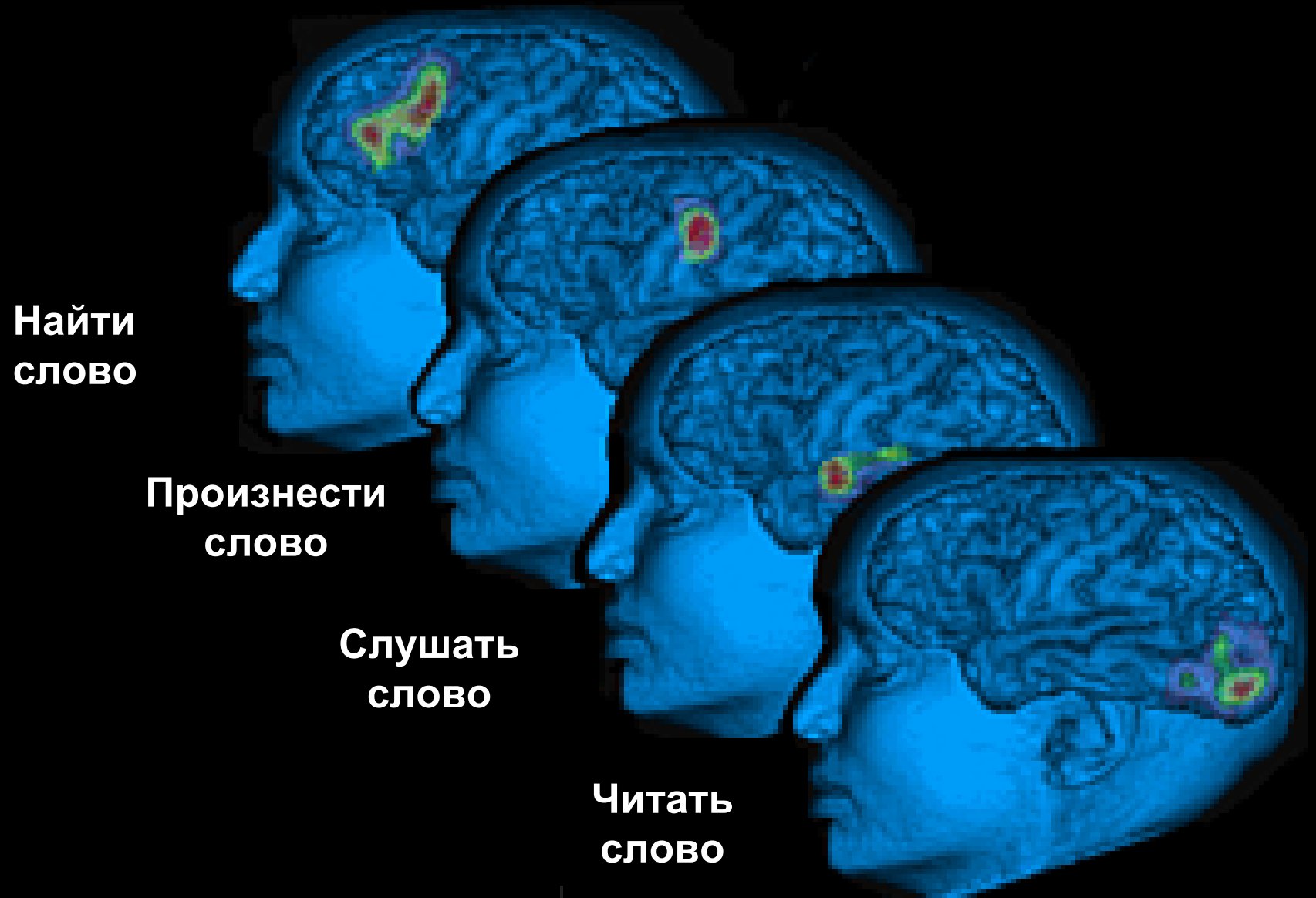

8
6
4
0
-10
0
10
20
30
40
50

Подвижность дендритных шипиков

Effect	*Correlate*	*Time-scale*
Motility 	Shape Change	Seconds
Growing 	New Spines	Minutes Hours

Возможный механизм формирования новых синапсов




Найти слово
Произнести слово
Слушать слово
Читать слово

На память:

- Синапсы - динамические самоформирующиеся молекулярные модули, обеспечивающие быструю передачу информации в нервной системе

- Ионные каналы ключевые белки, обеспечивающие транформацию химических, механических и др. воздействий в электрические сигналы

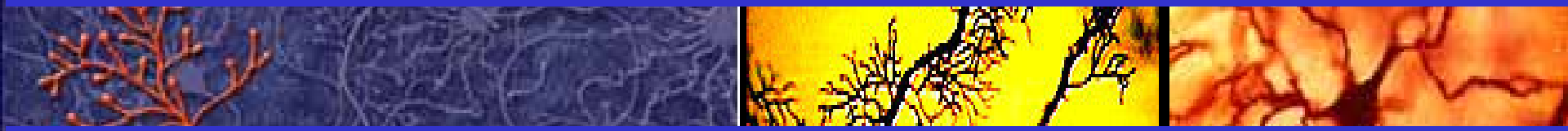
В будущем:

- формирование и пластичность нервной сети
- модели памяти
- нарушения функции каналов и синапсов

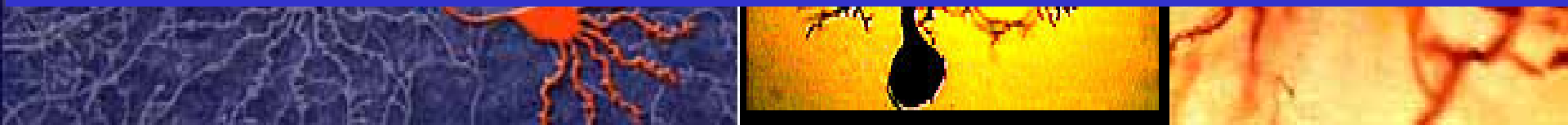
Фундаментальные вопросы

Как устроены молекулярные блоки и сети, обеспечивающие прием и обработку информации?

Какие функции выполняют молекулярные блоки нервной системы?



Как формируются блоки, обеспечивающие функцию мозга и нервной системы?

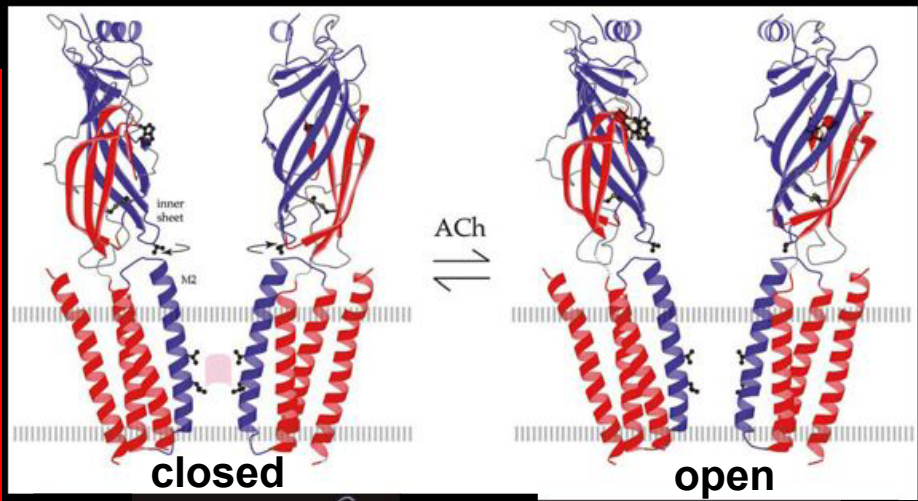


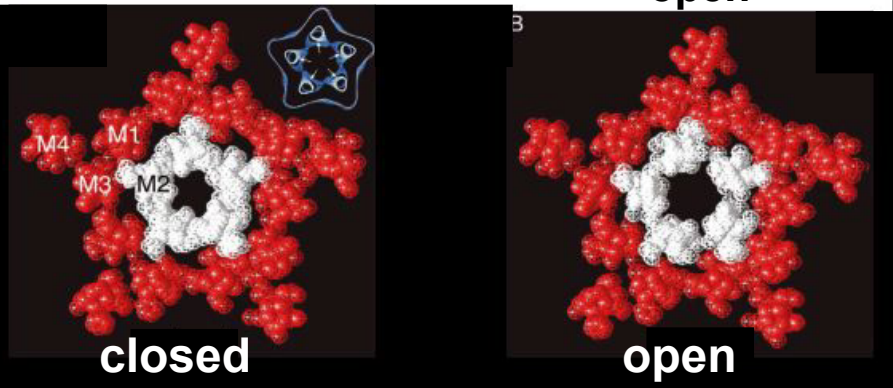
Ионные каналы и синапсы: ключевые молекулярные блоки биологической жизни

26 октября, 2009

Междисциплинарный курс лекций "Молекулярная физиология"

МГУ Москва

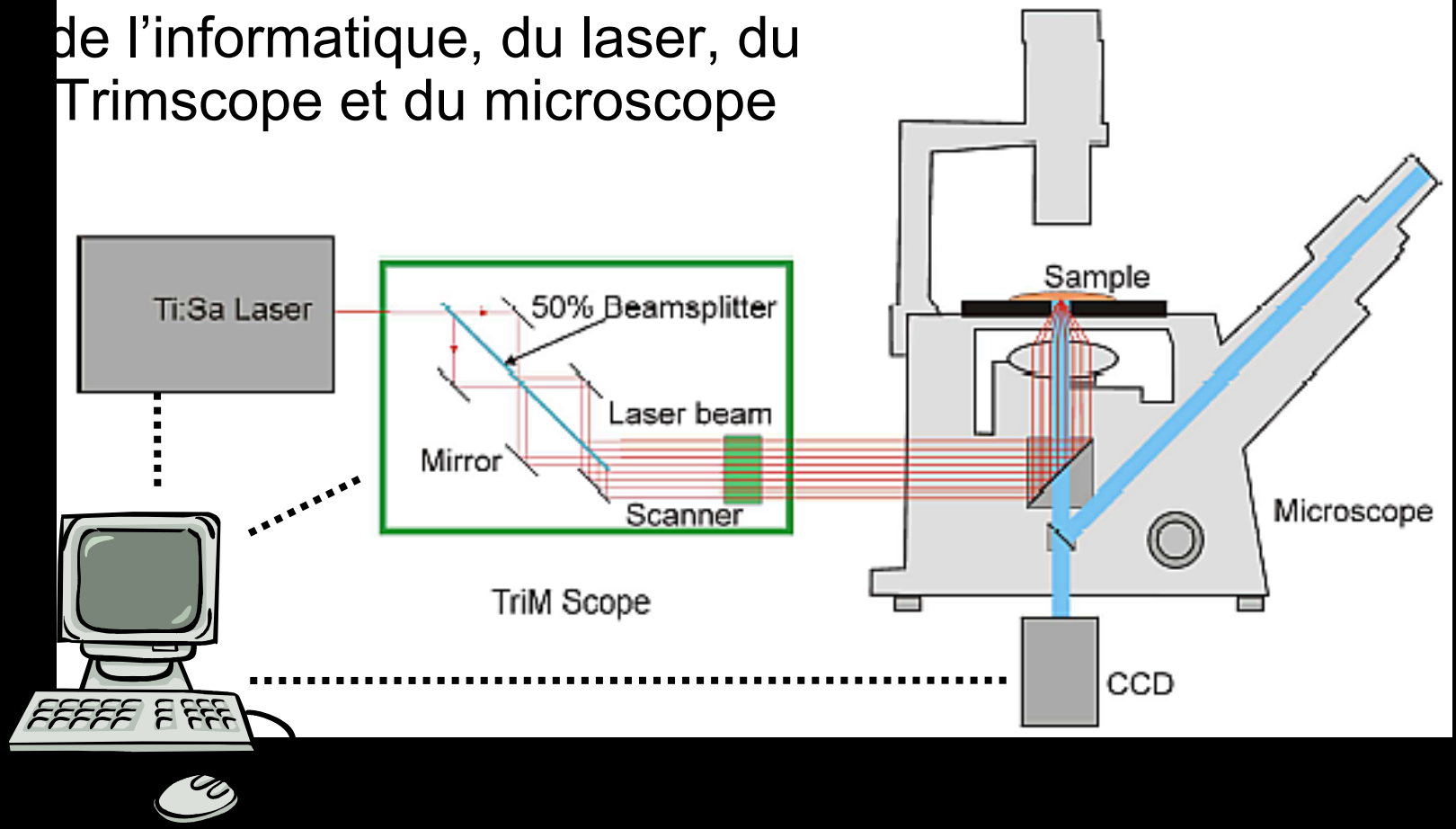




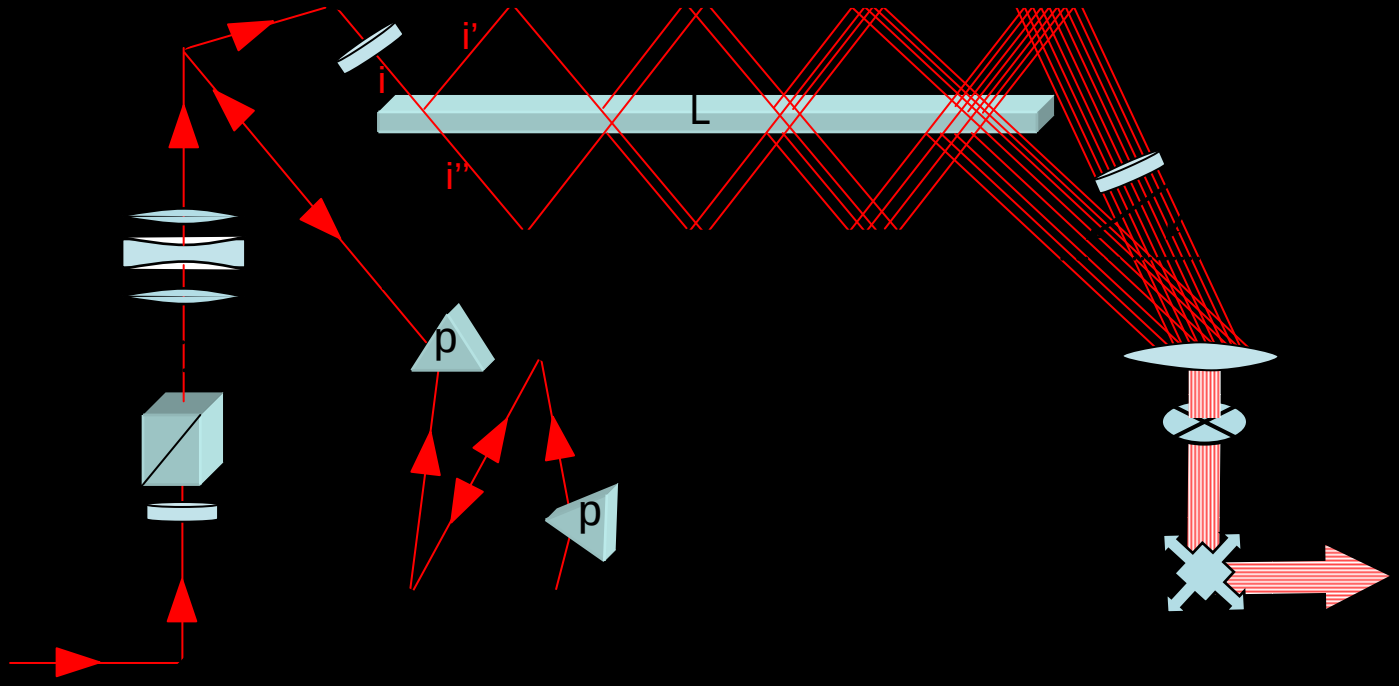
Петр Брежестовский
Средиземноморский Институт Нейробиологии
Марсель, Франция
pbreges@inmed.univ-mrs.fr

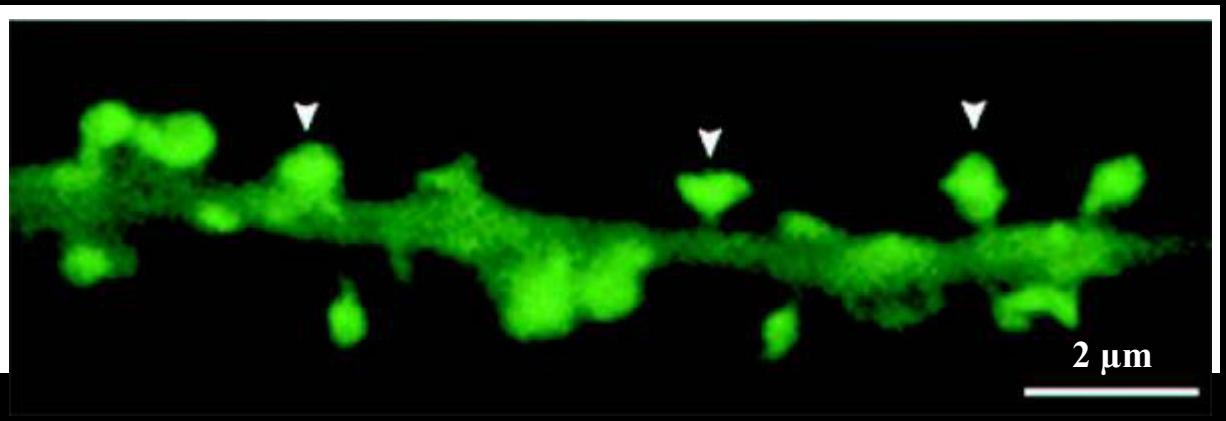
Быстрая 2-фотонная микроскопия

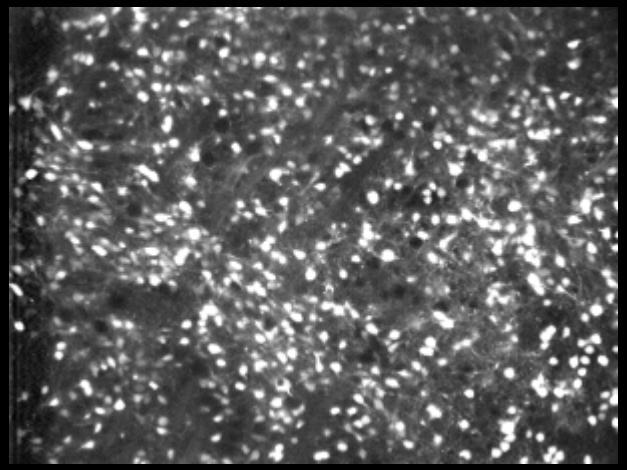
de l'informatique, du laser, du
Trimscope et du microscope



Fonctionnement du Trimscope



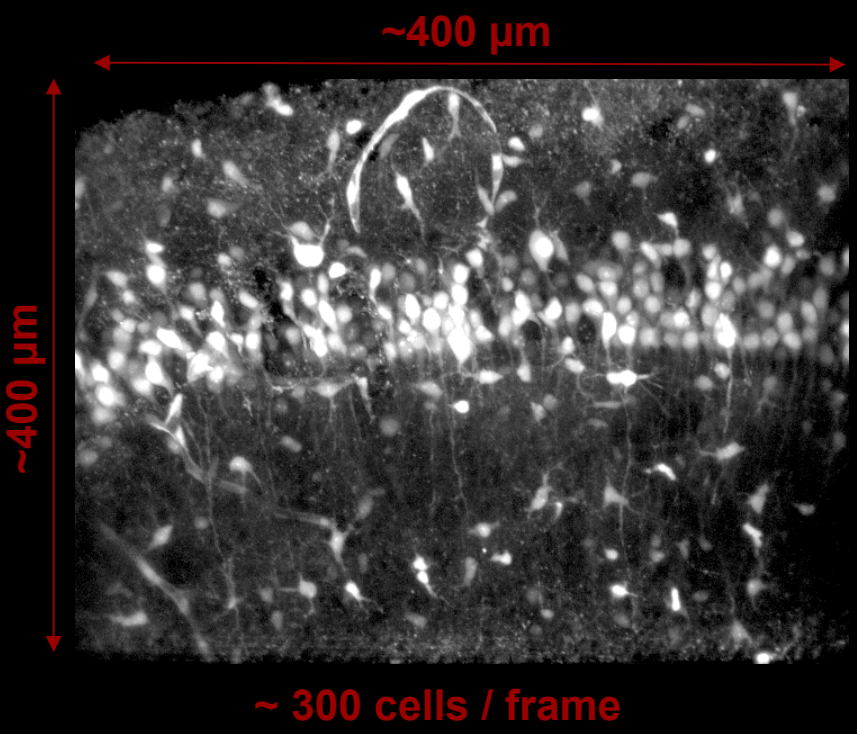




Experimental procedures

Hippocampal slices

Imaging experiments



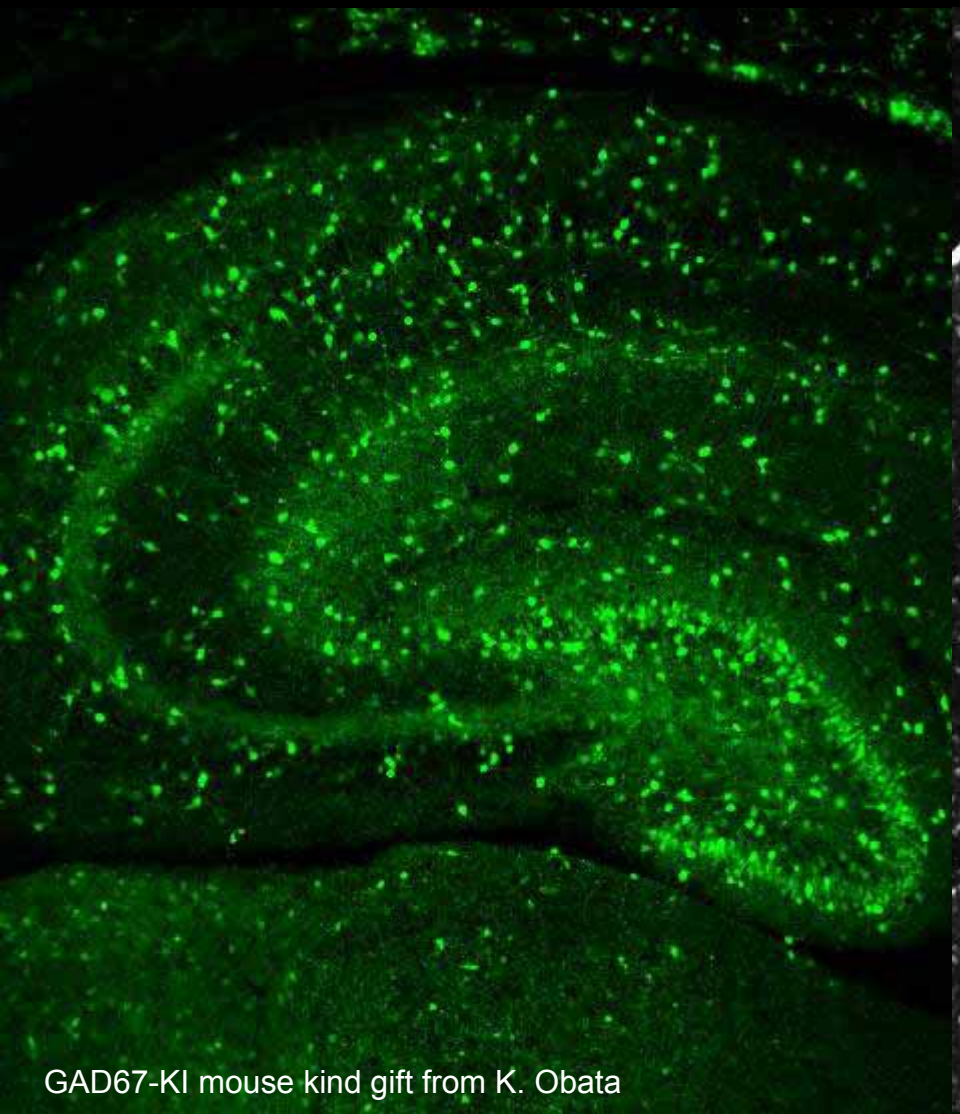
- E16 to 15-day old Swiss mice
- hippocampal slices (300 µm thick)
- Standart ACSF (submerged chamber, 30°C)

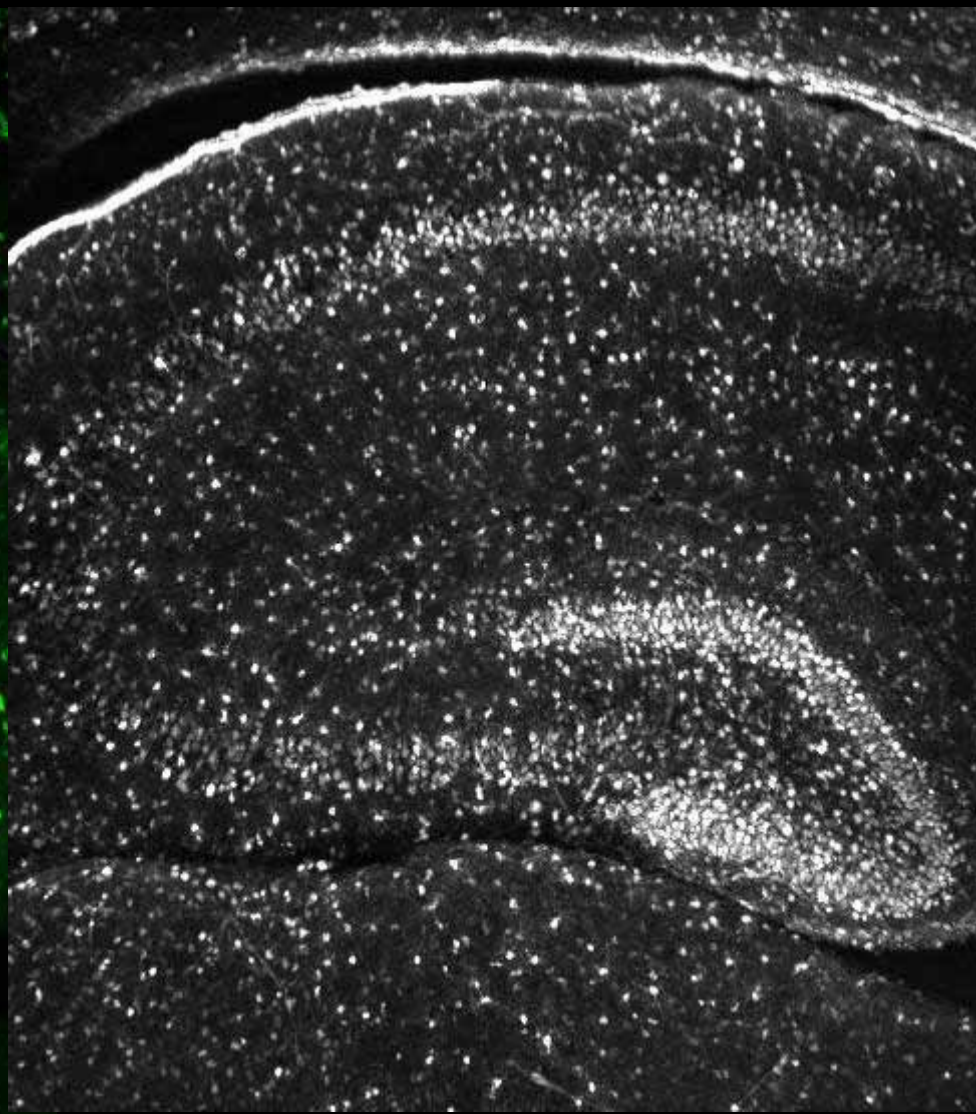
- Low-magnification (20X), high NA (0.95) objective
- Calcium-sensitive dye (Fura 2-AM)
- Two-photon excitation
- Multi-beam (64) : 100ms / frame

Imaging GABAergic neurons

GFP+ neurons=GABAergic neurons

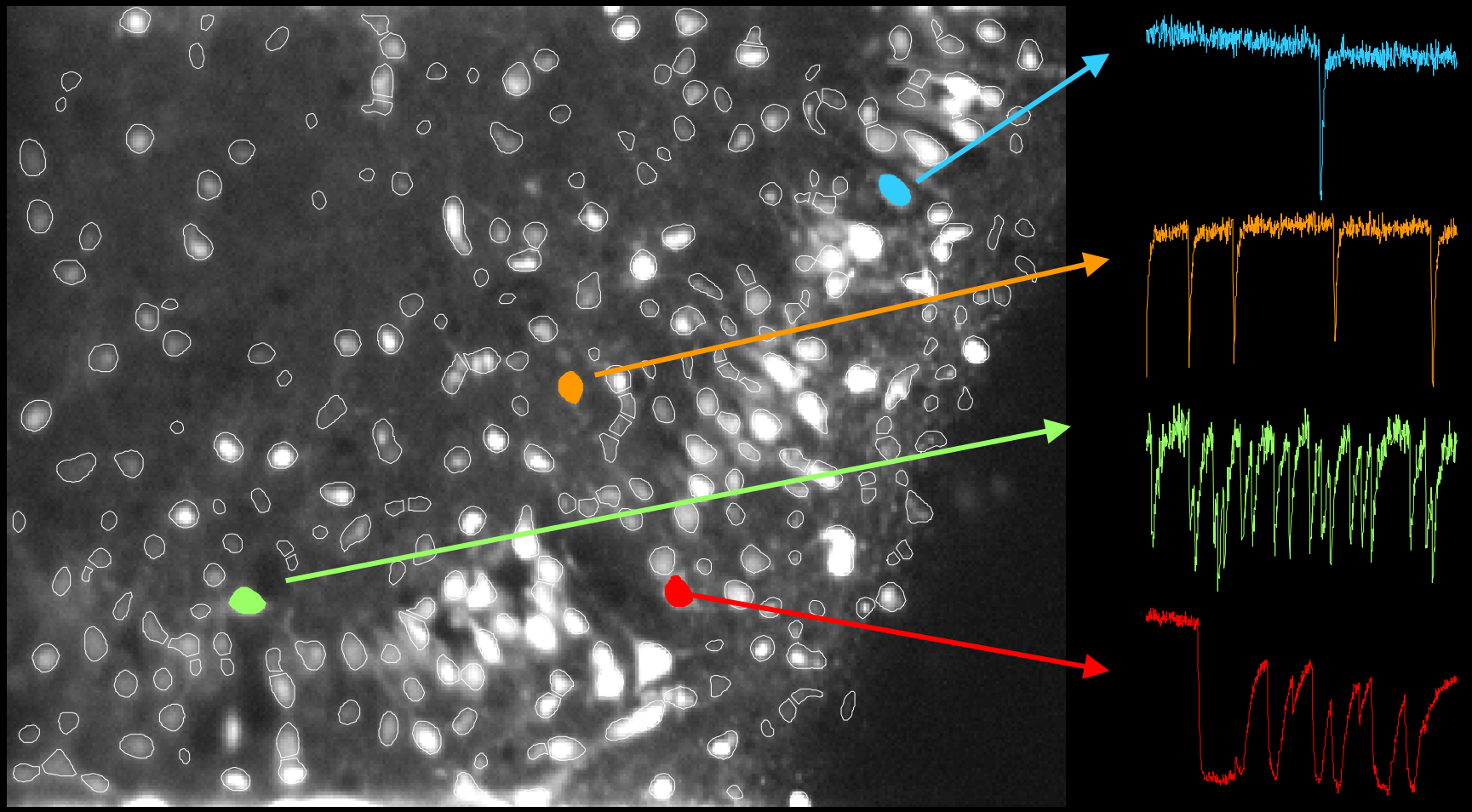
Calcium imaging





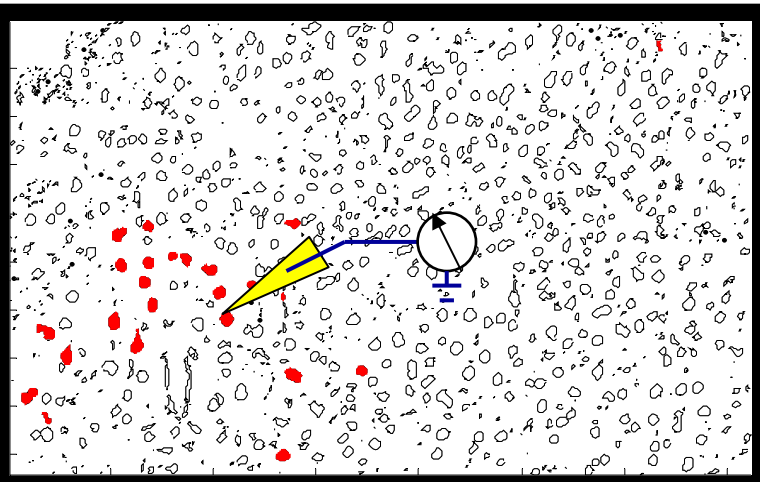
GAD67-KI mouse kind gift from K. Obata

Online analysis: cell detection

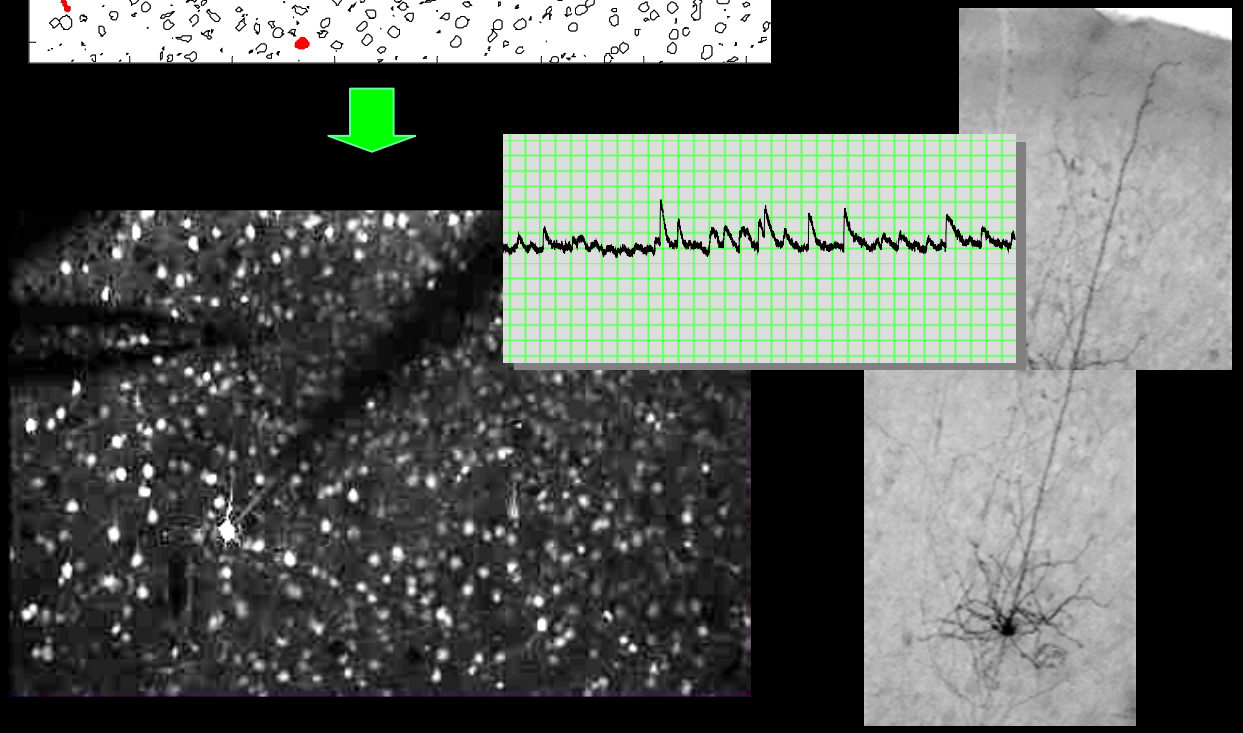


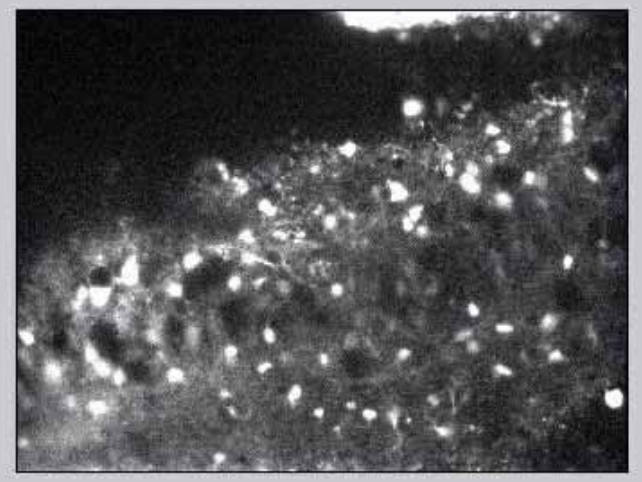
Patch-clamp recording of the active microcircuits

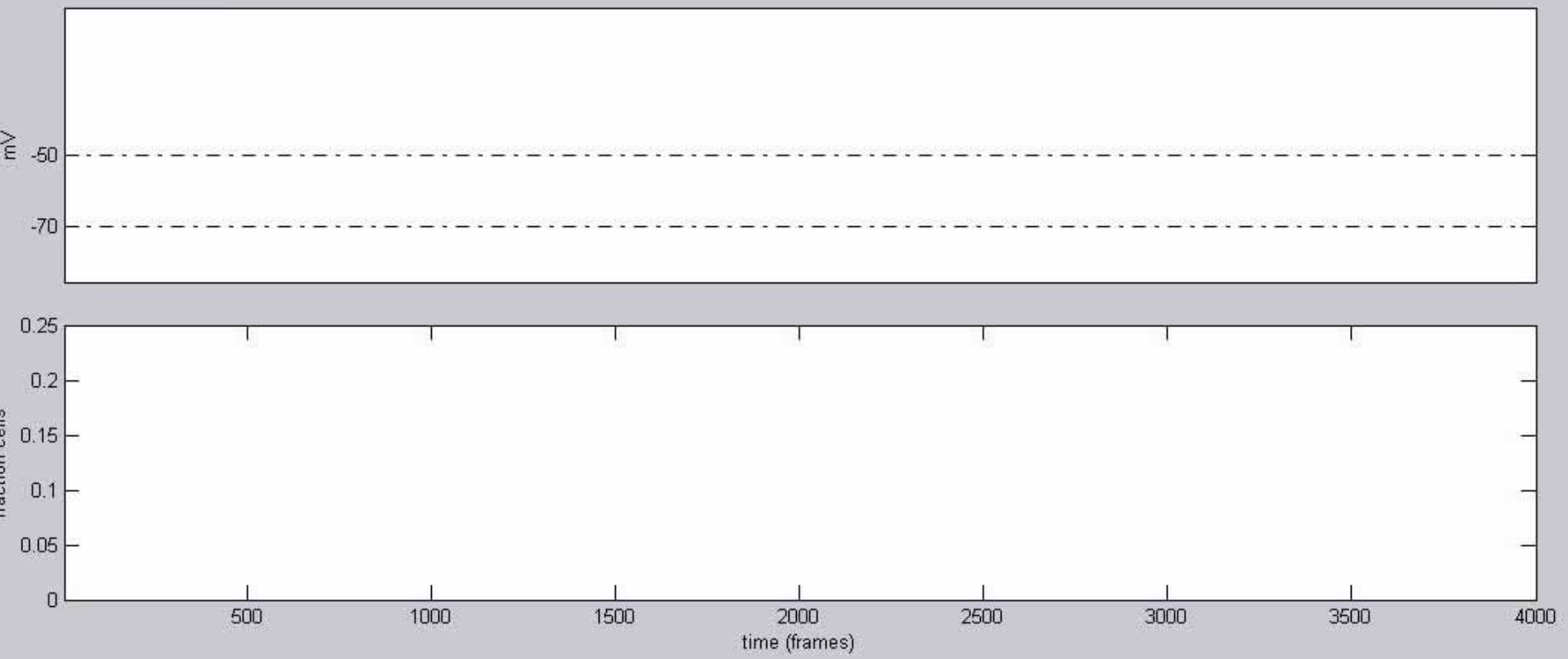
Localization of active microcircuits



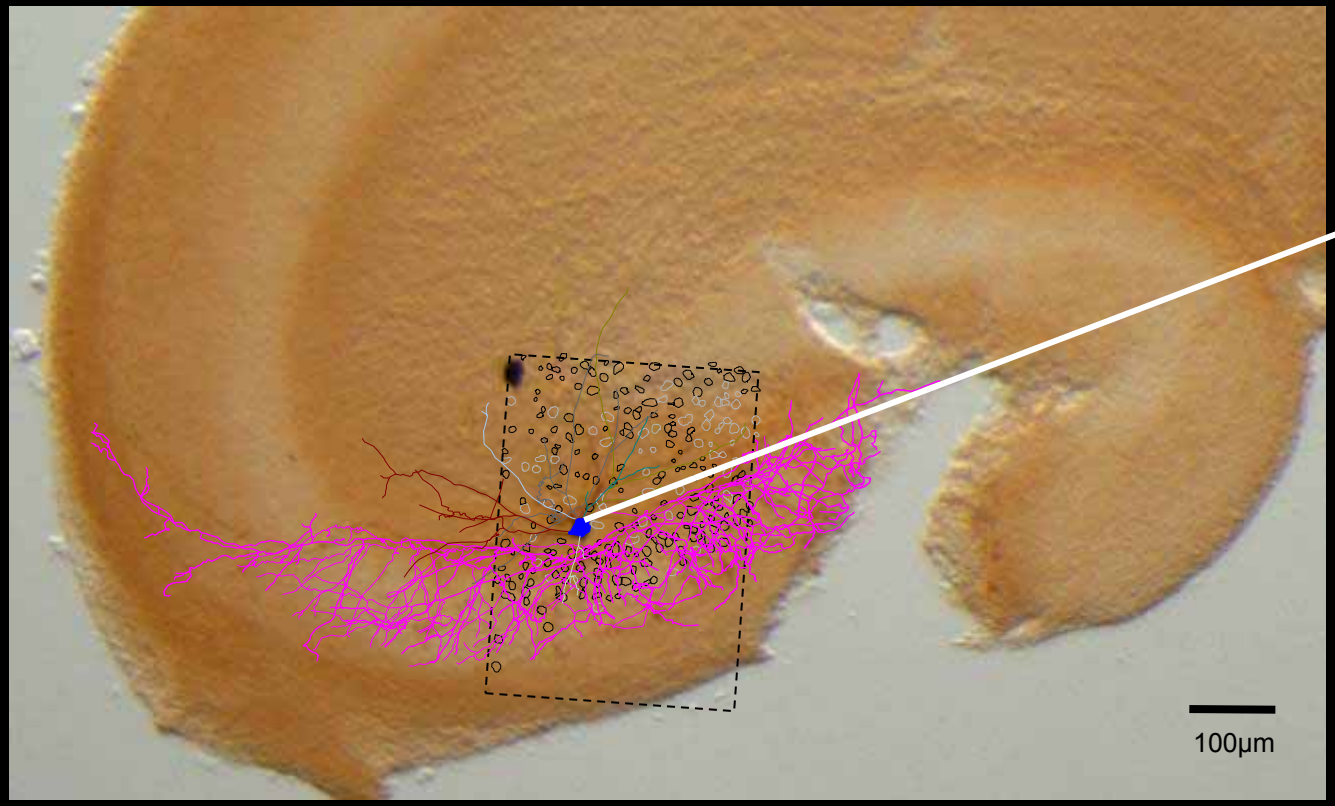
Patch clamp and morphological analysis

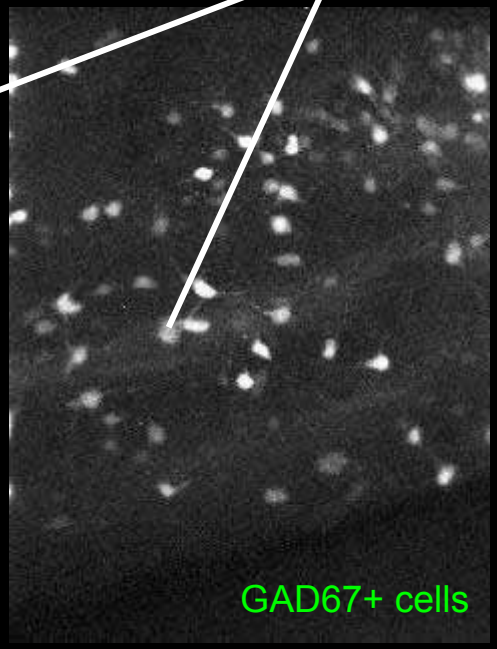





mV
-50
-70
0.25
0.2
0.15
0.1
0.05
0
500
1000
1500
2000
2500
3000
3500
4000
time (frames)

Hubs are GABA neurons with widespread axonal arborization





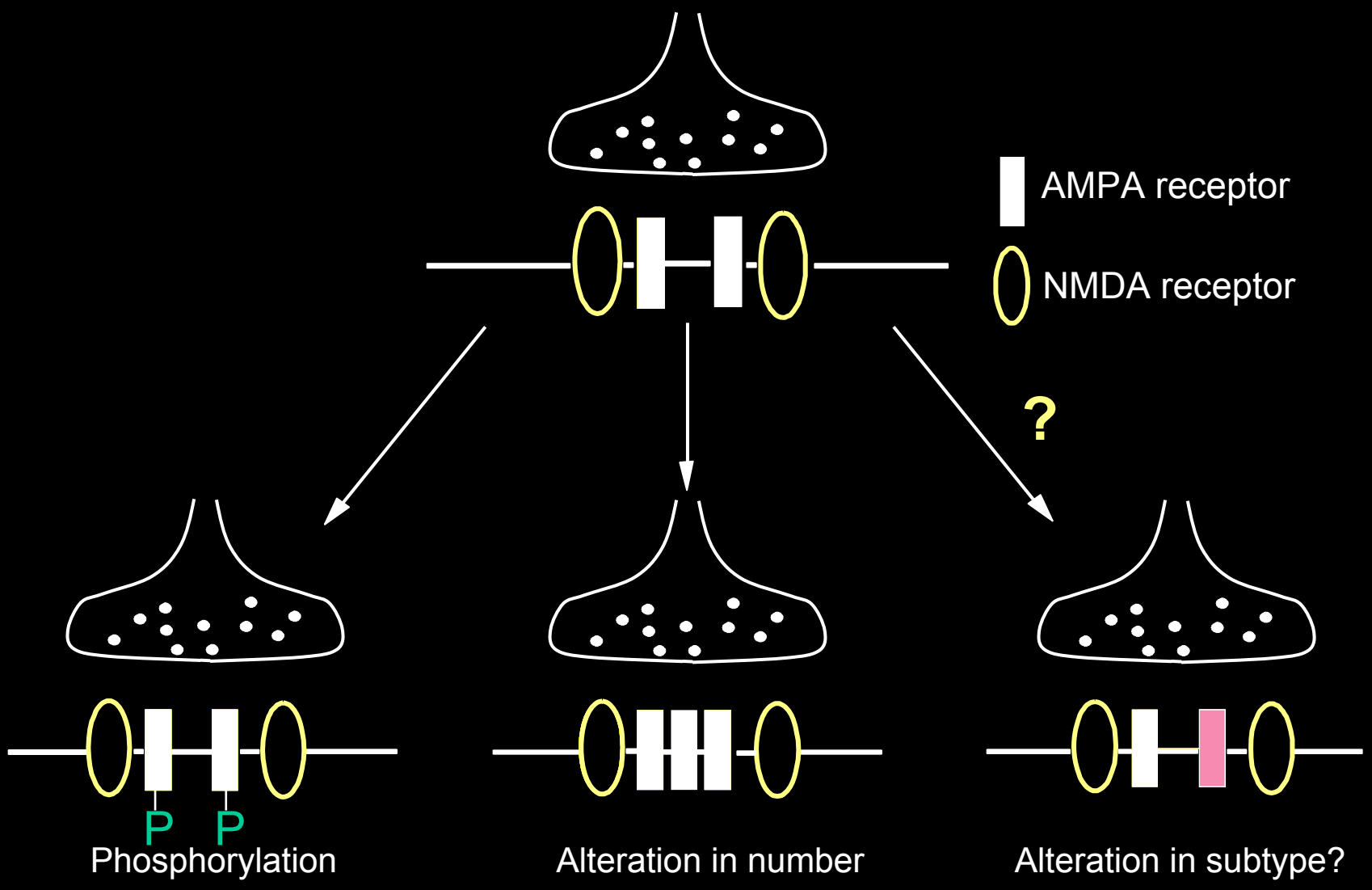
На память:

- Синапсы - динамические самоформирующиеся молекулярные модули, обеспечивающие быструю передачу информации в нервной системе

- Ионные каналы ключевые белки, обеспечивающие транформацию действия химических молекул, механических и др. воздействий в электрические сигналы

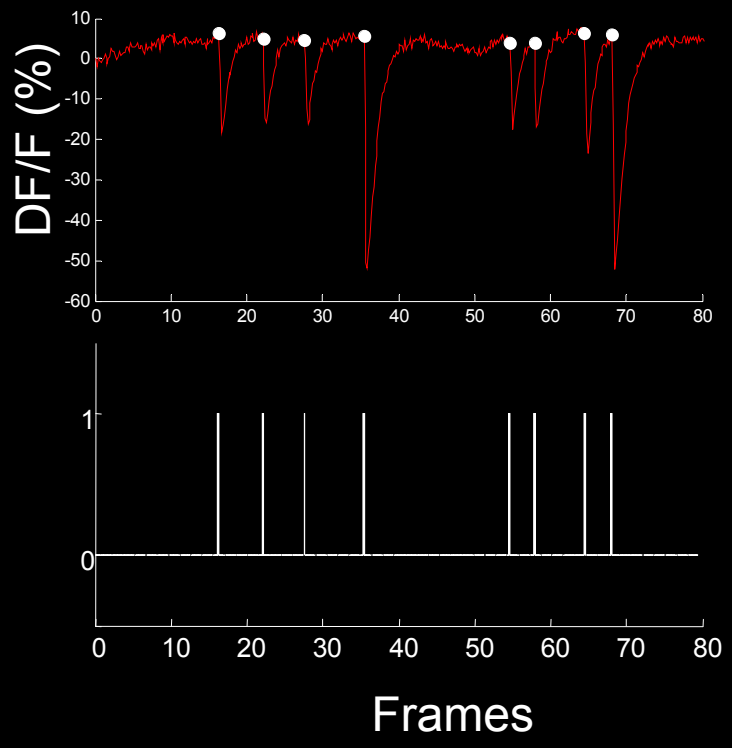
- формирование и пластичность нервной сети
- память
- патологии (каналопатии)

Plasticity of synapric channels

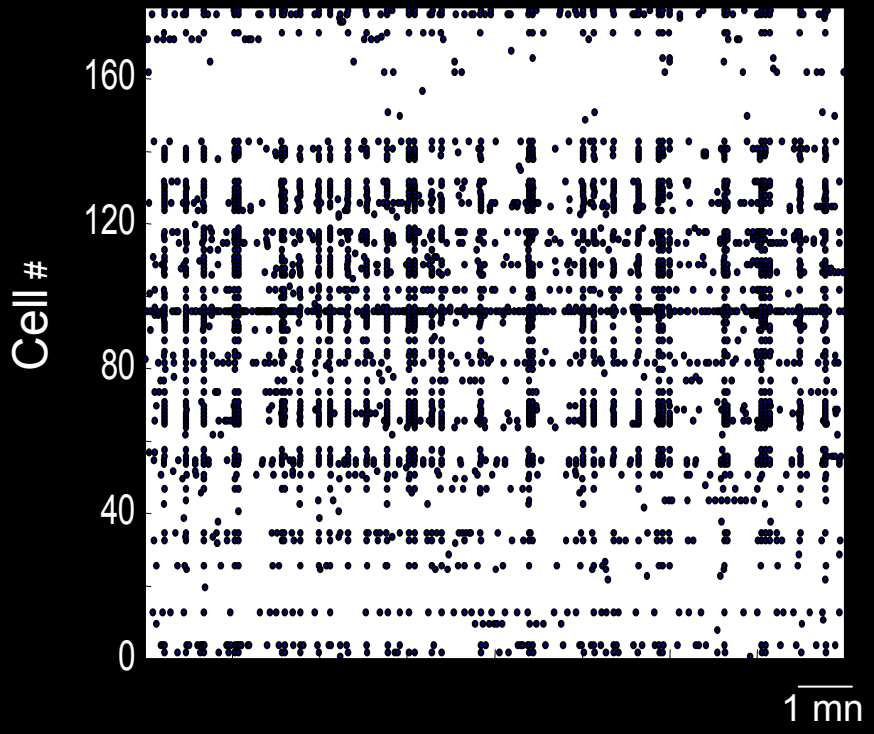


Online analysis: event detection

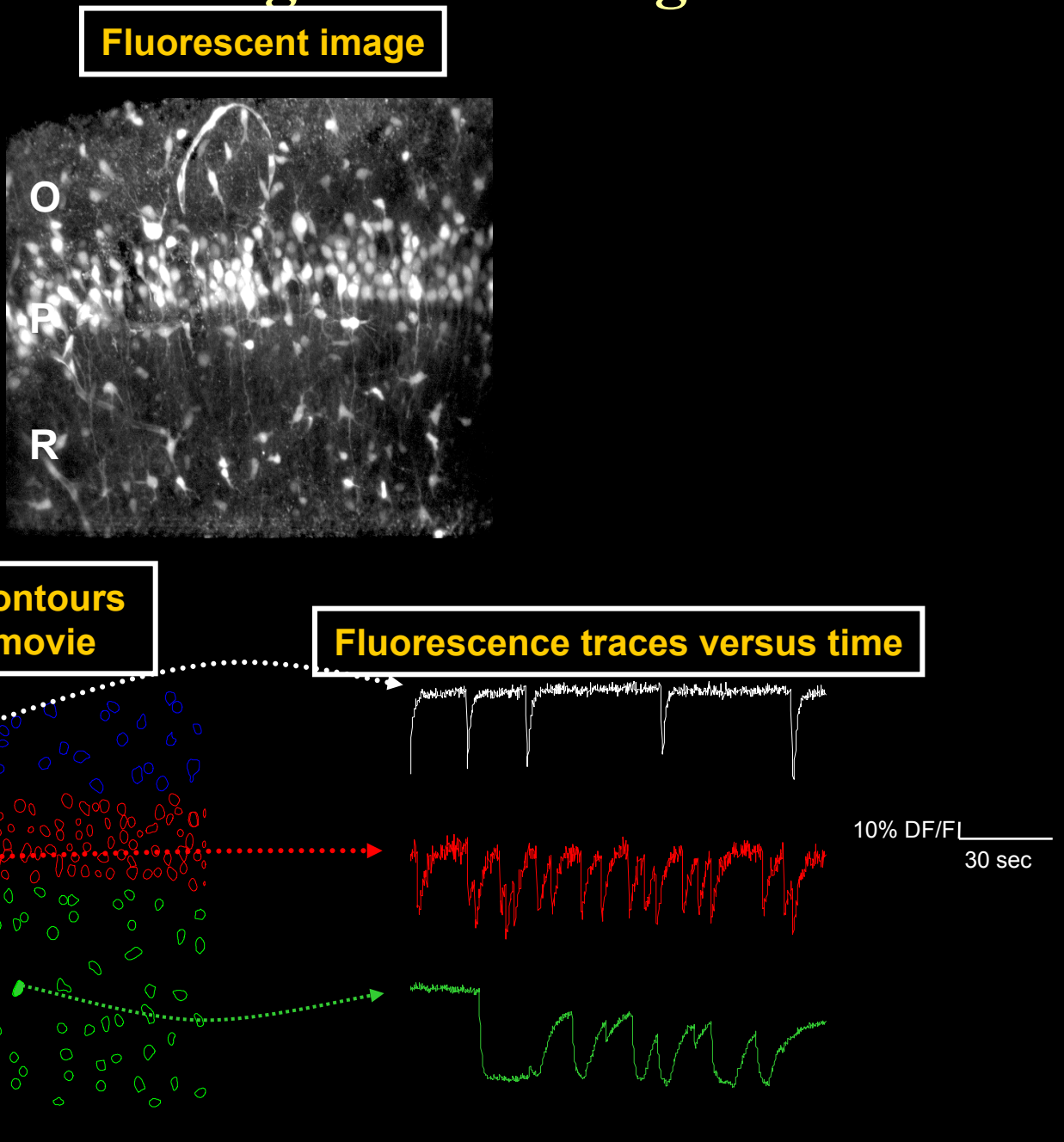
Event detection



Rasterplot of network activity

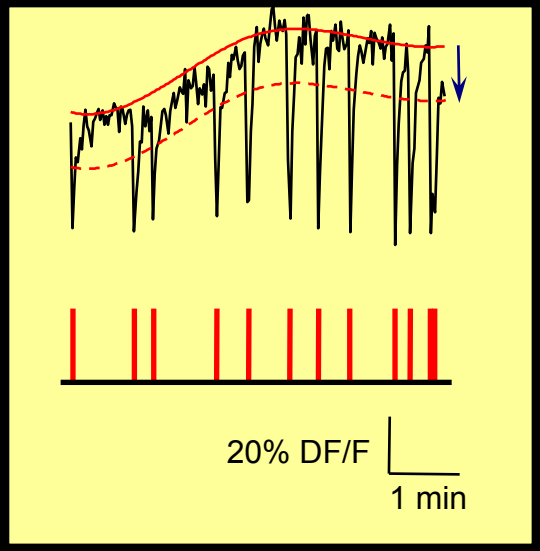


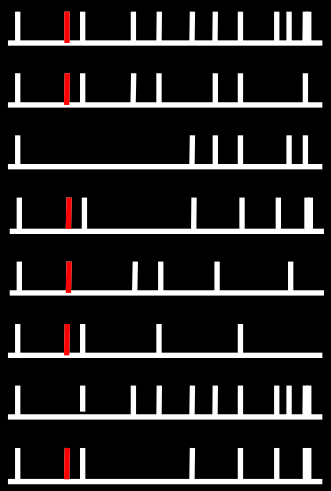
Detecting calcium signal from single neurons



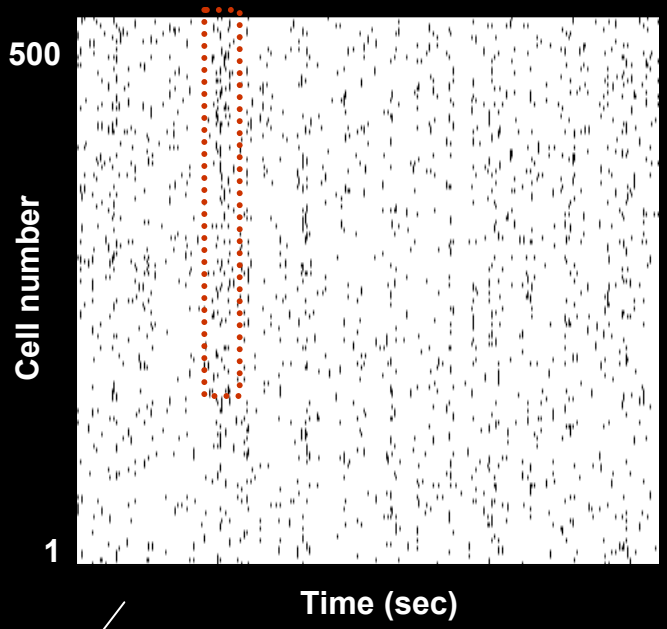
Analysis of the network activity

Signal detection





Rasterplots of the activity



Reconstruction of **network** activity

На память:

- Синапсы - динамические самоформирующиеся молекулярные модули, обеспечивающие быструю передачу информации в нервной системе

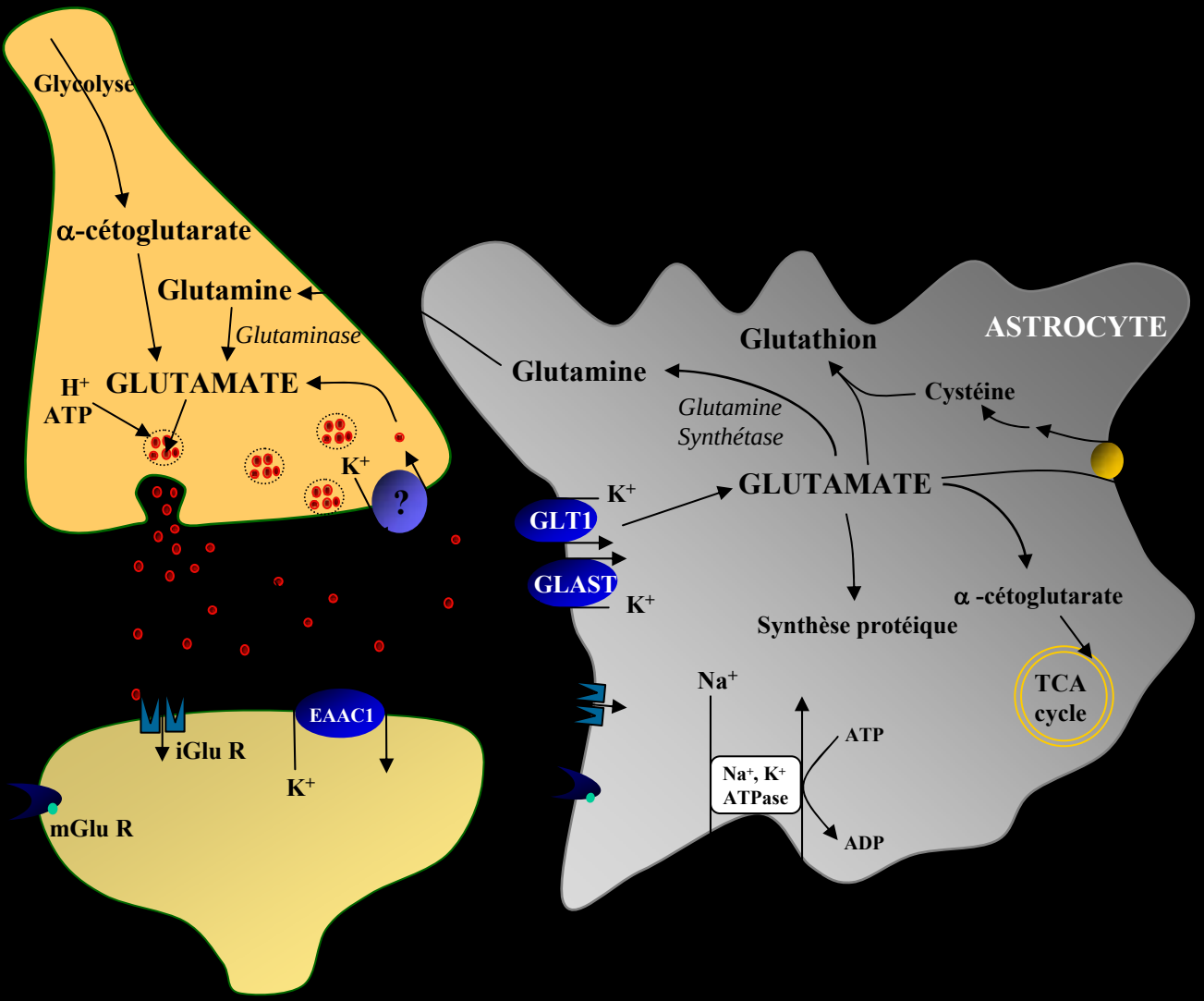
- Ионные каналы ключевые белки, обеспечивающие транформацию действия химических молекул, механических и др. воздействий в электрические сигналы

- How chemical molecules activates channels?
- How synaptic activation leads to "intelligent" operation of brain?

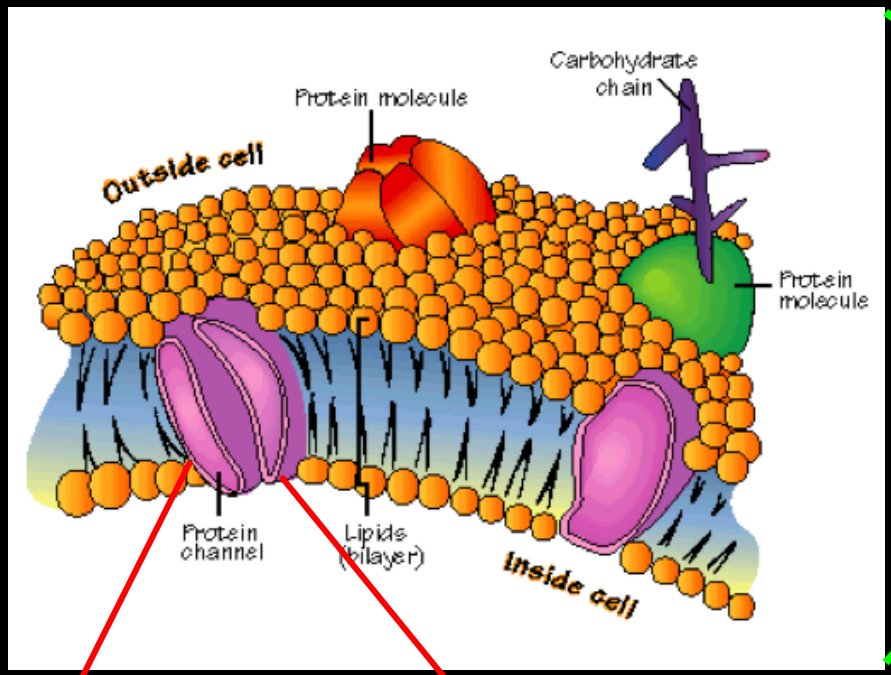
Molecules activating receptor-operated channels

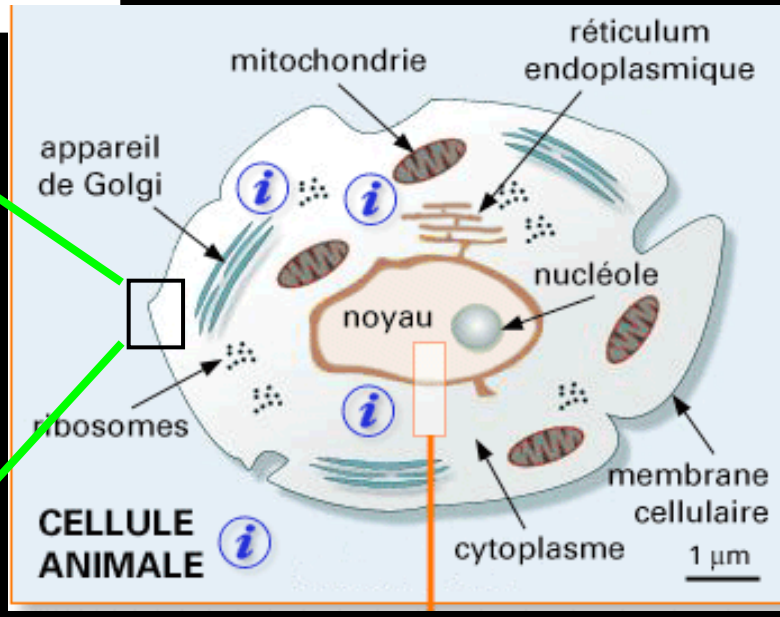
Excitatory - Cation-selective (Na +, K +):
- main neurotransmitter in CNS: **Glutamate**
- in neuro-muscular junction: **Acetylcholine**

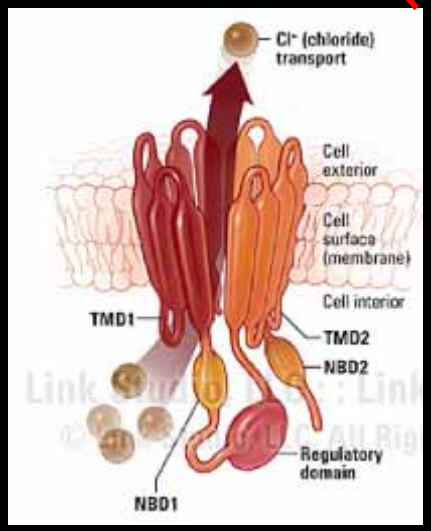
Inhibitory-Ation-selective (Cl-):
- main neurotransmitter in brain: **GABA**
- in spinal cord: **glycine**


Glycolyse
α-cétoglutarate
Glutamine
Glutaminase
H+
ATP
GLUTAMATE
K+
?
ASTROCYTE
Glutathion
Glutamine
Cystéine
Glutamine
Synthétase
GLUTAMATE
K+
GLT1
GLAST
K+
α -cétoglutarate
Synthèse protéique
TCA
cycle
Na+
ATP
Na+, K+
ATPase
ADP
EAAC1
iGlu R
K+
mGlu R

Membranes and Channels

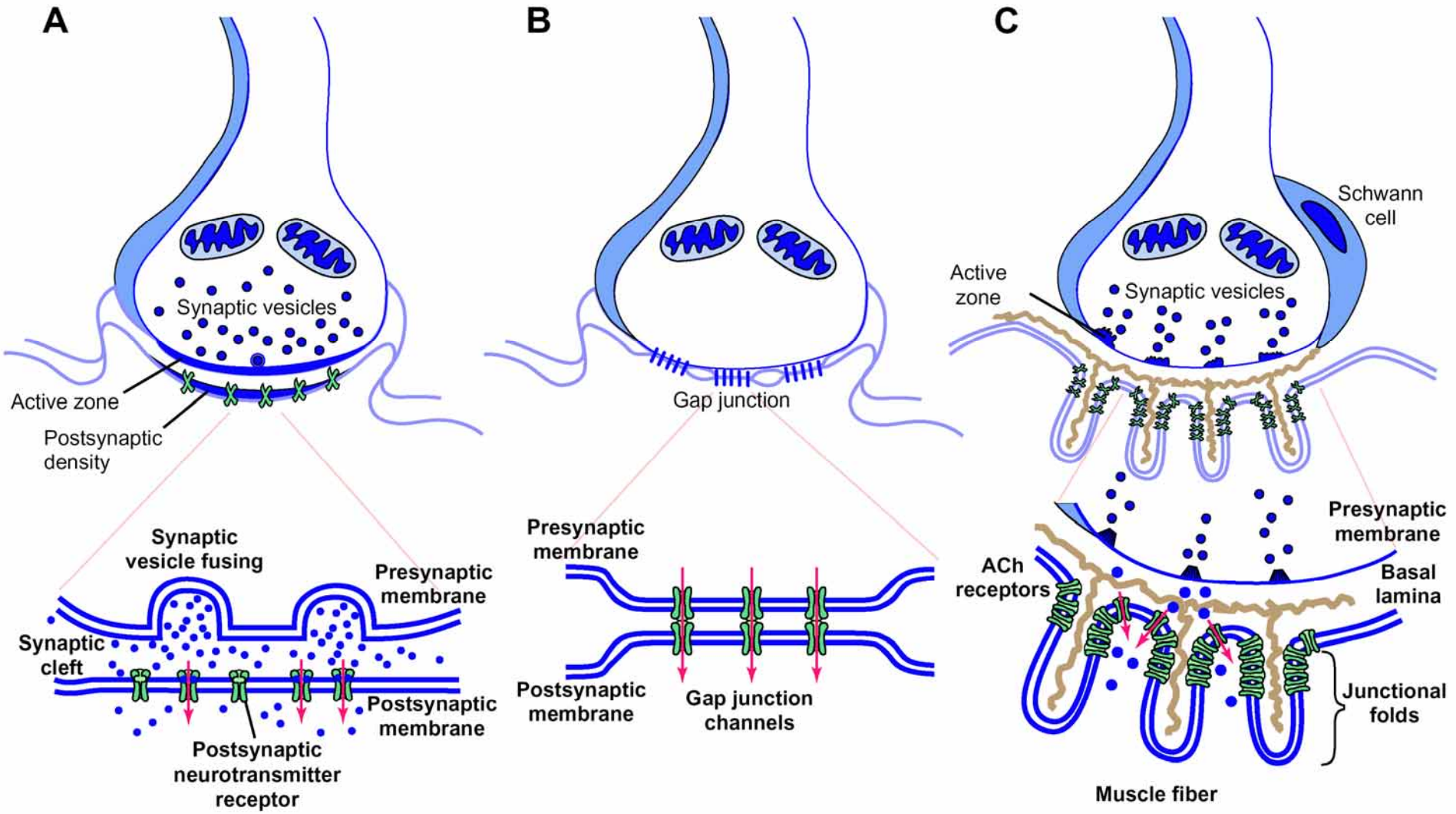




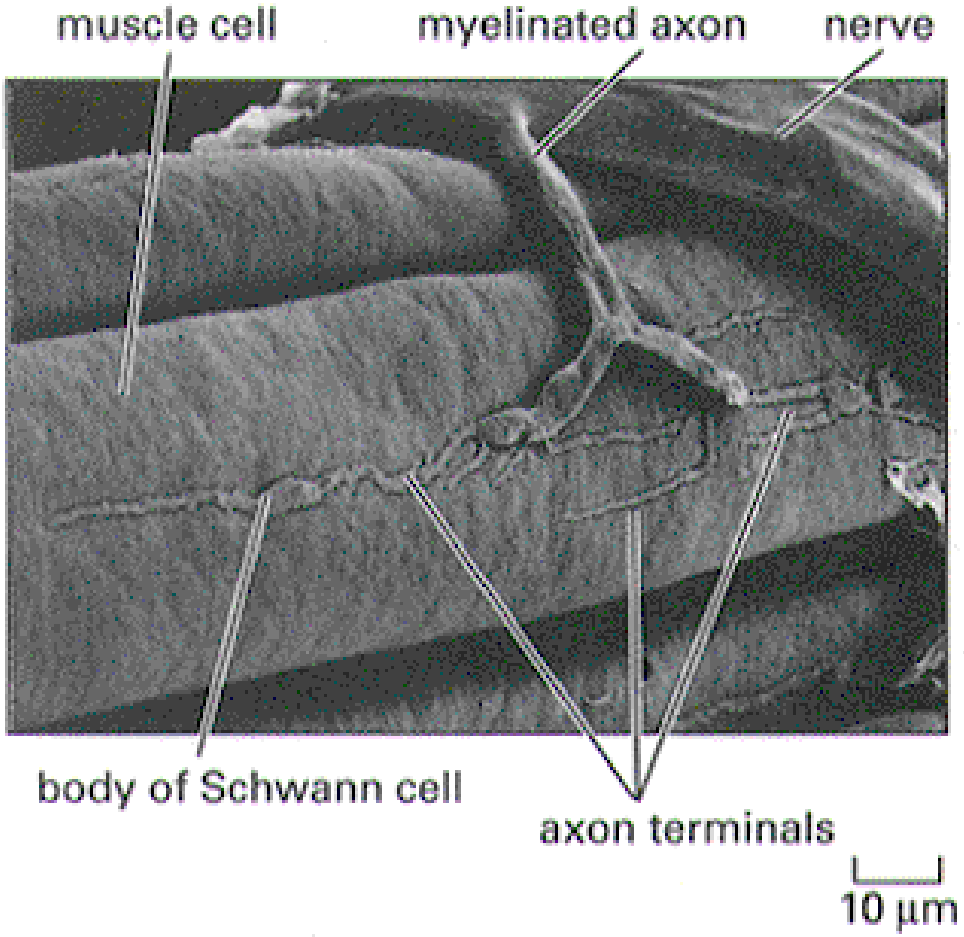


Ion channels mediate intracellular communication by converting the voltage, chemical or mechanical signals into ion fluxes

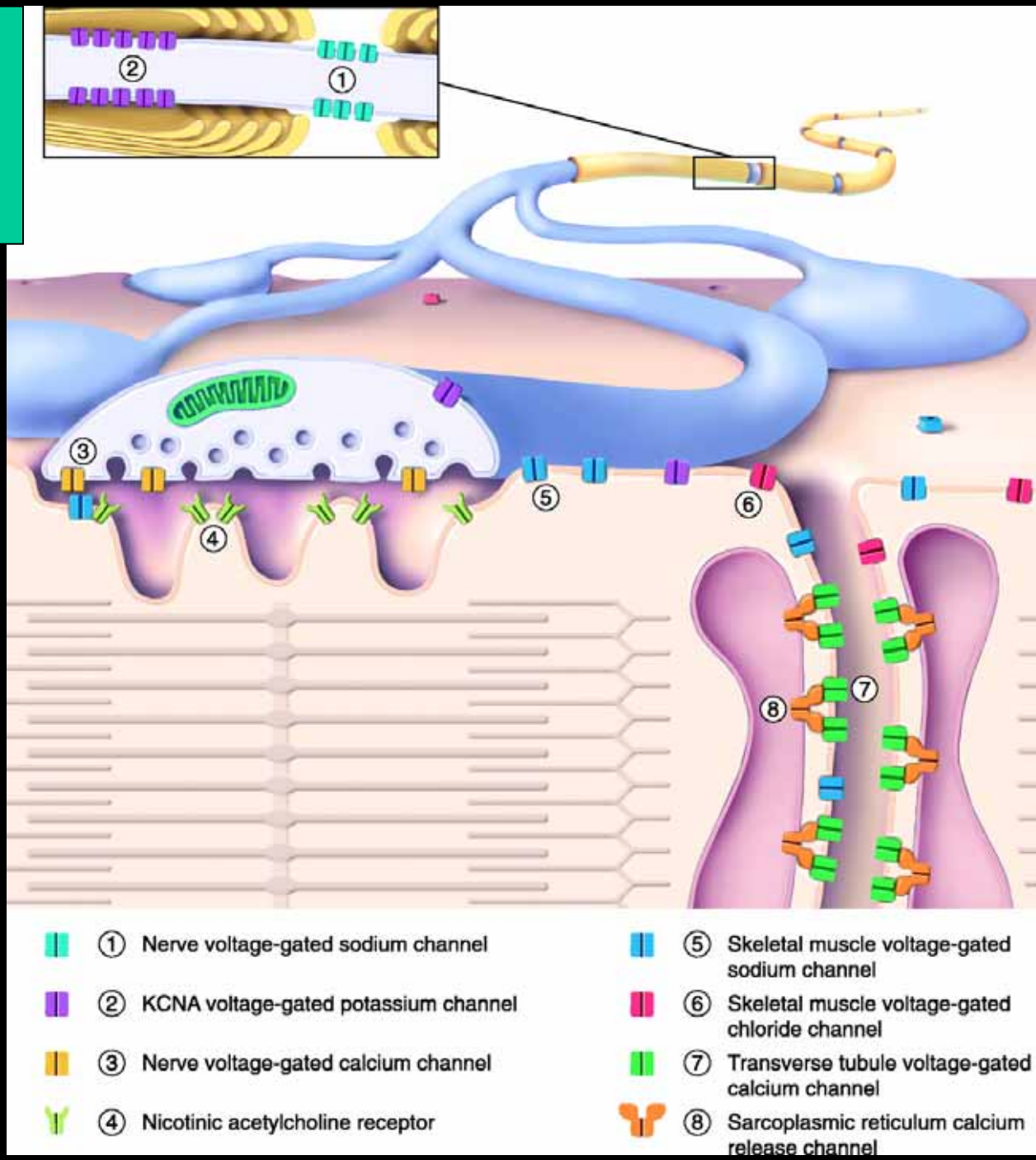
Interneuronal and neuromuscular synapses



Ion channel - protein interactions in neuromuscular synapses



Ion channels of end-plate

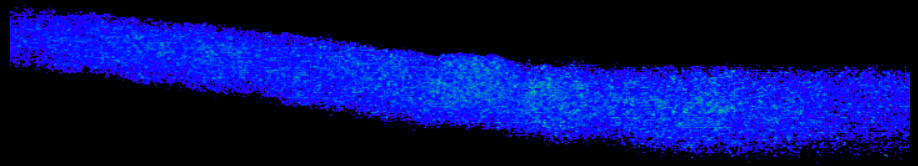


Ca^{2+} in muscle at application of 3μM ACh

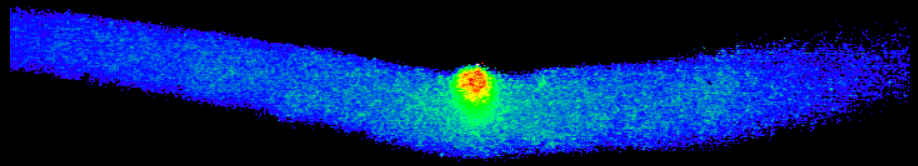
Before acetylcholine

Fura-2

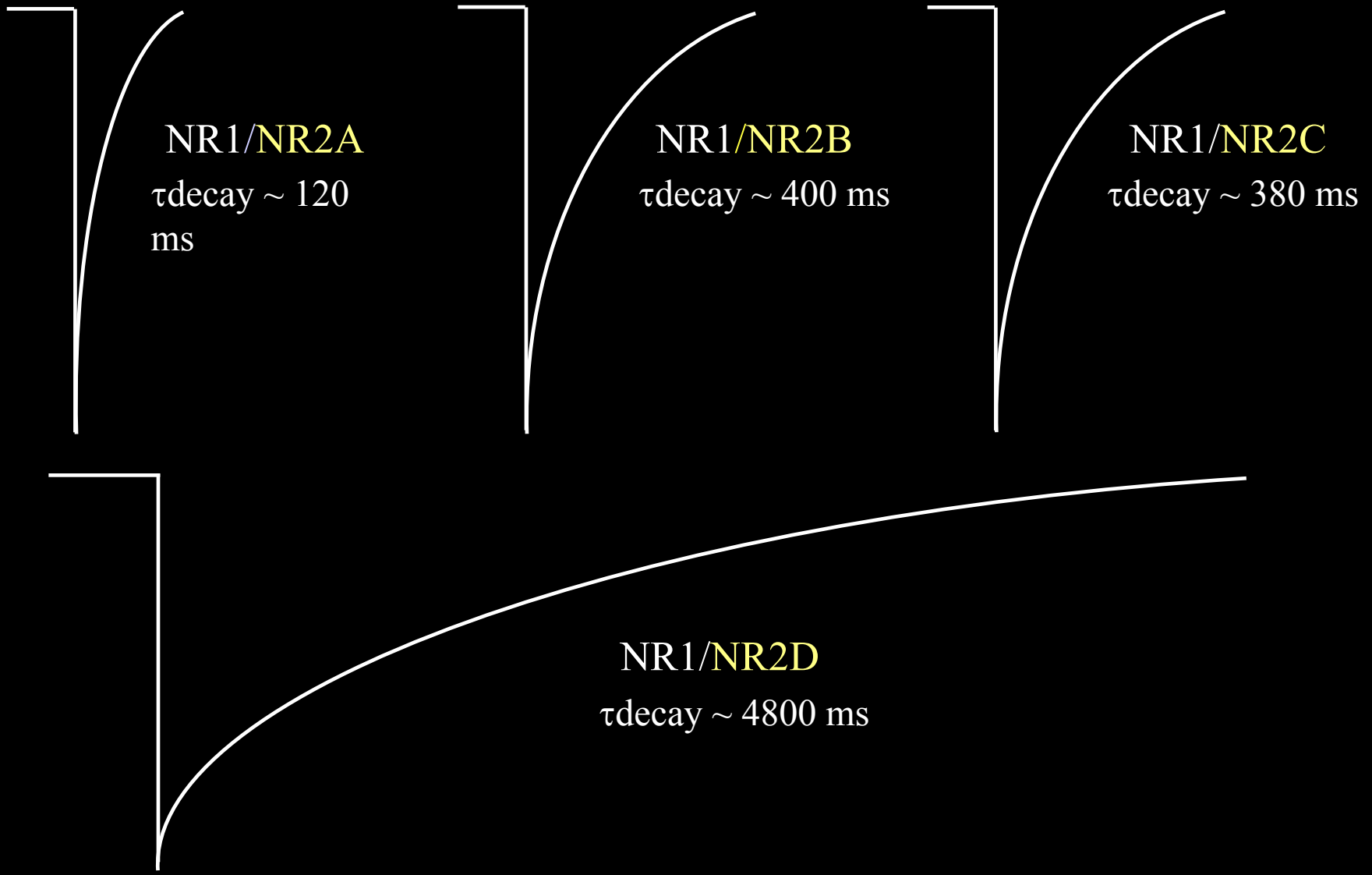
Ca-sensitive dye



After acetylcholine



Deactivation kinetics of NMDARs



We will discuss today:

- **Ionic channels:**
  - **general principles of organization;**
  - **how to record channels;**
  - **main functional parameters**

- **Neuronal plasticity:**
  - **Long-term potentiation;**
  - **Long-term inhibition;**
  - **Short-term modulation.**

- **Actin cytoskeleton and Ca^{2+} in formation and plasticity of spines**

Tim Bliss, Per Andersen and Terje Lømo at The Royal Society in London last May during the meeting "Long-term potentiation: enhancing neuroscience for 30 years". Bliss and Lømo discovered LTP while working in Andersen's laboratory in Oslo. Photo courtesy of J. Lisman.

J. Physiol. (1973), **232**, *pp.* 331–356
With 12 text-figures
Printed in Great Britain

LONG-LASTING POTENTIATION OF SYNAPTIC TRANSMISSION IN THE DENTATE AREA OF THE ANAESTHETIZED RABBIT FOLLOWING STIMULATION OF THE PERFORANT PATH

BY T. V. P. BLISS AND T. LØMO

From the National Institute for Medical Research, Mill Hill, London NW7 1AA and the Institute of Neurophysiology, University of Oslo, Norway

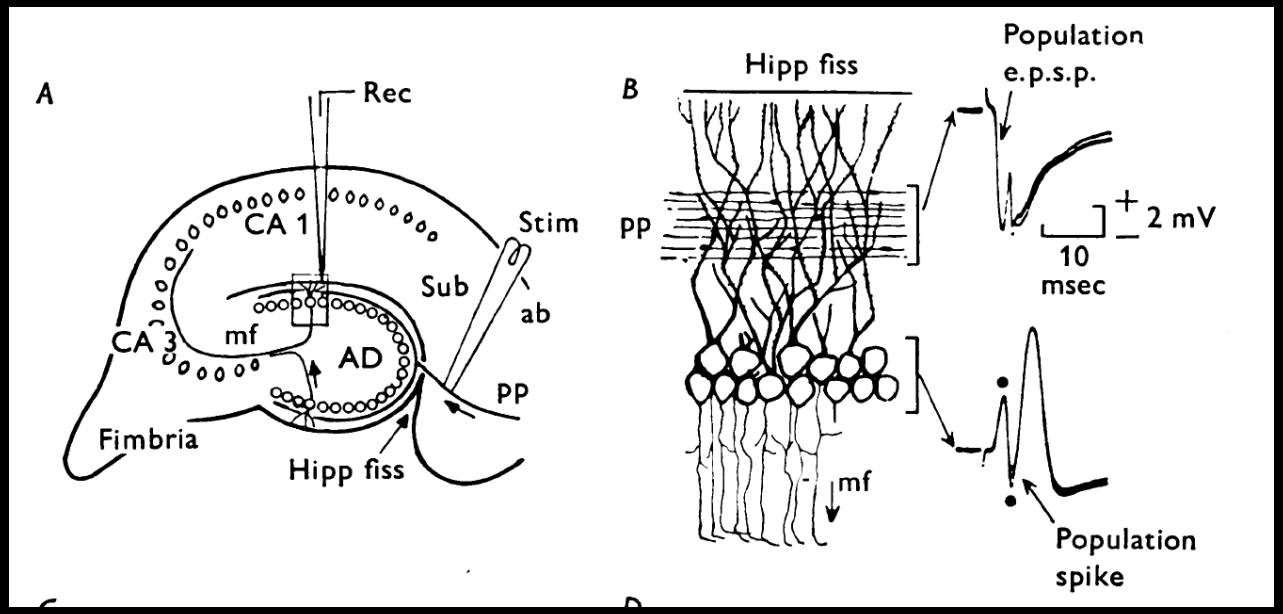
(*Received 12 February 1973*)

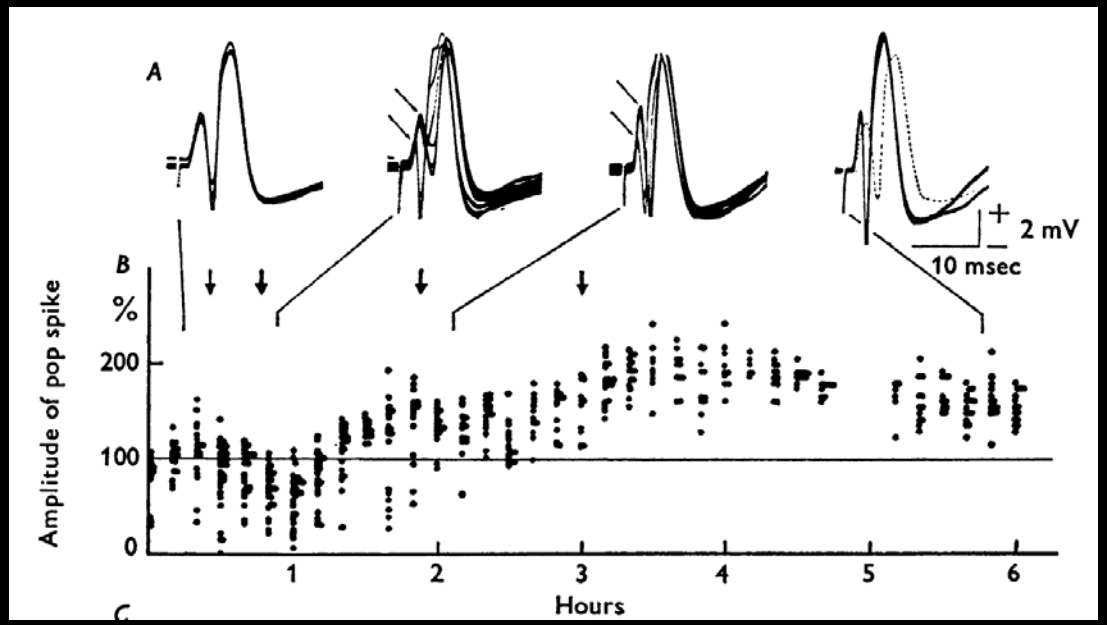
SUMMARY

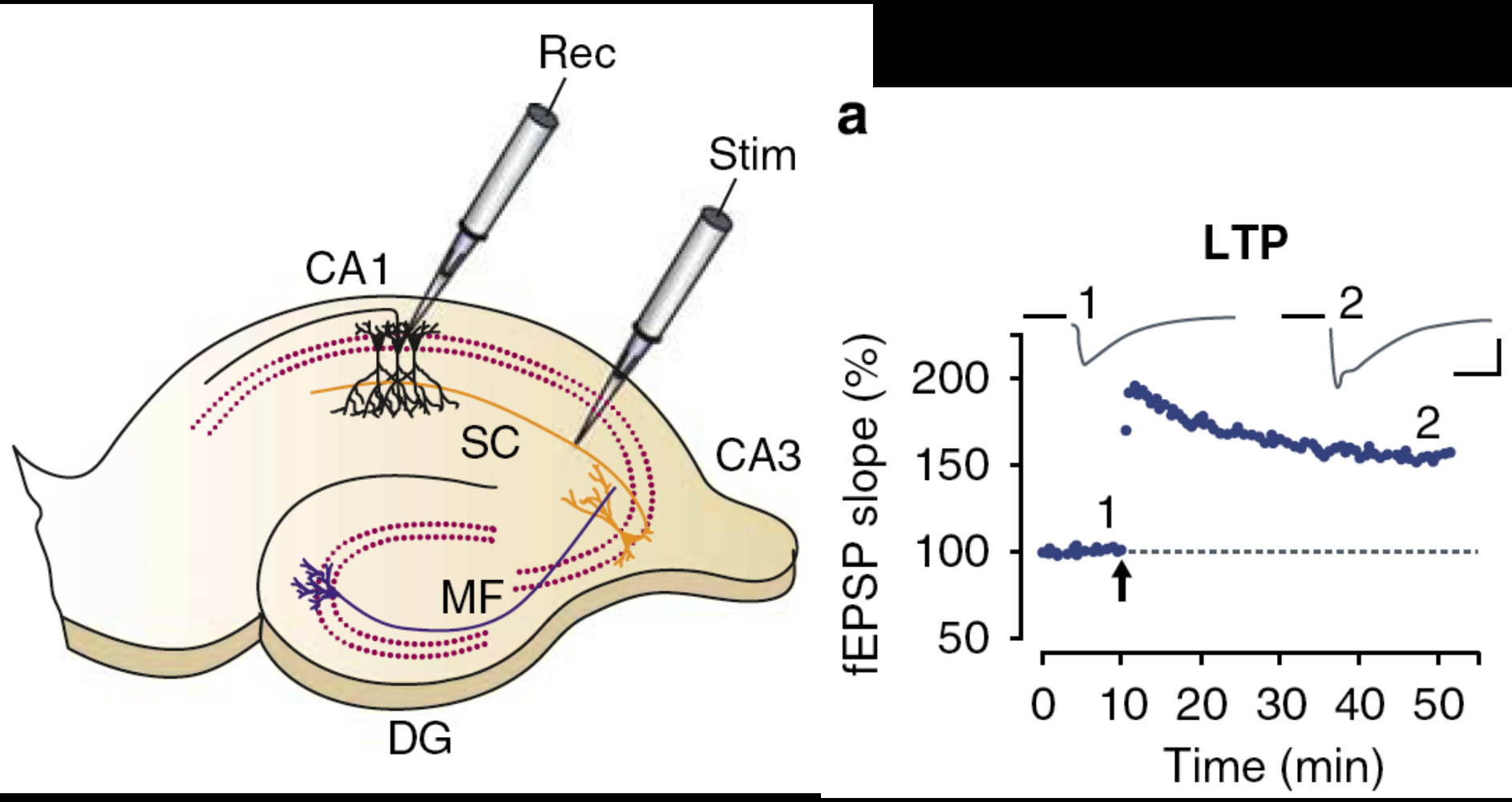
1. The after-effects of repetitive stimulation of the perforant path fibres to the dentate area of the hippocampal formation have been examined with extracellular micro-electrodes in rabbits anaesthetized with urethane.

2. In fifteen out of eighteen rabbits the population response recorded from granule cells in the dentate area to single perforant path volleys was potentiated for periods ranging from 30 min to 10 hr after one or more conditioning trains at 10–20/sec for 10–15 sec, or 100/sec for 3–4 sec.

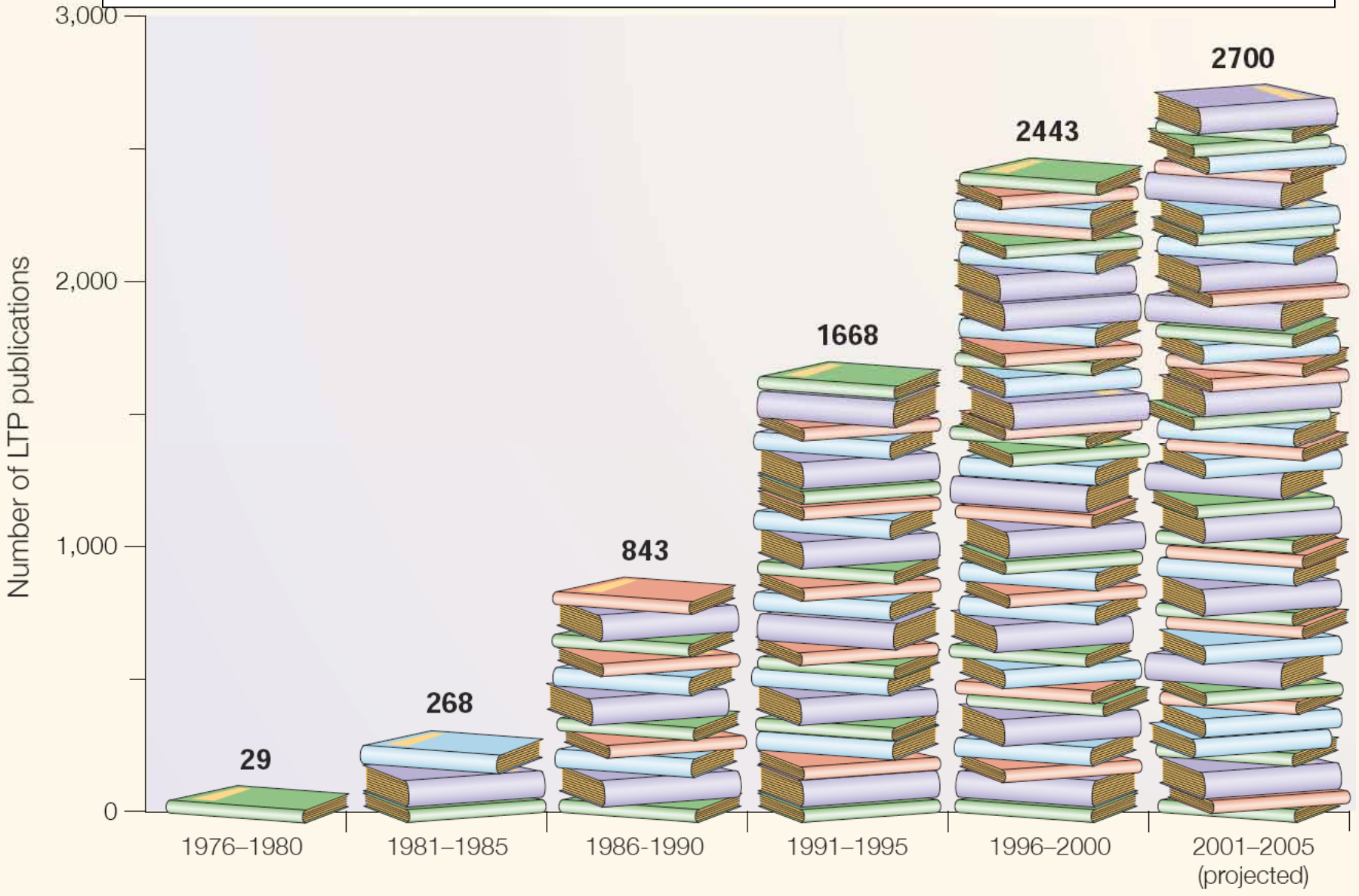
Discovery of Long-Term Potentiation (LTP)

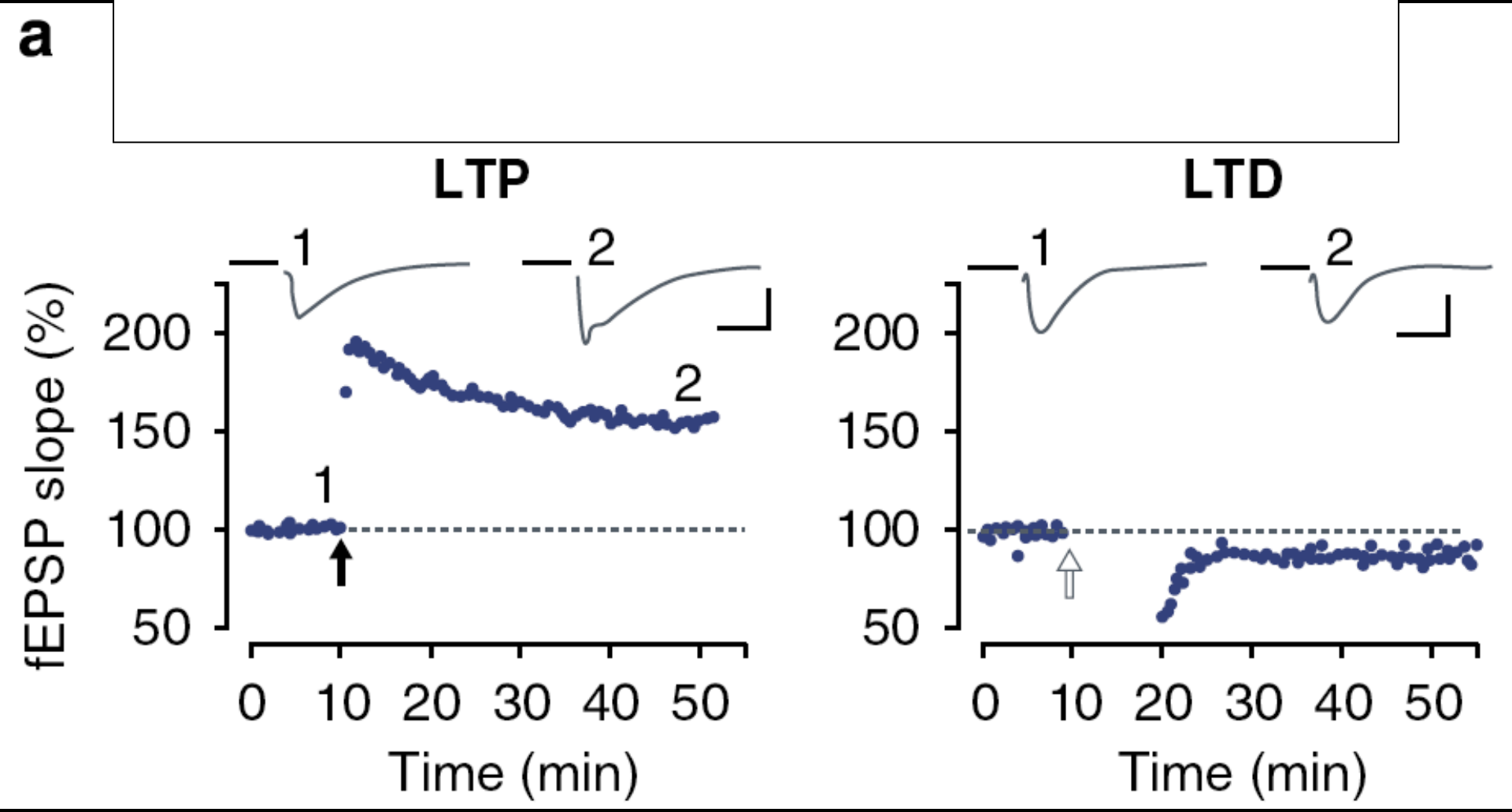


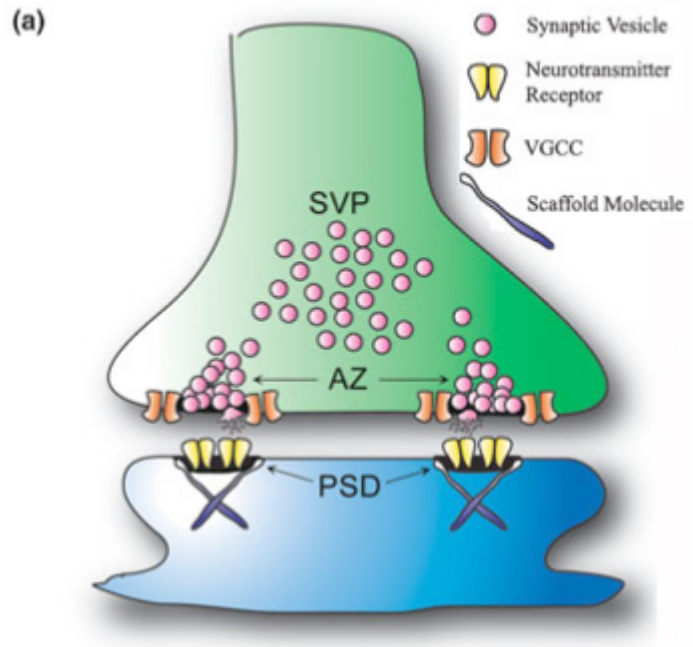



Rec
Stim
CA1
SC
CA3
MF
DG
a
LTP
1
2
fEPSP slope (%)
200
150
100
50
0
10
20
30
40
50
Time (min)

Number of LTP publications




a
LTP
LTD
1
2
fEPSP slope (%)
200
150
100
50
0
10
20
30
40
50
Time (min)



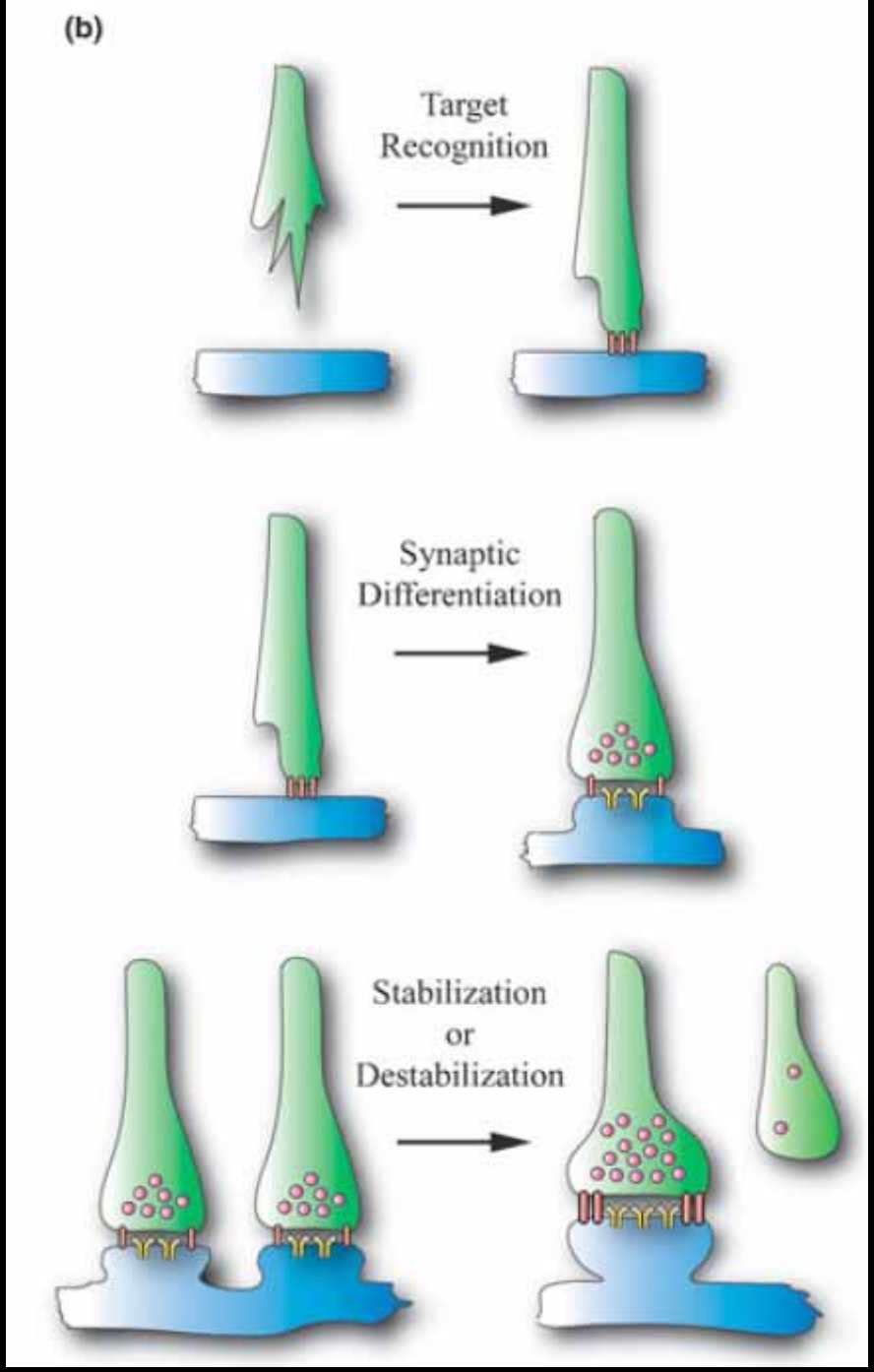


Fig. 1 (a) Anatomy of a synapse. The presynaptic terminal contains multiple active zones (AZ) that contain densely packed synaptic vesicles and are flanked by VGCCs. The active zone is the site of neurotransmitter release, depicted here by the release of a neurotransmitter into the synaptic cleft. In addition to synaptic vesicles clustering at active zones, several synaptic vesicle pools (SVP) accumulate elsewhere in the presynaptic terminal during presynaptic differentiation. The number and type of synaptic vesicle pools depends on the type of synapse. Across the synaptic cleft, the postsynaptic apparatus contains postsynaptic densities (PSD) precisely apposed to the presynaptic active zones. Postsynaptic densities contain clustered neurotransmitter receptors stabilized by synaptic scaffolding molecules, such as PSD-95 and gephyrin. (b) Three steps of synapse formation (see the text for details).

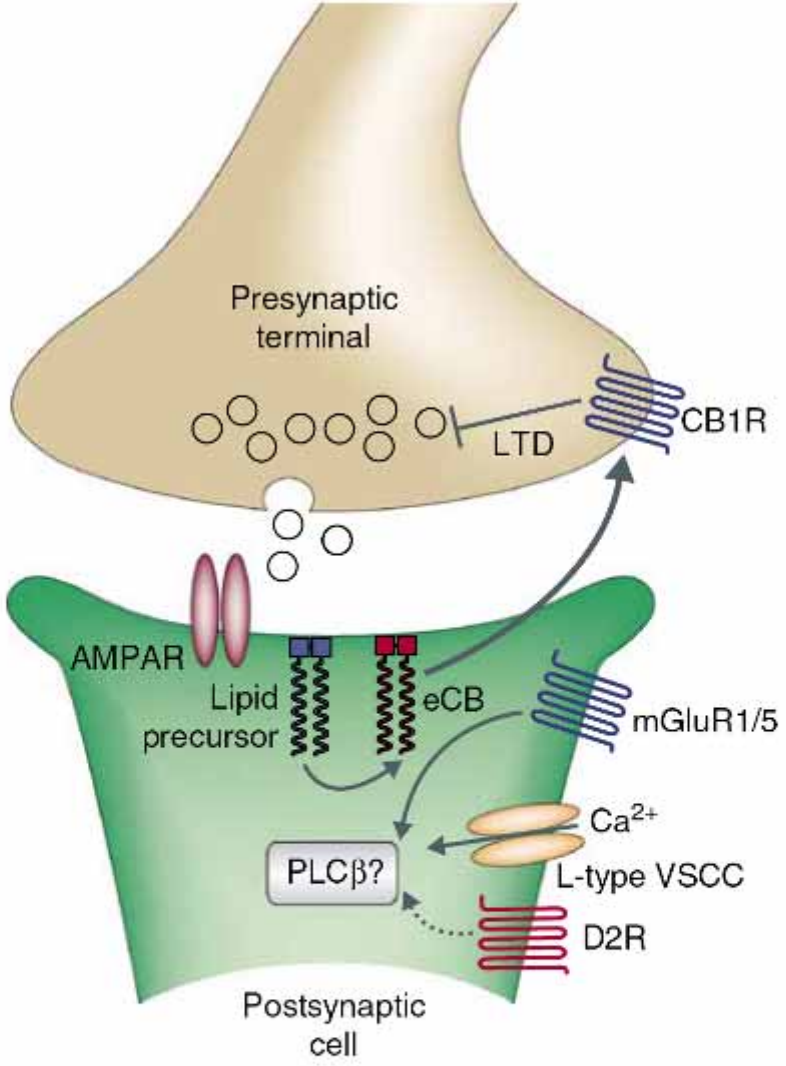


Figure 3 Model of eCB-LTD at excitatory synapses onto medium spiny neurons in the striatum. Activation of postsynaptic type I mGluRs, along with coincident subthreshold depolarization of medium spiny neurons sufficient to activate L-type voltage-sensitive calcium channels (VSCCs), stimulates the postsynaptic synthesis and release of endocannabinoids. What enzyme generates the endocannabinoids is not known; one candidate is PLCβ. Co-activation of postsynaptic dopamine D2-type receptors (D2R) enhances endocannabinoid production and the subse-

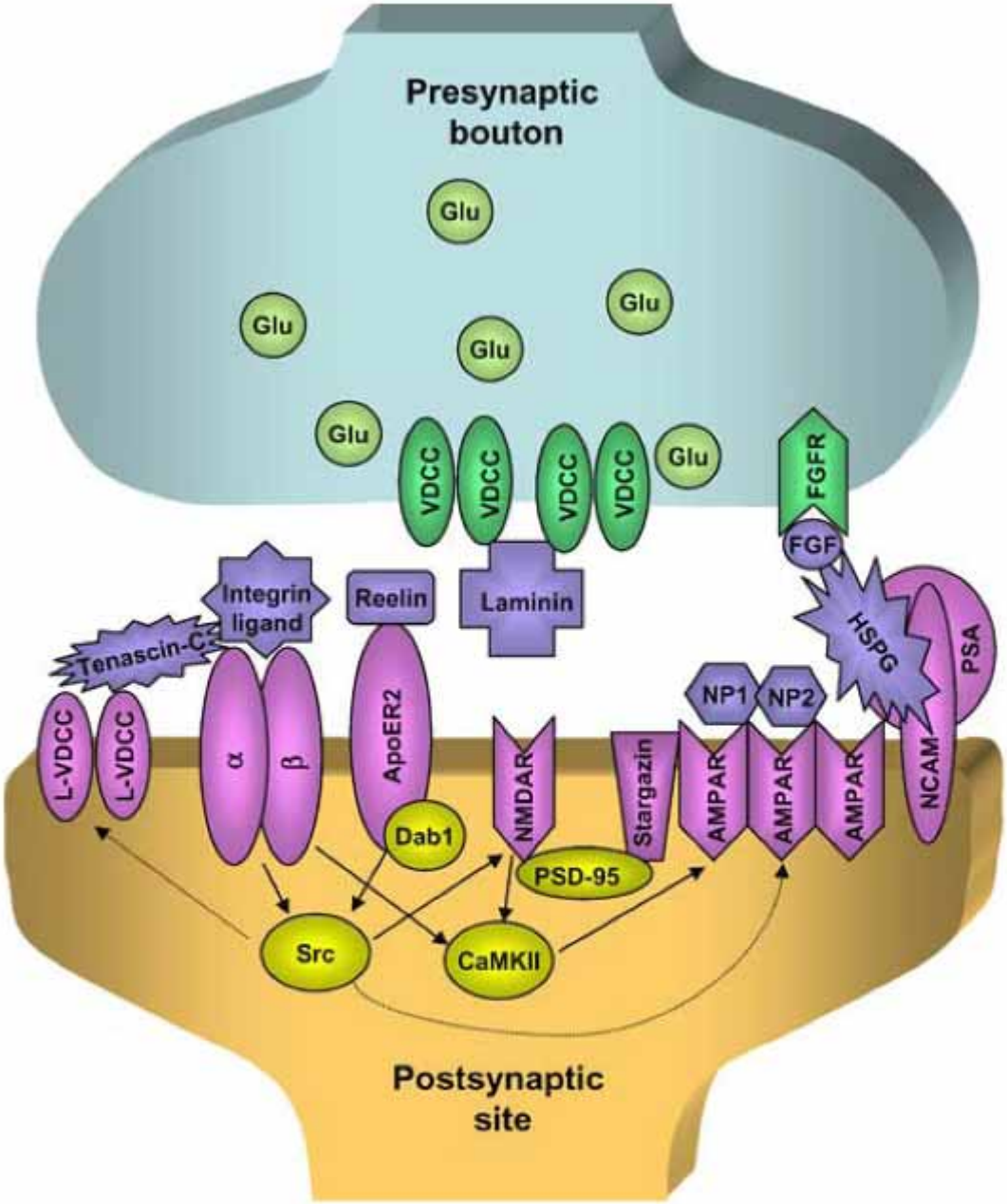
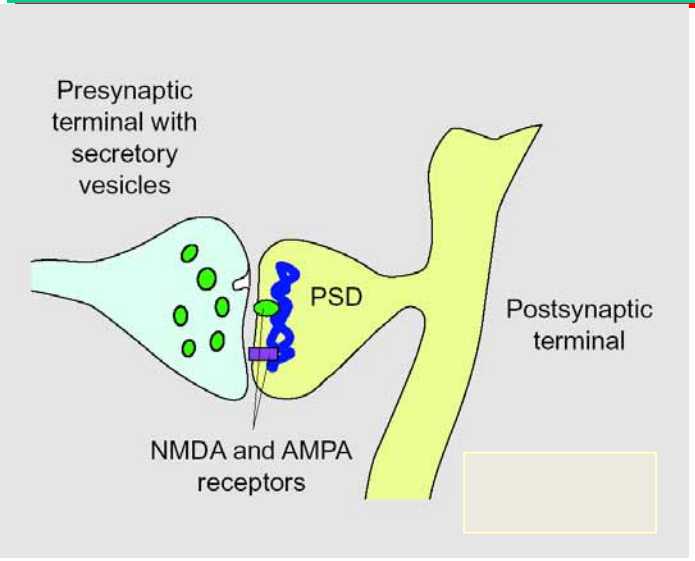
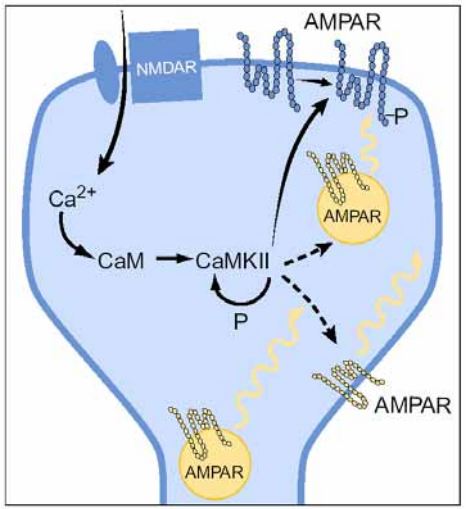
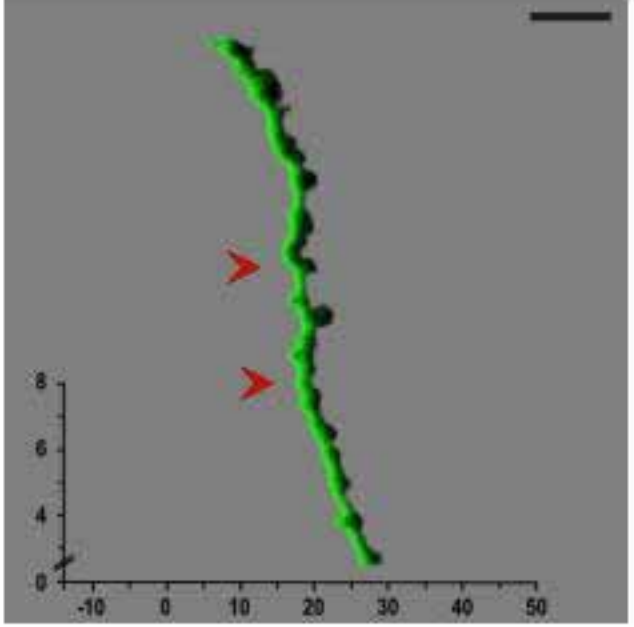


Fig. 1 Summary of mechanisms by which ECM molecules may shape synaptogenesis and synaptic plasticity. Neuronal pentraxins *NP1* and *NP2* interact with each other and form a scaffold in the extracellular

Ca^{2+}-induced modulation

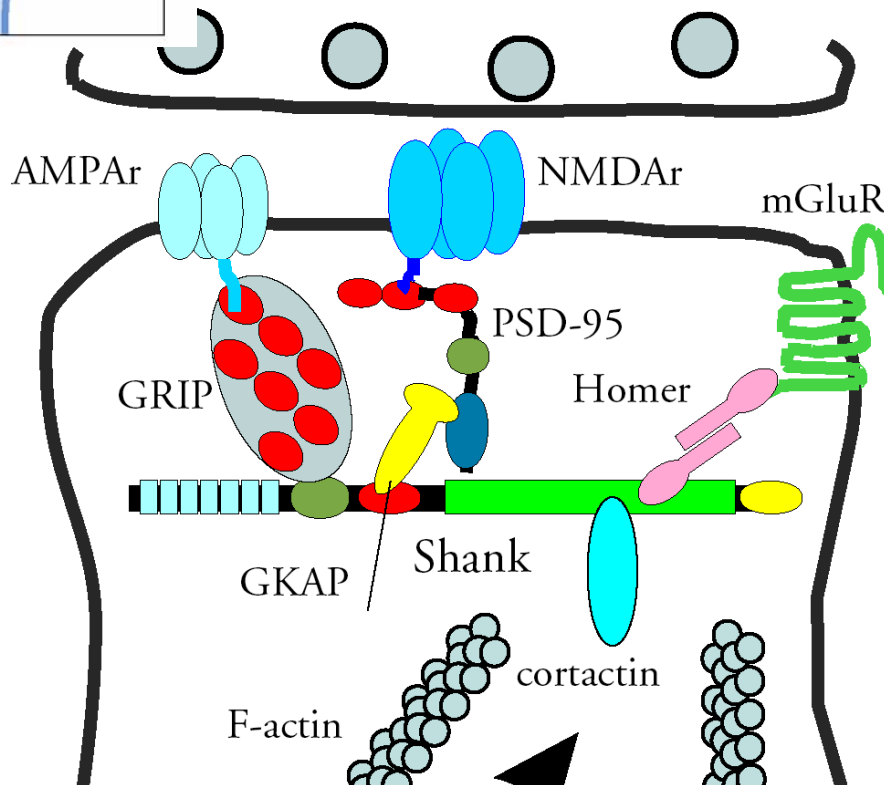






- Movement of spines

- Formation of specialized synaptic structures



Actin

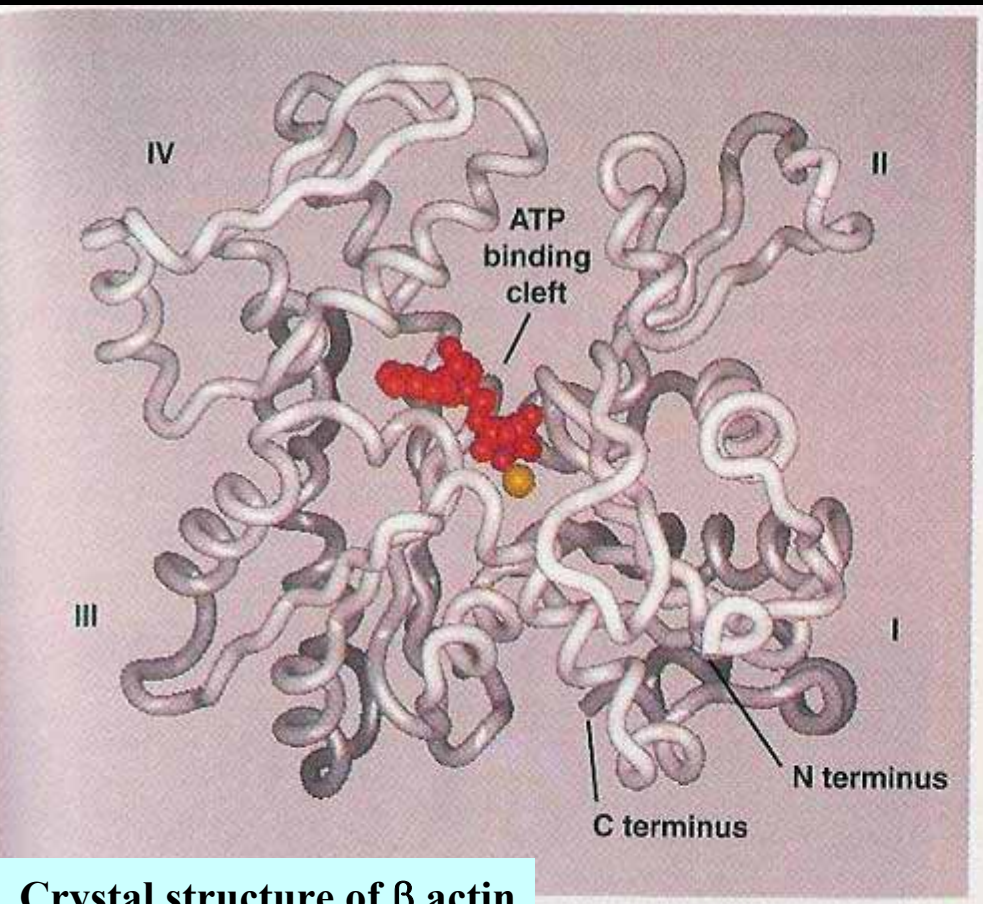
-The most abundant protein (10% in muscle cells & 1-5% in non-muscle

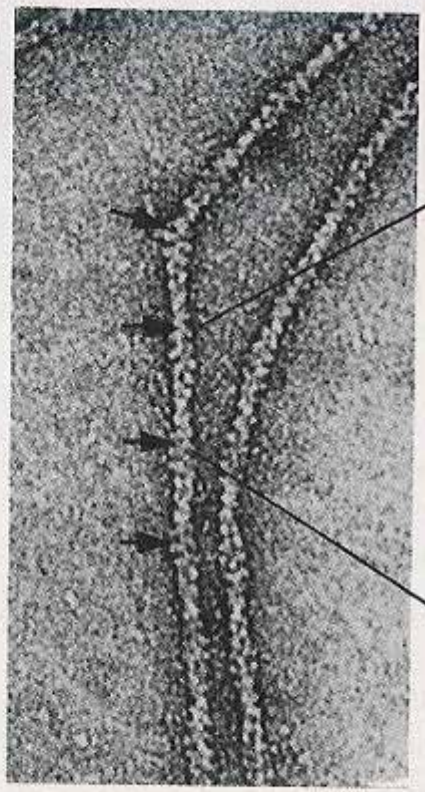
- 375 amino acids protein

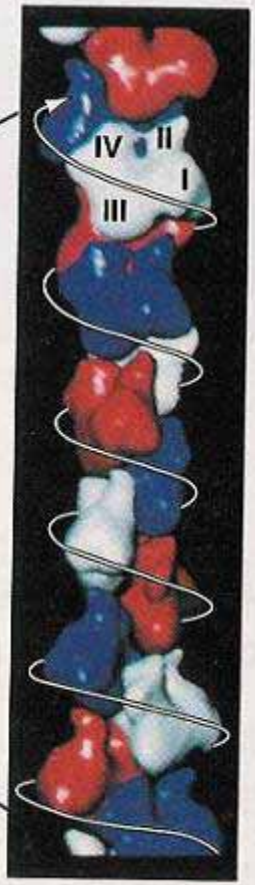
- 6 types of actin in mammals: 3 – α actins, β and γ. Neuronal is mainly β

– Exist in two main forms: G-actin- globuler and F-actin – filaments

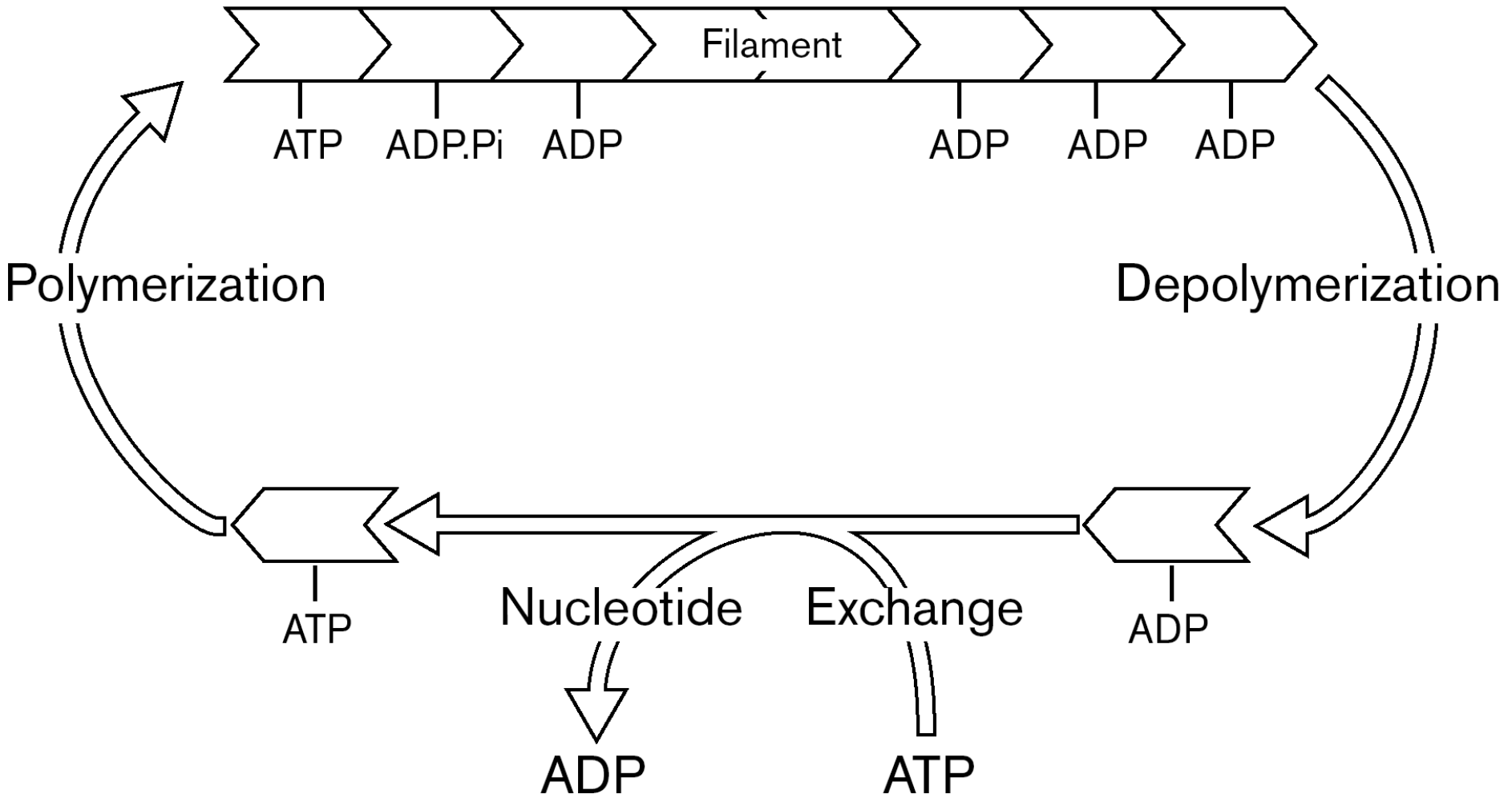
- Each actin contains MgATP or MgADP

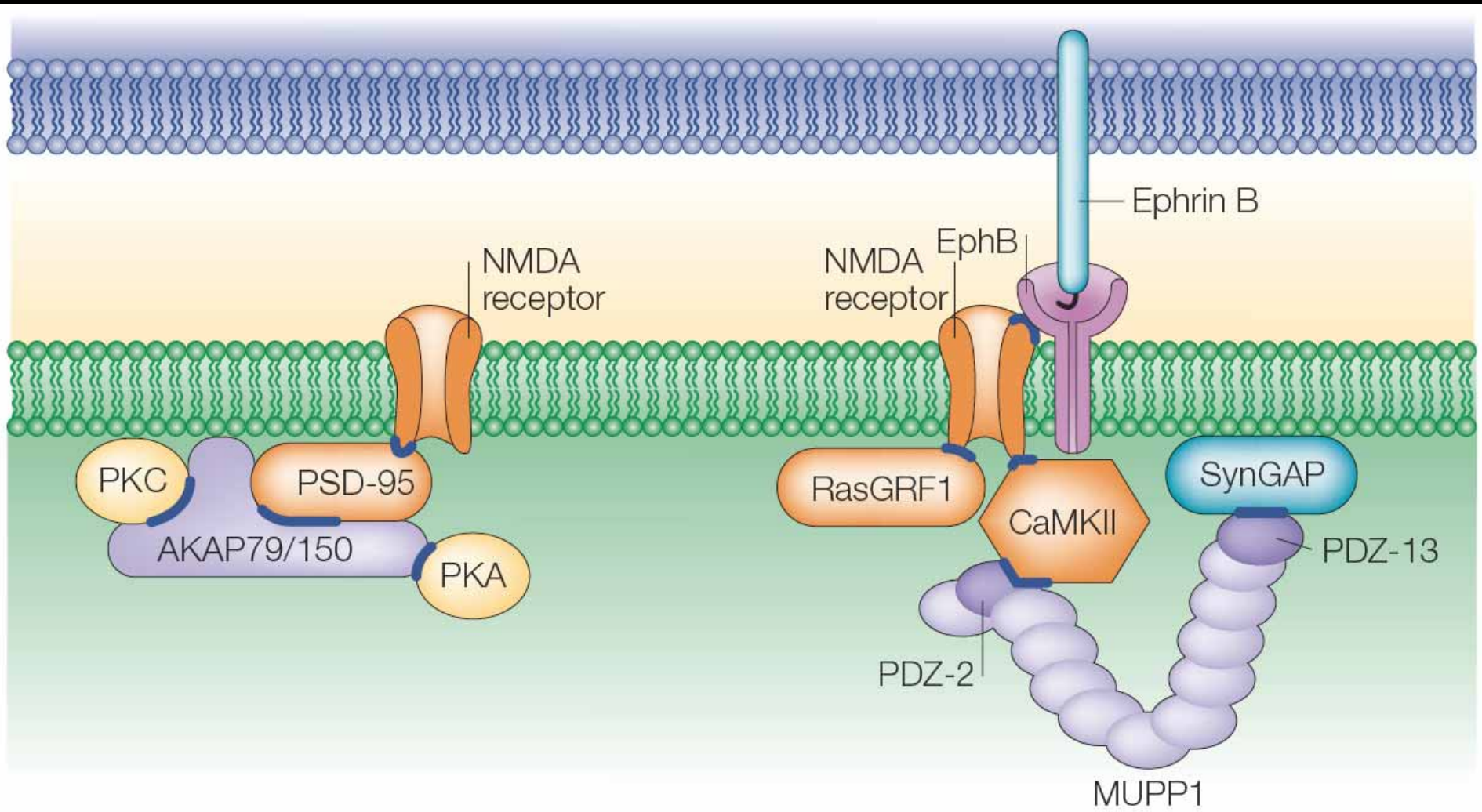






Crystal structure of β actin

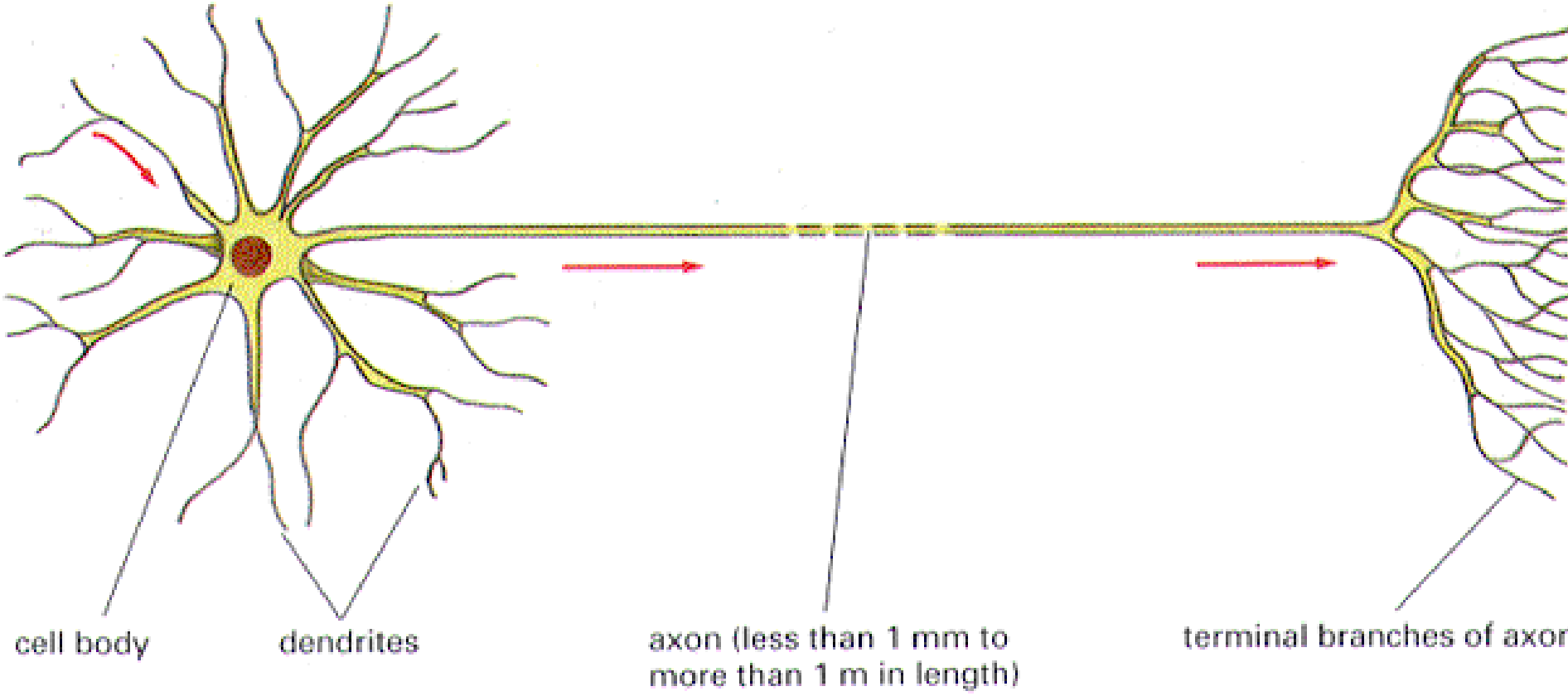

Filament
ATP
ADP.Pi
ADP
ADP
ADP
ADP
Polymerization
Depolymerization
ATP
Nucleotide
Exchange
ADP
ADP
ATP


Ephrin B
EphB
NMDA receptor
NMDA receptor
PKC
PSD-95
AKAP79/150
PKA
RasGRF1
CaMKII
SynGAP
PDZ-13
PDZ-2
MUPP1

Neuron - key cell of nervous system

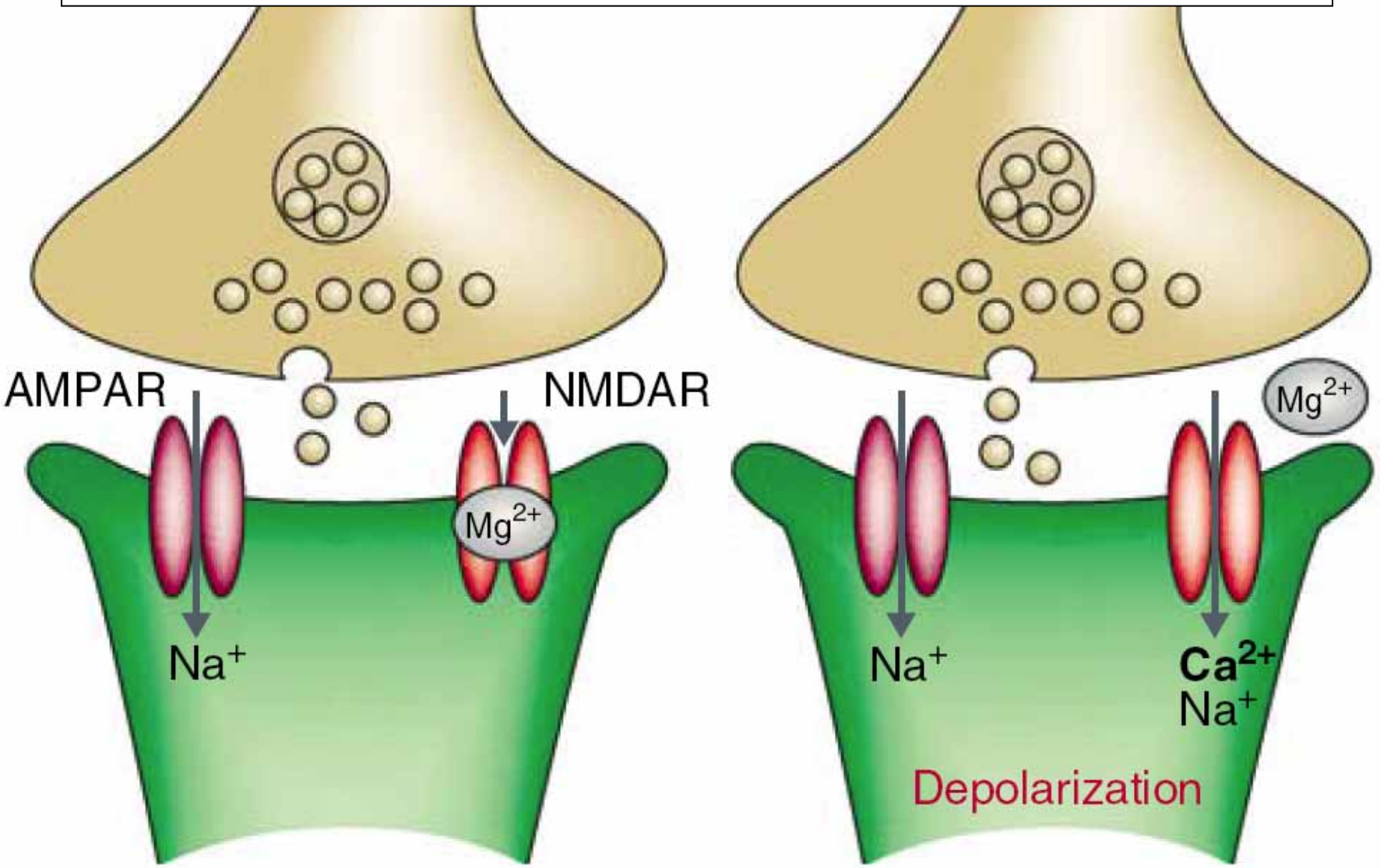
10^{11} neurons

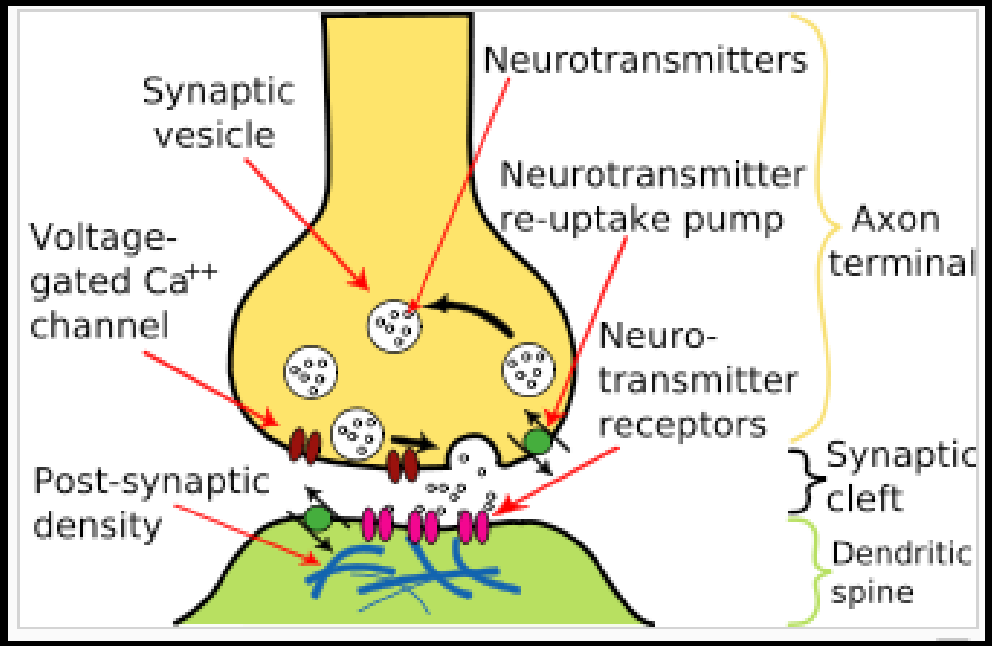
10^{15} synapses

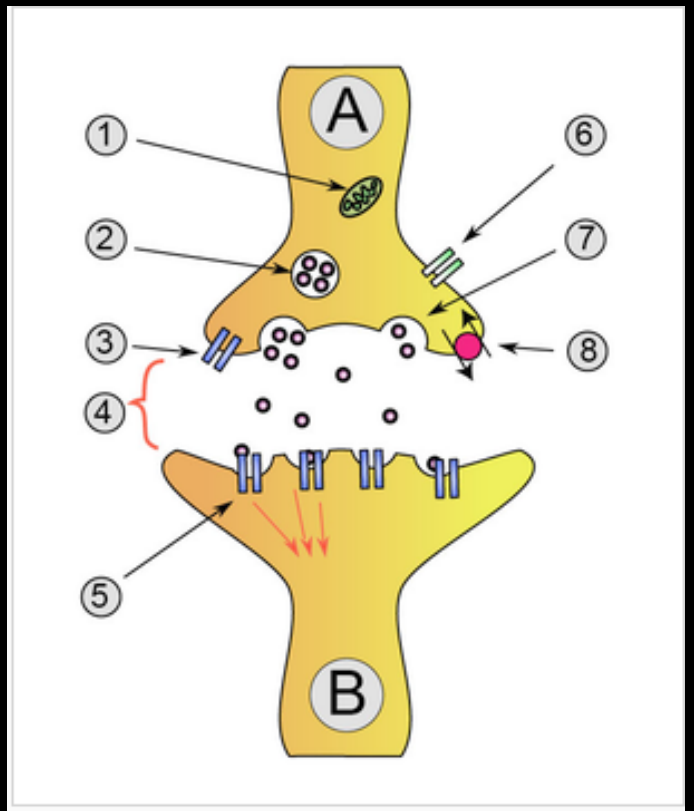


Channels are selectively permeable

- Example: potassium channel
  - K^+ ion (1.33 Å) vs. Na^+ ion (0.95 Å)
  - ratio of permeabilities is 10,000
  - 10^8 ions/second

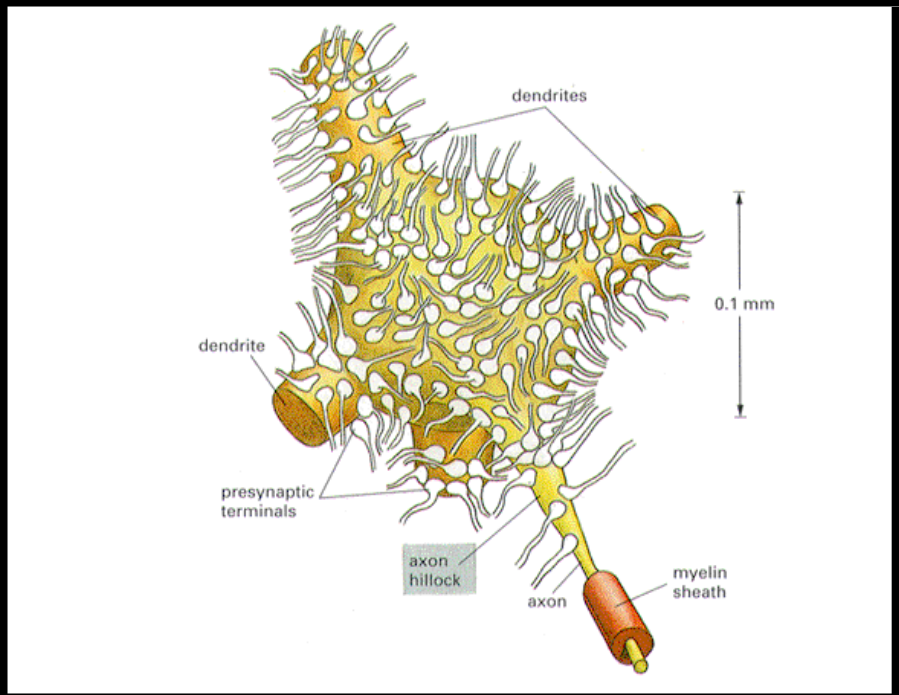

AMPAR
NMDAR
Mg2+
Na+
Mg2+
Na+
Ca2+
Na+
Depolarization



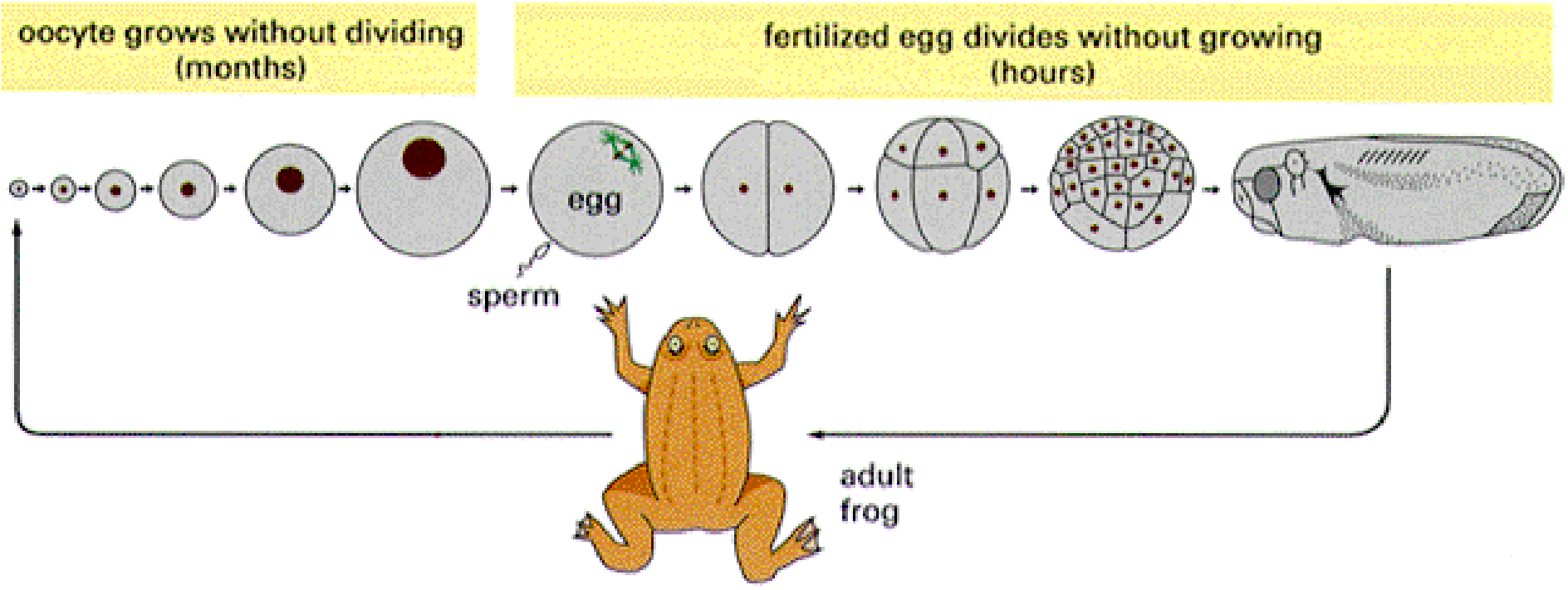


Neuron A (transmitting) to neuron B (receiving)

1. Mitochondria
2. **synaptic vesicle** with neurotransmitters
3. Autoreceptor
4. Synapse with neurotransmitter released (serotonin)
5. Postsynaptic receptors activated by neurotransmitter (induction of a postsynaptic potential)
6. Calcium channel
7. Exocytosis of a vesicle
8. Recaptured neurotransmitter



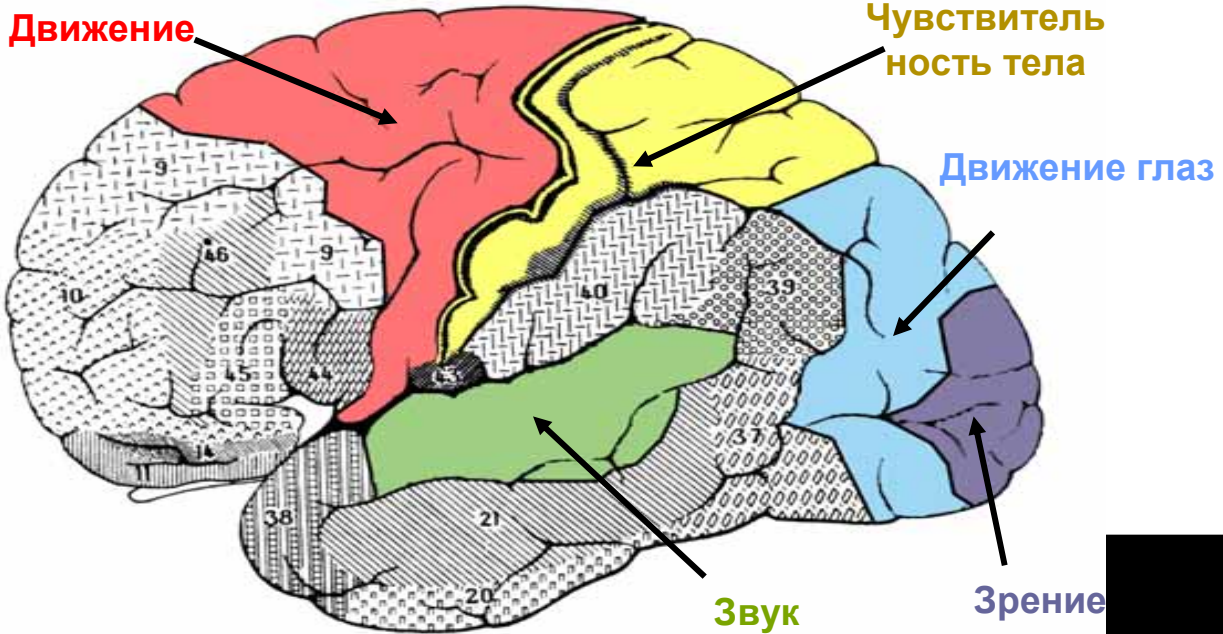
Main stages of development

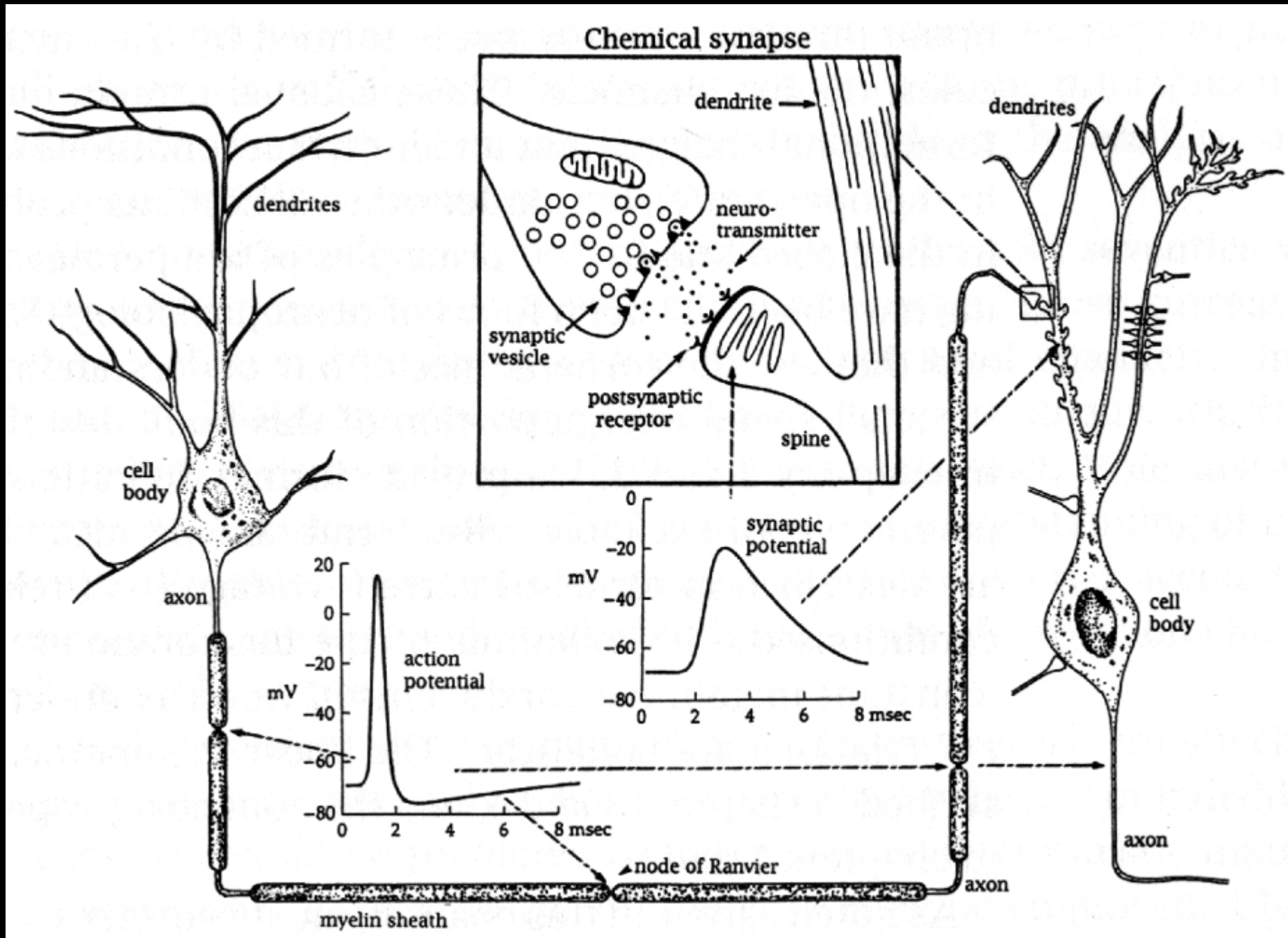


Основные чувства и органы чувств








Chemical synapse
dendrite
neuro-
transmitter
synaptic
vesicle
postsynaptic
receptor
spine
dendrites
cell
body
axon
myelin sheath
node of Ranvier
axon
dendrites
cell
body
axon
synaptic
potential
mV
0
-20
-40
-60
-80
0
2
4
6
8 msec
action
potential
mV
20
0
-20
-40
-60
-80
0
2
4
6
8 msec

Эксперимент аспиранта Турпаева (1946), ставший отправной точкой для цикла работ по холинорецептору

Турпаев-1946

ждение свободных сульфгидрильных групп путем внесения цистеина полностью восстанавливает исчезнувший эффект (рис.13). Конечно, этой способностью не обладает испытанный нами цистин.

Уже этот опыт с достаточной ясностью показывает, что нервное воздействие, а также воздействие тех химических агентов, которые в качестве специфических продуктов обмена

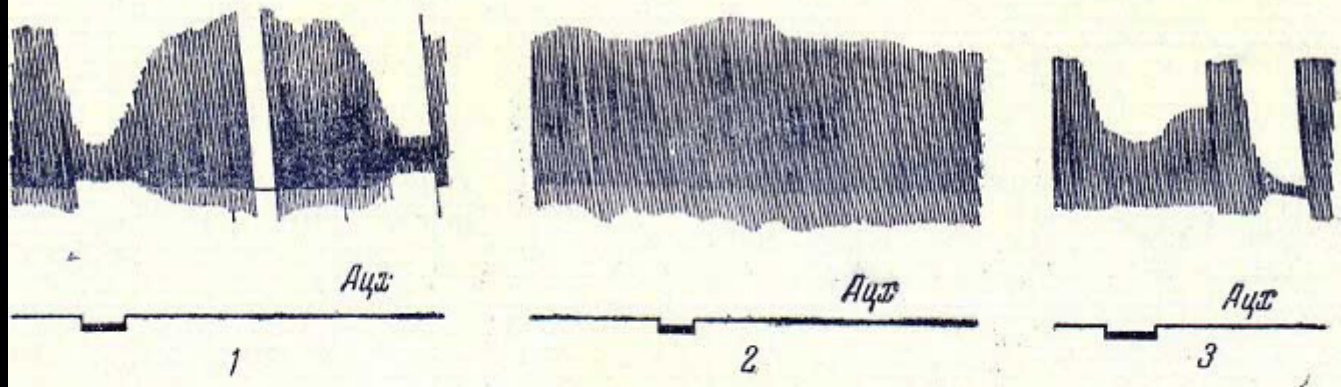


Рис. 13.

1 — действие раздражения блуждающего нерва (и ацетилхолина для сравнения) на сокращения миокарда желудочка сердца лягушки в норме; *2* — после связывания сульфгидрильных групп сулемой ($5 \cdot 10^{-5}$); *3* — после действия цистеина ($5 \cdot 10^{-4}$)

Сулема - $HgCl_2$
- дихлорид ртути

gift of D.A. Sakharov

Холинорецептор - белок

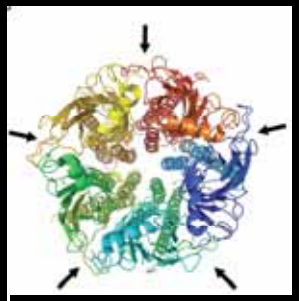
Хачатур Седракович Коштоянц

Letters to Editor

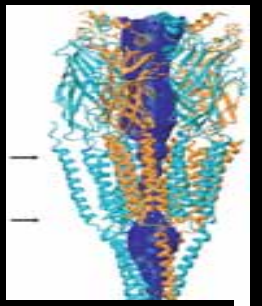
nature 158, 837-838 (07 December 1946) | doi:10.1038/158837b0

Role of Sulphydril Groups in the Action of Acetylcholine and Inhibition of the Vagus Nerve

Ch. S. KOSCHTOJANZ & T. M. TURPAJEW



Some key events in AChR's life



1946 - evidences that AChR is a protein

T.M. Turpaev & C.S. Koschtojanz

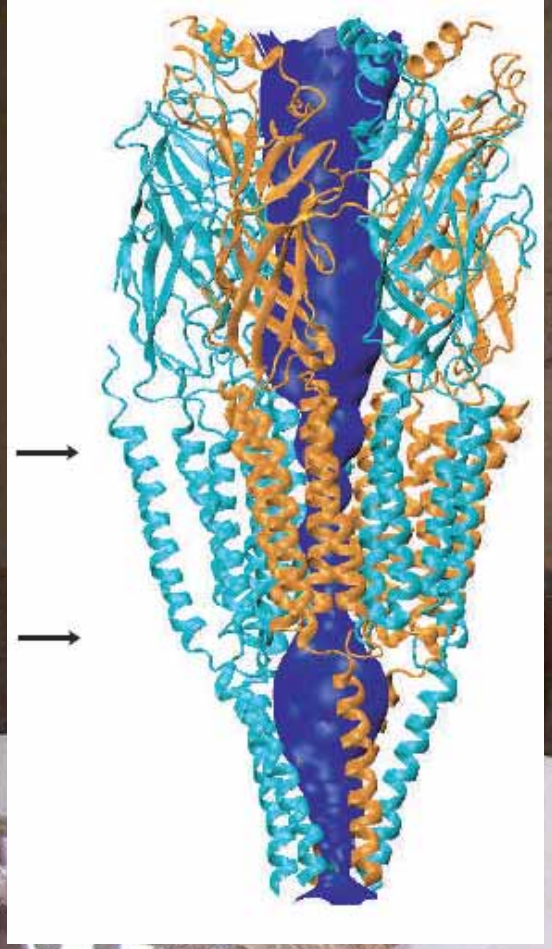
1958-60-th - Homogenate of AChR Turpaev & co-authors

АХ смещает кривую меркуриметрического титрования SH- групп в тканевом гомогенате

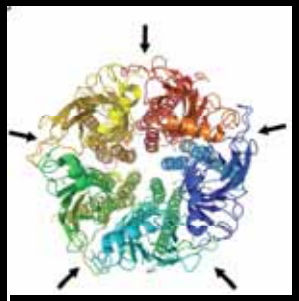
1972 - purification of nAChR from Torpedo using affinity chromatography J-P. Changeux & co-authors

1974 - AChR - pentameric protein A. Karlin & co-authors

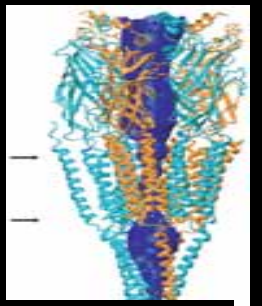
1979 - first 20 amino acids of AChR sequence from *T.Marmorata* J-P. Changeux & co-authors



J-P Chanqeux - Storogevsky monastery Oct-2008



Some key events in AChR's life



1946 - evidences that AChR is a protein

T.M. Turpaev & C.S. Koschtojanz

1960-th - isolation of AChR T.M. Turpaev & co-authors

АХ смещает кривую меркуриметрического титрования SH- групп в тканевом гомогенате

1972 - purification of AChR from Torpedo using affinity chromatography J-P. Changeux & co-authors

1974 - AChR - pentameric protein A. Karlin & co-authors

1979 - first 20 amino acids of AChR sequence from *T.Marmorata* J-P. Changeux & co-authors

1983 - complete sequence of AChR from *T.Californica*

S. Numa & co-authors

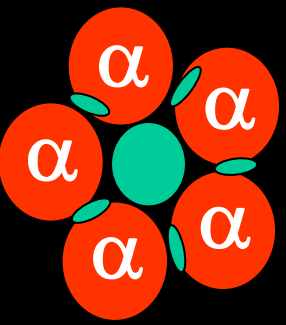
Cys-Loop ligand-gated channels

Cation-selective

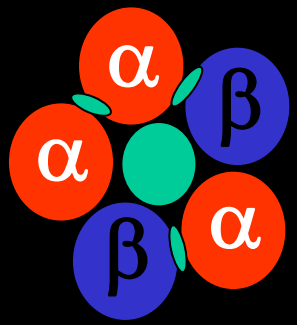
- Acetylcholine
- Serotonine (5-HT)

Anion-selective

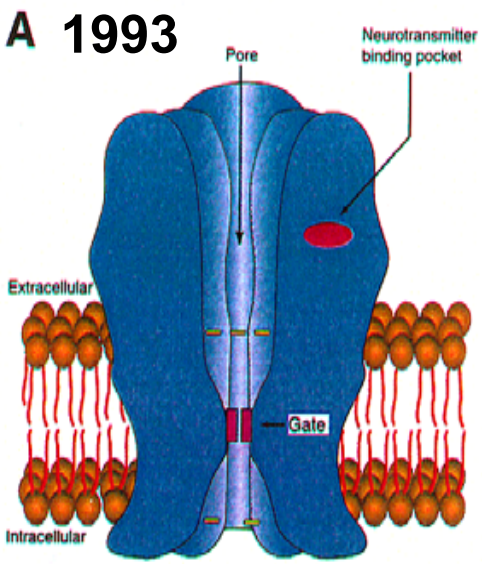
- GABA
- Glycine



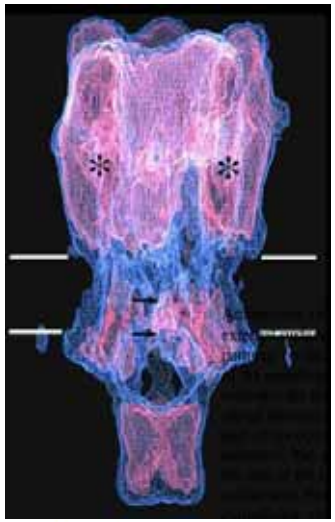
homo-

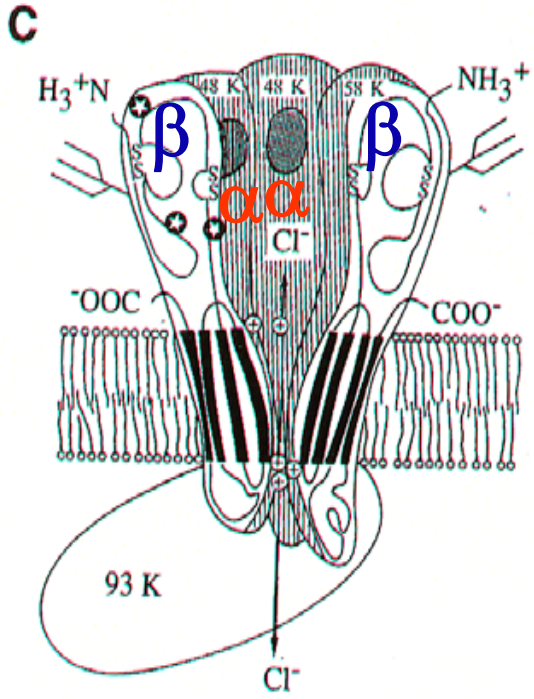


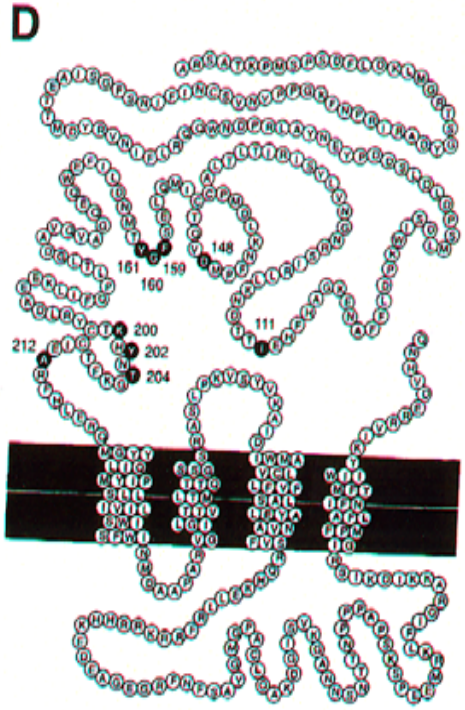
hetero-oligomeric



B 1999







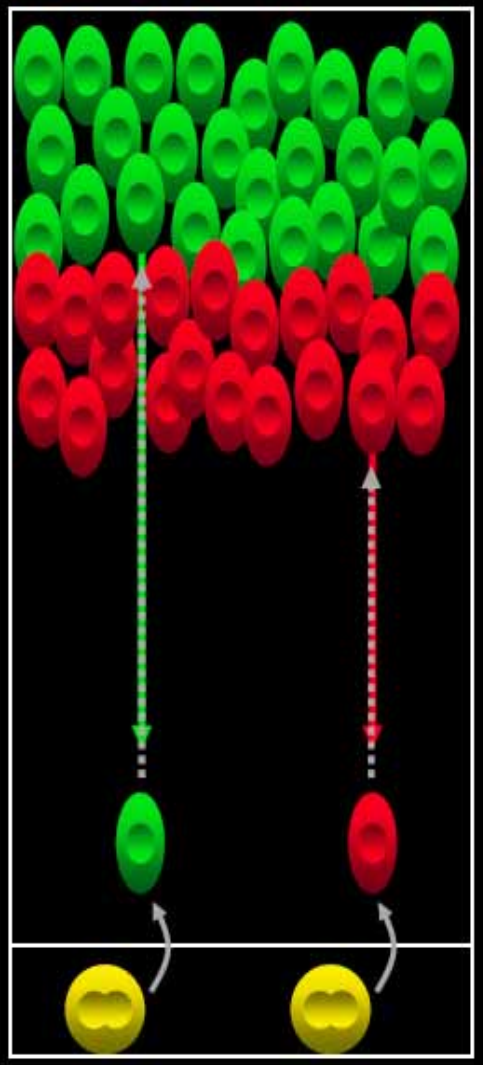
Glycine Receptor

GlyR α subunit

Ligand-gated 4-TM channels:

- What we know about:
  - Structure
  - Conformational transitions during activation

Steps in the early development of the nervous system



Neurons

Progenitors

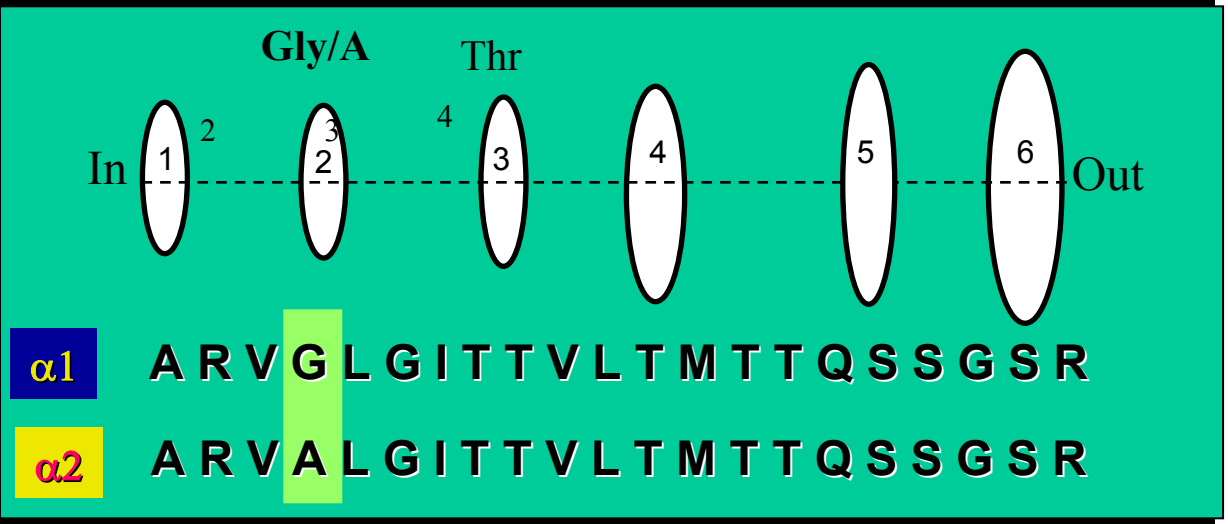
Guidance

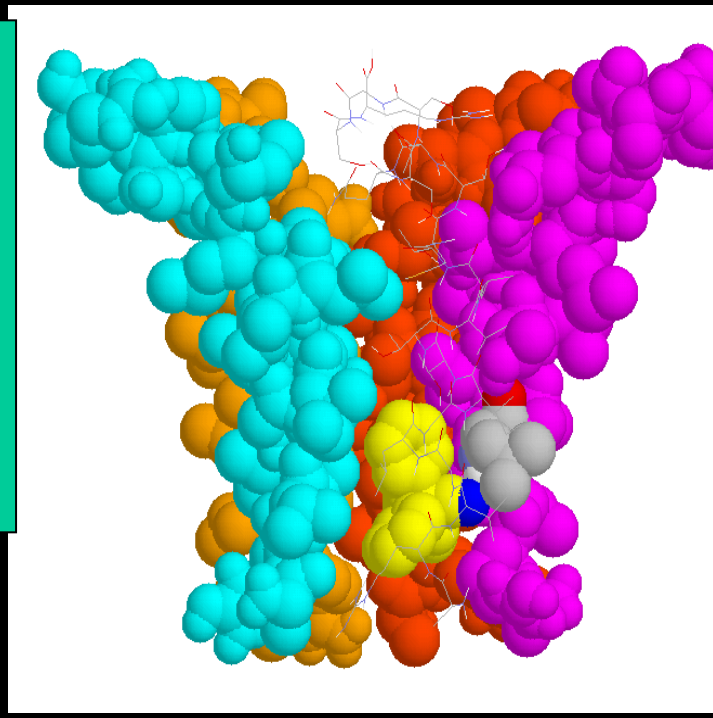
3. Formation of axonal connections

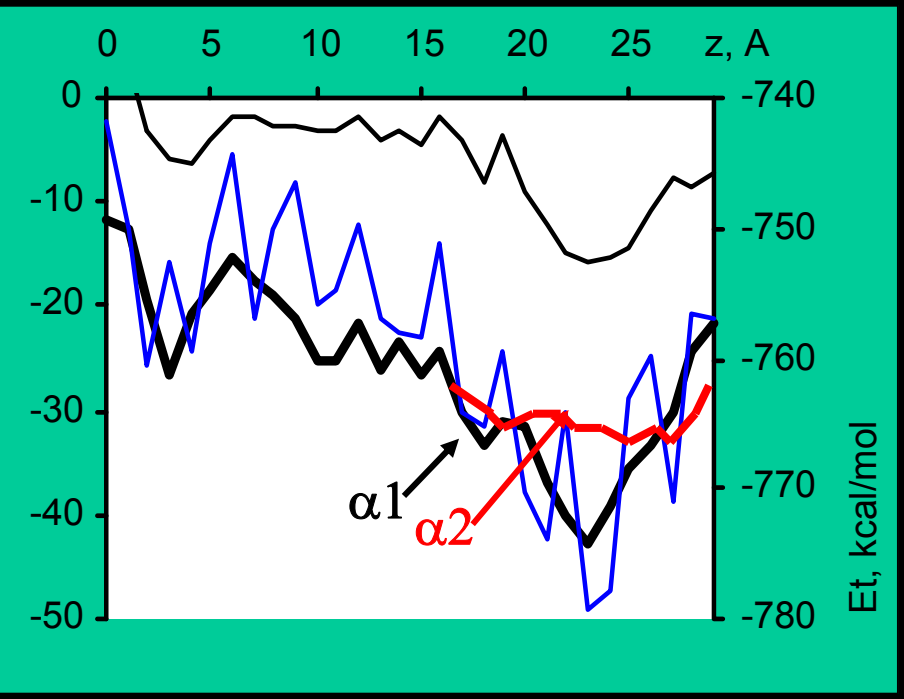
2. Migration

1. Specification

Energy Profile of CTB in a1 and a2 GlyRs

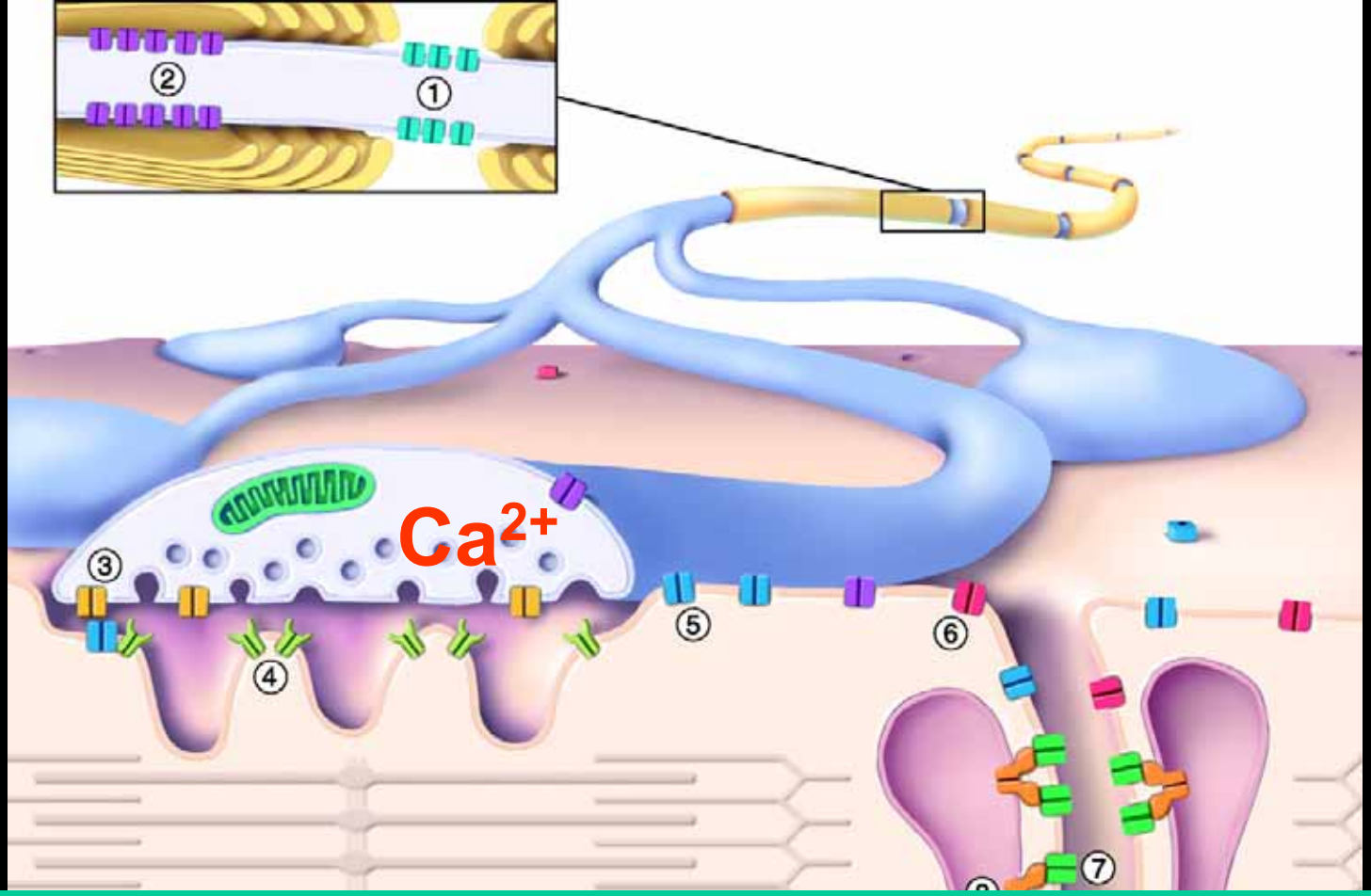






Ligand-receptor energy for a1
Ligand-receptor energy for a2
Electrostatic ligand-receptor energy
Total energy

Zhorov and Bregestovski, Biophys. J., 2000



Calcium - key trigger of neurotransmitter release in chemical synapses

chloride channel

③ Nerve voltage-gated calcium channel

⑦ Transverse tubule voltage-gated

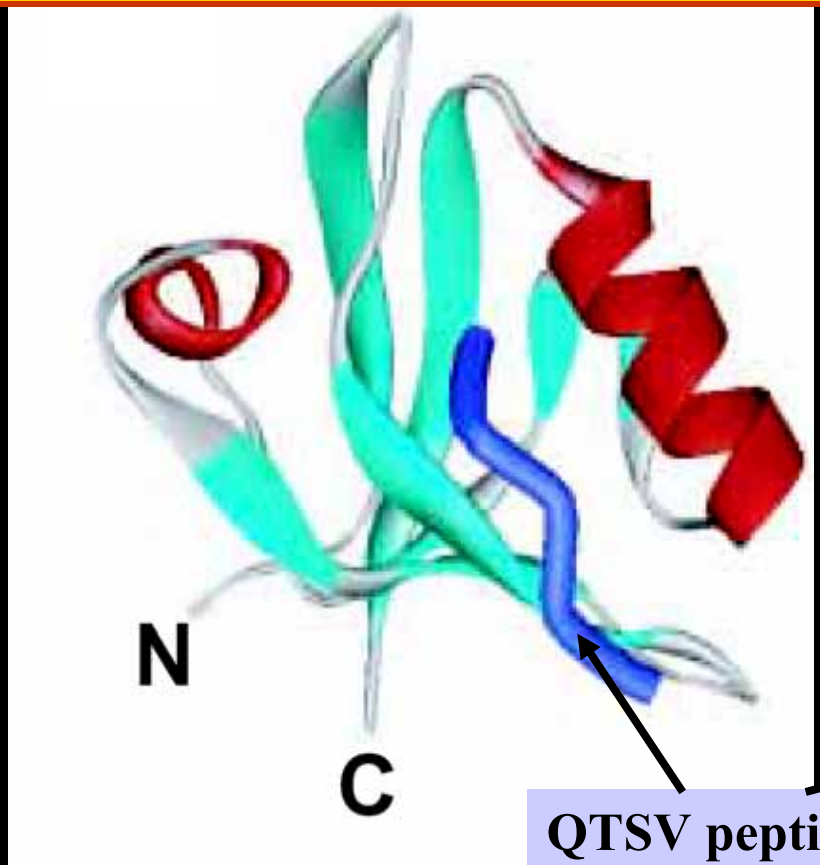
Interaction of peptides with PDZ domains

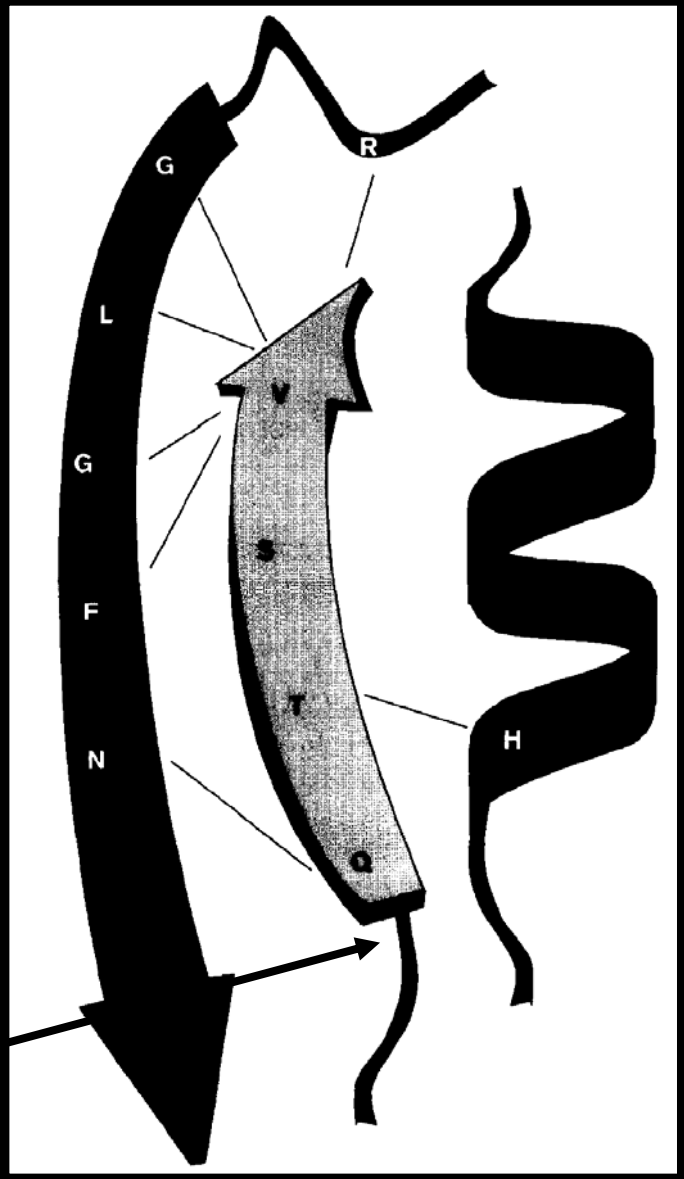
- 90 amino acids

-Six β-strands and two α-helices

PDZ domains- modular protein-interaction domains that are specified for binding a short peptide motifs at the extreme caboxy (C) termini of other proteins.

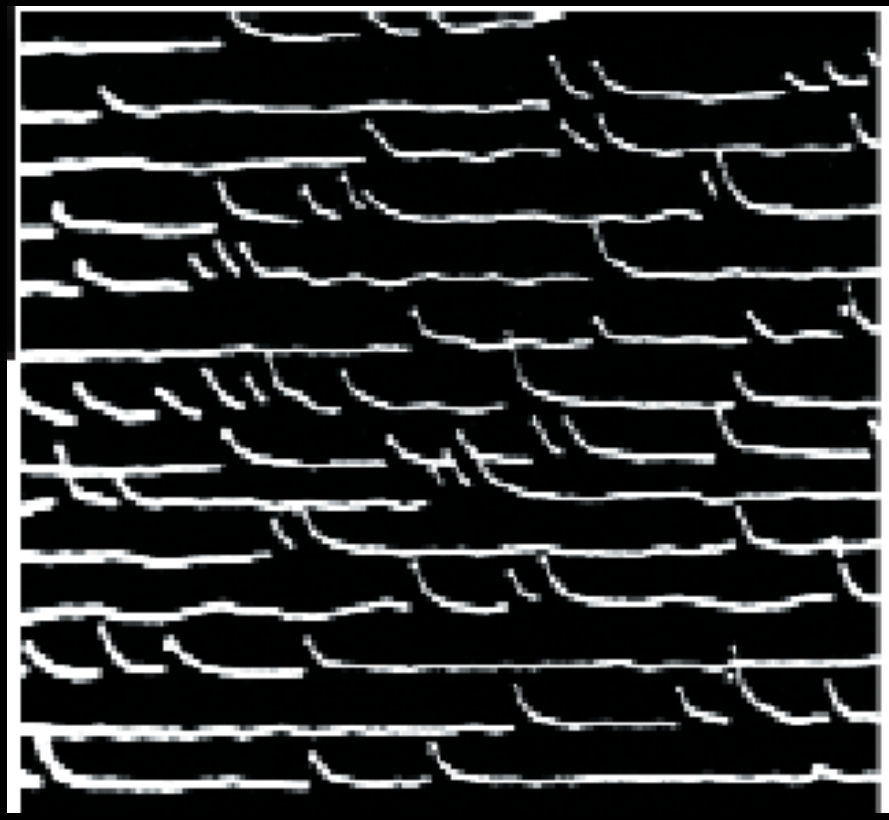
PDZ domains-are located at about 12 nm below postsynaptic membrane.

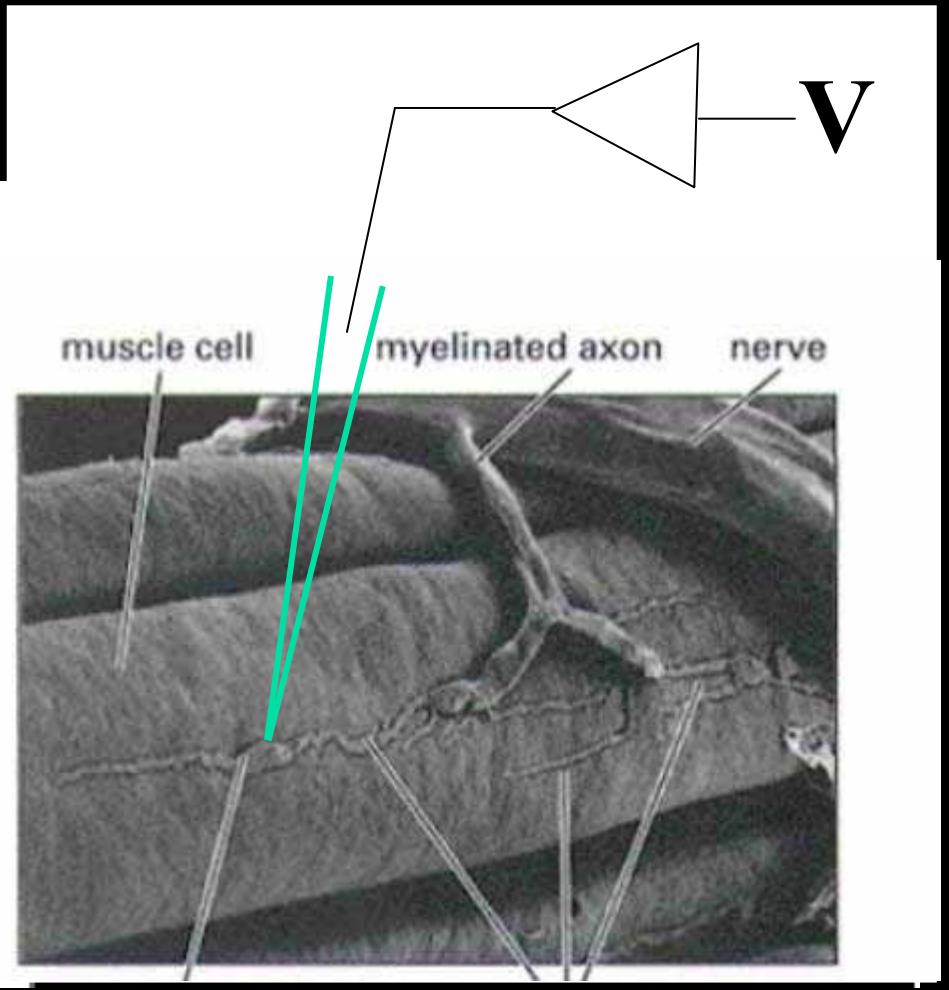




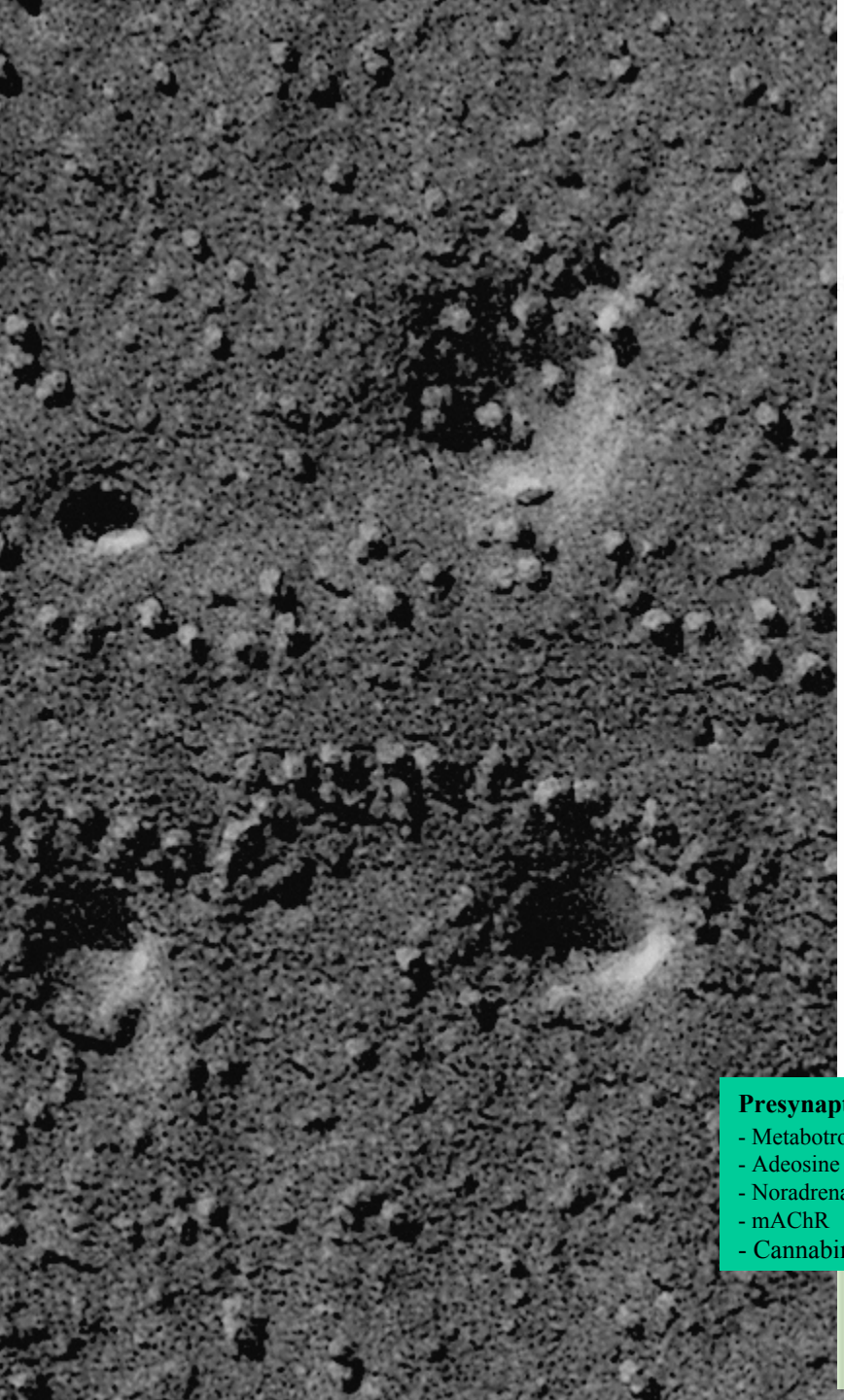
Миниатюрные синаптические потенциалы

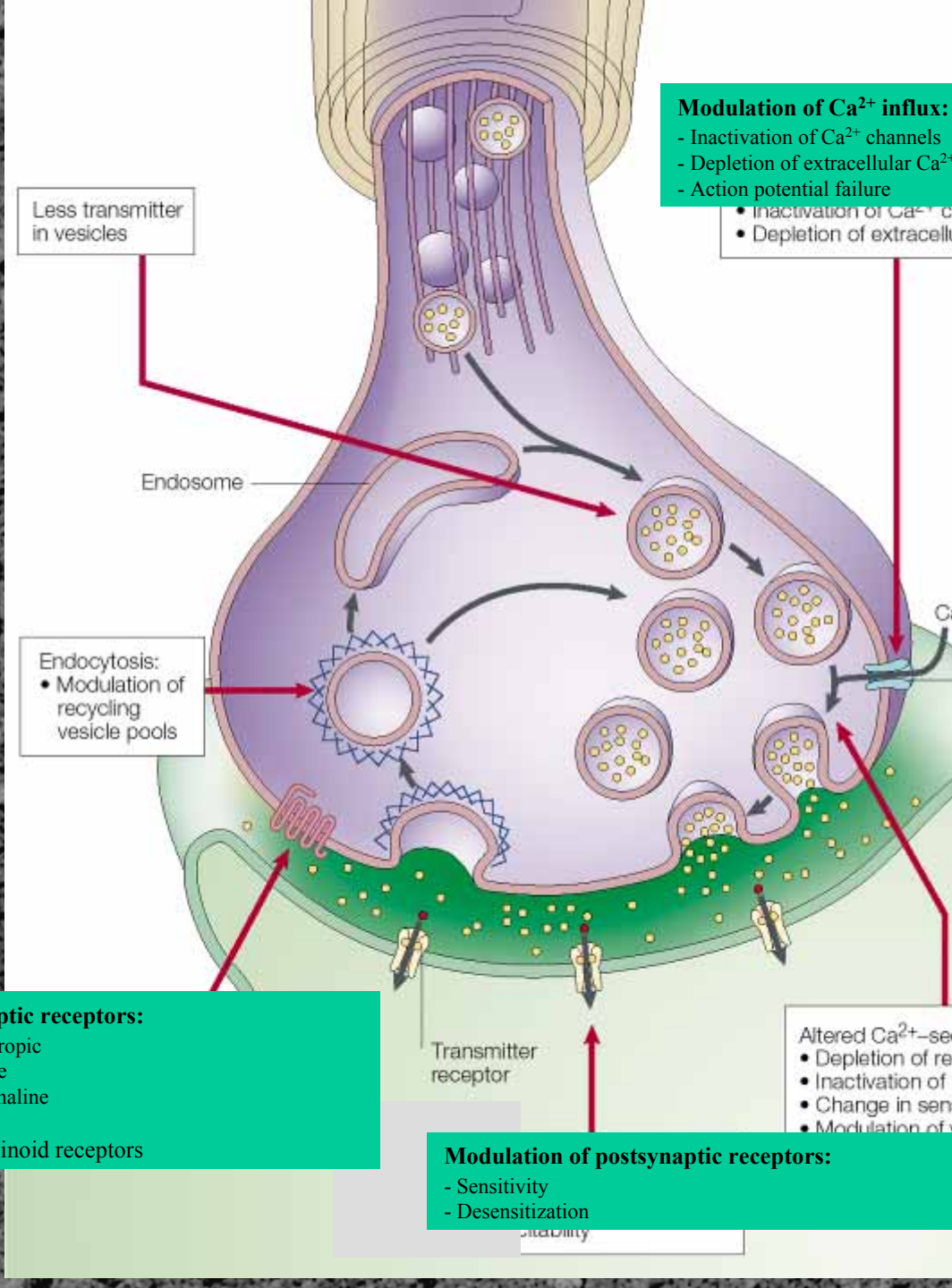
CONTROL



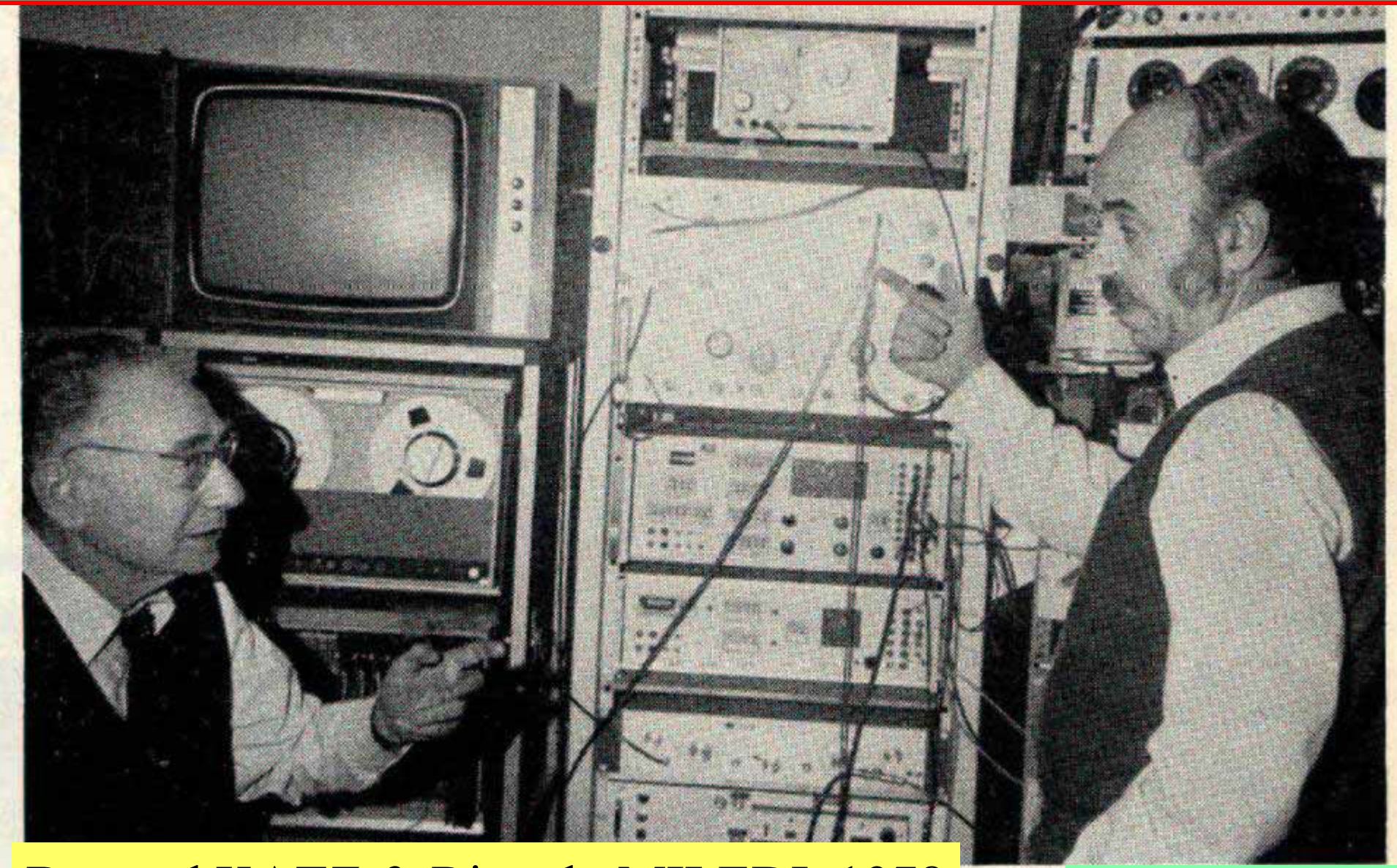


P. Fatt & B. Katz, *J. Physiol.*, 117:109, 1952




Modulation of Ca^{2+} influx:
- Inactivation of Ca^{2+} channels
- Depletion of extracellular Ca^{2+}
- Action potential failure
Less transmitter in vesicles
Endosome
Endocytosis:
• Modulation of recycling vesicle pools
Transmitter receptor
Presynaptic receptors:
- Metabotropic
- Adeosine
- Noradrenaline
- mAChR
- Cannabinoid receptors
Modulation of postsynaptic receptors:
- Sensitivity
- Desensitization

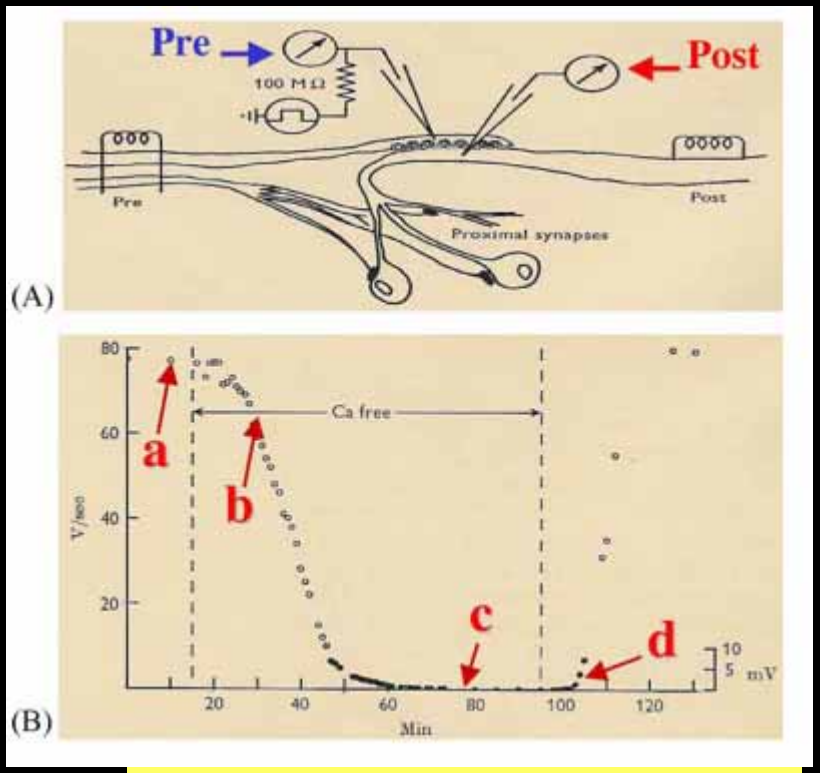
Ca^{2+} - ключевая роль в выбросе нейромедиатора



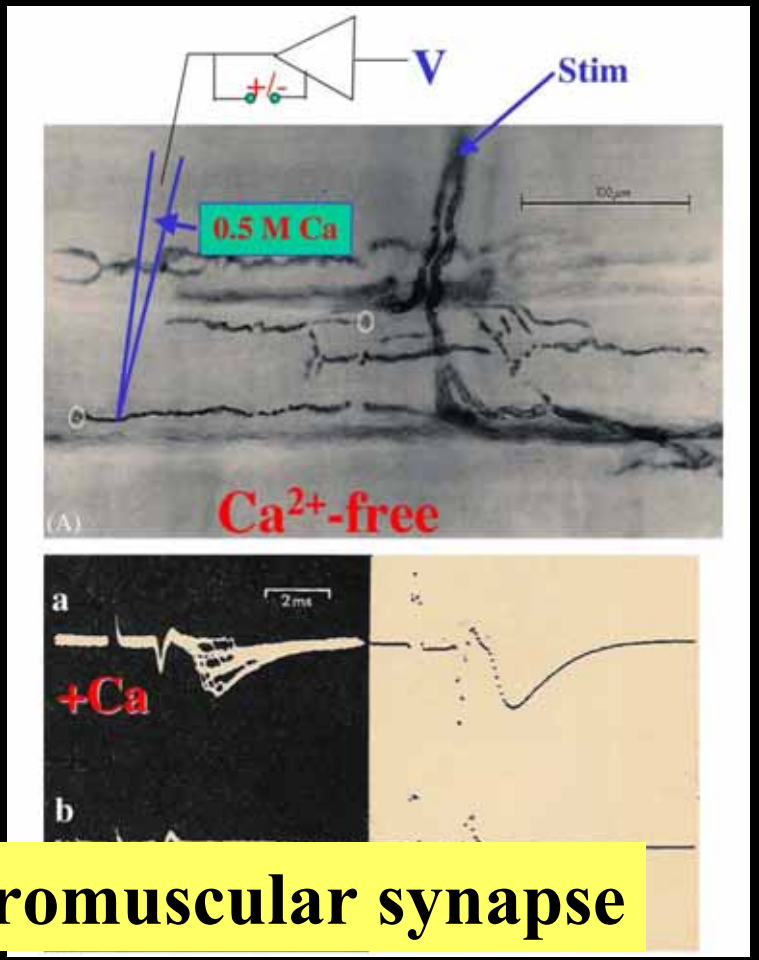
Bernard KATZ & Ricardo MILEDI, 1978

Photo: T. Takahashi

Доказательство ключевой роли Ca^{2+}



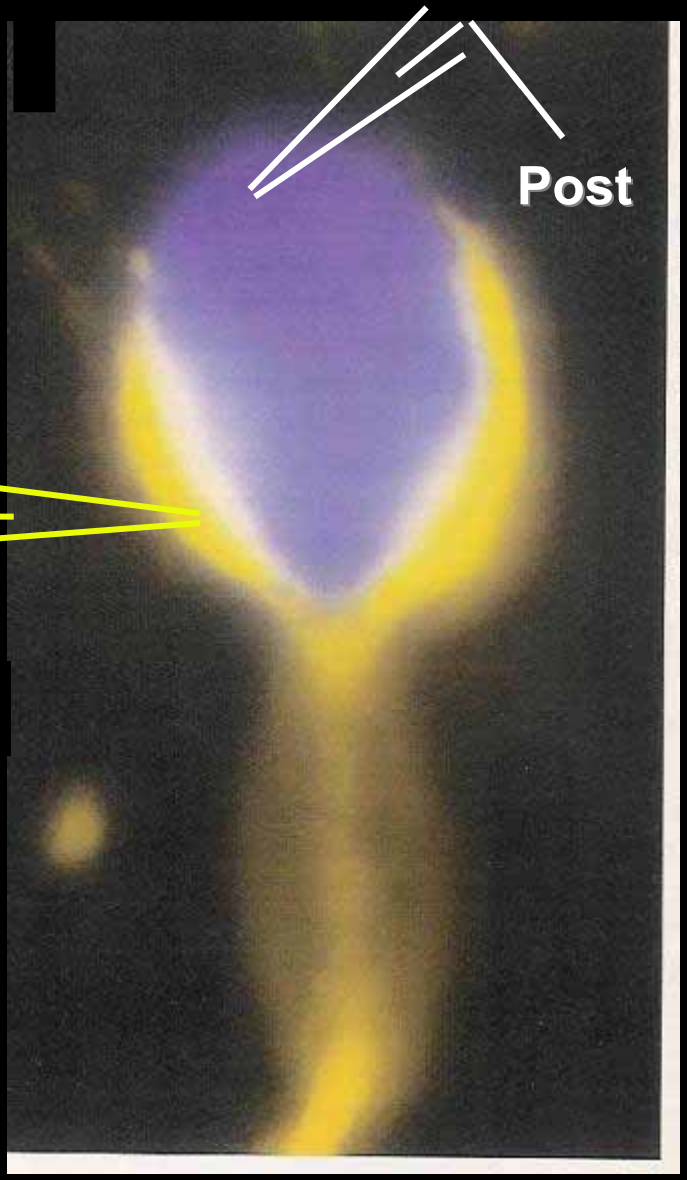
Squid giant synapse

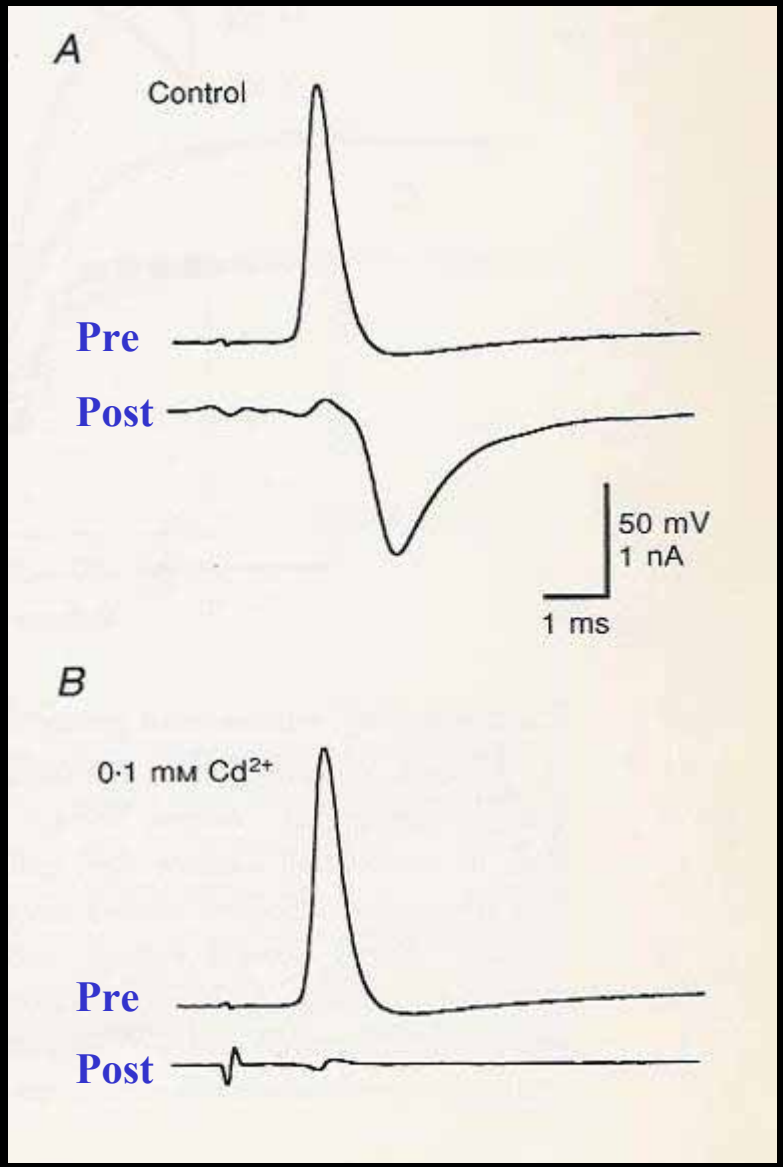


Neuromuscular synapse

Katz & Miledi, *Nature*, 212: 1242-1245, 1966:
"At the squid giant synapse as well as at the neuromuscular junction, external calcium ions are indispensable for synaptic activity"

Calyx of Held – giant mammalian synapse





Borst, Helmehen & Sakmann, *J.Physiol*, 489: 825-840, 1995