O Lado pré-sináptico

- Exocitose e endocitose das vesículas sinápticas.
- Modulação pré-sináptica da neurotransmissão.

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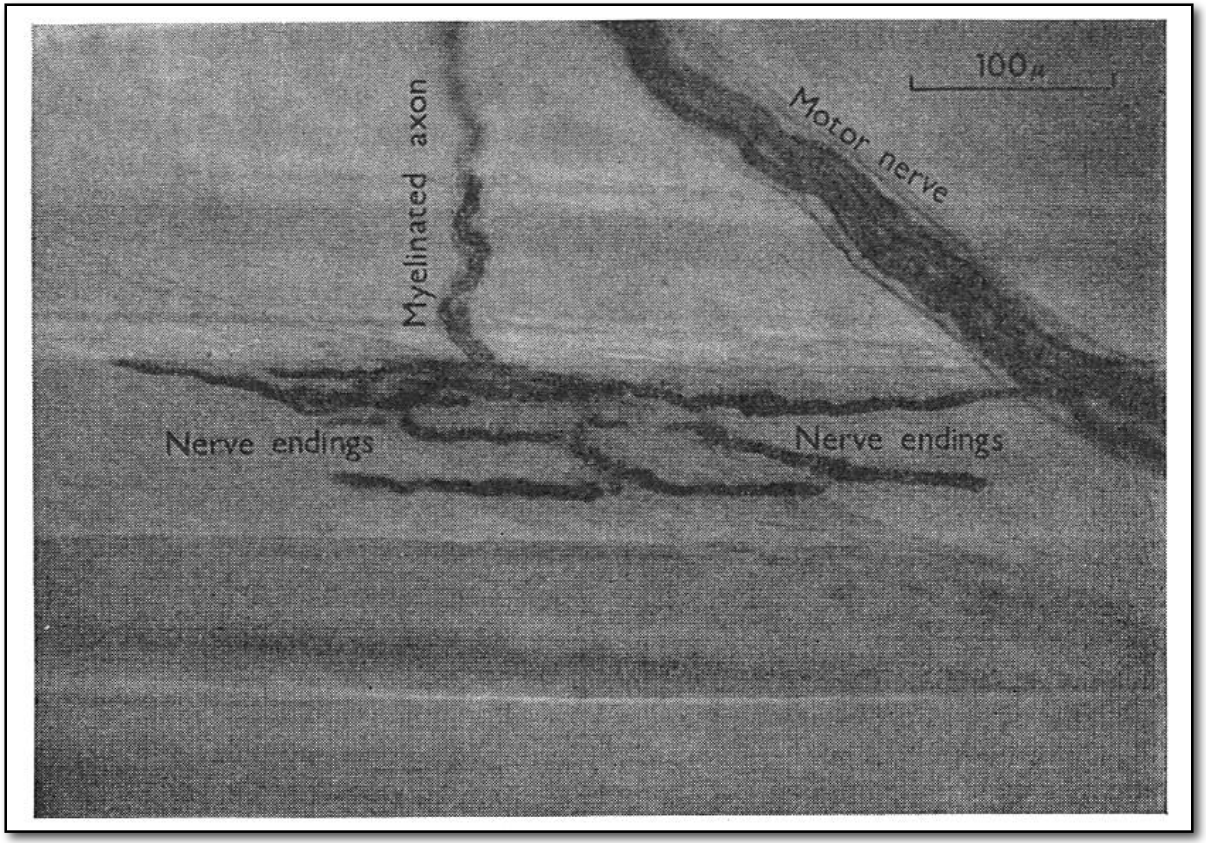
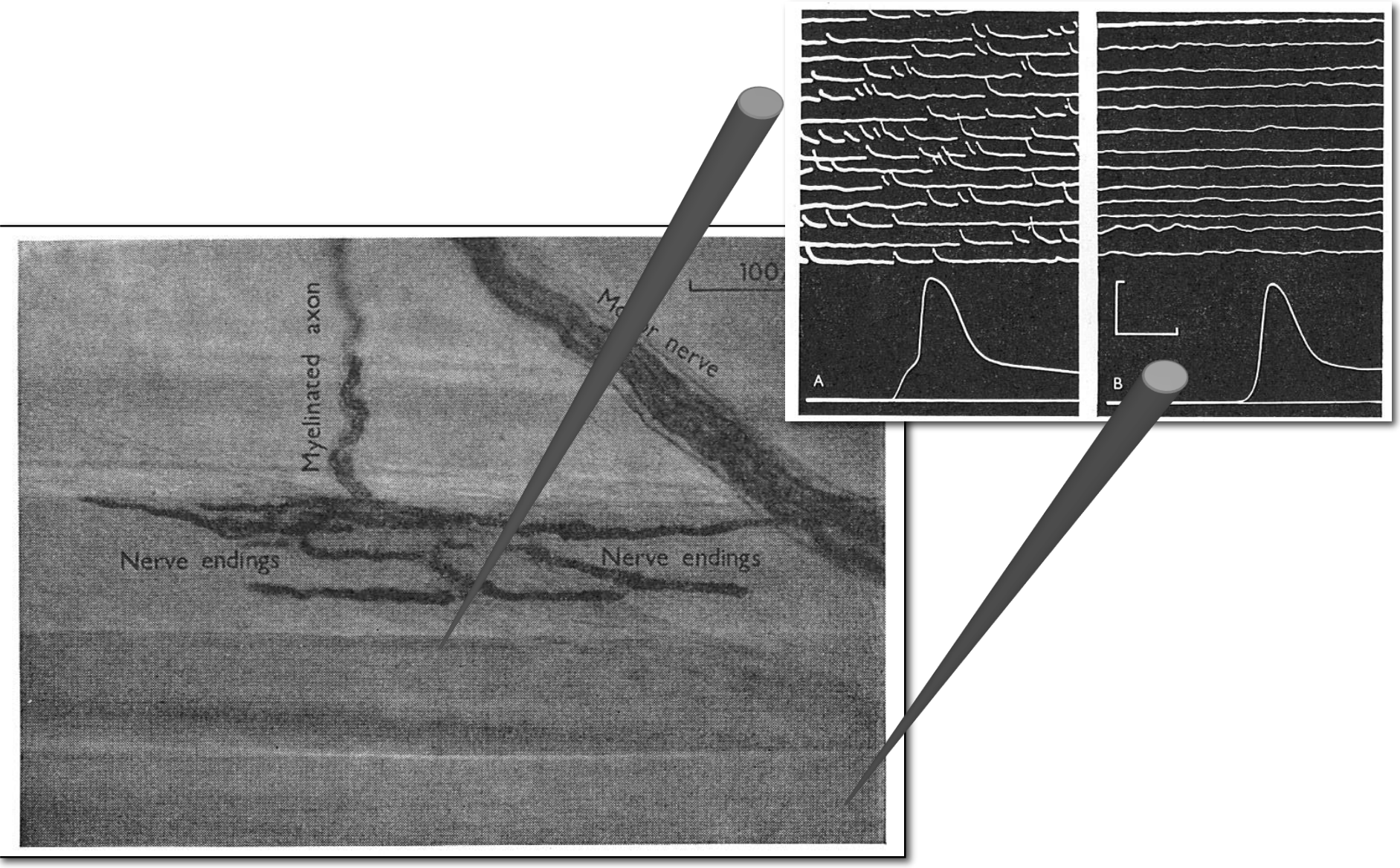
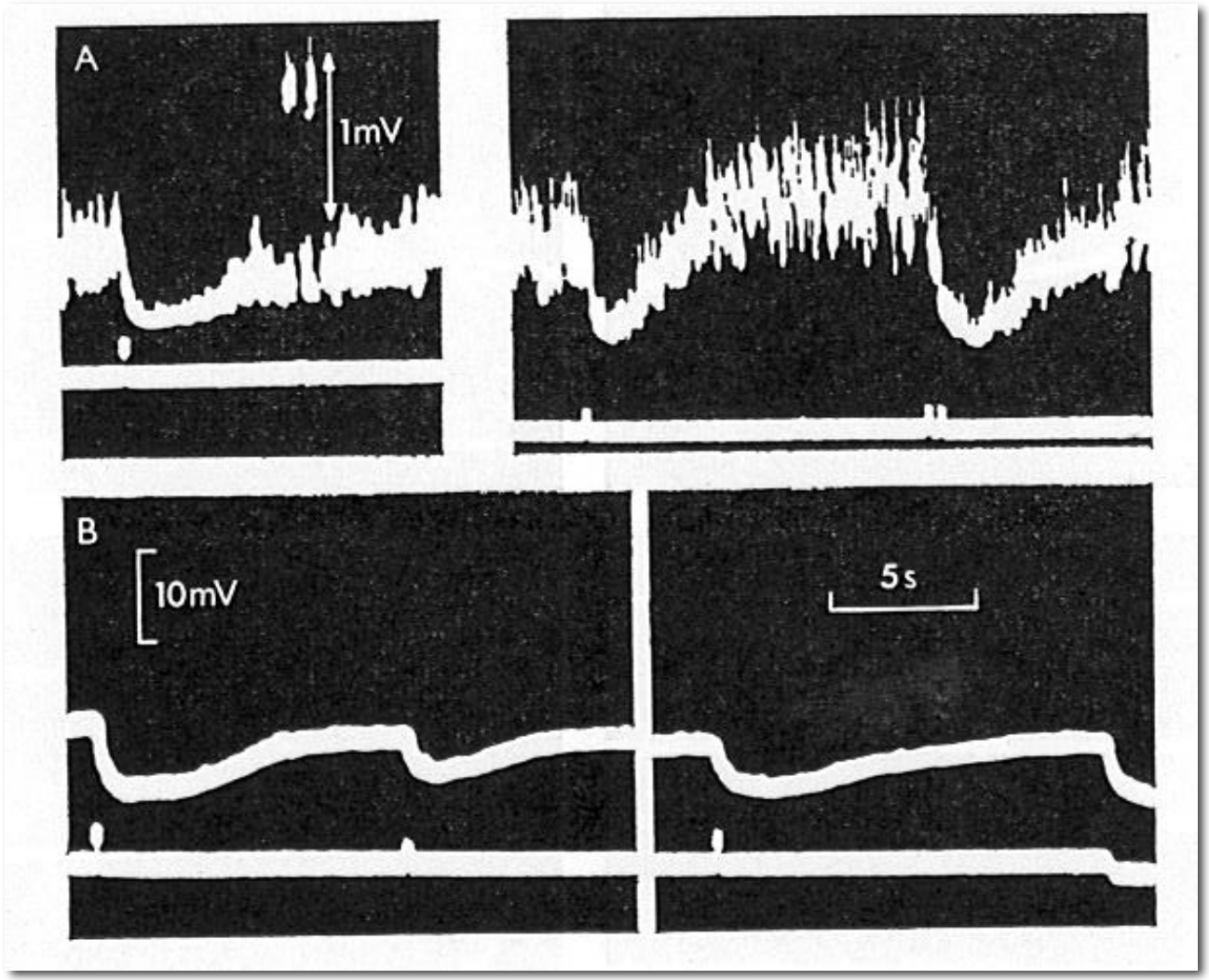
Bernard Katz e a neurotransmissão quantal na junção neuromuscular da rã (1952)



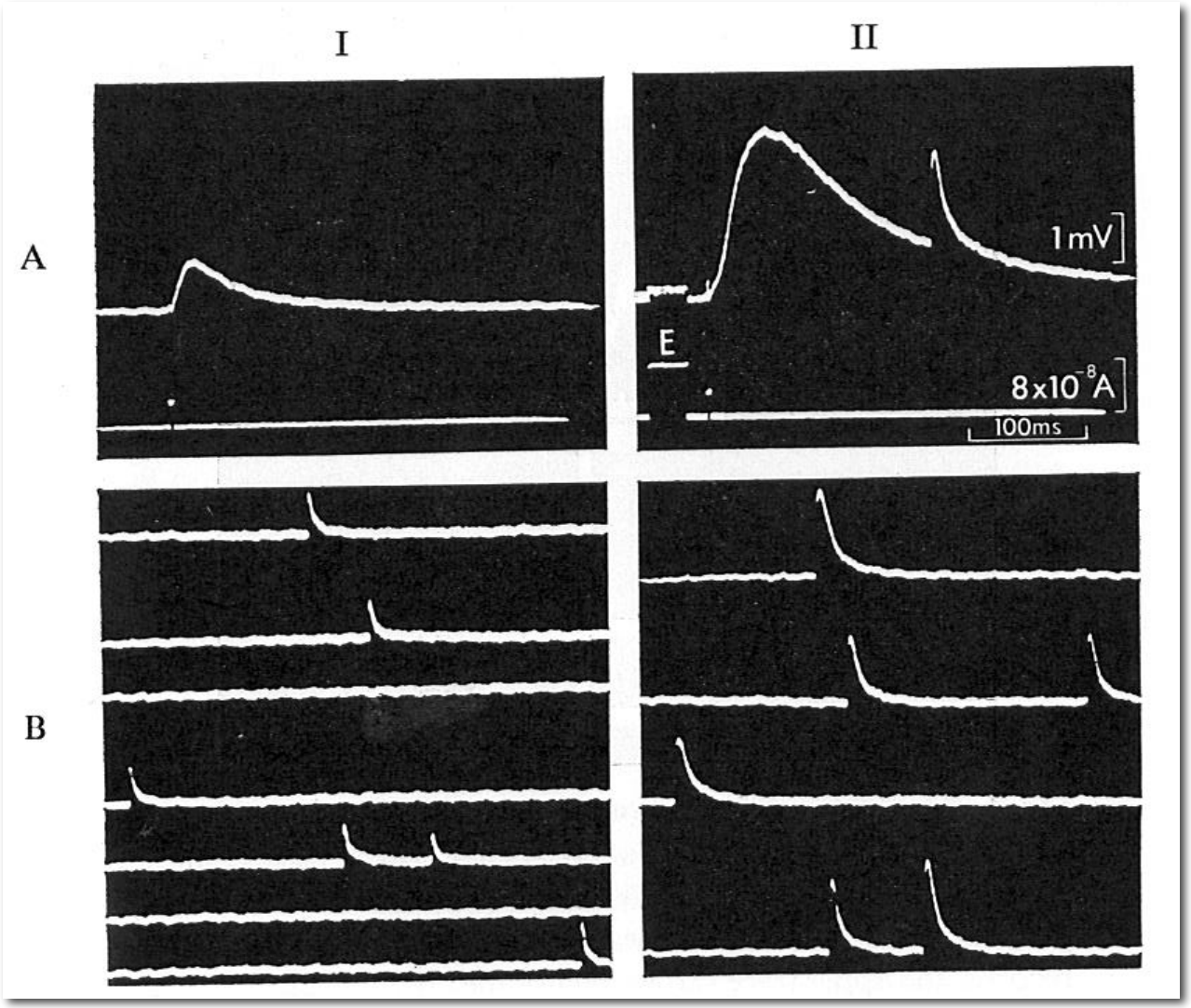
Bernard Katz e a neurotransmissão quantal na junção neuromuscular da rã



Bloqueio de “bursts” de minis (A) e de despolarizações evocadas por aplicação de acetilcolina (B) por aplicação iontoforética de curarina

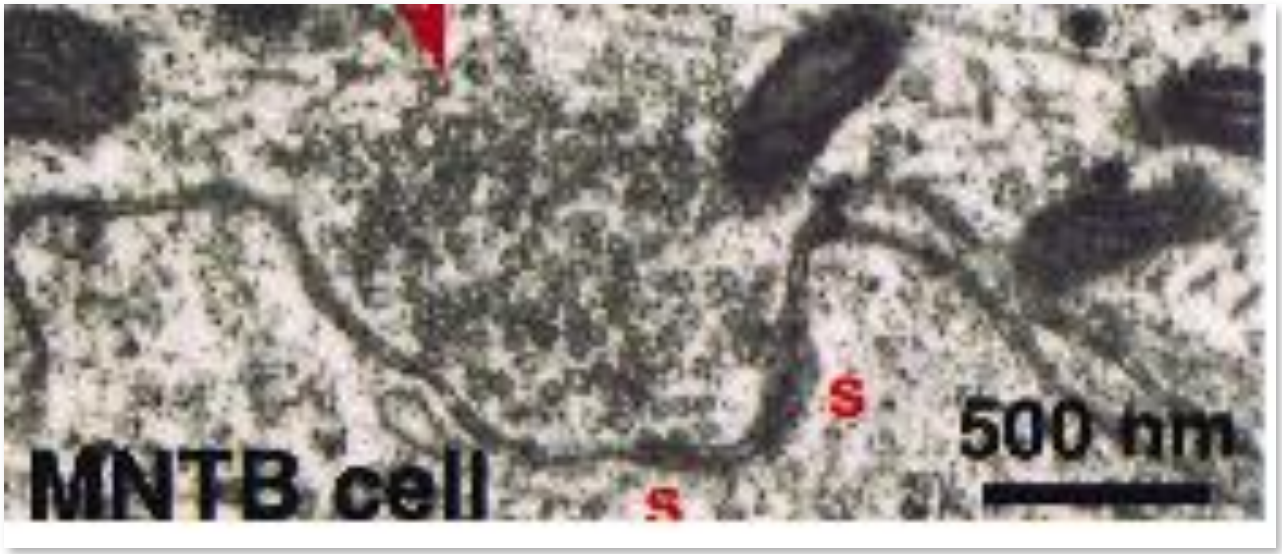


Inibição da acetilcolinaesterase por eserina (II) aumentou a amplitude e duração de tanto os mEPSPs (A) quanto dos EPSPs (B)

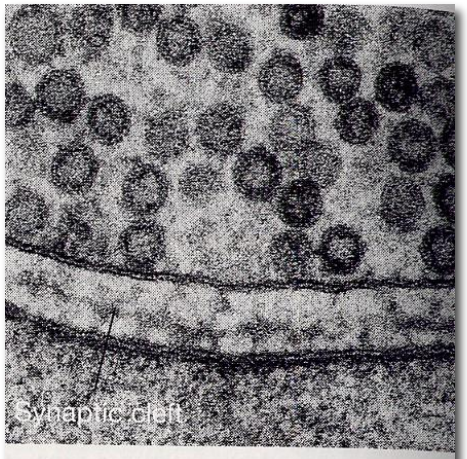


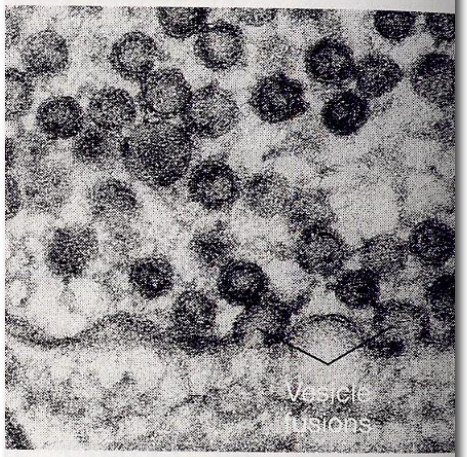
Katz postulou que a acetilcolina era liberada não continuamente, mas em “pacotes”que ele chamou de *quanta*

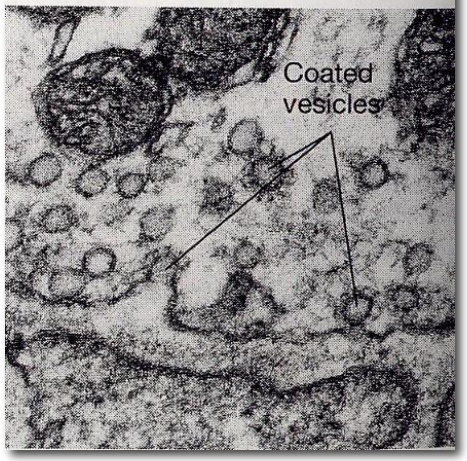
Qual seria a natureza física do *quanta*?

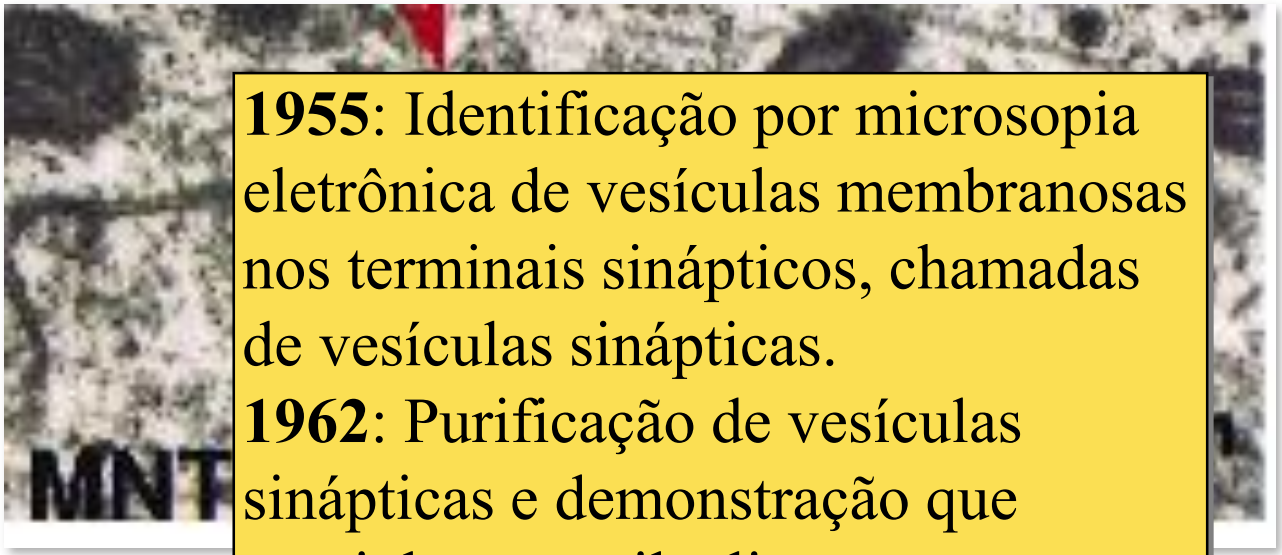

MNTB cell
S
S
500 nm


200 nm


Synaptic cleft


Vesicle fusions

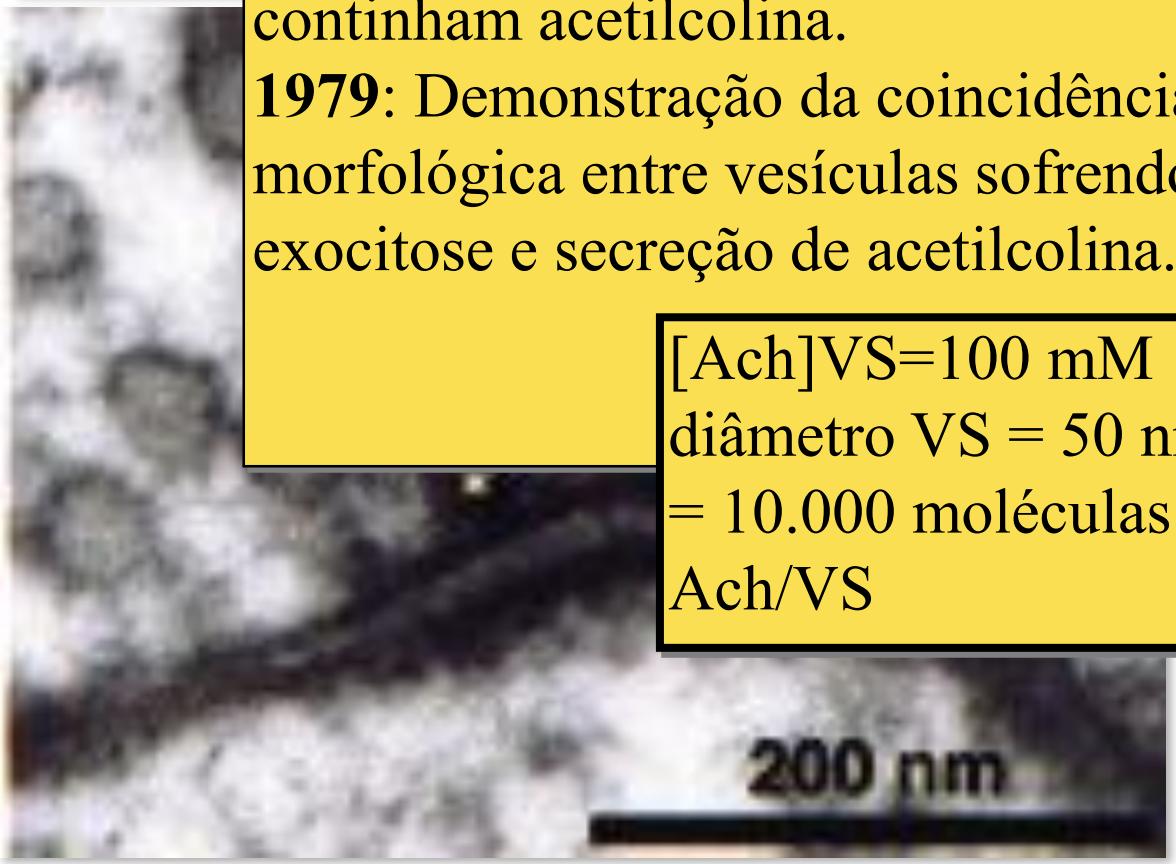

Coated vesicles



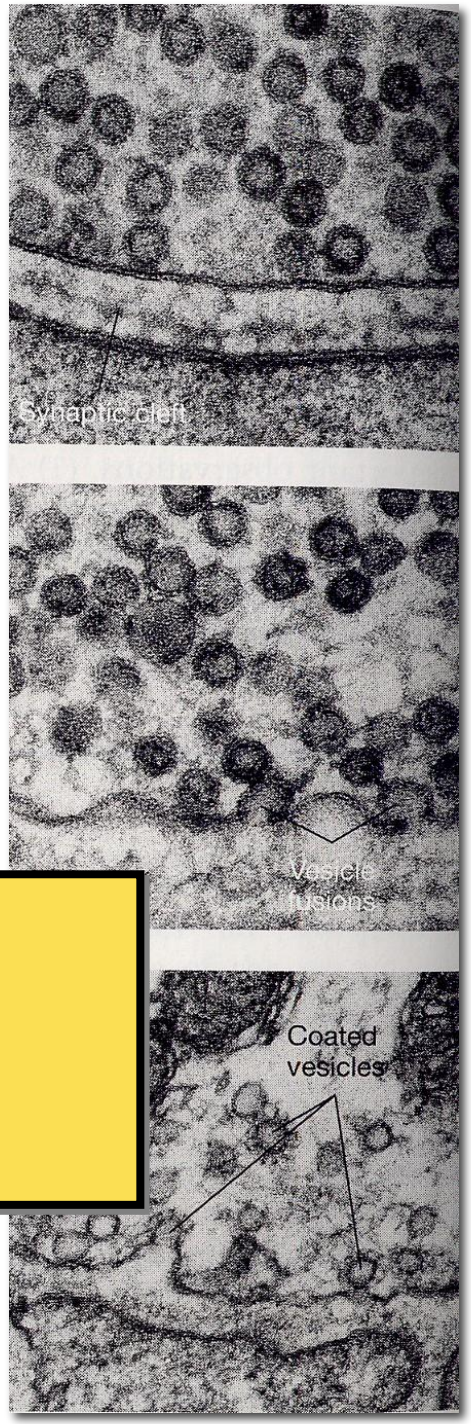
1955: Identificação por microsopia eletrônica de vesículas membranosas nos terminais sinápticos, chamadas de vesículas sinápticas.

1962: Purificação de vesículas sinápticas e demonstração que continham acetilcolina.

1979: Demonstração da coincidência morfológica entre vesículas sofrendo exocitose e secreção de acetilcolina.



[Ach]VS=100 mM
diâmetro VS = 50 nm
= 10.000 moléculas de Ach/VS



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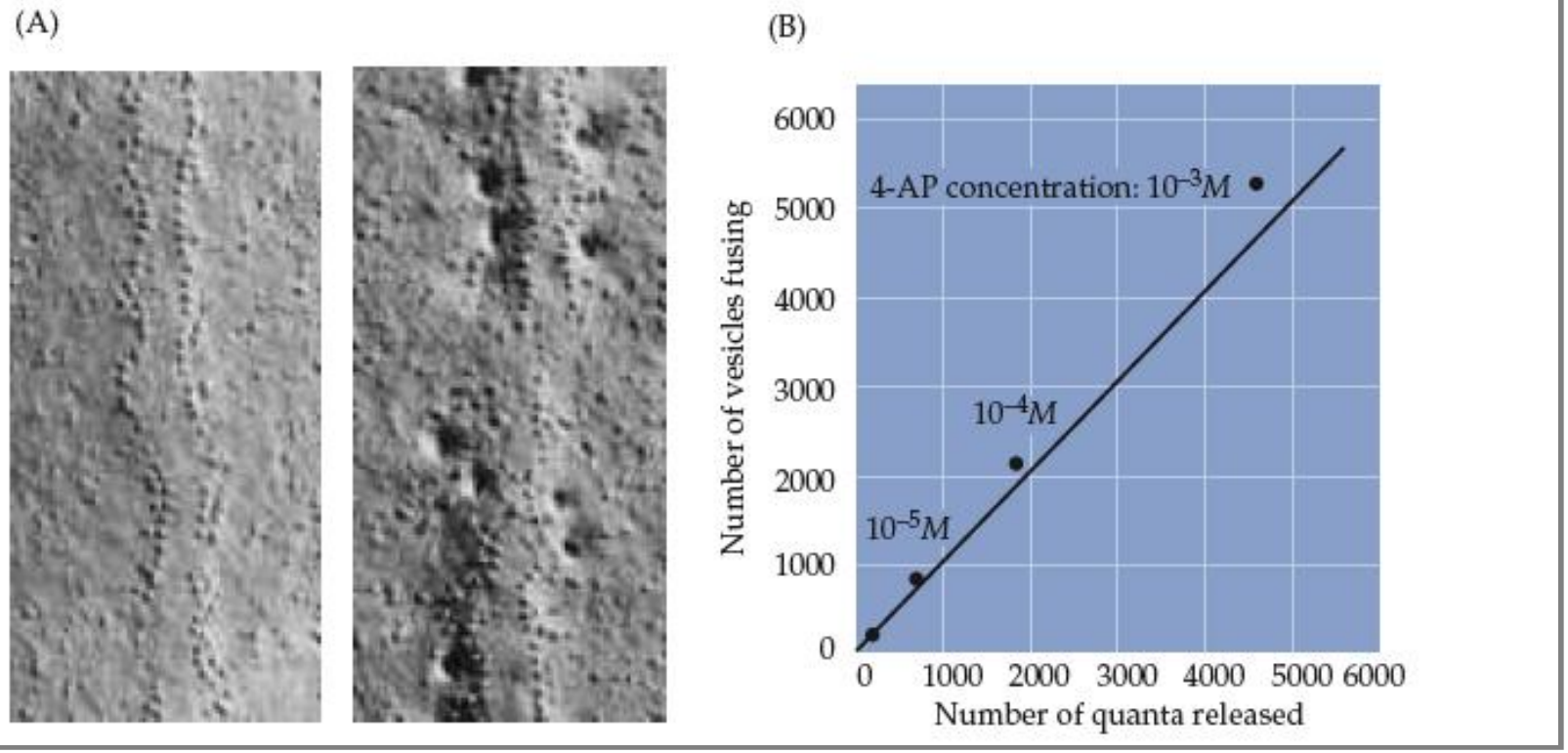
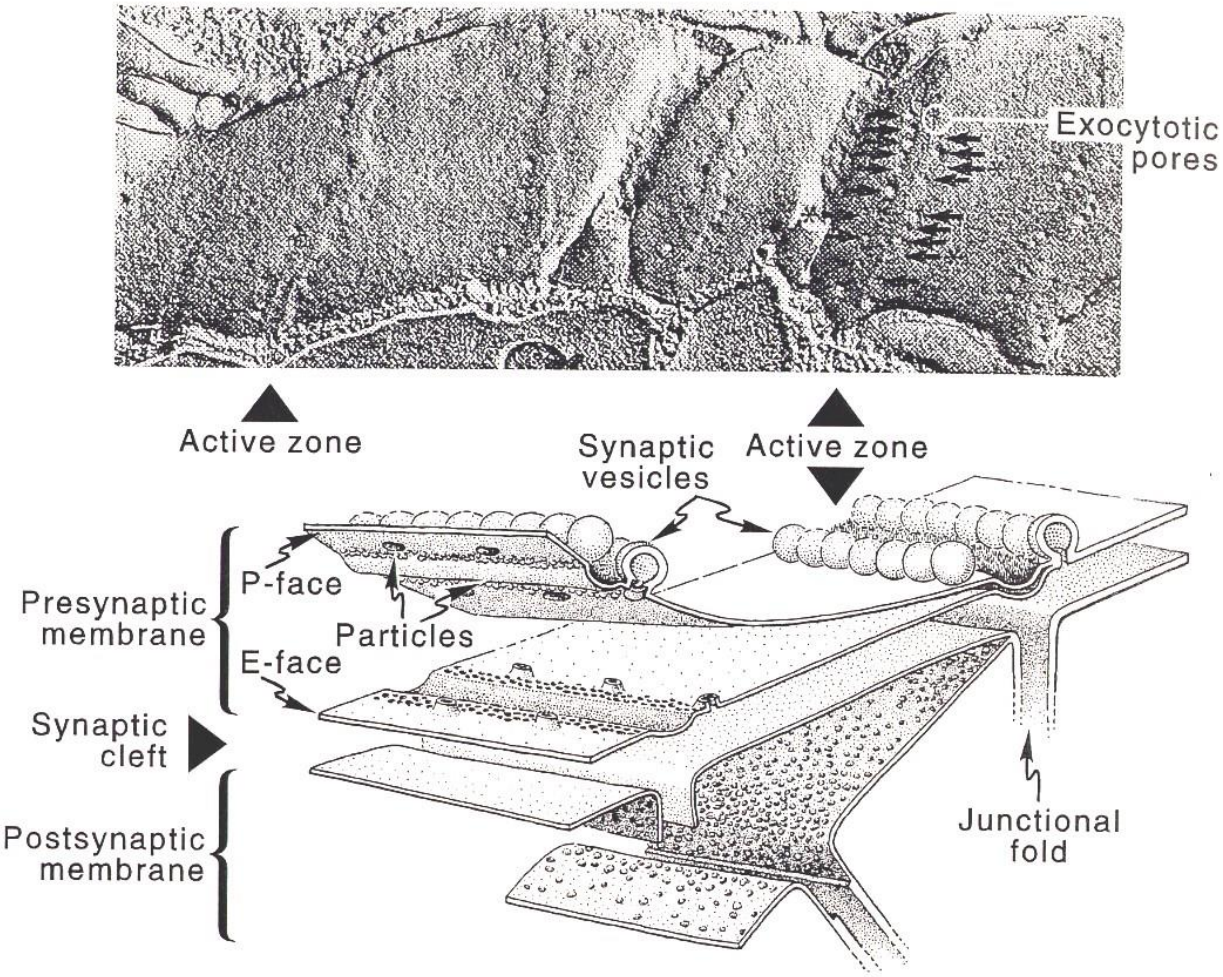
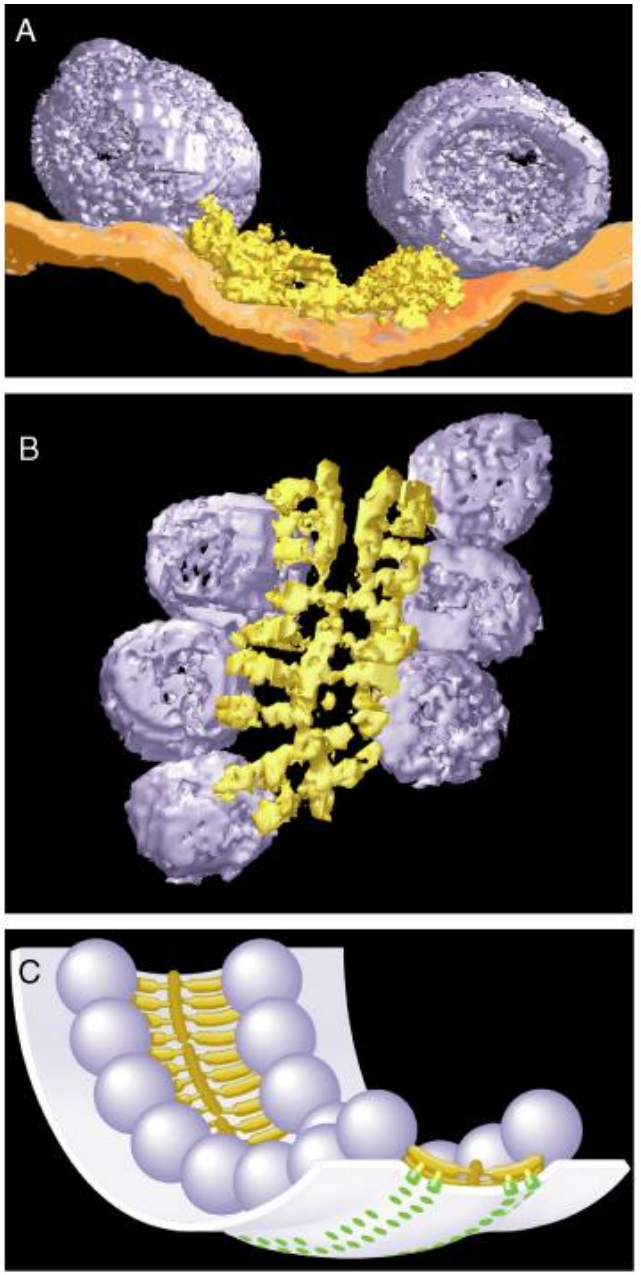


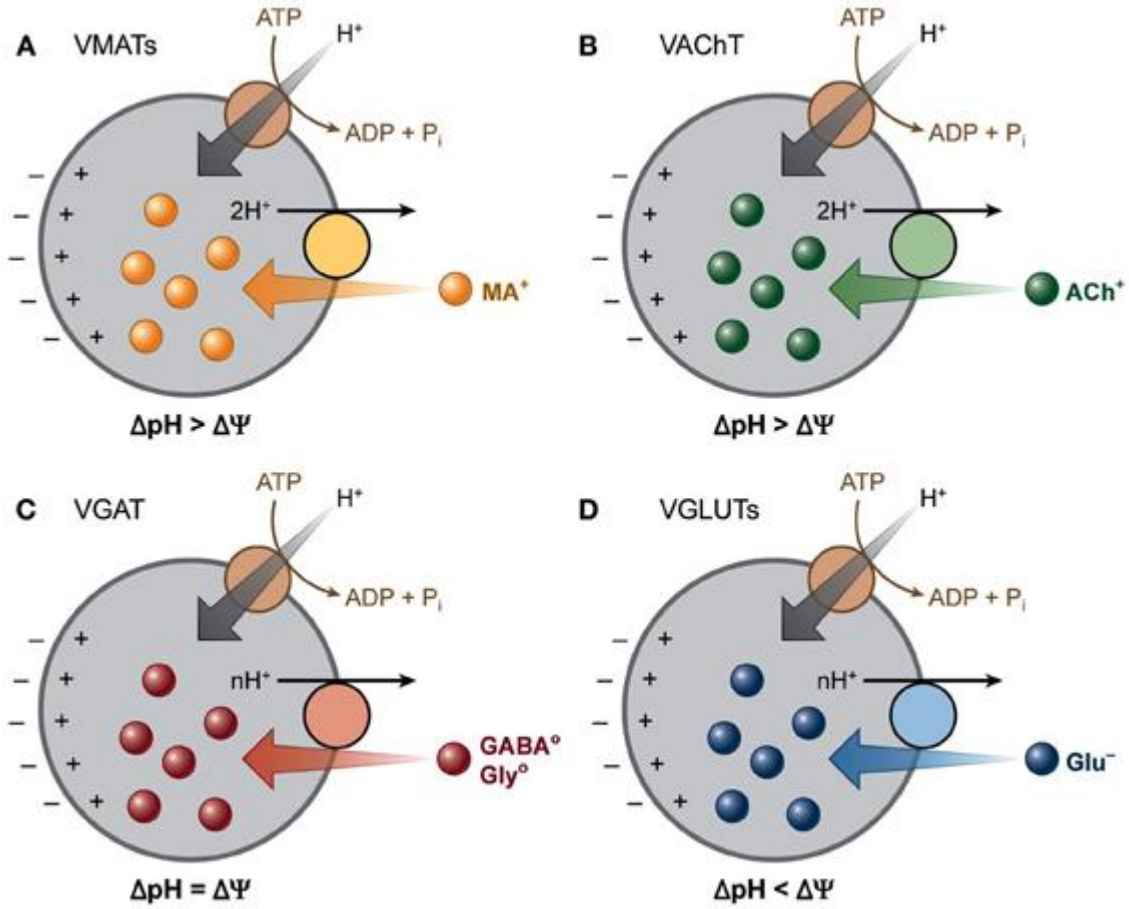
A organização das vesículas sinápticas na junção neuromuscular (JNM)



As vesículas sinápticas se ancoram nas **zona ativas**



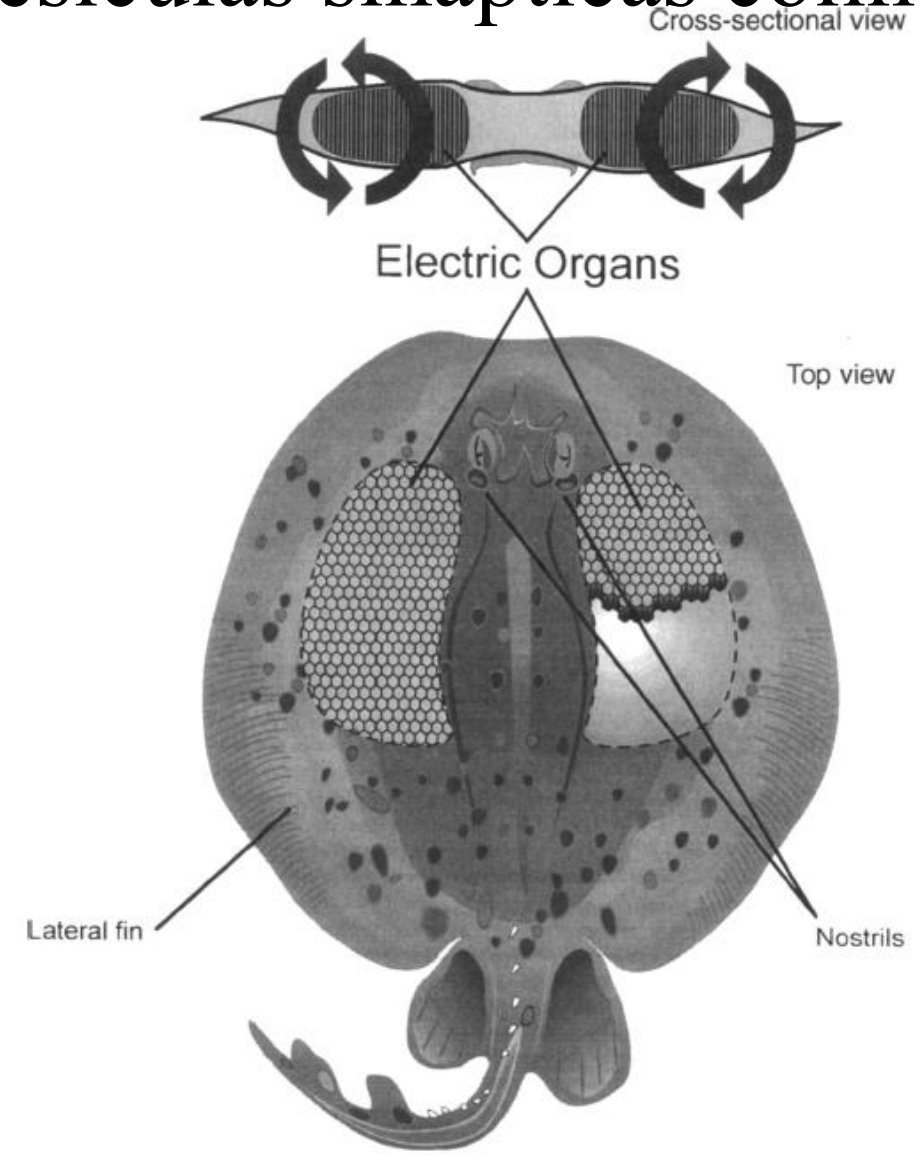
Os neurotransmissores são concentrados na vesícula por transporte ativo secundário



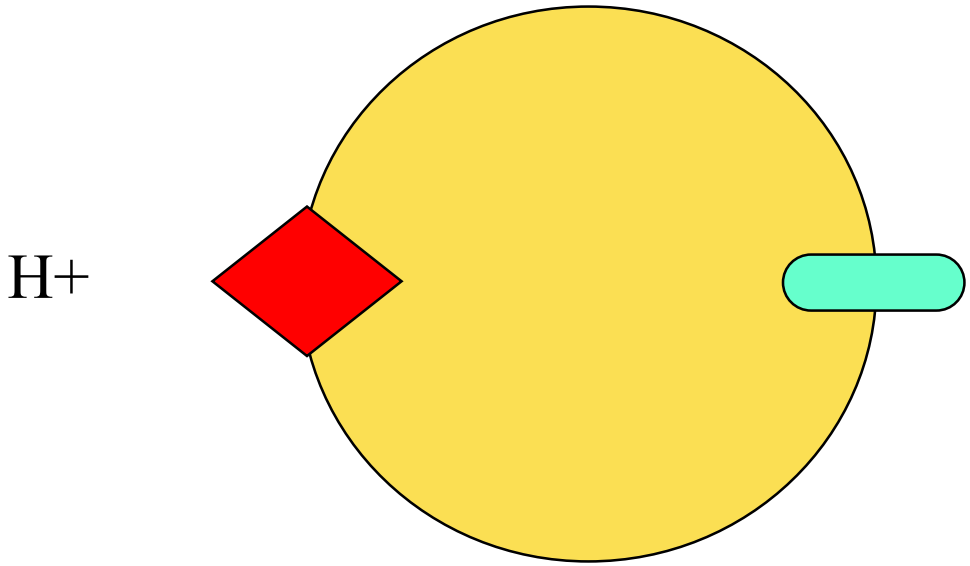
Evidências neuroquímicas lançavam dúvidas quanto a teoria vesicular...

- Experimentos neuroquímicos entre 1969-1974 sugeriam que a origem da acetilcolina liberada era citoplasmática

O órgão elétrico da raia *Torpedo* é rico em vesículas sinápticas colinérgicas



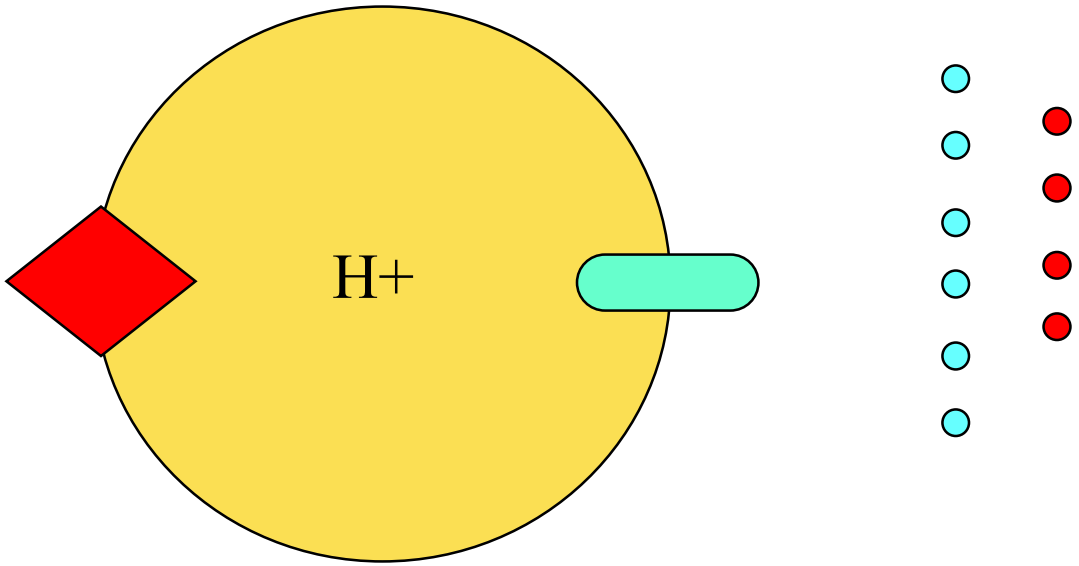
Marcando as vesículas sinápticas isoladas do órgão elétrico do *Torpedo* com acetilcolina radioativa (“quente”)



Vh-ATPase

Transportador de Ach

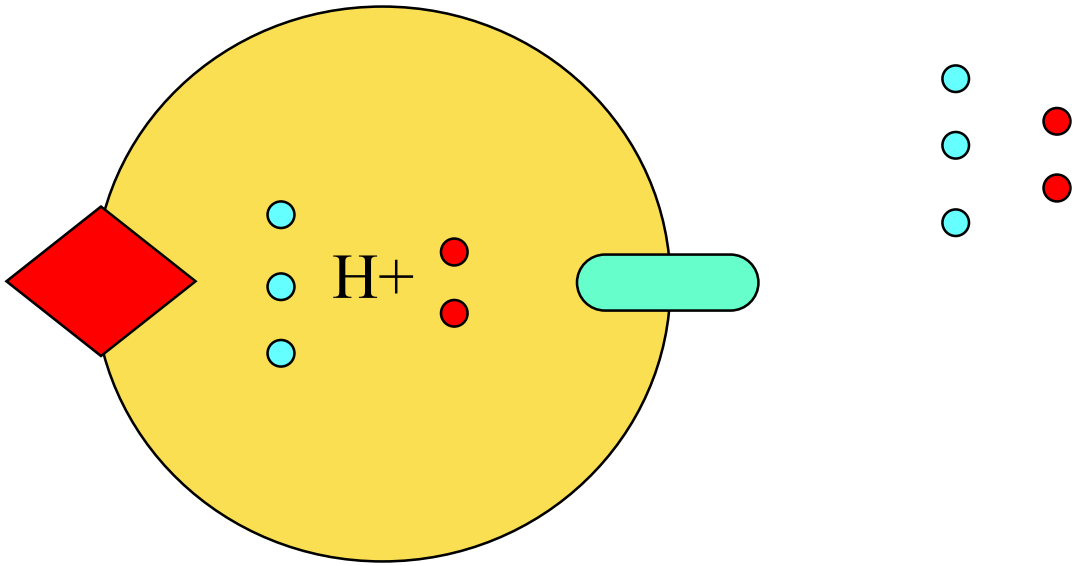
A mesma proporção de Ach “fria’/”quente” deve ser encontrada no citoplasma...



Ach “fria”

Ach “quente”

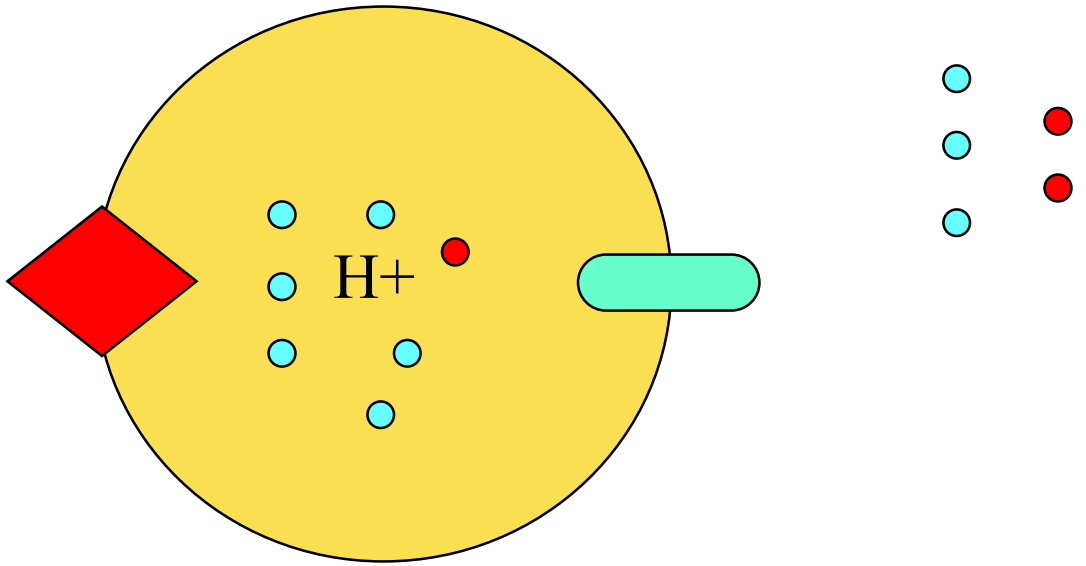
... e dentro das vesículas sinápticas



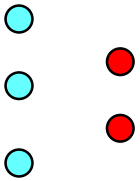
Ach “fria”

Ach “quente”

Entretanto essa razão é MENOR dentro das vesículas...



E a acetilcolina liberada tem a atividade especifica da Ach citoplasmática

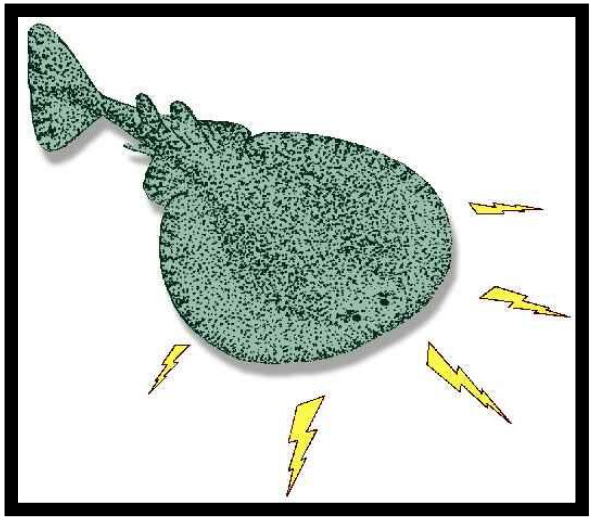


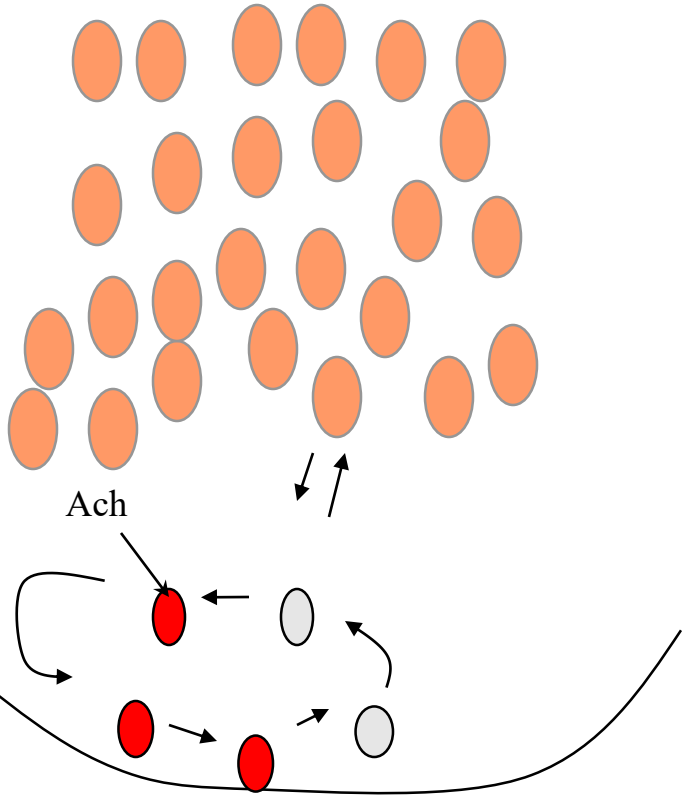
Ach “fria”

Ach “quente”

Resposta = Apenas uma fração das vesículas participam na exocitose

"Pools" vesiculares na eletroplaca (1977)





vesícula reciclante com Ach "quente"

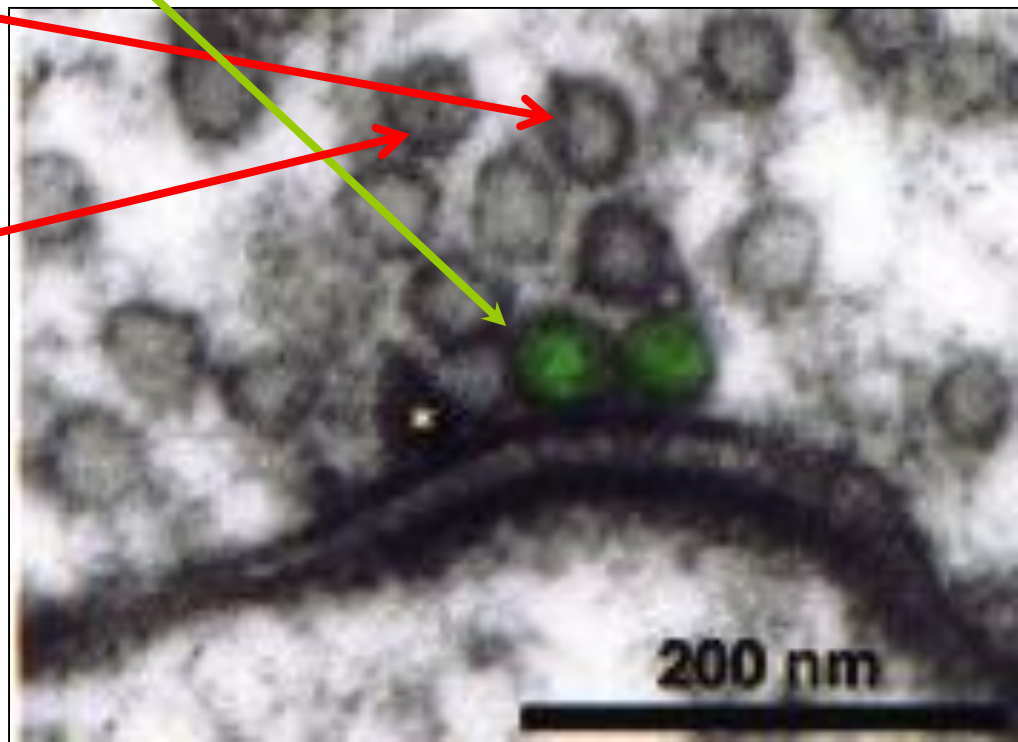
vesícula não-reciclante com Ach "fria"

"Pools" vesiculares

- *Pool liberável (20-25 vesículas-hipocampo)*
  - *– Liberação imediata-RRP (5-8)*
  - *– reciclável (17-20)*
- *Pool reserva*

(*repouso*)

(~180 vesículas)



“Pools” vesiculares

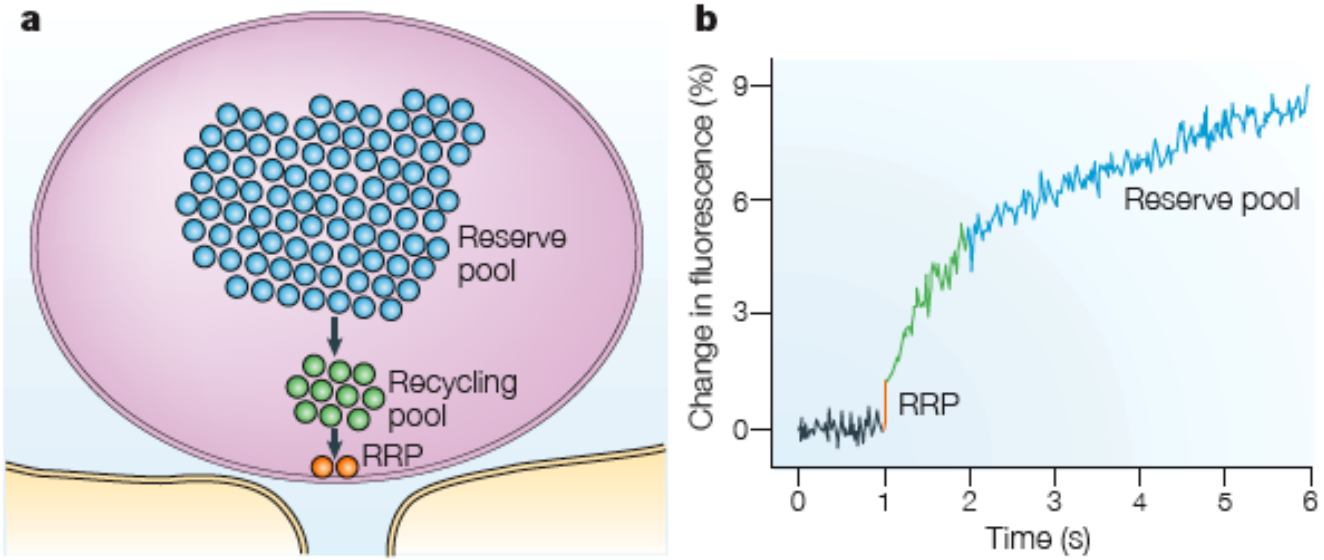


Figure 1 | **Three vesicle pools. a** | The classic three-pool model. The reserve pool makes up ~80–90% of the total pool, and the recycling pool is significantly smaller (~10–15%). The readily releasable pool (RRP) consists of a few vesicles (~1%) that seem to be docked and primed for release. **b** | Three kinetic components of release (indicating release of three vesicle pools) on depolarization of goldfish bipolar cells. The cell was stimulated in the presence of the styryl dye FM 1-43, and the increase in fluorescence gives a direct measure of exocytosis. Panel **b** modified, with permission, from REF. 12 © (1999) Blackwell Scientific Publishing.

Rizzoli e Betz, Nature Reviews Neuroscience, 2005

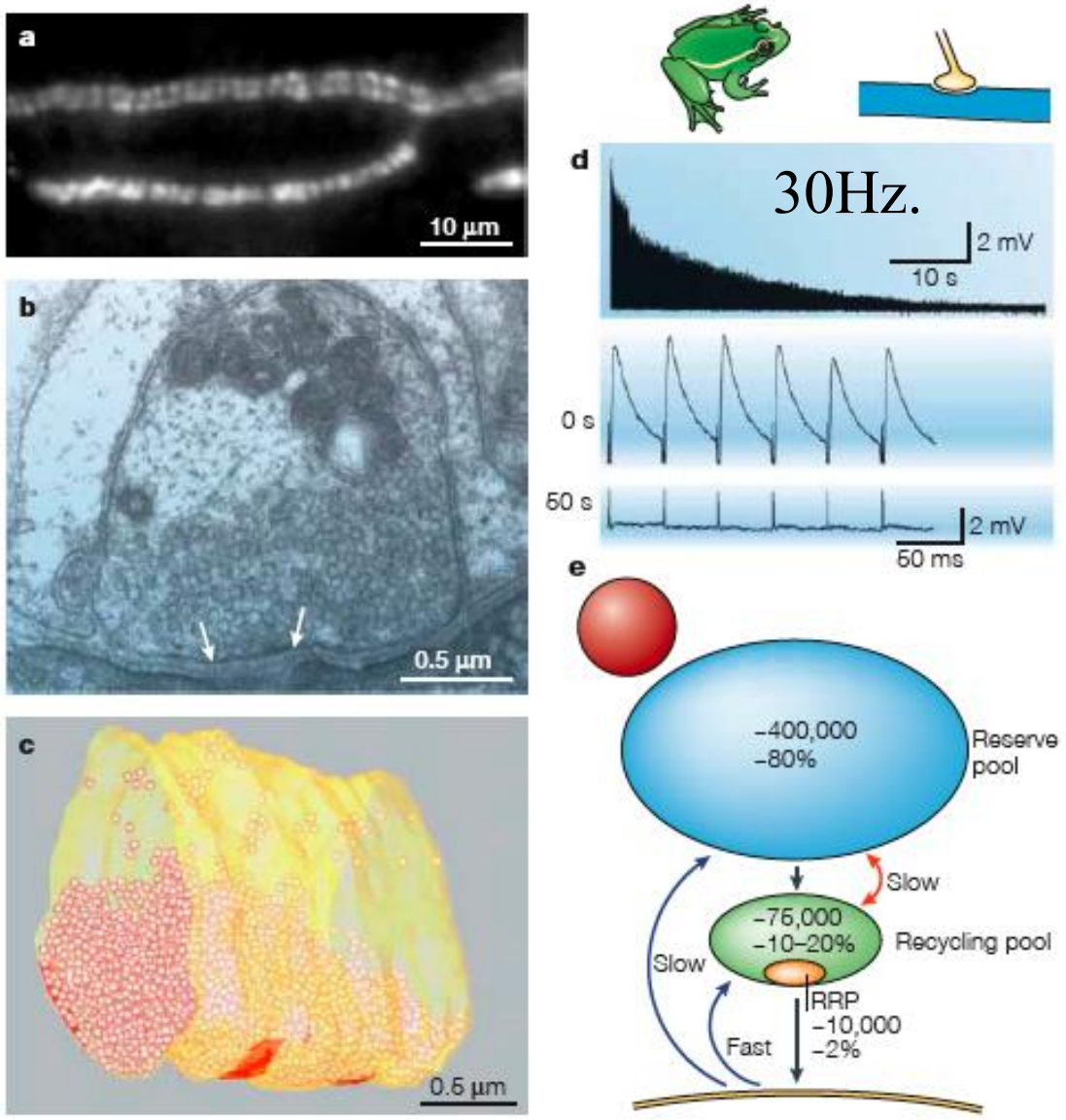
"Pools" vesiculares

Table 1 | **Characteristics of the vesicle pools**

Pool	Readily releasable pool (RRP)	Recycling pool	Reserve pool
Size (% of all vesicles)	~1–2%	~10–20%	~80–90%
Location	Docked	Scattered	Scattered (bulk of vesicle cluster)
Released within	<1 second	A few seconds	Tens of seconds, minutes
Recycling	Fast (seconds)	Fast (seconds)	Slow (minutes)
Mixing with other pools	Fast mixing with recycling vesicles	Slow mixing with reserve	Slow mixing with other vesicles
Mobility in resting terminals	None — docked	High	Low (high in bipolar cells)

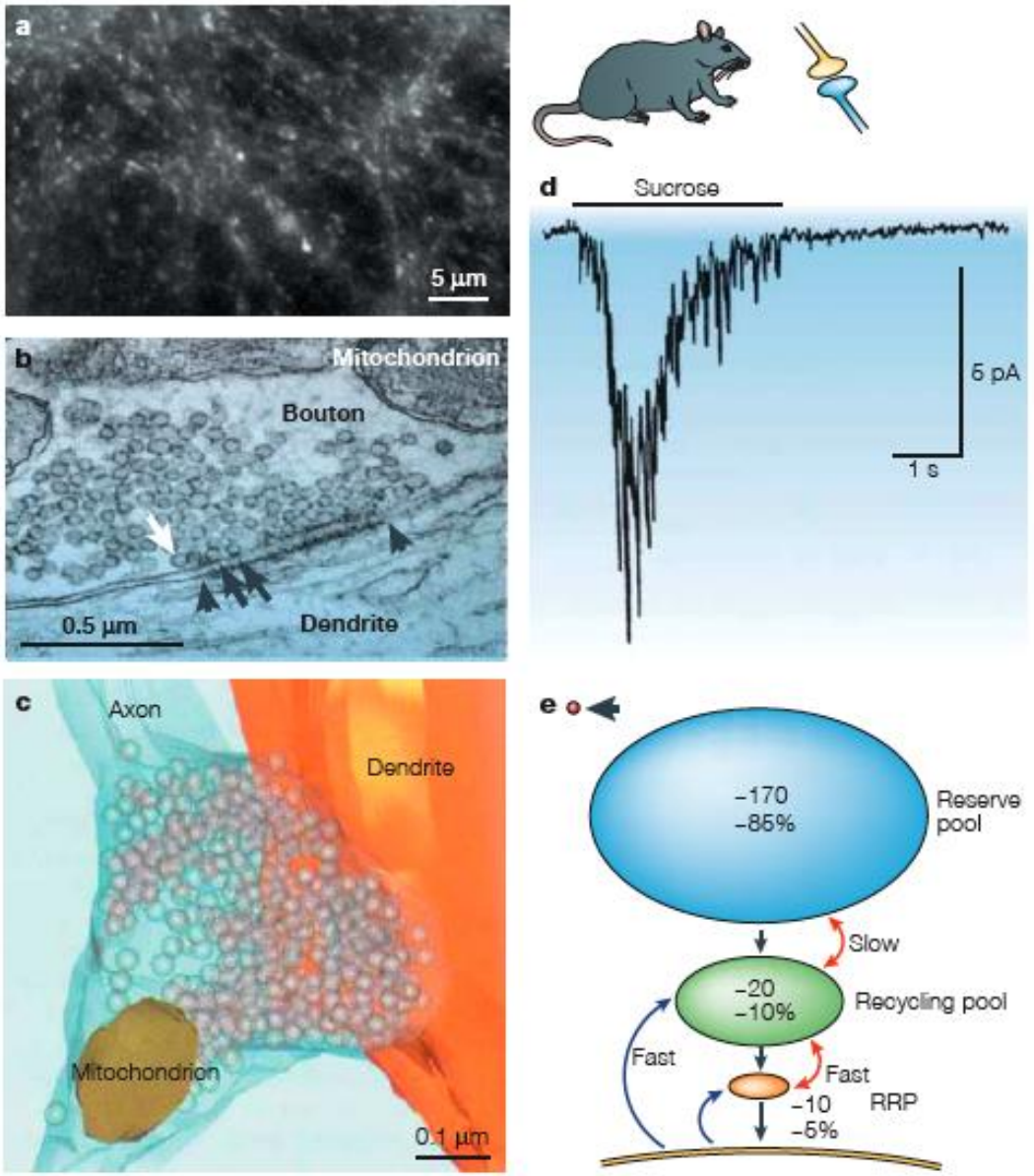
Rizzoli e Betz, Nature Reviews Neuroscience, 2005

“Pools” vesiculares na JNM



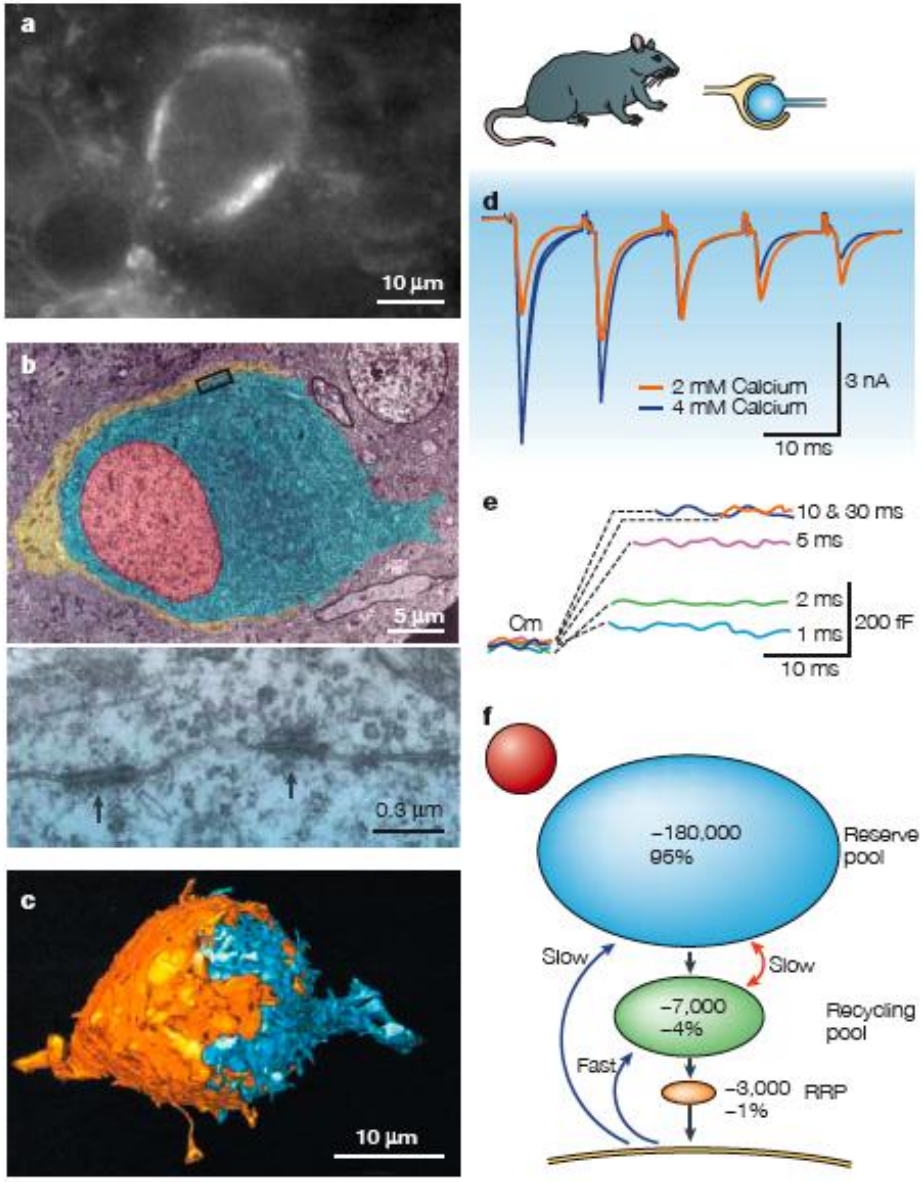
Rizzoli e Betz, Nature Reviews Neuroscience, 2005

“Pools” vesiculares no hipocampo



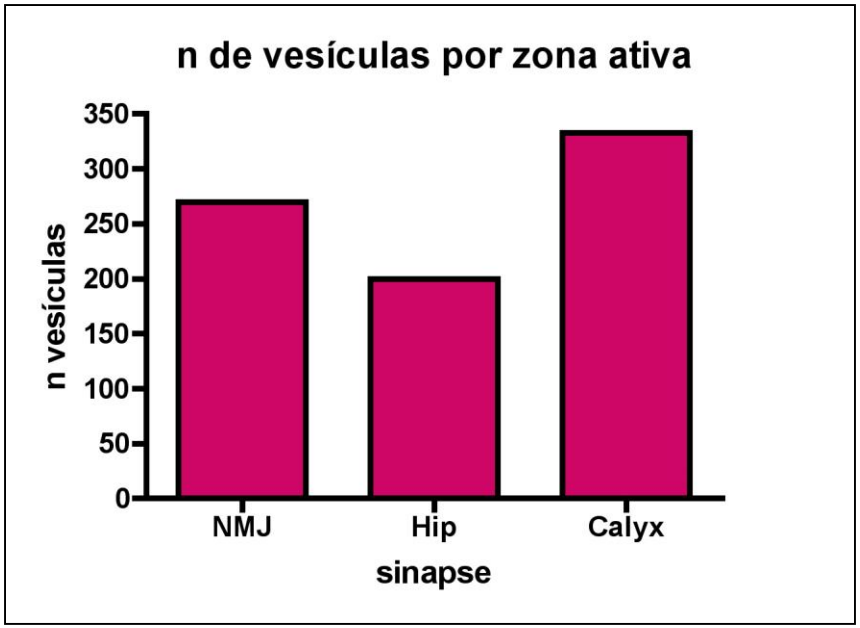
Rizzoli e Betz, Nature Reviews Neuroscience, 2005

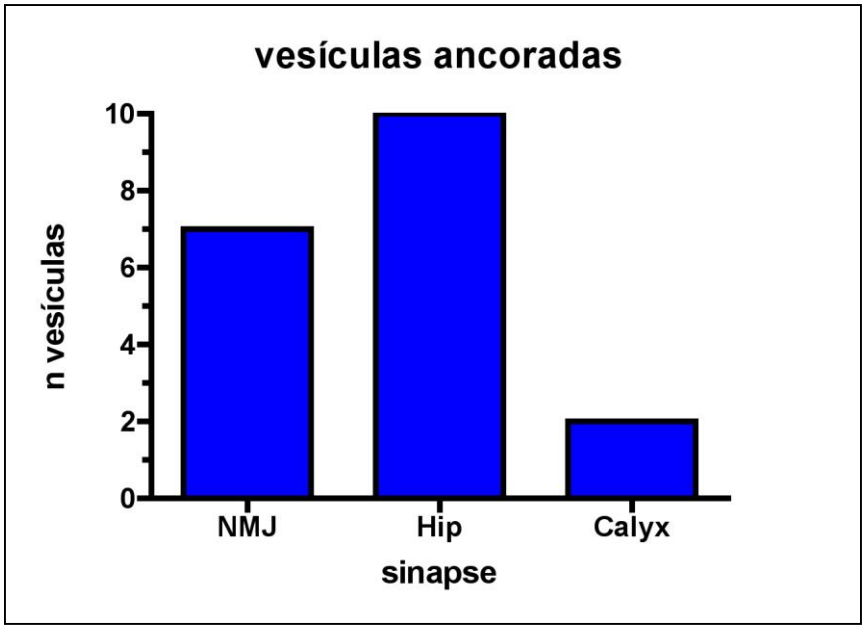
Pools vesiculares no cálice de Held



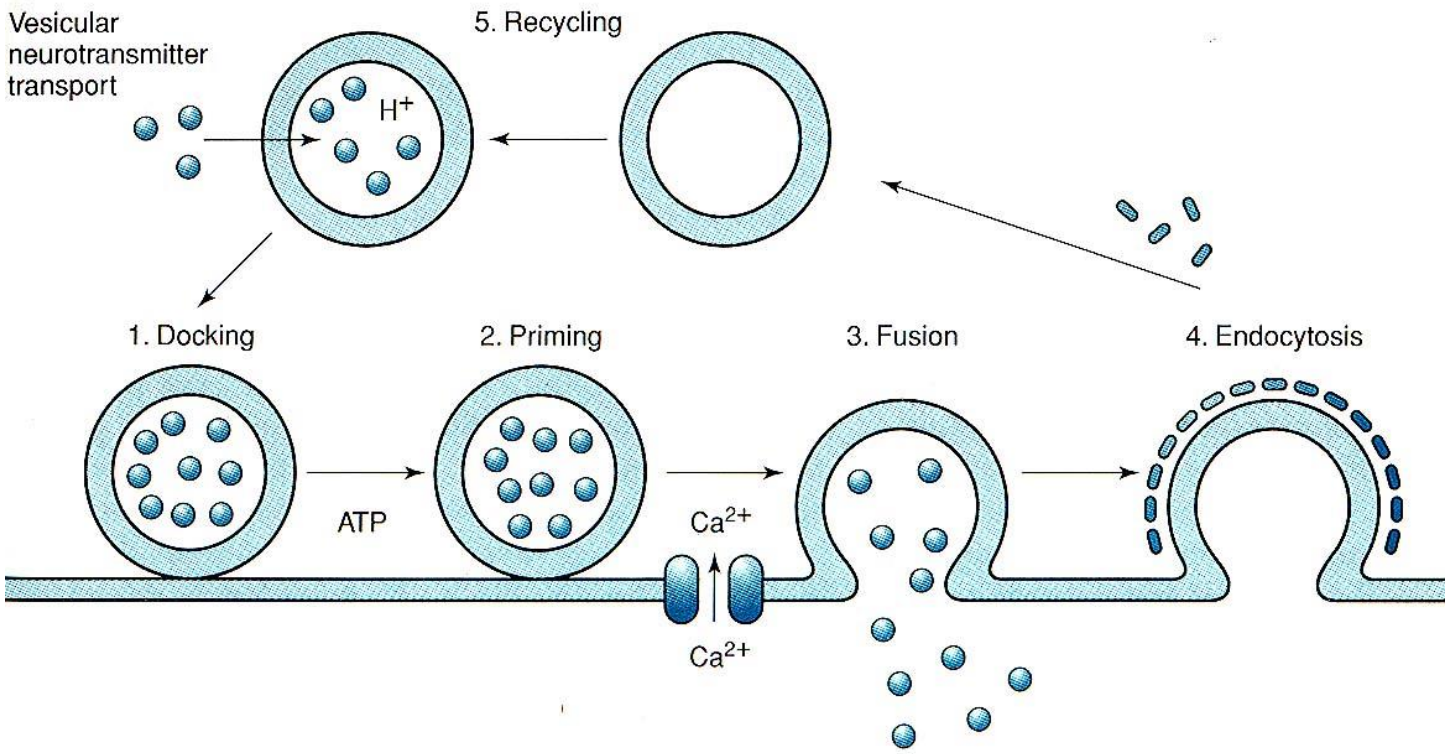
, 2005

Quantas vesículas uma sinapse tem por zona ativa?

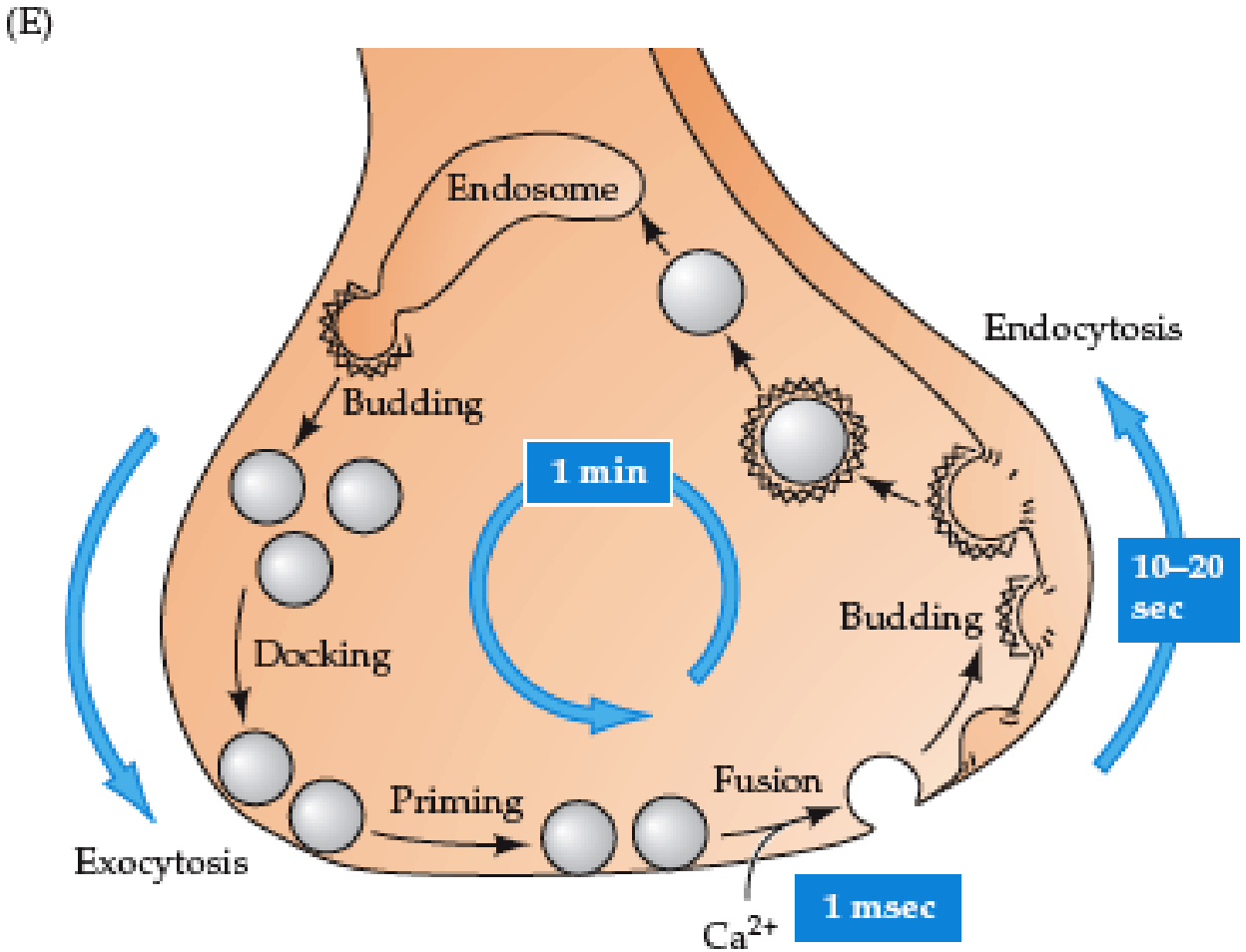




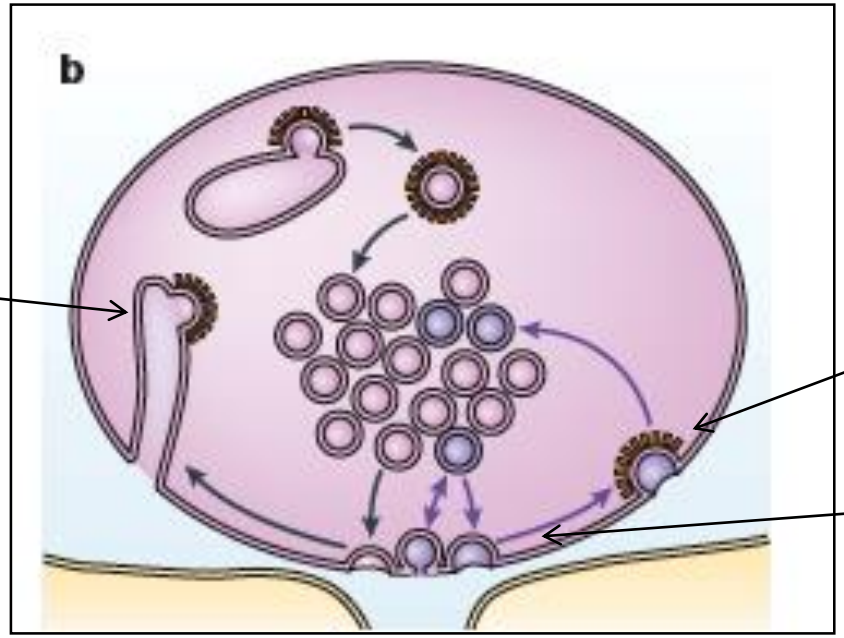
O Ciclo das vesículas sinápticas



O Ciclo das vesículas sinápticas



Vias de recuperação dos *pools* vesiculares



Composição protéica das vesículas do órgão elétrico de *Torpedo*.

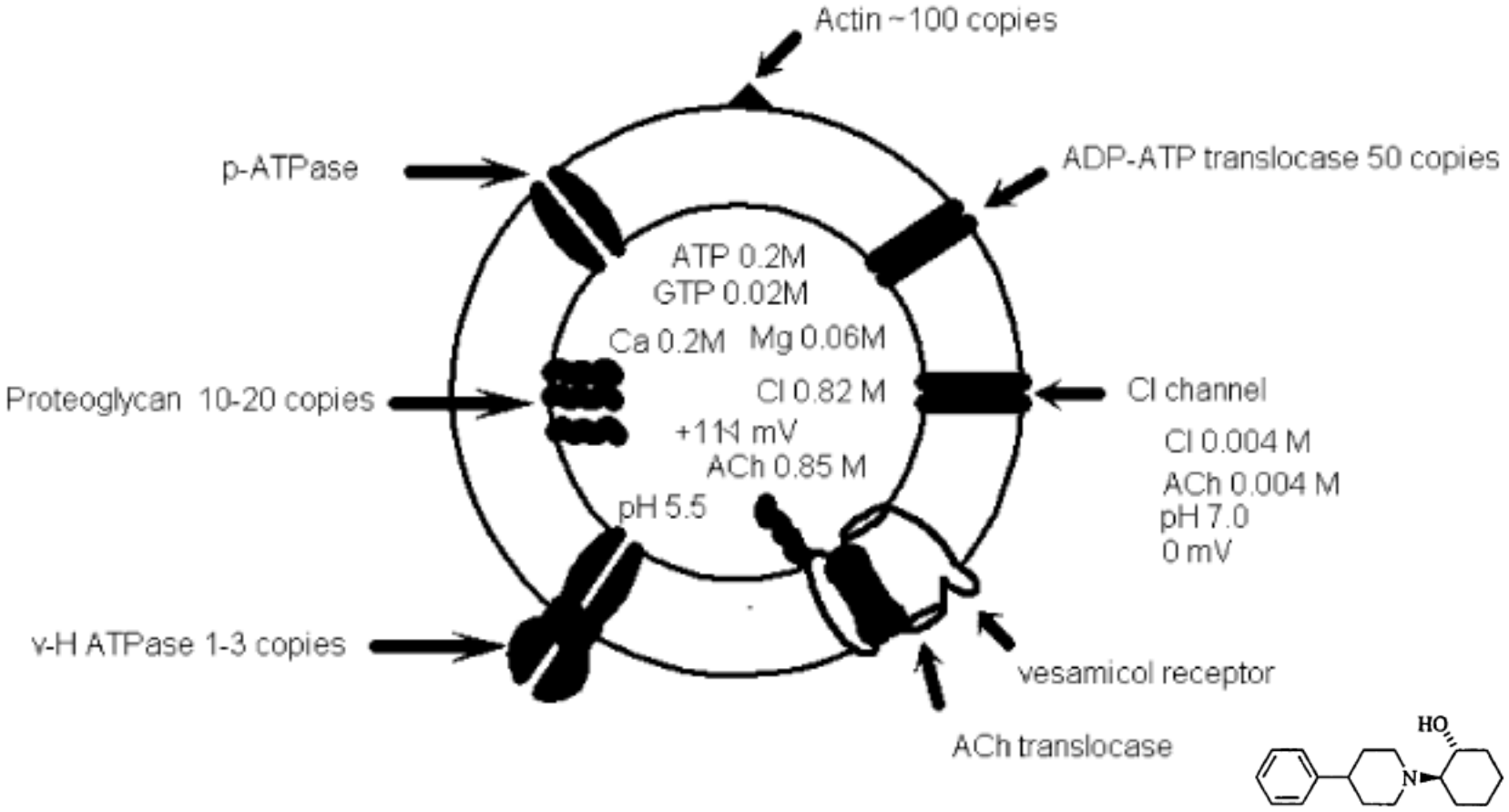
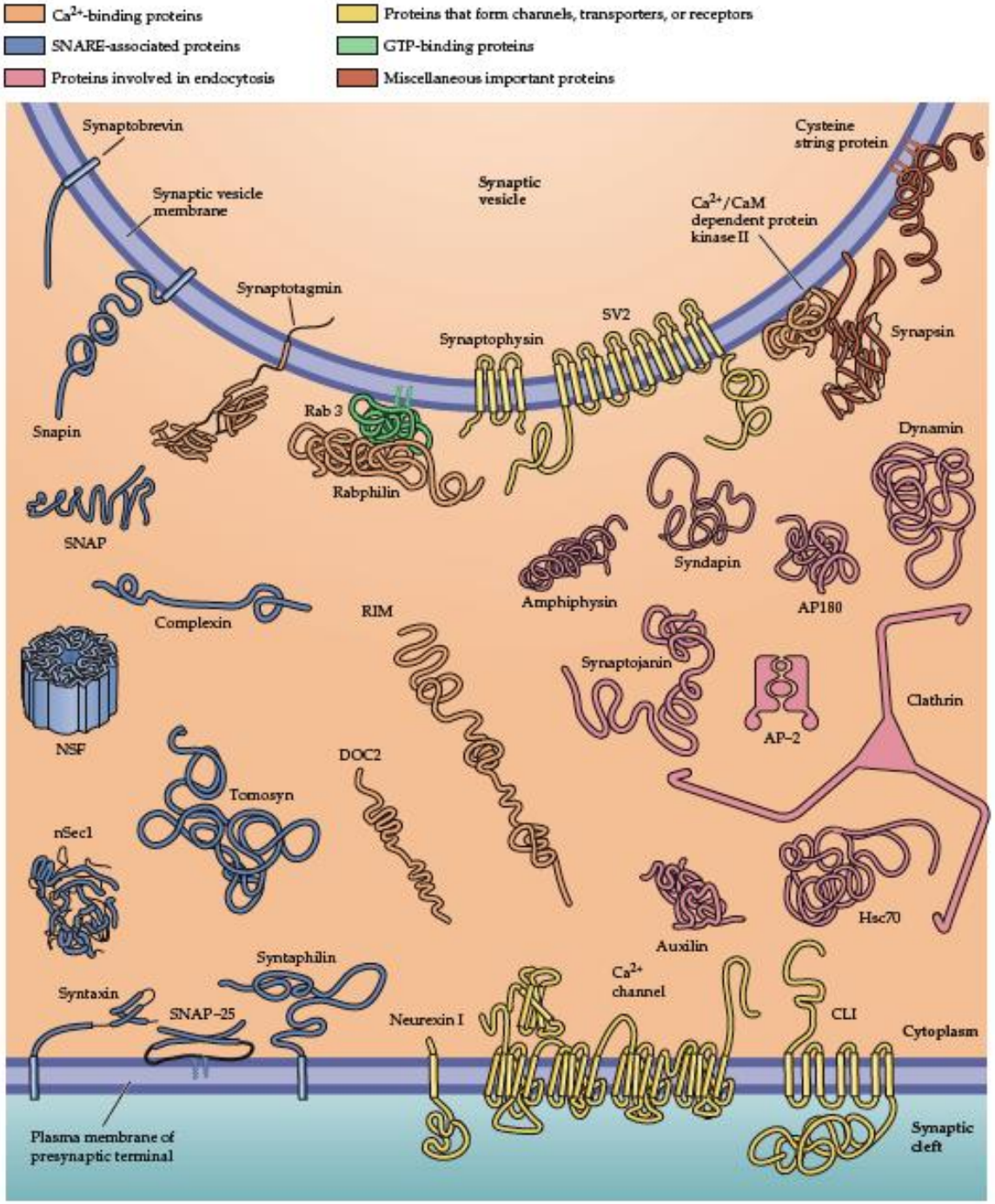
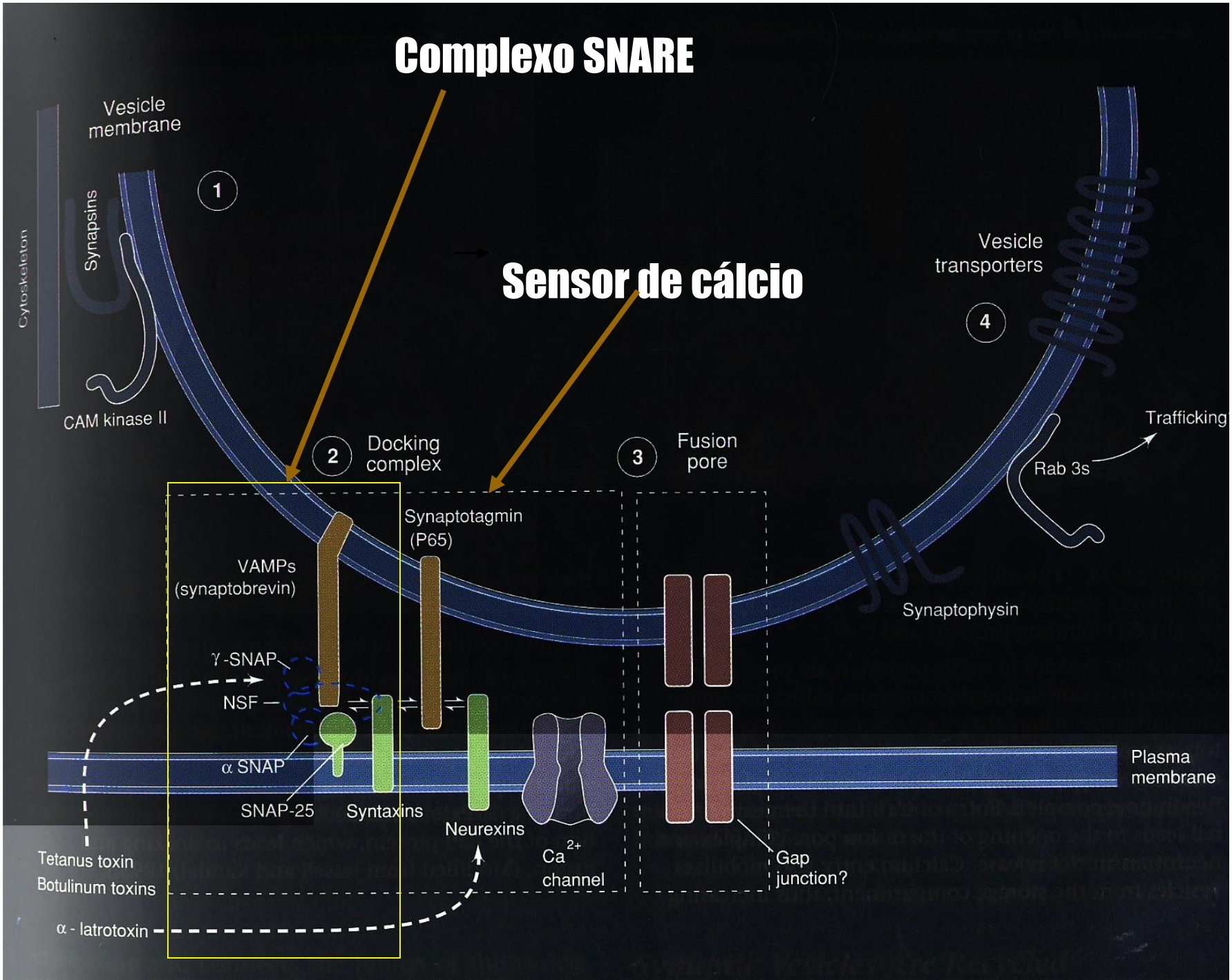
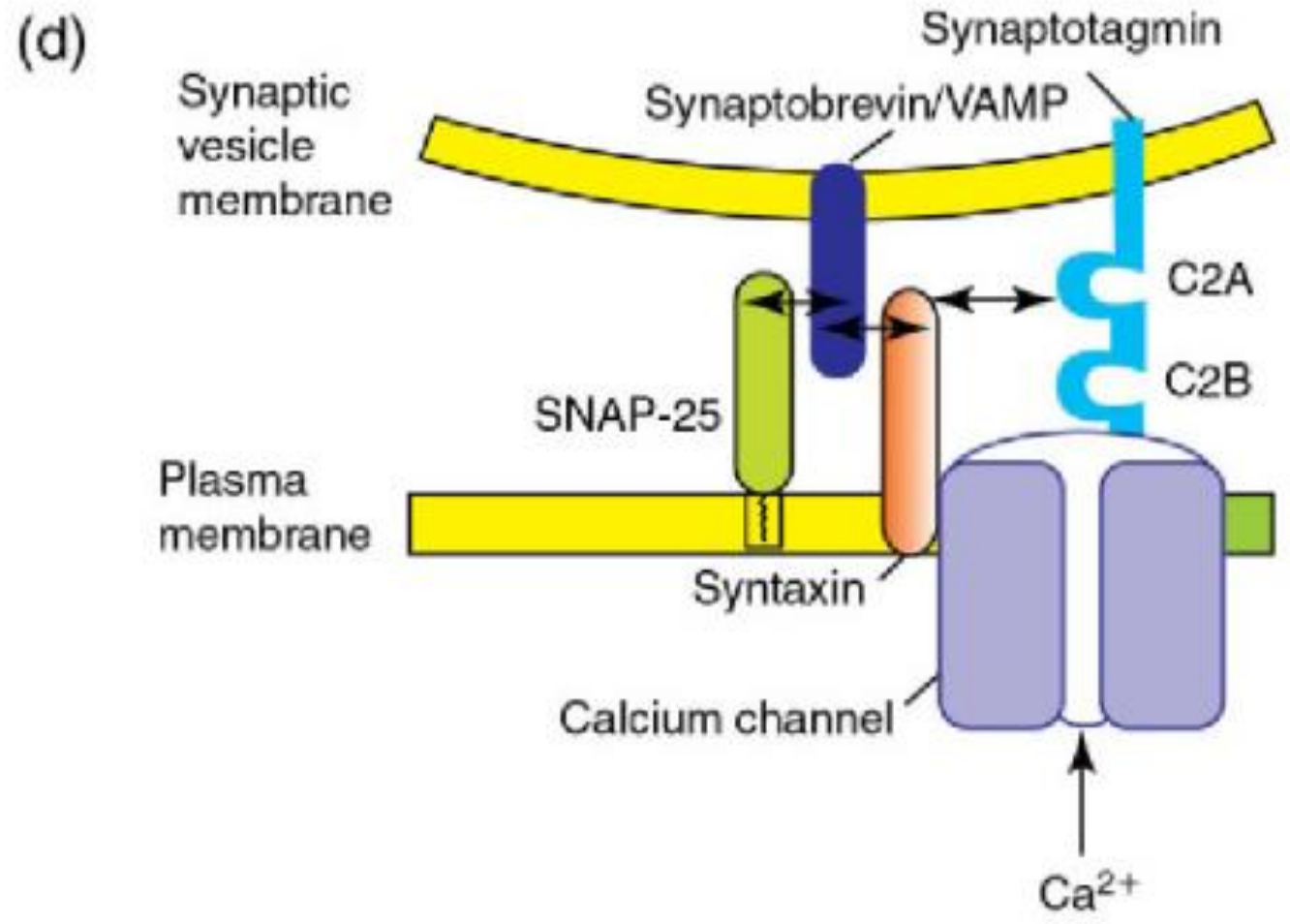


Fig. 2. (−)-Vesamicol.

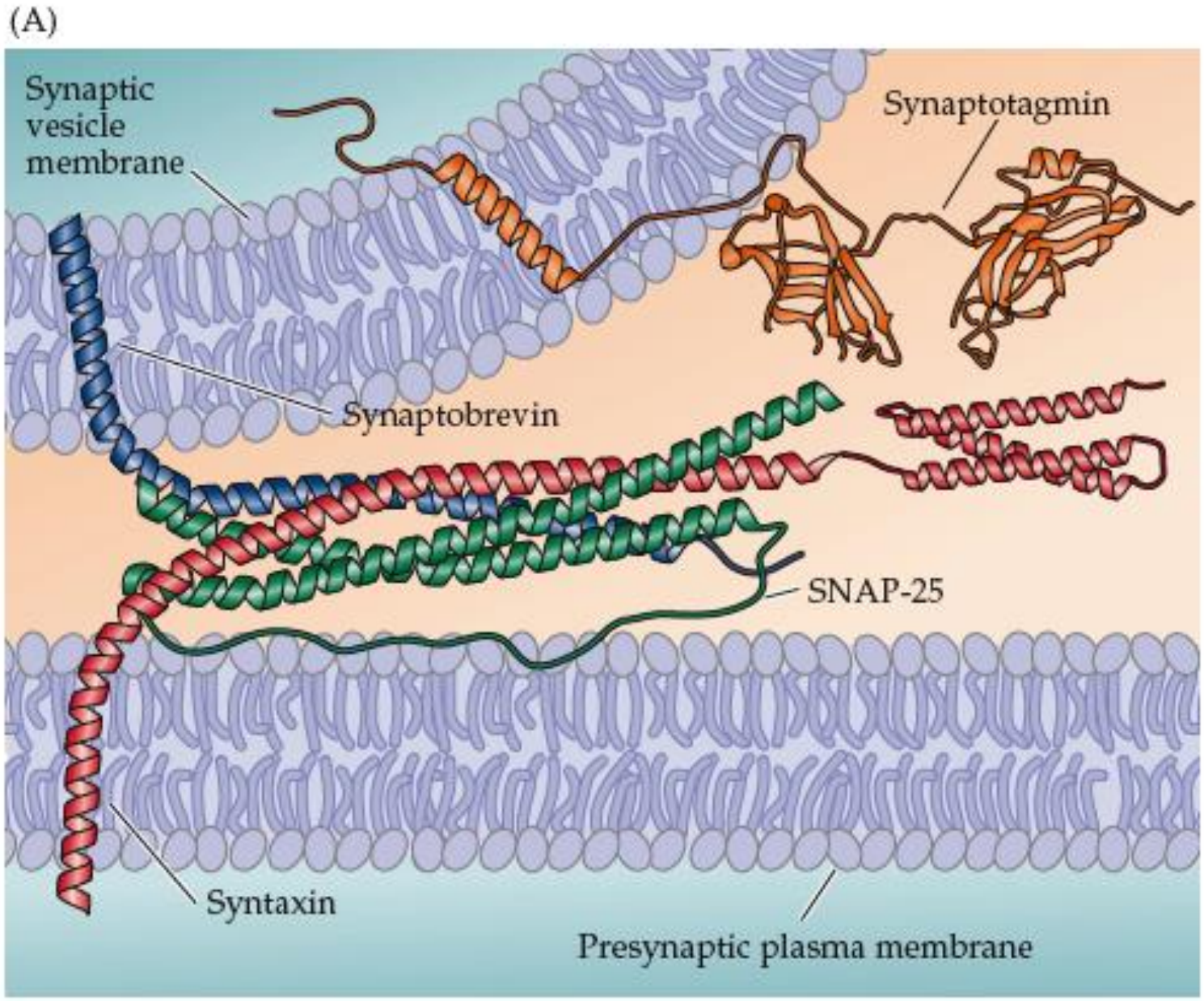

Ca^{2+}-binding proteins
SNARE-associated proteins
Proteins involved in endocytosis
Proteins that form channels, transporters, or receptors
GTP-binding proteins
Miscellaneous important proteins
Synaptobrevin
Synaptic vesicle membrane
Synaptic vesicle
Cysteine string protein
Ca^{2+}/CaM dependent protein kinase II
Synaptotagmin
SV2
Synaptophysin
Synapsin
Snapin
Rab 3
Rabphilin
Dynamin
SNAP
Syndapin
Amphiphysin
AP180
Complexin
RIM
Synaptojanin
Clathrin
AP-2
NSF
DOC2
Tomosyn
nSec1
Hsc70
Auxilin
Syntaphilin
Syntaxin
SNAP-25
Ca^{2+} channel
Neurexin I
CLI
Cytoplasm
Plasma membrane of presynaptic terminal
Synaptic cleft


Complexo SNARE
Sensor de cálcio
Vesicle membrane
Synapsins
Cytoskeleton
1
CAM kinase II
2
Docking complex
3
Fusion pore
4
Vesicle transporters
Trafficking
Rab 3s
Synaptotagmin (P65)
VAMPs (synaptobrevin)
Synaptophysin
γ-SNAP
NSF
α SNAP
SNAP-25
Syntaxins
Neurexins
Ca2+ channel
Gap junction?
Plasma membrane
Tetanus toxin
Botulinum toxins
α - latrotoxin

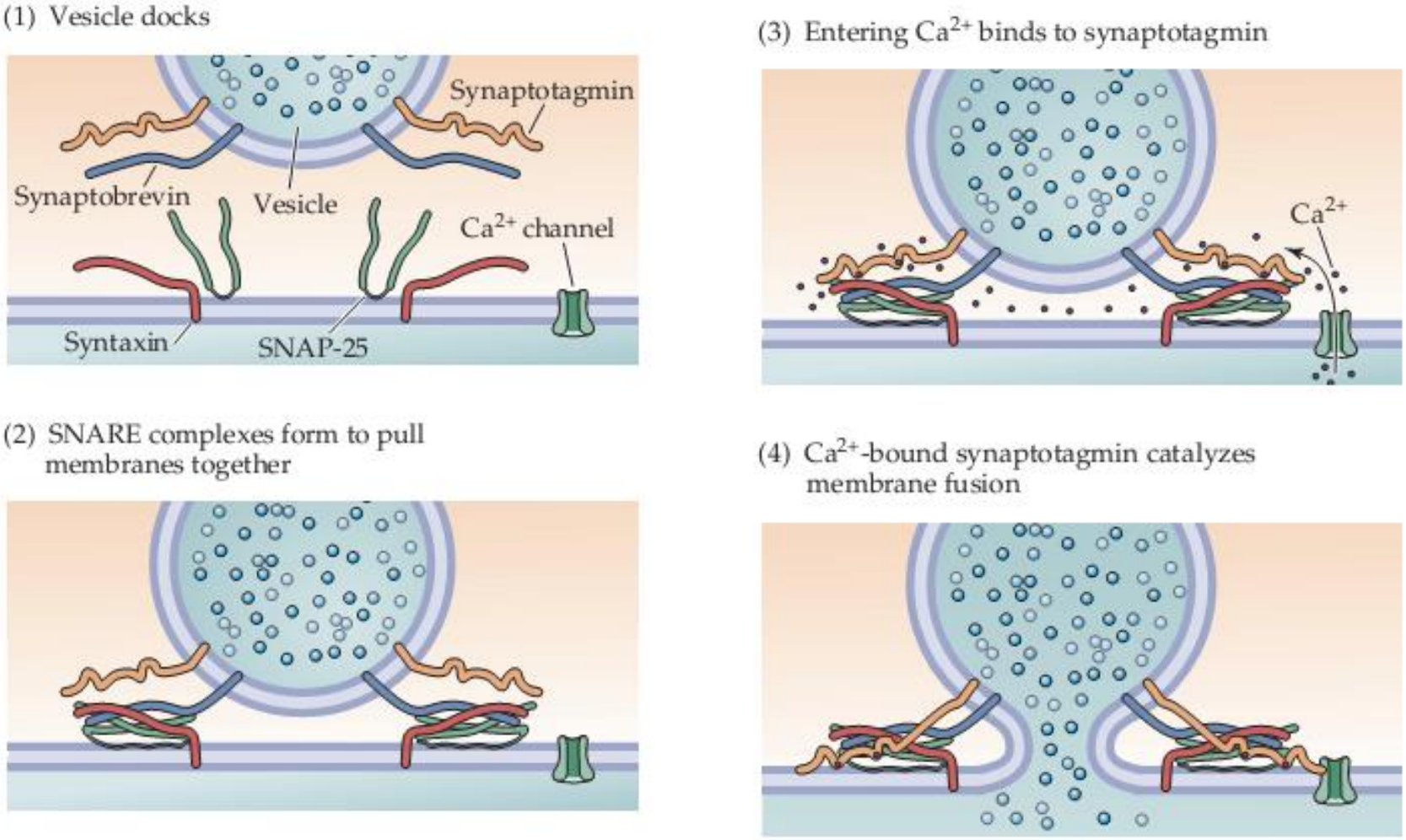
O complexo SNARE ancora as vesículas a membrana e a sinaptotagmina é o sensor de cálcio



Atuação do complexo SNARE

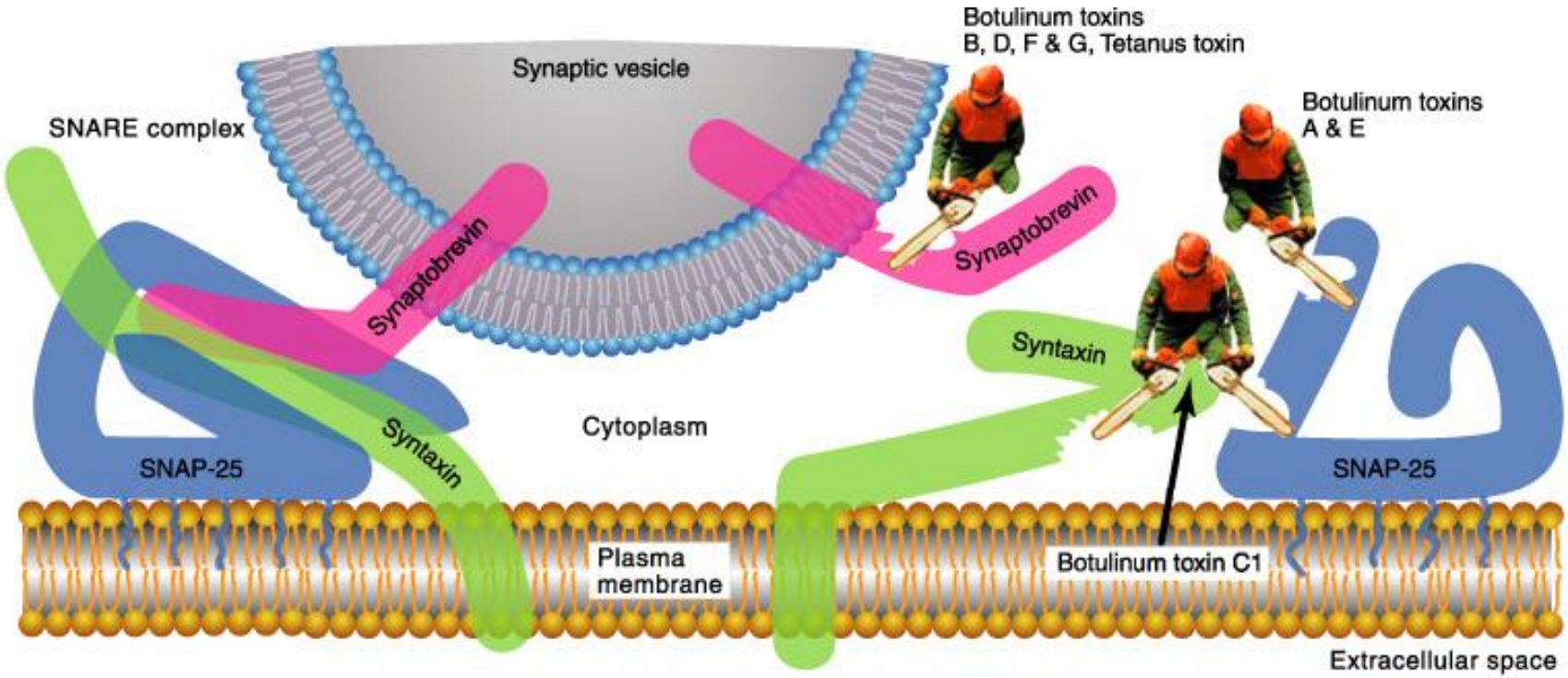


O complexo SNARE ancora as vesículas na membrana e participa do processo de fusão

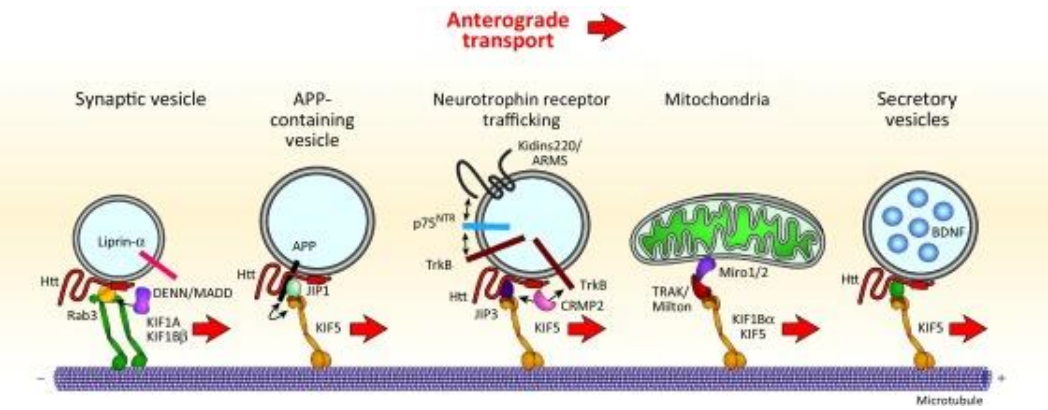


NEUROSCIENCE: Third Edition

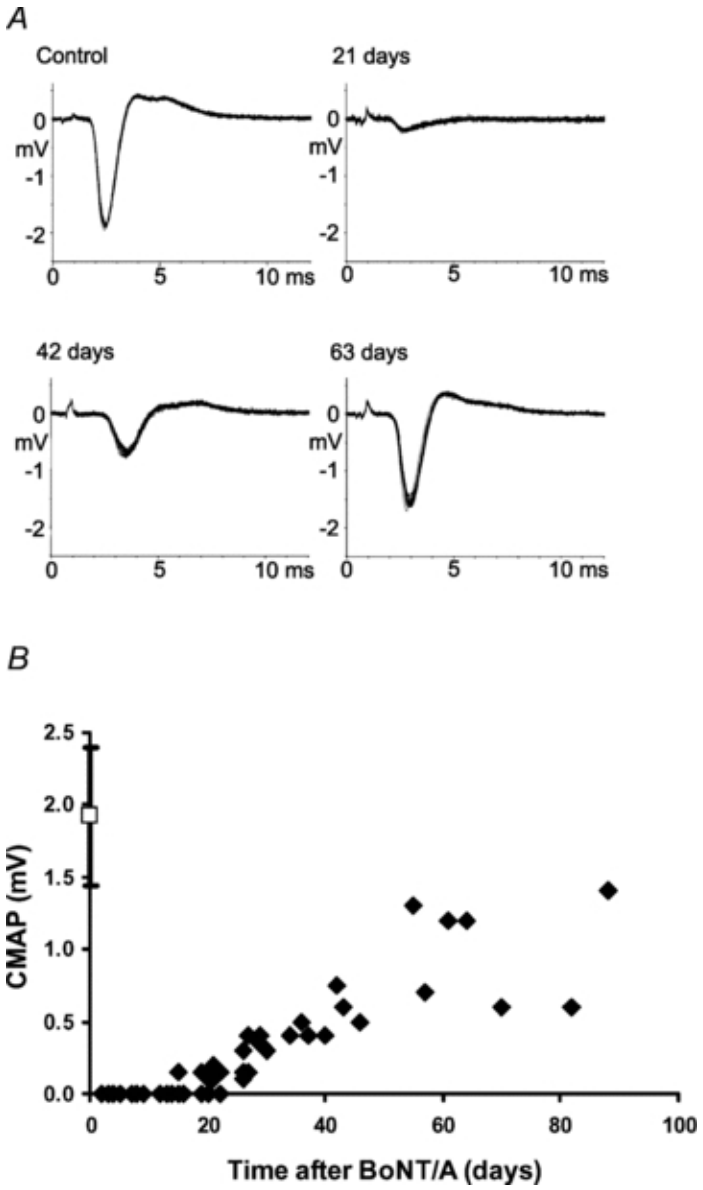
Ação das toxinas clostridiais



Toxina botulinica inibe a neurotransmissão muscular por um longo prazo pois as novas vesículas com novas proteinas SNARE vem do corpo celular por **transporte axonal anterógrado**.



Trends in Biochemical Sciences 2015 40, 597-610DOI: (10.1016/j.tibs.2015.08.003)

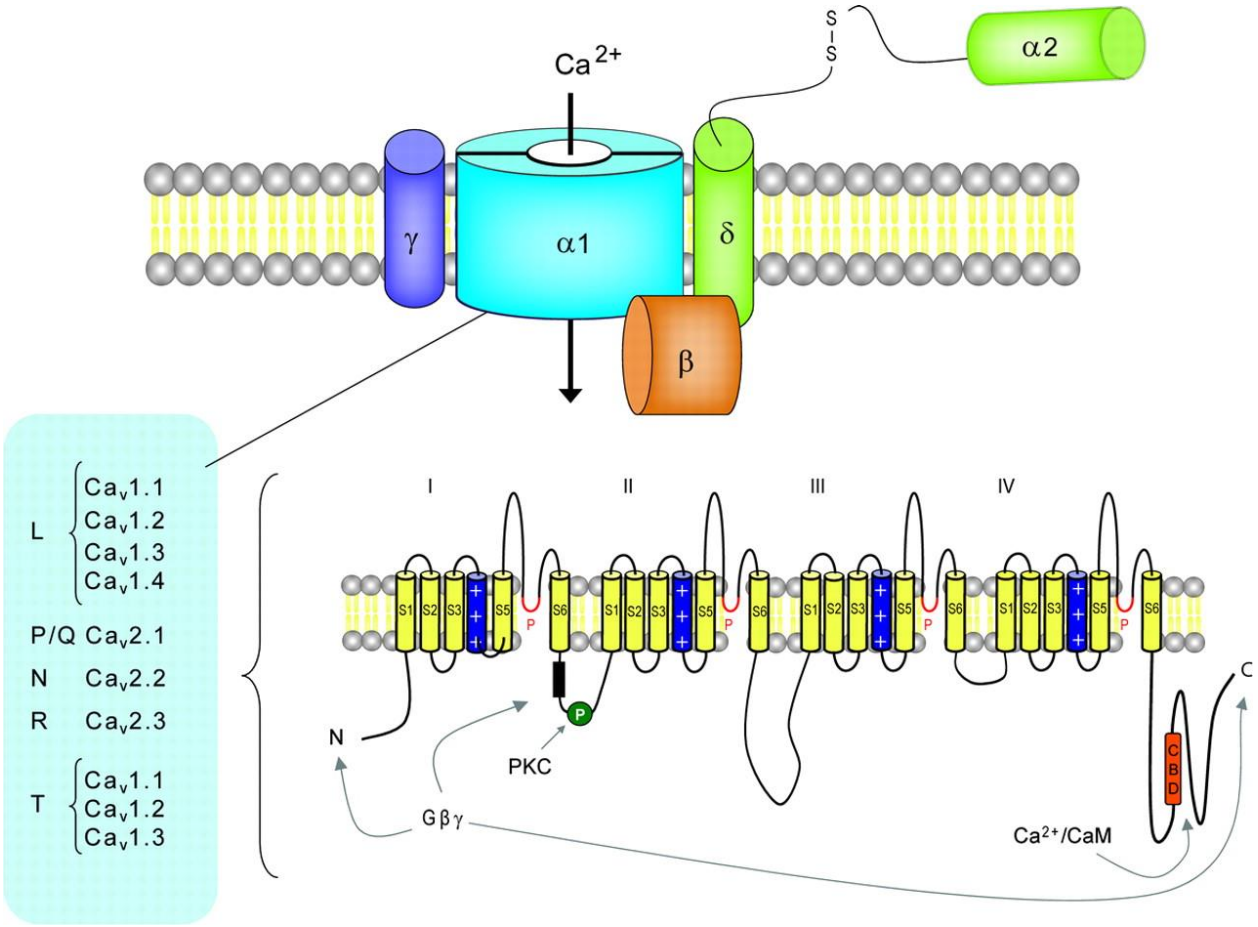


J Physiol 586.13 2008


COffe? café?

A neurotransmissão é TOTALMENTE dependente do cálcio externo!

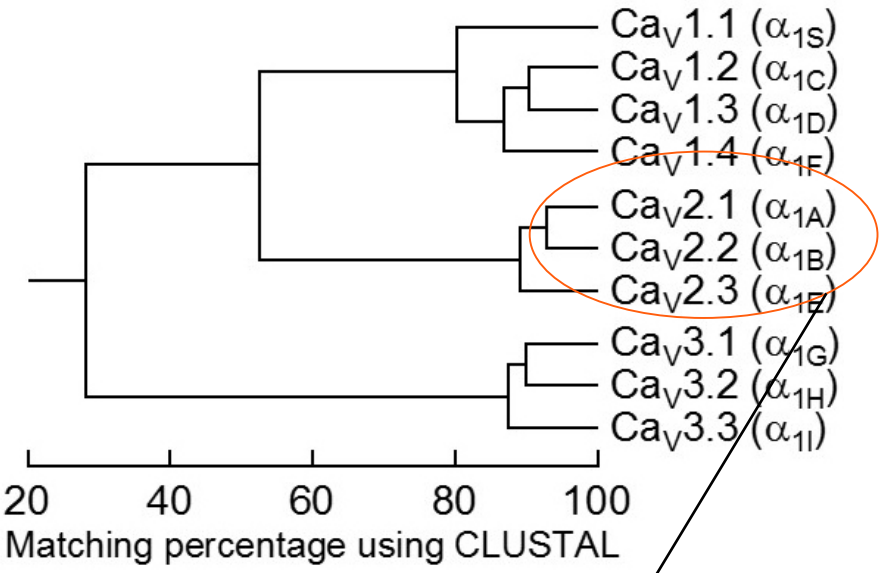
- O cálcio entra por canais de cálcio sensíveis a voltagem localizados na membrana pré-sináptica



Neurology, april 20, 2010; 74 (16)

Diversidade dos canais de cálcio dependentes de voltagem (Ca_v)

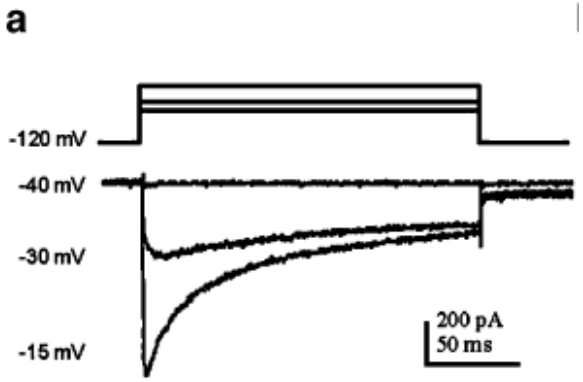
Channel	Current	Localization	Specific Antagonists*	Cellular Functions
$Ca_V1.1$	L	Skeletal muscle; transverse tubules	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; excitation-transcription coupling
$Ca_V1.2$	L	Cardiac myocytes; smooth muscle myocytes; endocrine cells; neuronal cell bodies; proximal dendrites	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; hormone release; regulation of transcription; synaptic integration
$Ca_V1.3$	L	Endocrine cells; neuronal cell bodies and dendrites; cardiac atrial myocytes and pacemaker cells; cochlear hair cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Hormone release; regulation of transcription; synaptic regulation; cardiac pacemaking; hearing; neurotransmitter release from sensory cells
$Ca_V1.4$	L	Retinal rod and bipolar cells	Dihydropyridines (low affinity)	Neurotransmitter release from photoreceptors
$Ca_V2.1$	P/Q	Nerve terminals and dendrites; neuroendocrine cells	ω-Agatoxin IVA	Neurotransmitter release; dendritic Ca^{2+} transients; hormone release
$Ca_V2.2$	N	Nerve terminals and dendrites; neuroendocrine cells	ω-Conotoxin-GVIA	Neurotransmitter release; dendritic Ca^{2+} transients; hormone release
$Ca_V2.3$	R	Neuronal cell bodies and dendrites	SNX-482	Repetitive firing; dendritic Ca^{2+} transients
$Ca_V3.1$	T	Neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
$Ca_V3.2$	T	Neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
$Ca_V3.3$	T	Neuronal cell bodies and dendrites	None	Pacemaking; repetitive firing

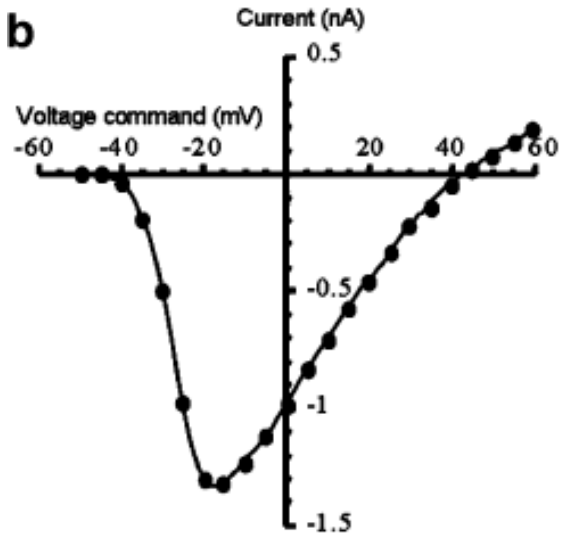


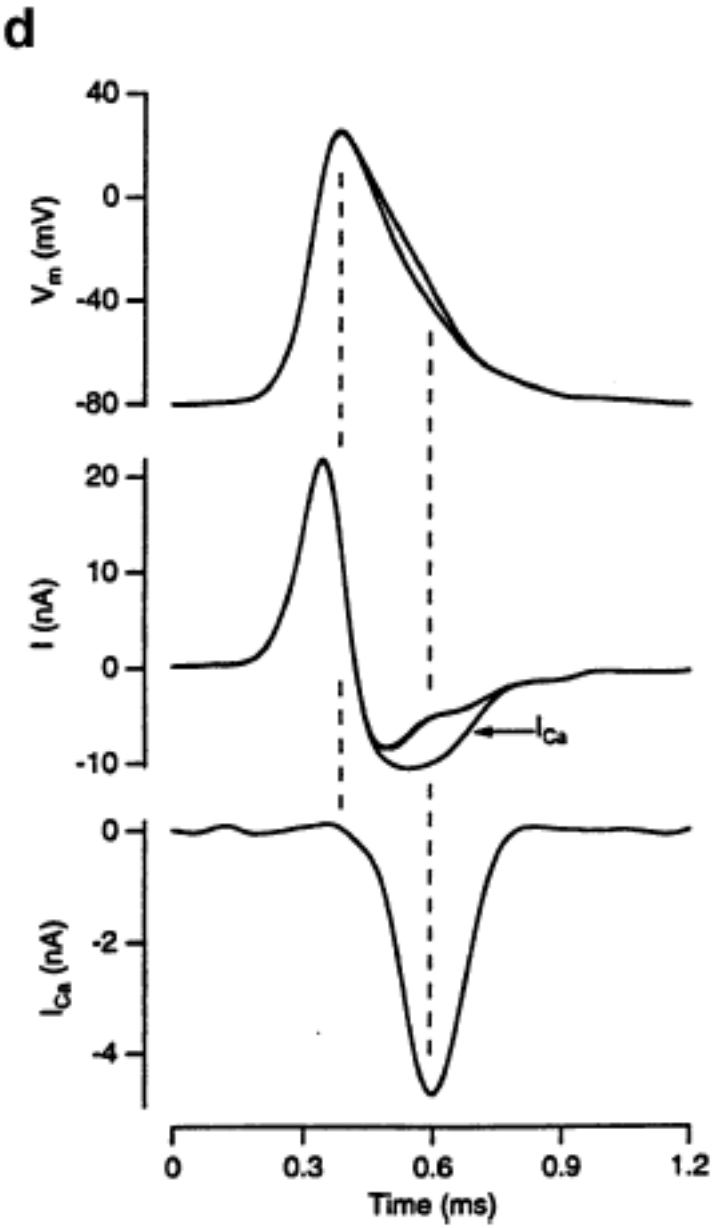
neurotransmissão

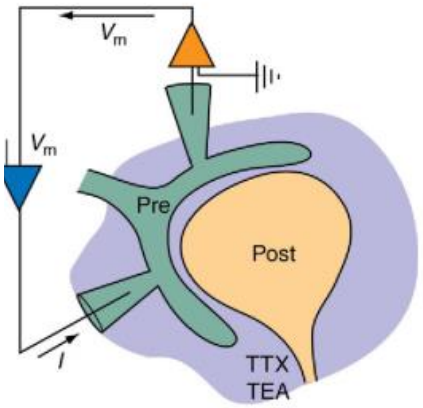
Correntes de cálcio pré-sinápticas

Calyx of Held

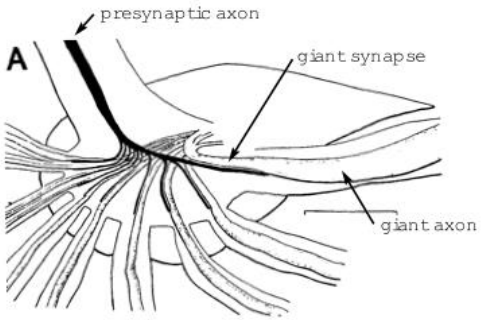


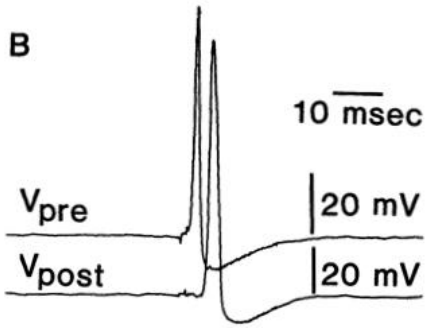


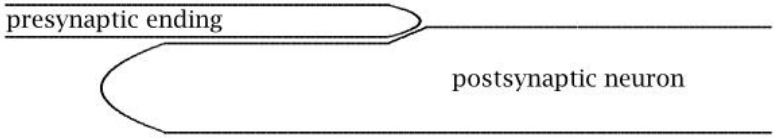




Sinapse gigante da lula (gânglio estrelado) – década de 70/80



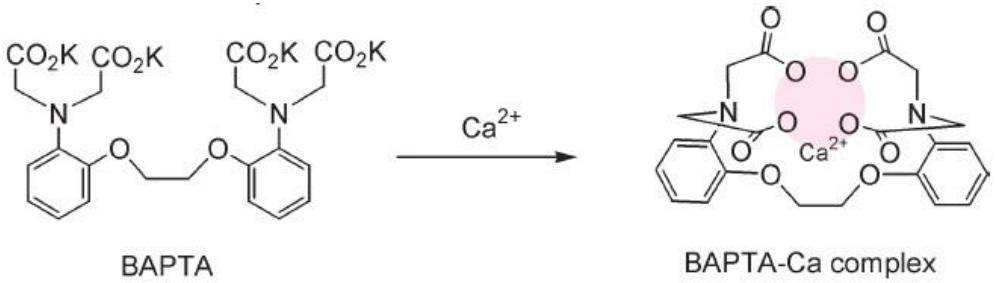


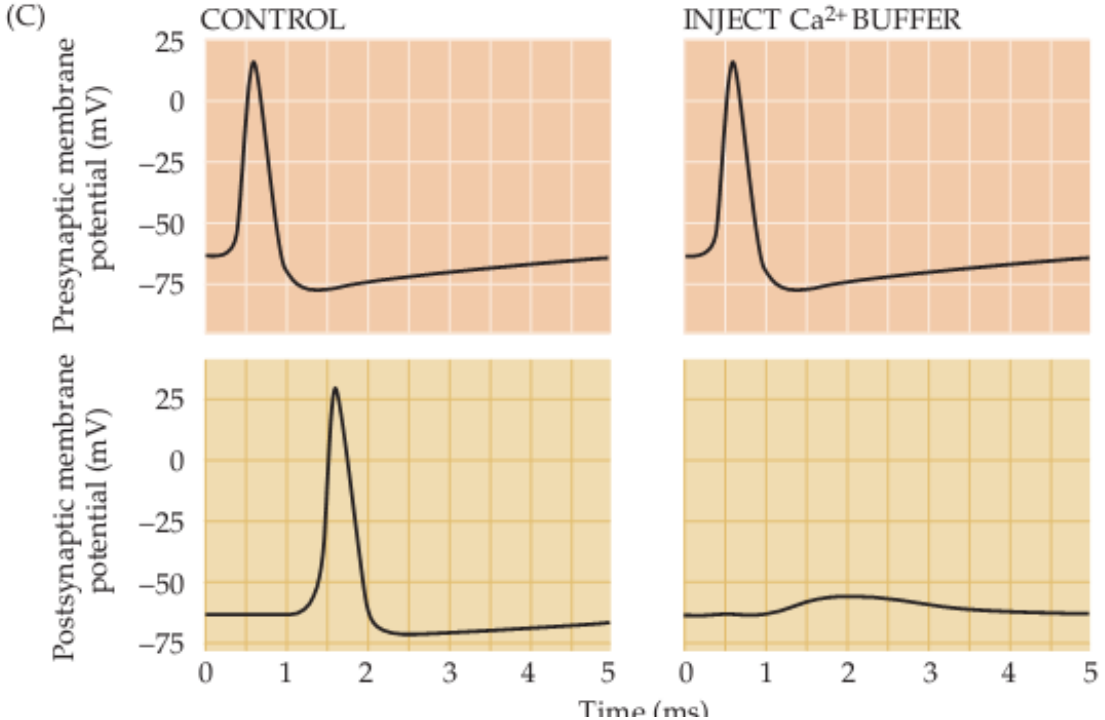


RODOLFO LLINÁS, IZCHAK Z. STEINBERG*, AND KERRY WALTON

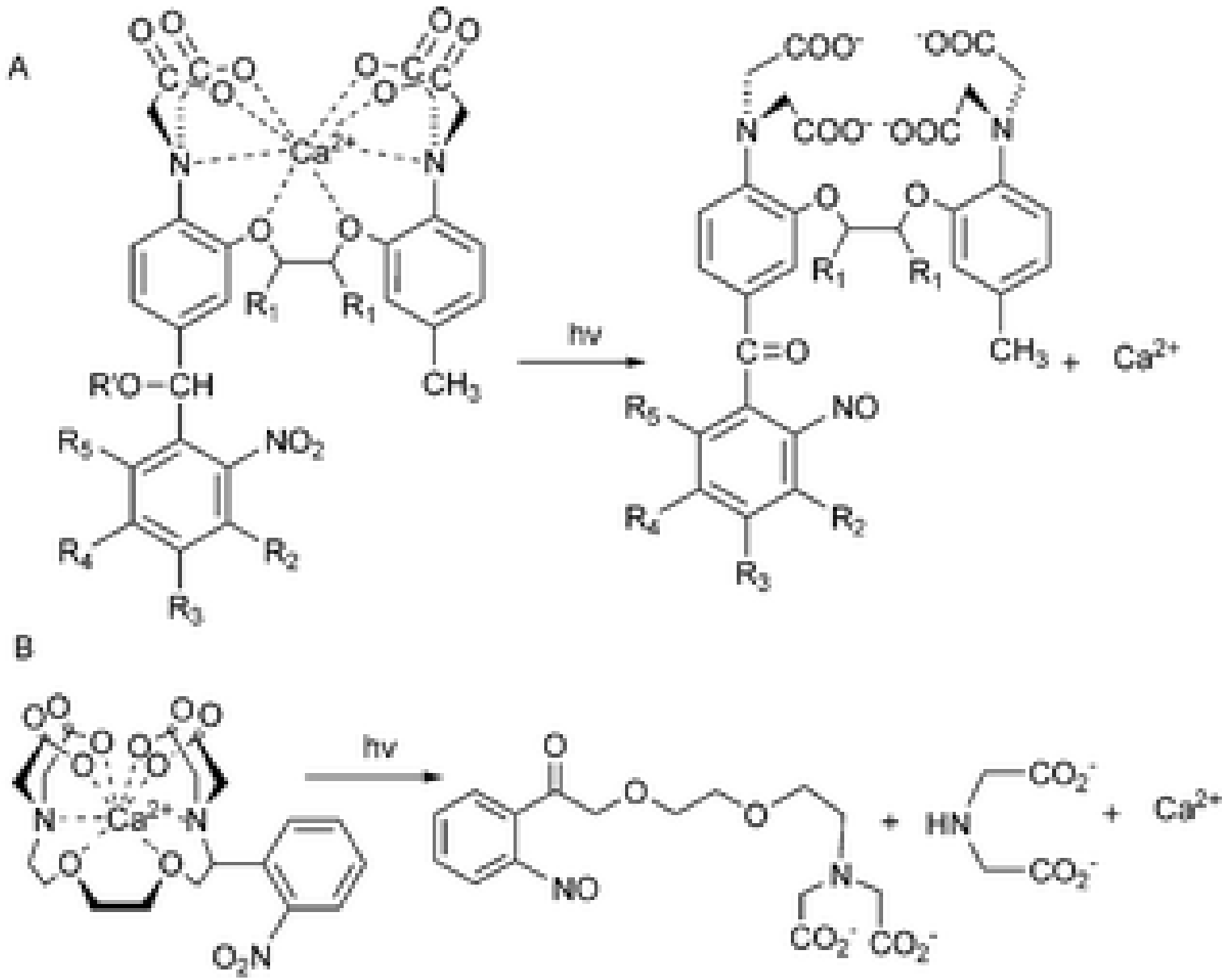
Division of Neurobiology, University of Iowa, Oakdale Iowa 52319, and Neurosciences Research Program, Massachusetts Institute of Technology, Boston, Mass. 02130

A neurotransmissão é dependente da entrada de cálcio no terminal

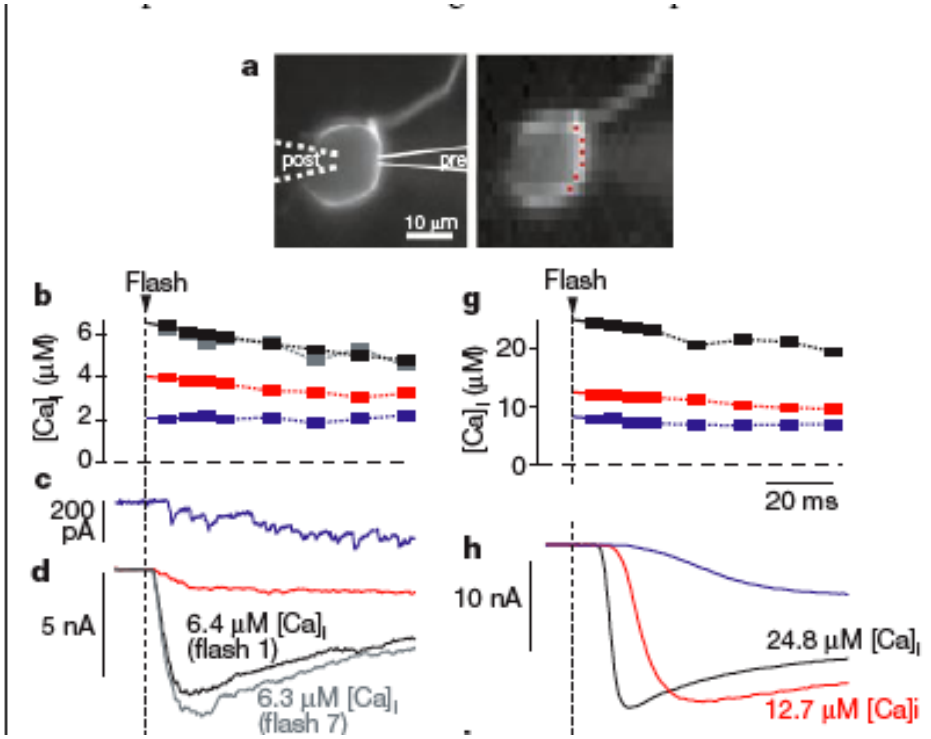


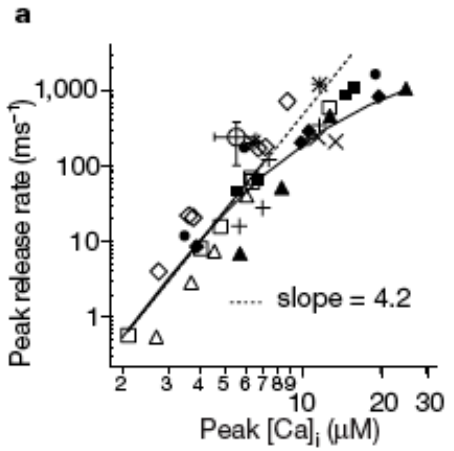


Experimentos de fotólise de cálcio no cálice de Held revelaram a sensitividade ao cálcio dessa sinapse central



Experimentos de fotólise de cálcio no cálice de Held revelaram a sensitividade ao cálcio dessa sinapse central





$$V \xrightleftharpoons[k_{off}\, b^0]{Ca^{2+}\ 5k_{on}} V_{Ca} \quad \cdots \quad V_{4Ca} \xrightleftharpoons[5k_{off}\, b^4]{Ca^{2+}\ k_{on}} V_{5Ca} \xrightarrow{\gamma} \text{fused}$$

Intracellular calcium dependence of transmitter release rates at a fast central synapse

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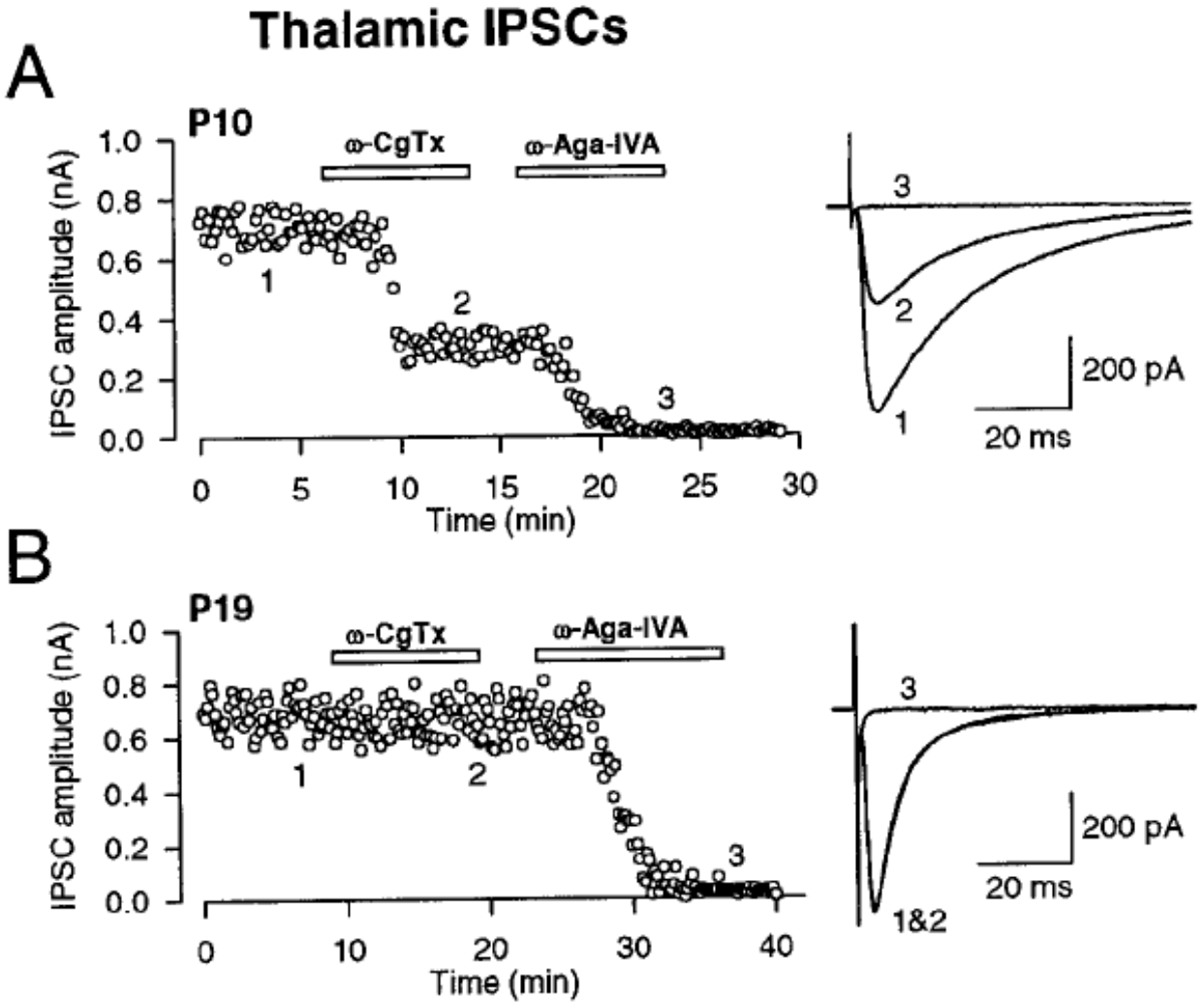
Abteilung Membranbiophysik, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg 11, D-37077 Göttingen, Germany

NATURE | VOL 406 | 24 AUGUST 2000 | www.nature.com

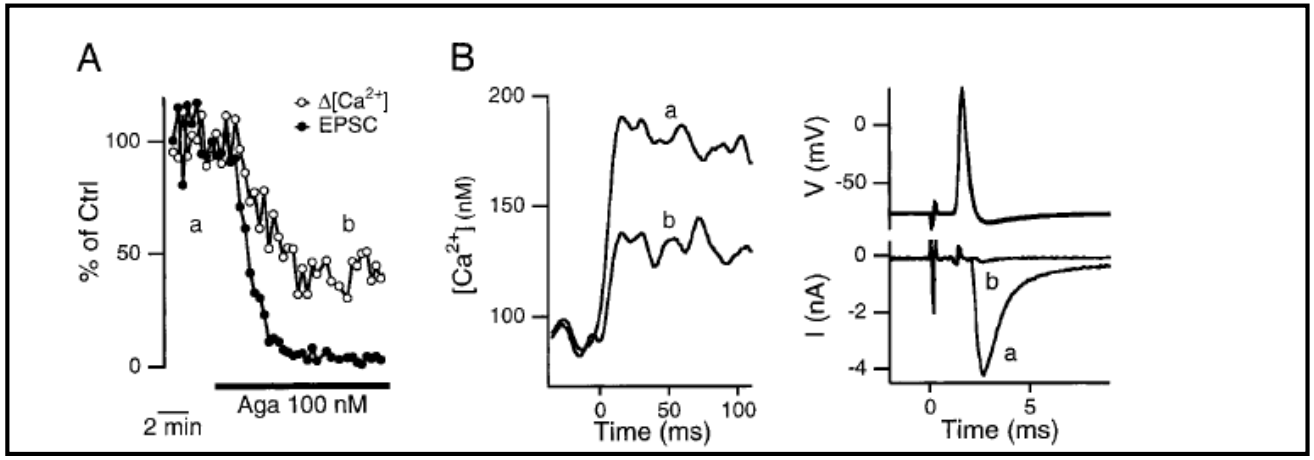
Canais de cálcio pré-sinápticos possuem farmacologia distinta

Canais N ($Ca_v2.2$): ω-conotoxina GVIA
Canais P/Q ($Ca_v2.1$): ω-Agatoxina IVA / ω-conotoxina MVIIC
Canais R ($Ca_v2.3$): sem bloqueador específico

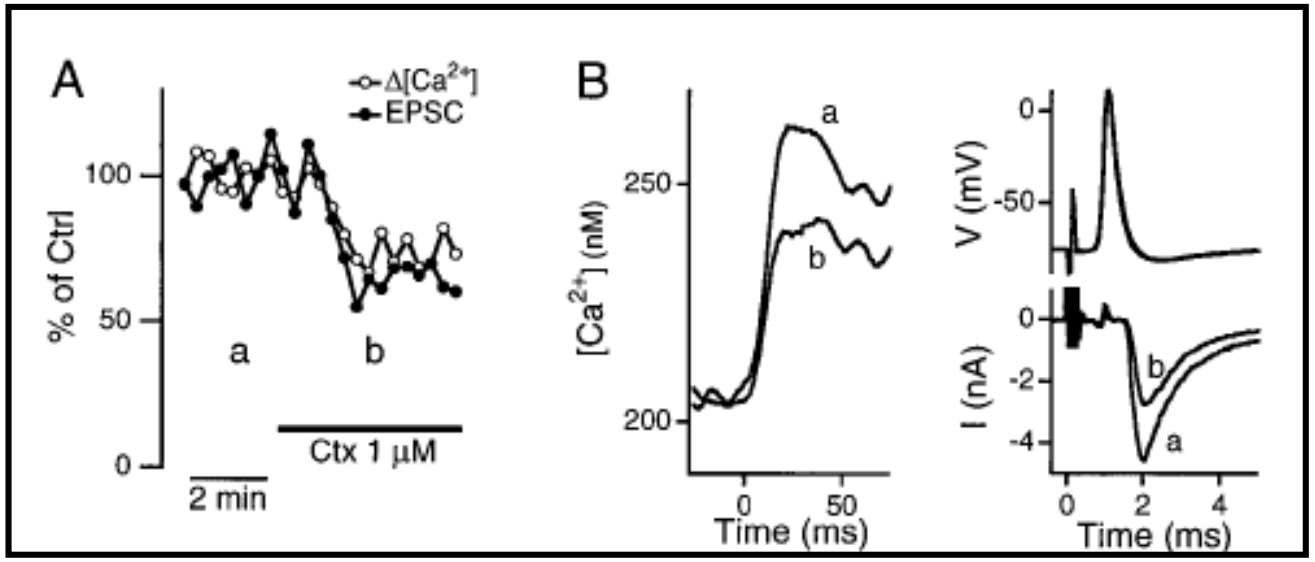
Alterações desenvolvimentais na dependência da neurotransmissão dos canais de cálcio



Diferentes eficiências da acoplação cálcio-exocitose na sinapse Calyx de Held

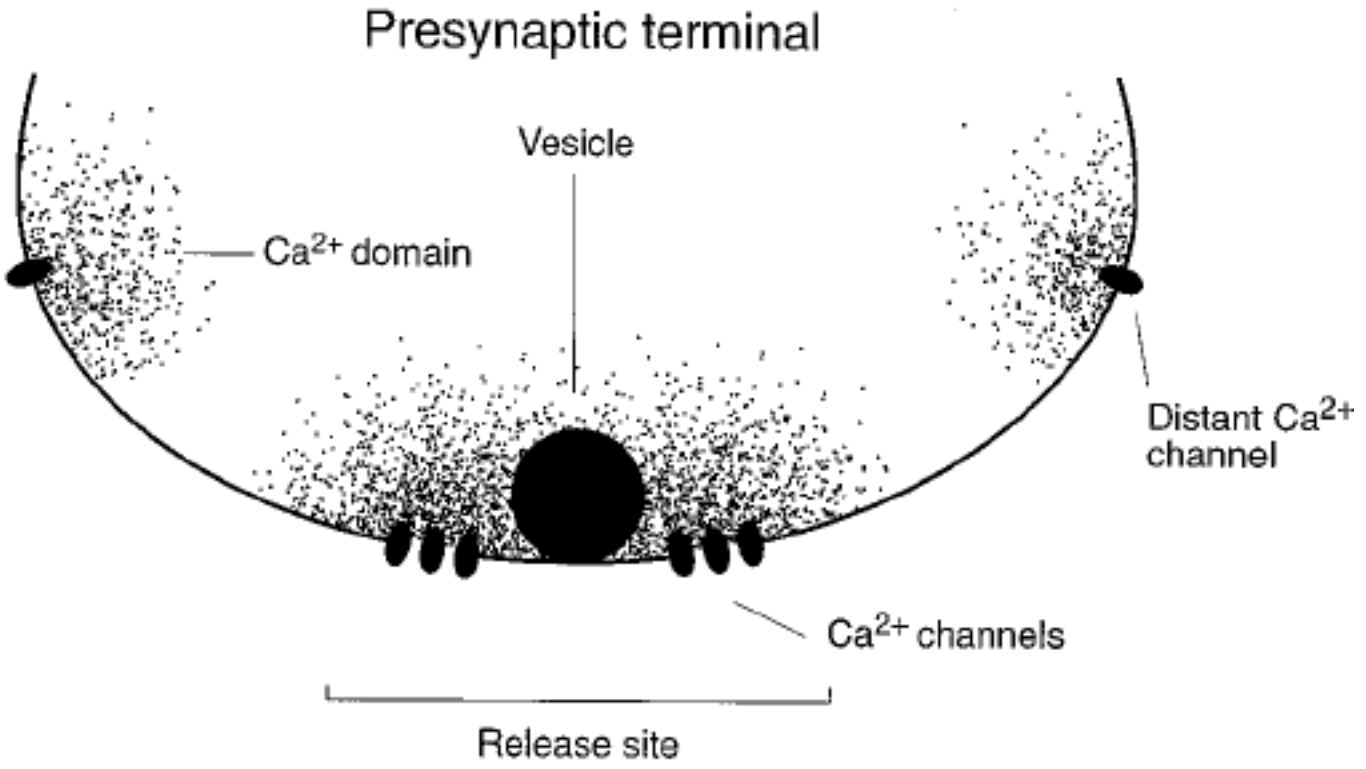


Bloqueio dos canais P/Q pela ω–AgaIV

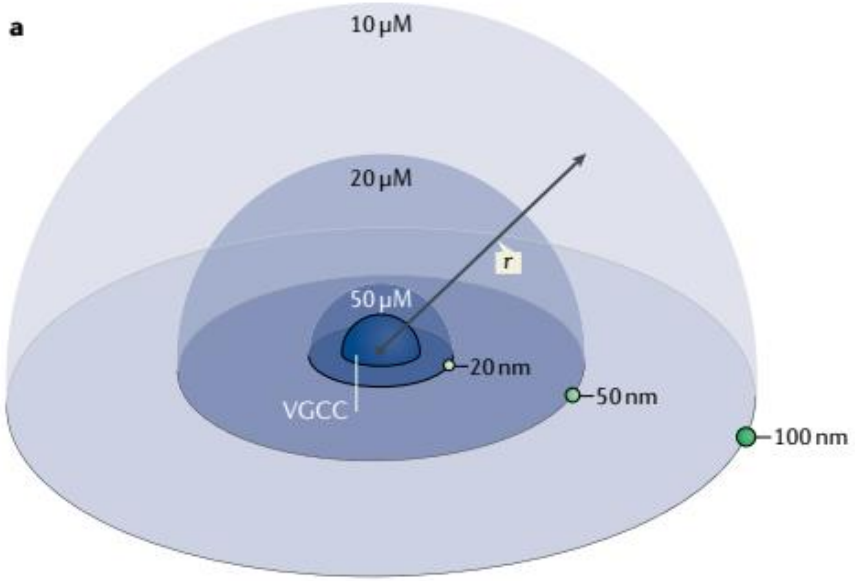


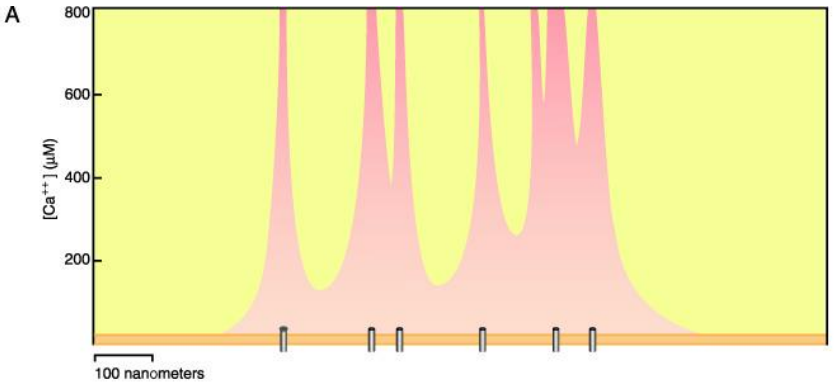
Bloqueio dos canais NQ pela ω–Ctx GVIA

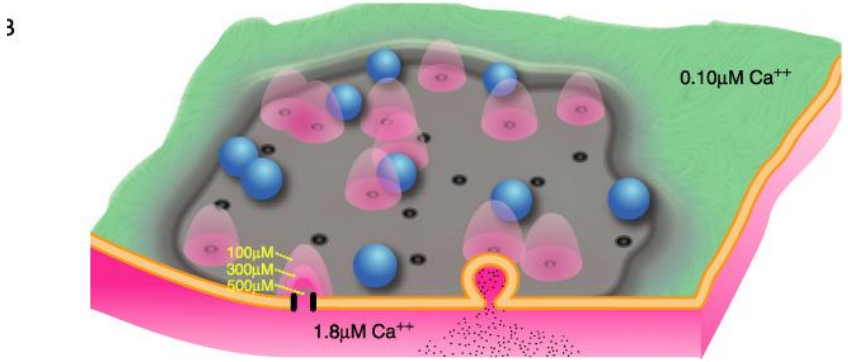
Canais de cálcio mais e menos acoplados as vesículas

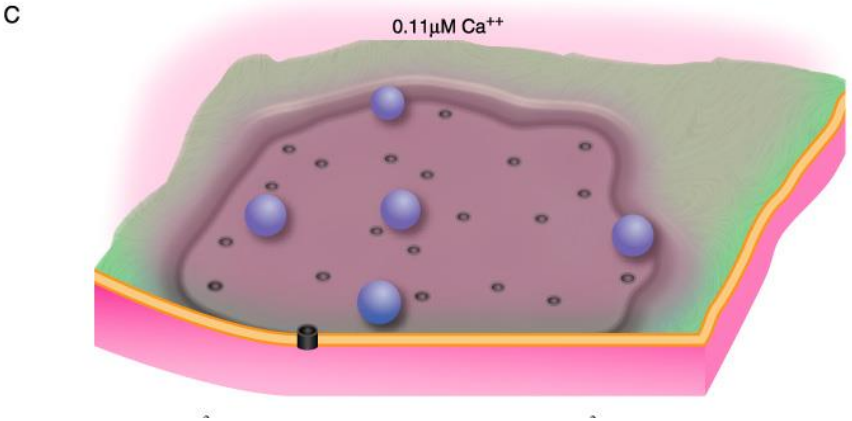


As vesículas são expostas a microdomínios de cálcio

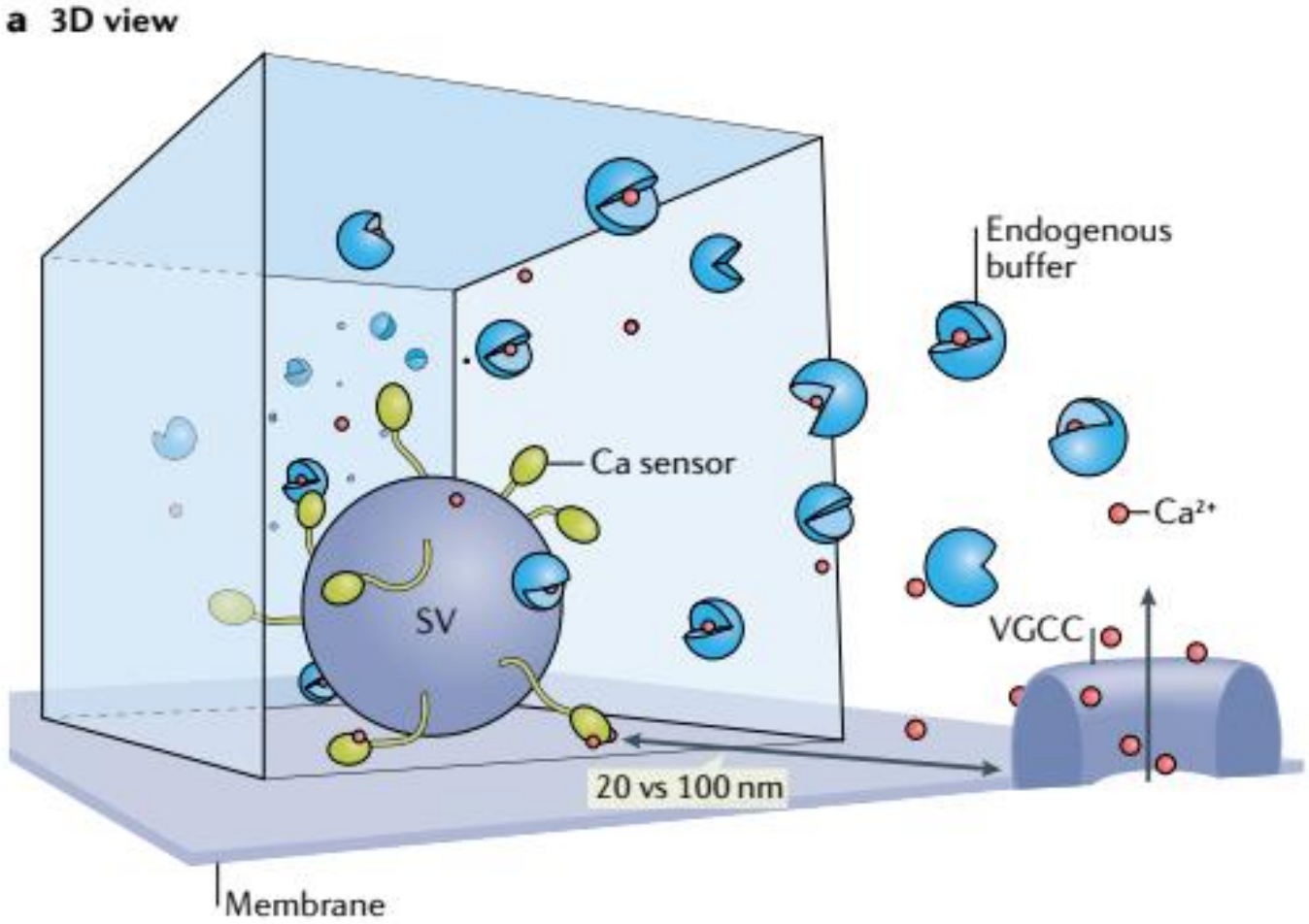




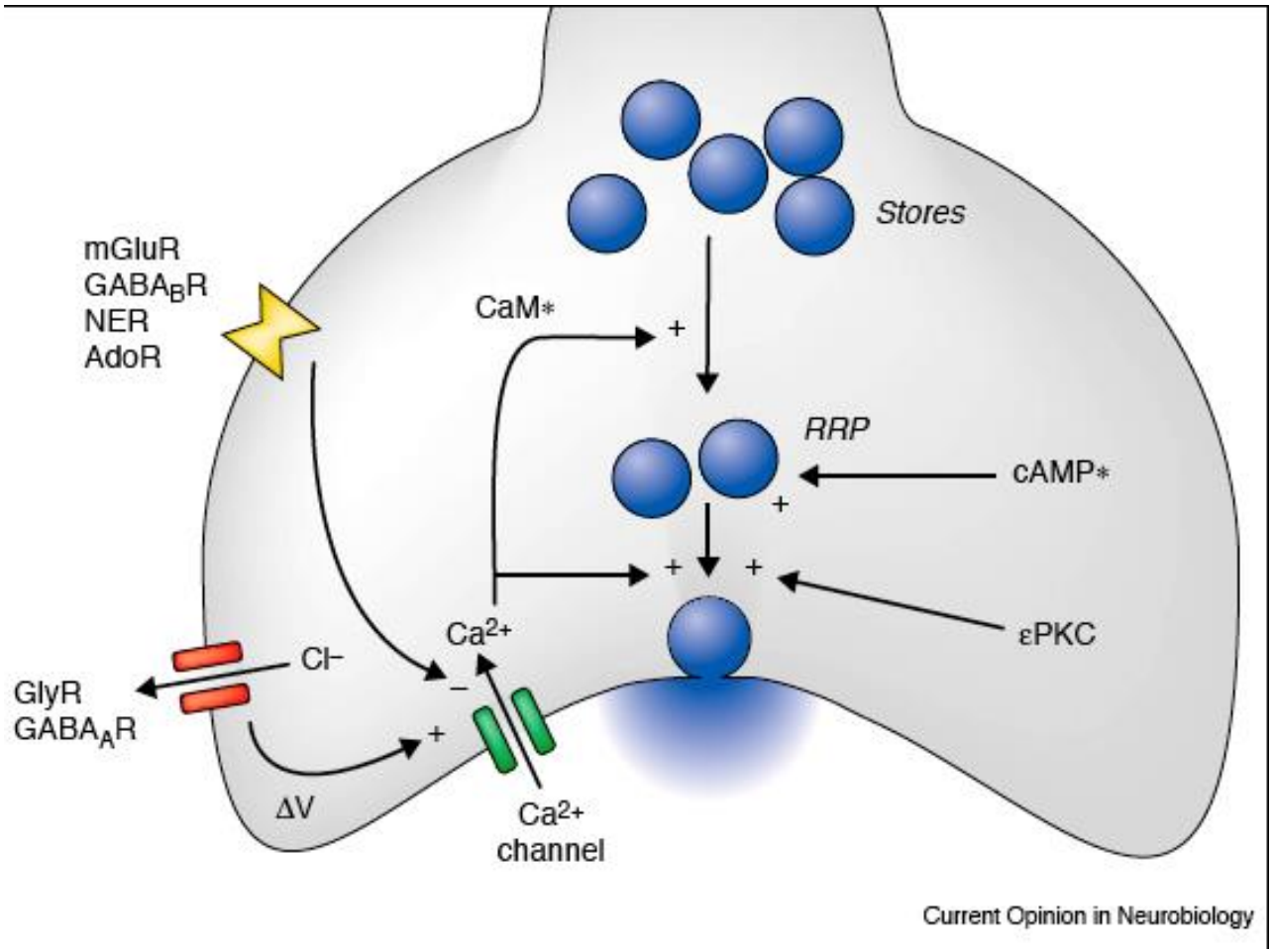




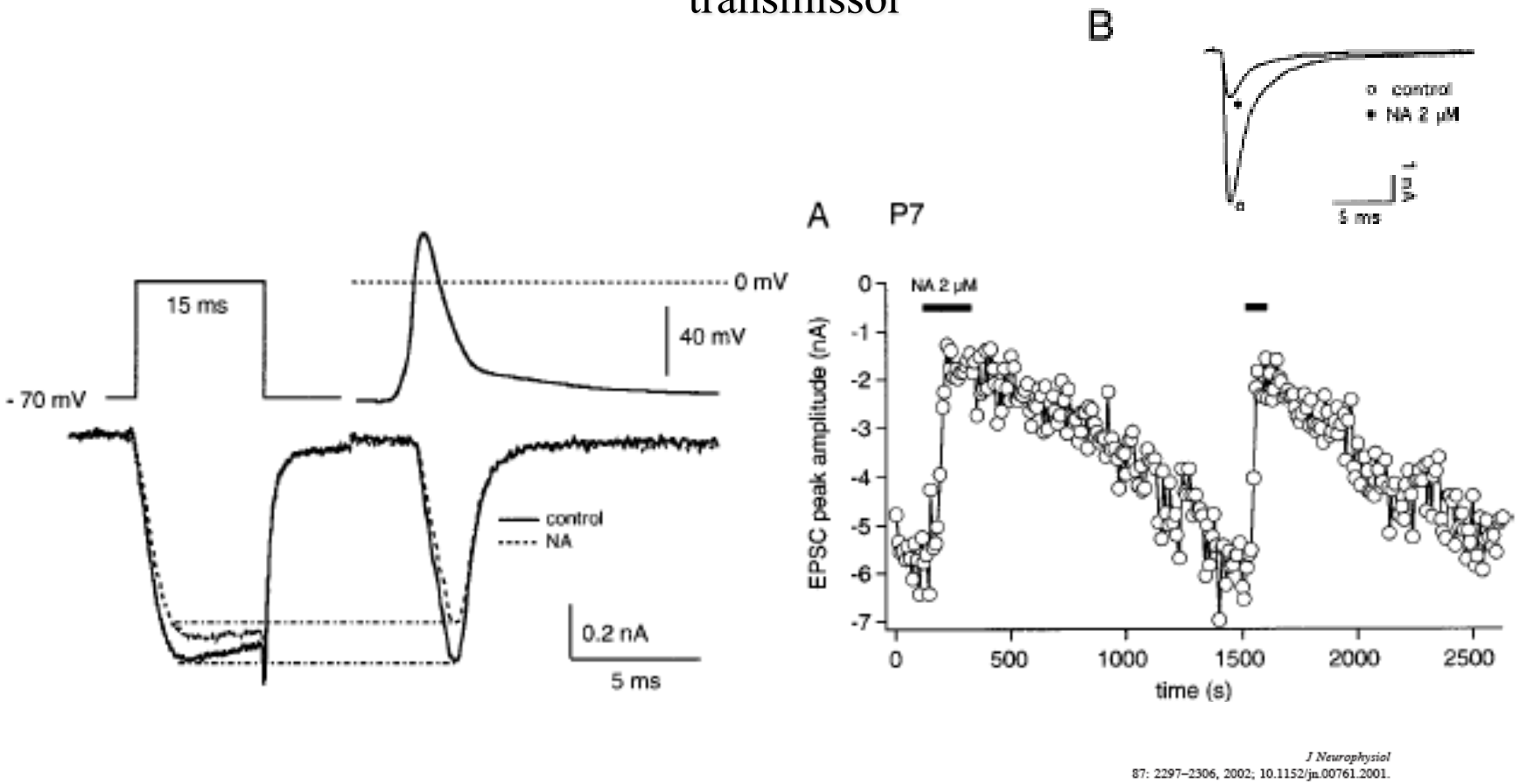
Proteínas ligantes de cálcio competem com a sinaptotagmina pelo cálcio



A liberação de neurotransmissores é intensamente modulada



Uma pequena redução das correntes de cálcio pré-sinápticas por receptores ligados a proteínas G diminuem bastante a liberação de transmissor



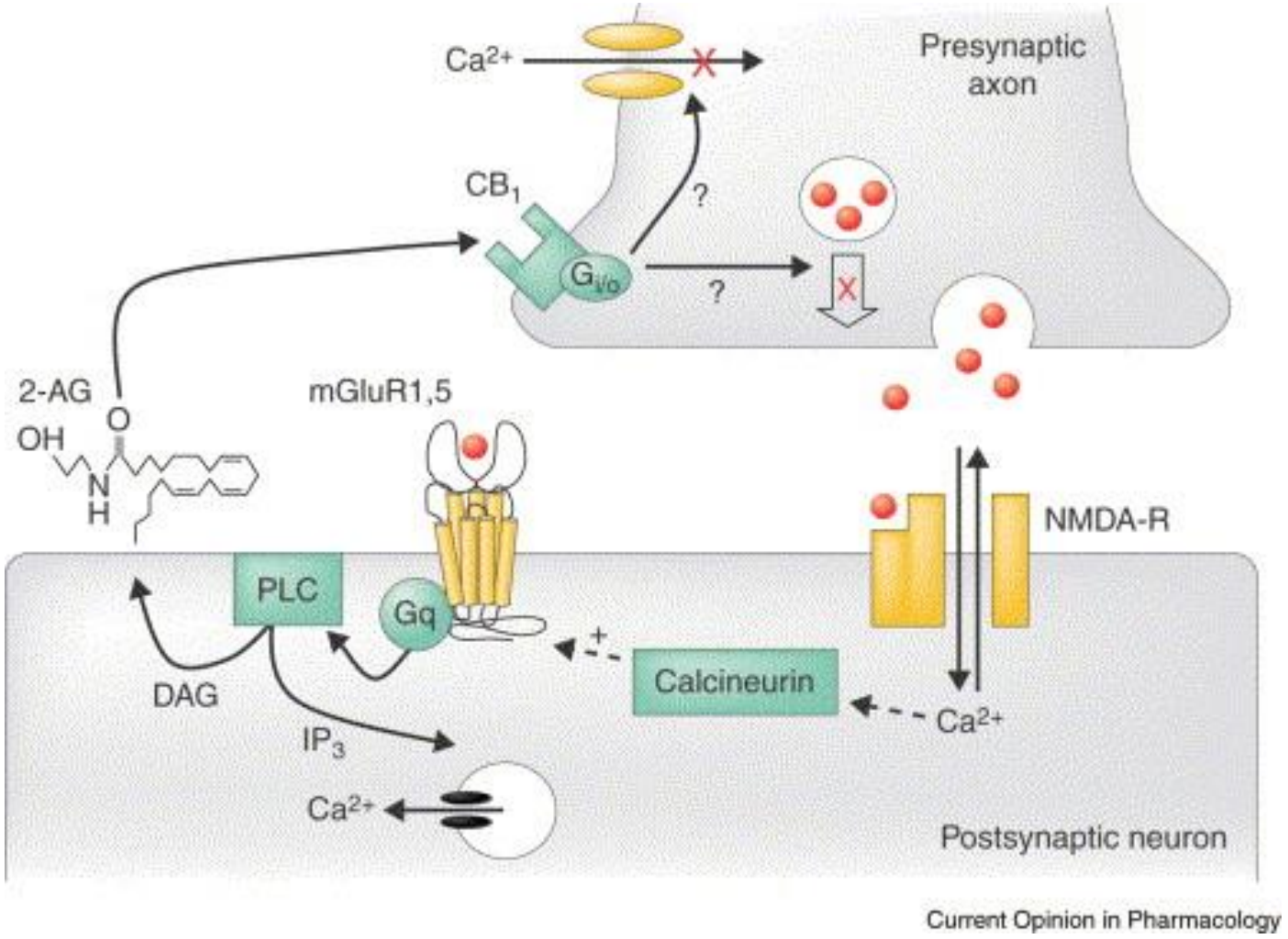
J Neurophysiol
87: 2297–2306, 2002; 10.1152/jn.00761.2001.

Noradrenaline Increases High-Frequency Firing at the Calyx of Held Synapse During Development by Inhibiting Glutamate Release

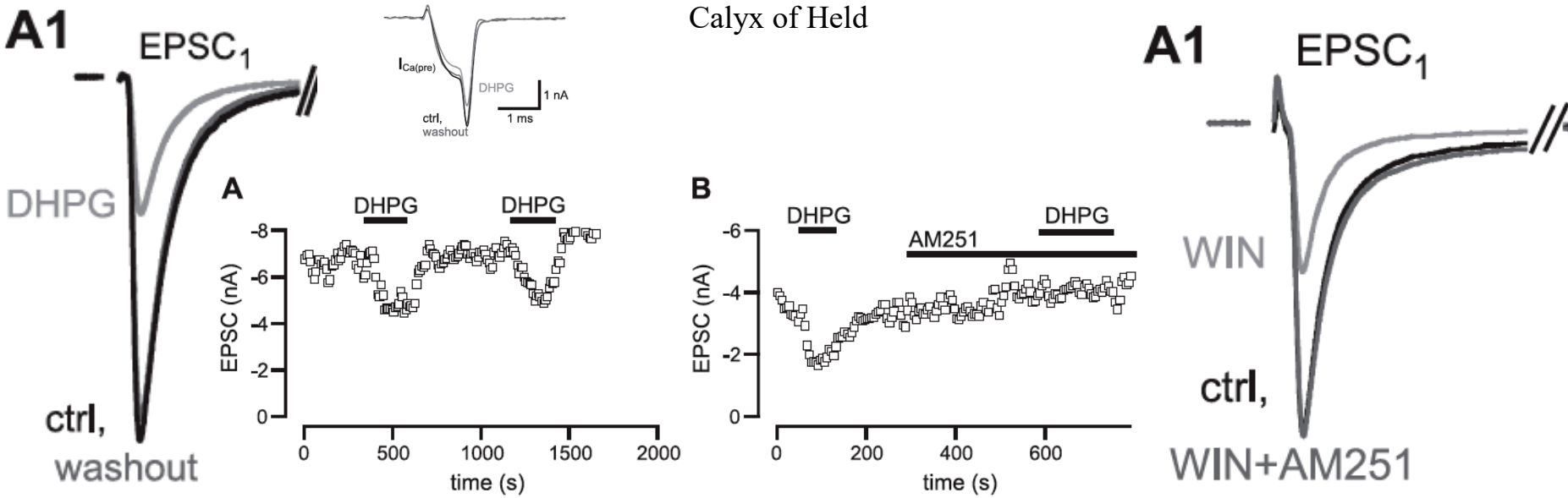
RICARDO M. LEÃO AND HENRIQUE VON GERSDORFF
The Vollum Institute, Oregon Health and Science University, Portland, Oregon 97201-3098

Inibição retrógrada da neurotransmissão por endocanabinóides produzidos no neurônio pós-sináptico

Calyx of Held



Inibição retrógrada da neurotransmissão por endocanabinóides produzidos no neurônio pós-sináptico



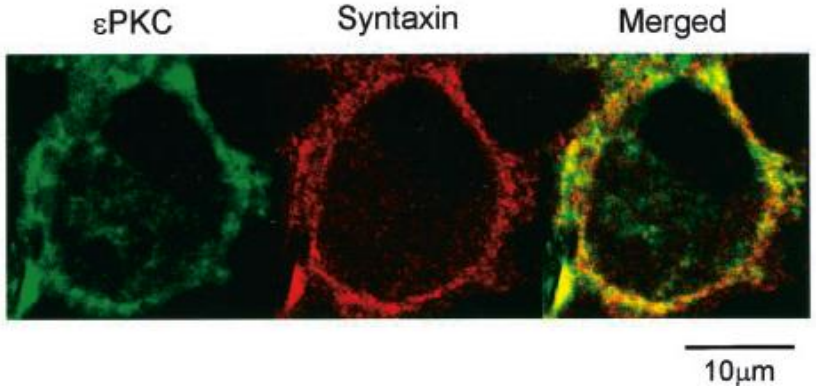
Cellular/Molecular

Retroinhibition of Presynaptic Ca^{2+} Currents by Endocannabinoids Released via Postsynaptic mGluR Activation at a Calyx Synapse

Christopher Kushmerick,[1]* Gareth D. Price,[1]* Holger Taschenberger,[2] Nagore Puente,[4] Robert Renden,[1] Jacques I. Wadiche,[1] Robert M. Duvoisin,[3] Pedro Grandes,[4] and Henrique von Gersdorff[1]

[1]The Vollum Institute, Oregon Health and Science University, Portland, Oregon 97239, [2]Max Planck Institute for Biophysical Chemistry, D-37077 Göttingen, Germany, [3]The Neurological Sciences Institute, Oregon Health and Science University, Beaverton, Oregon 97006, and [4]Department of Neurosciences, Faculty of Medicine and Dentistry, Basque Country University, 699-48080 Bilbao, Spain

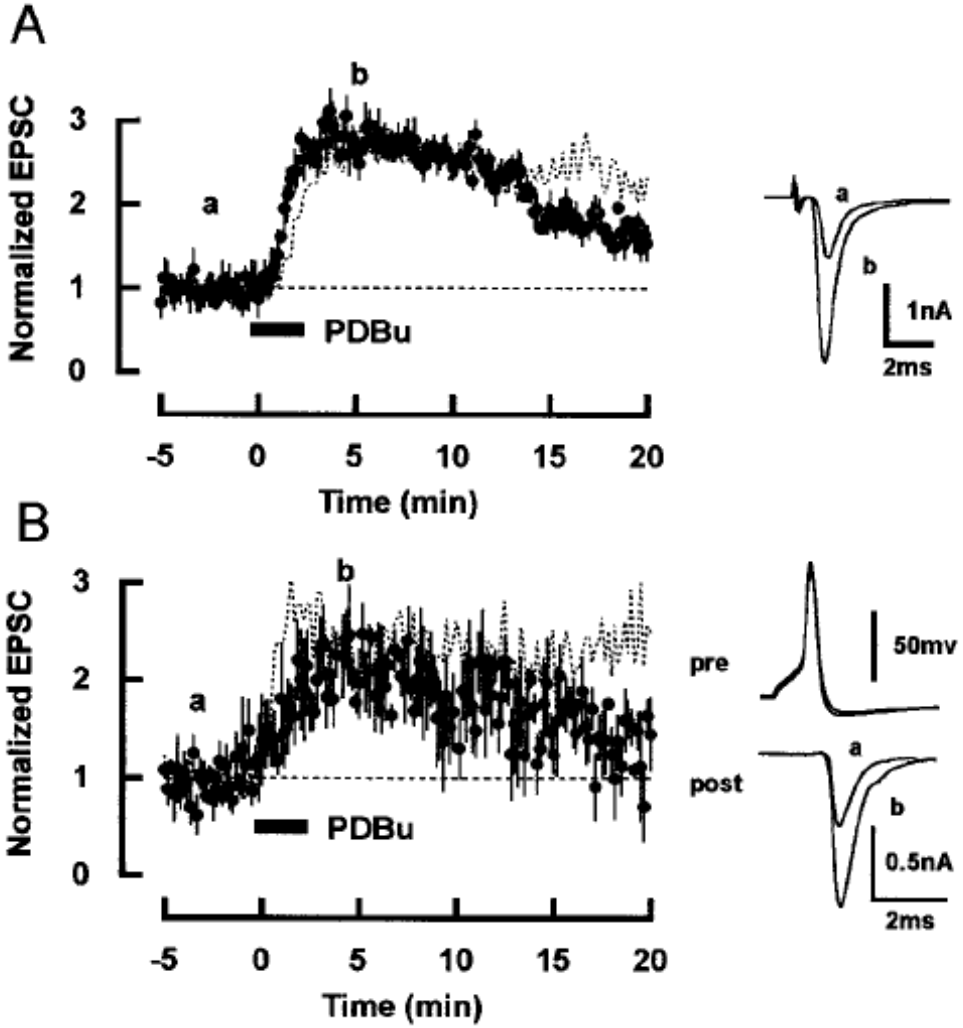
Potenciação independente de cálcio da neurotransmissão pela PKC



Activation of the epsilon isoform of protein kinase C in the mammalian nerve terminal

Naoto Saitoh, Tetsuya Hori, and Tomoyuki Takahashi*

Department of Neurophysiology, University of Tokyo, Graduate School of Medicine, Tokyo 113-0033, Japan

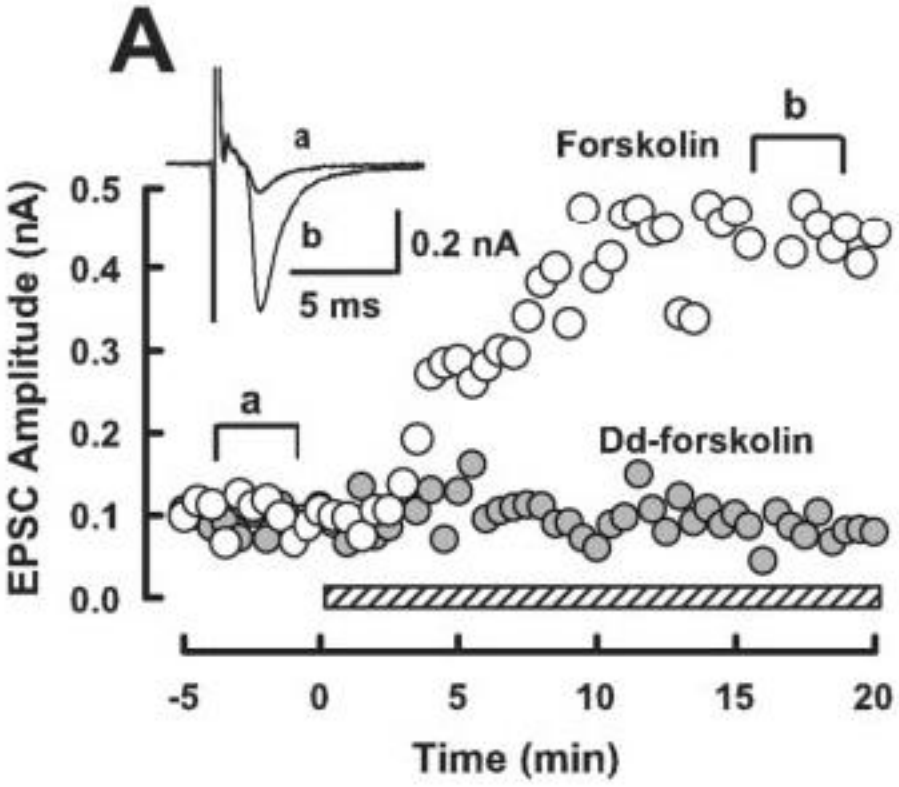


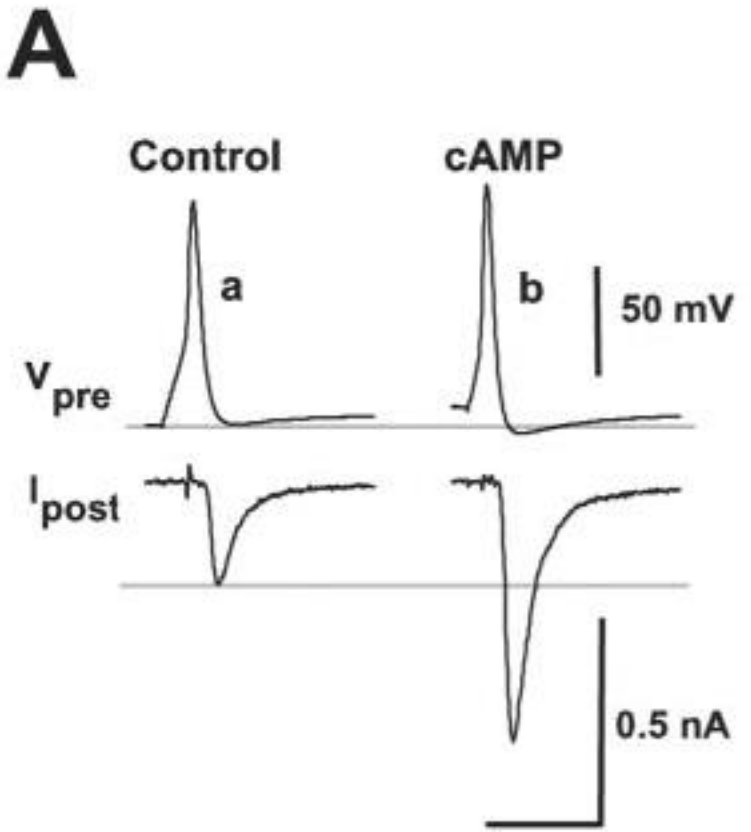
Potenciação da neurotransmissão pelo cAMP

Calyx of Held

Presynaptic Mechanism Underlying cAMP-Dependent Synaptic Potentiation

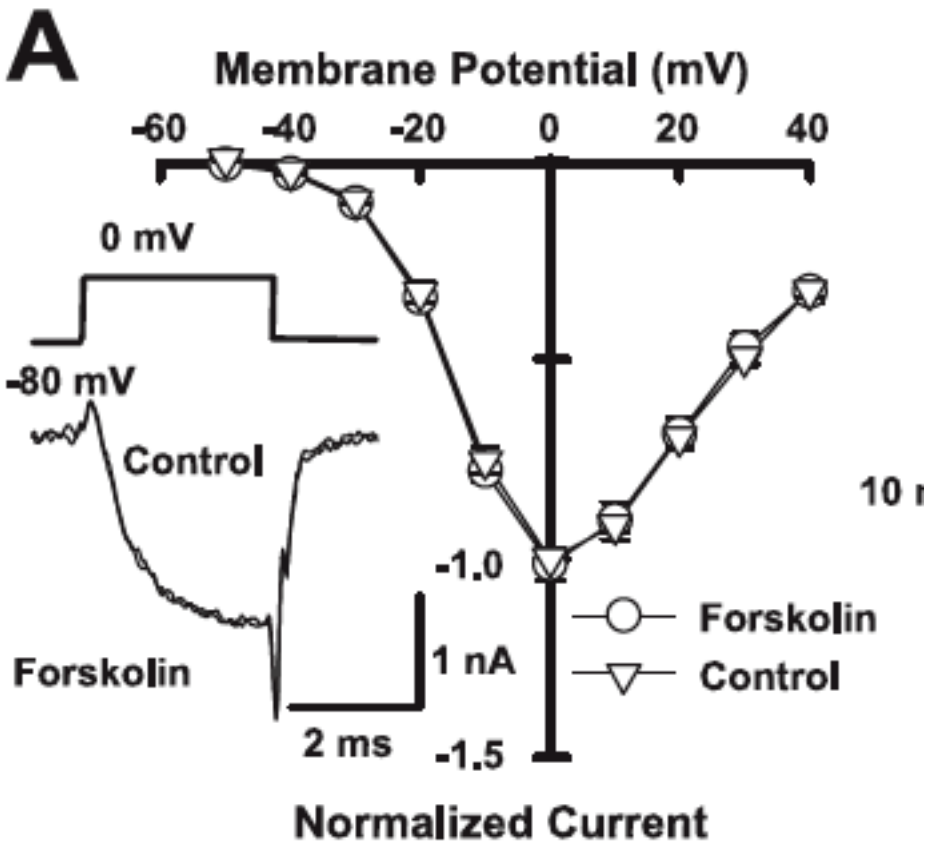
Masahiro Kaneko and Tomoyuki Takahashi
Department of Neurophysiology, University of Tokyo Graduate School of Medicine, Tokyo 113-0033, Japan





Potenciação da neurotransmissão pelo cAMP não involve potenciação das correntes de cálcio

Calyx of Held



CONCLUSÕES

-Modulação negativa de canais de cálcio por receptores ligados a proteína G inibe a liberação de neurotansmissores.

- Eficiente mecanismo de retroalimentação negativa.

-cAMP e PKC aumentam a probabilidade de liberação vesicular sem afetar as correntes de cálcio.

- Mecanismo *downstream* ao cálcio.