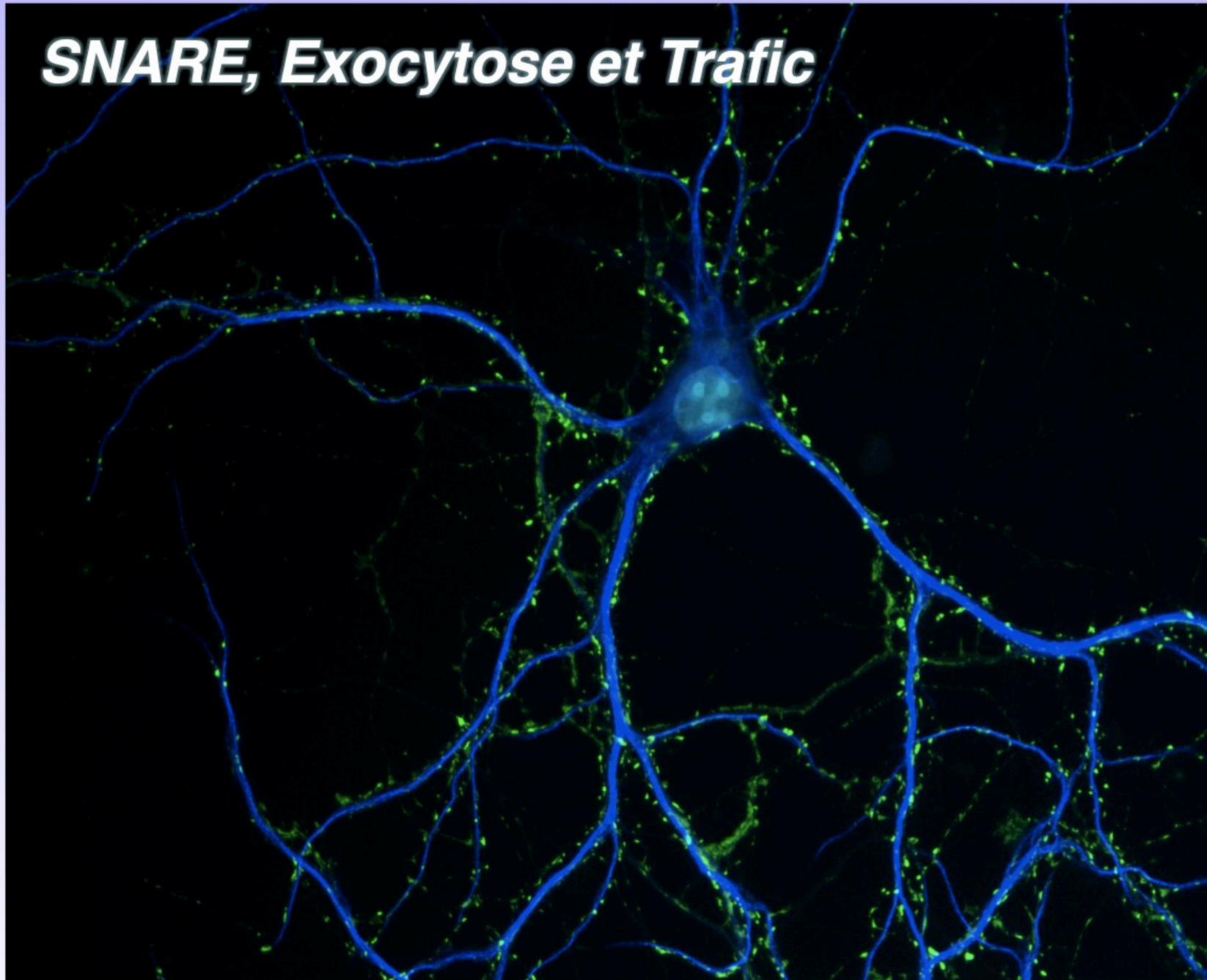
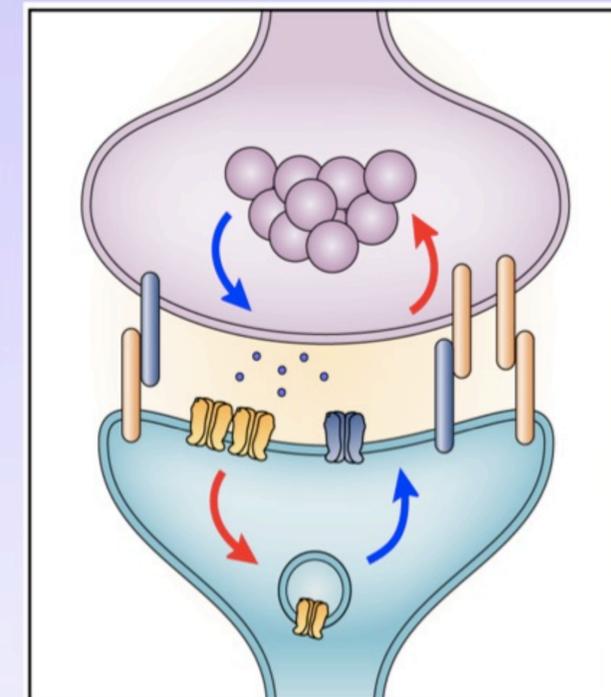




SNARE, Exocytose et Trafic



Lydia DANGLLOT



*Télécharger le cours sur
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Lydia Danglot - Cours de neurosc...

Lydia Danglot web page
Life Science & Imaging

Octobre 31, 2010

Thème de recherche

Publications

Enseignement

Liens favoris

CONTACT

French English

Enseignement

Cours

- [Master2 de Neurosciences - UE Synapse et synaptogenèse](#) (code UE : MBIP5019) - Université Pierre et Marie Curie (Paris 6): [Planning Neuritogenèse et polarité neuronale.](#)
- [Master2 de Neurosciences - UE Communication Cellulaire](#) (code UE : MBIP5003) - Université Pierre et Marie Curie (Paris 6): [Les protéines SNARE et l'exocytose](#) : classification des SNAREs, voie de recyclage des VS, comment mesurer l'exocytose, comment mesurer le recyclage, les protéines régulant l'assemblage des SNARE (Munc18, munc13, Syt, complexine), souris KO Syb2, souris mocha,...
- [Master2 de Génétique - Université Paris Diderot \(Paris 7\)](#), UE Neurobiologie cellulaire et développementale. [Développement de l'hippocampe et synaptogenèse:](#) Neuroanatomie générale, présentation du SNC, présentation du télencéphale et de l'hippocampe, développement de l'hippocampe, migration des neurones excitateurs et inhibiteurs, modèle des neurones dissociés d'hippocampe en culture, polarité neuronale, formation des synapses.
- [Ecole doctorale Frontières du Vivant \(Universités Paris V, VI, VII\)](#) Club Neurobiologie & Optique: [Diversité et usage des protéines fluorescentes en Neurosciences.](#)
- [Master2 de Biothérapies Tissulaires Cellulaires et Génétique- Faculté de Médecine Hôpital Henri Mondor \(Paris12\)](#),
[1. Animaux mutants: identifications, entretien, analyse.](#)
[2. Modèles en psychiatrie: addiction, schizophrénie, Hyperactivité et trouble de l'attention, et anxiété.](#)
- [Master1 de Biologie - Ecole Normale Supérieure](#), UE Du neurone au système. Module Neurobiologie n°2: [La machinerie d'exocytose.](#)

Travaux pratiques

- [Master2 de Génétique - Université Paris Diderot \(Paris 7\)](#), UE Neurobiologie cellulaire et développementale. Travaux pratiques d'imagerie: microscopie à épifluorescence, trajet optique, présentation des filtres dichroïques, acquisition d'images. Mesure d'exocytose (FM1-43) par vidéo-microscopie.

Travaux dirigés

MANUEL de cours

- Master2- Paris 6 Neuritogenèse et polarité neuronale. [Download](#)
- Master2- Paris 6 Complexe SNARE et communication cellulaire. [Download](#)
- Master2- Paris 7 Développement de l'hippocampe et synaptogenèse. [Download](#)
- Doctorat-Paris 5, 6, 7 Usage et diversité des protéines fluorescentes en Neurosciences. [Download](#)
- Master1- ENS- N2 Machinerie d'exocytose. [Download](#)

Trafic, exocytose et modèles physiopathologiques

1. Exocytose et complexe SNARE

Les voies d'exocytose régulée
 Définition du complexe SNARE
 Nomenclature v/t-SNARE et R/Q-SNARE

2. Exemple de la synapse

Découverte de NSF et SNAP
 Isolement des SNARE
 Rôle de NSF & SNAP dans la fusion

3. Le cycle des vésicules synaptiques

Voie lente: endocytose médiée par la clathrine
 Voie courte: kiss and run
 Les différents « pool » vésiculaires

4. Comment mesurer l'exocytose ?

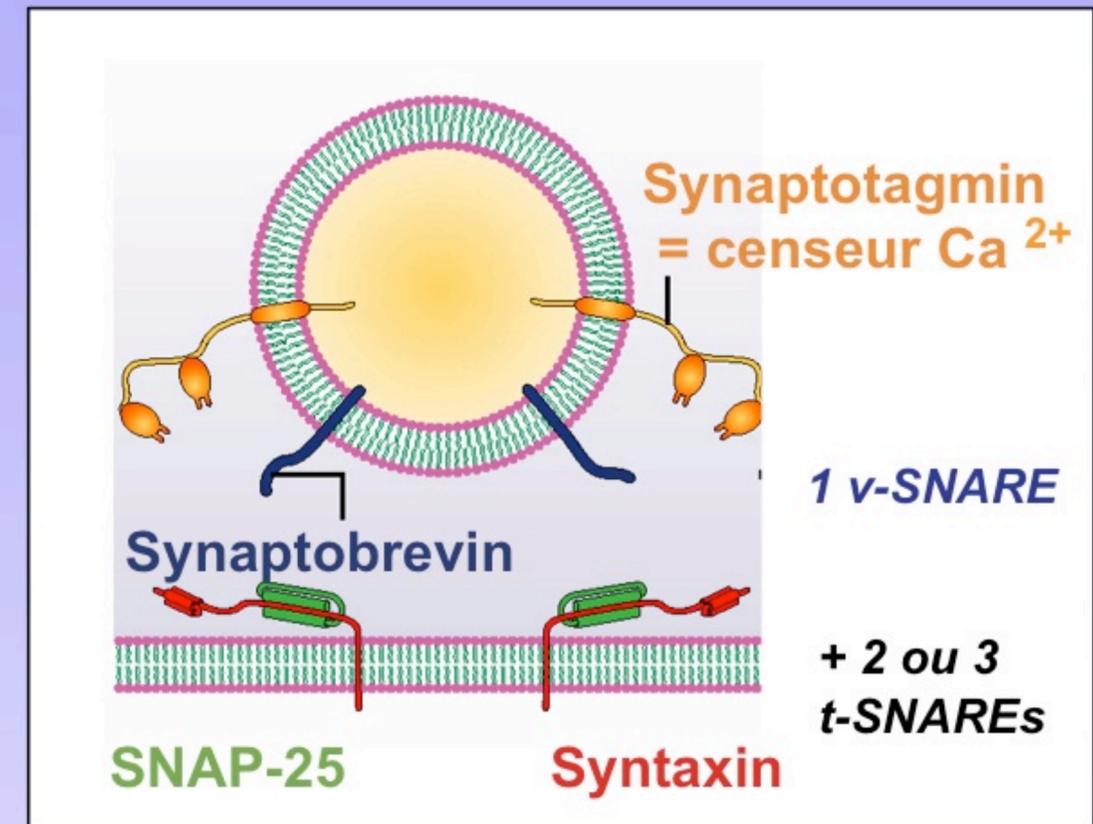
Capacitance
 Ampérométrie
 GFP pH sensible: la Phluorin
 Utilisation des SNARE inversée

5. Comment mesurer le recyclage ?

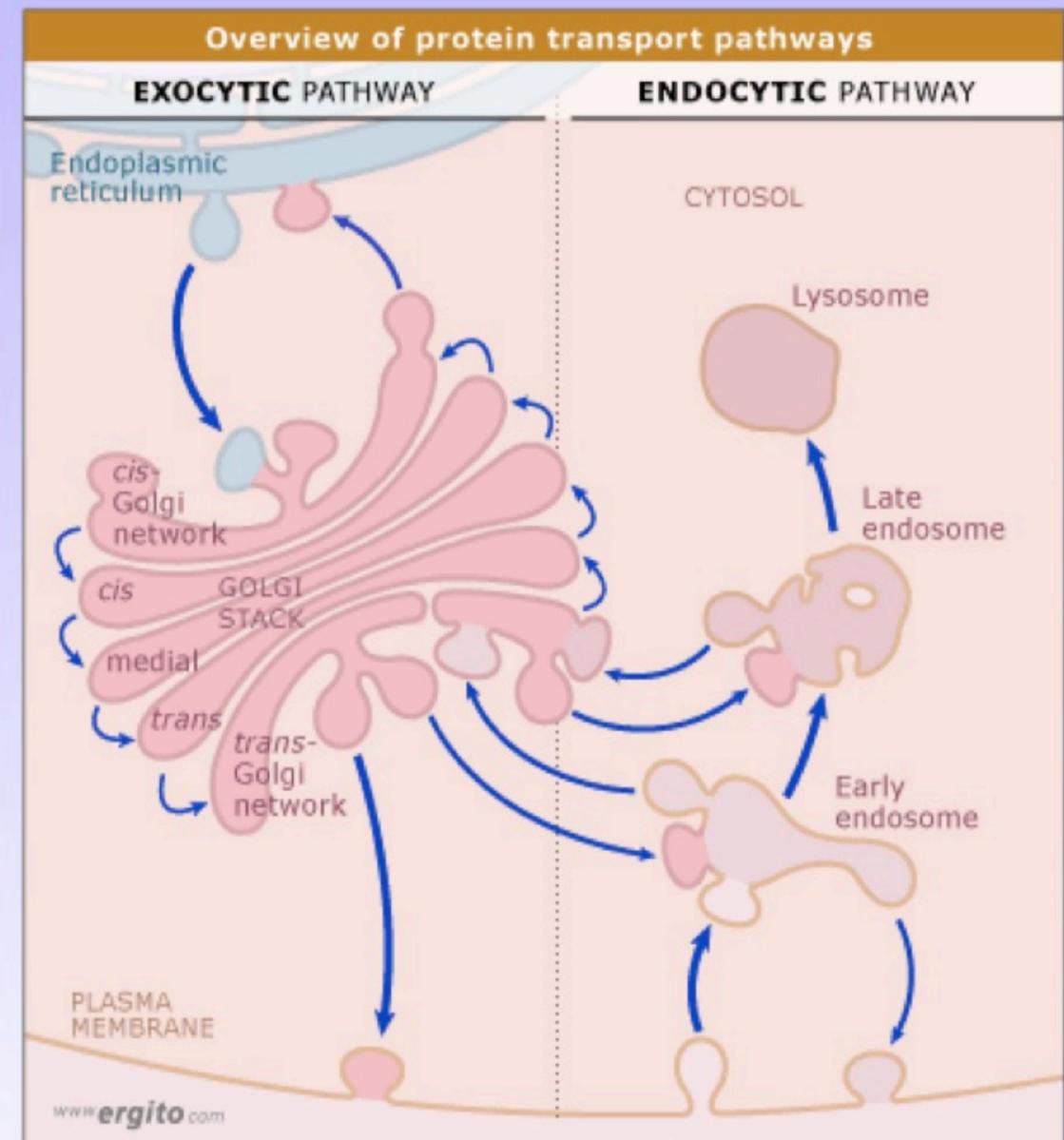
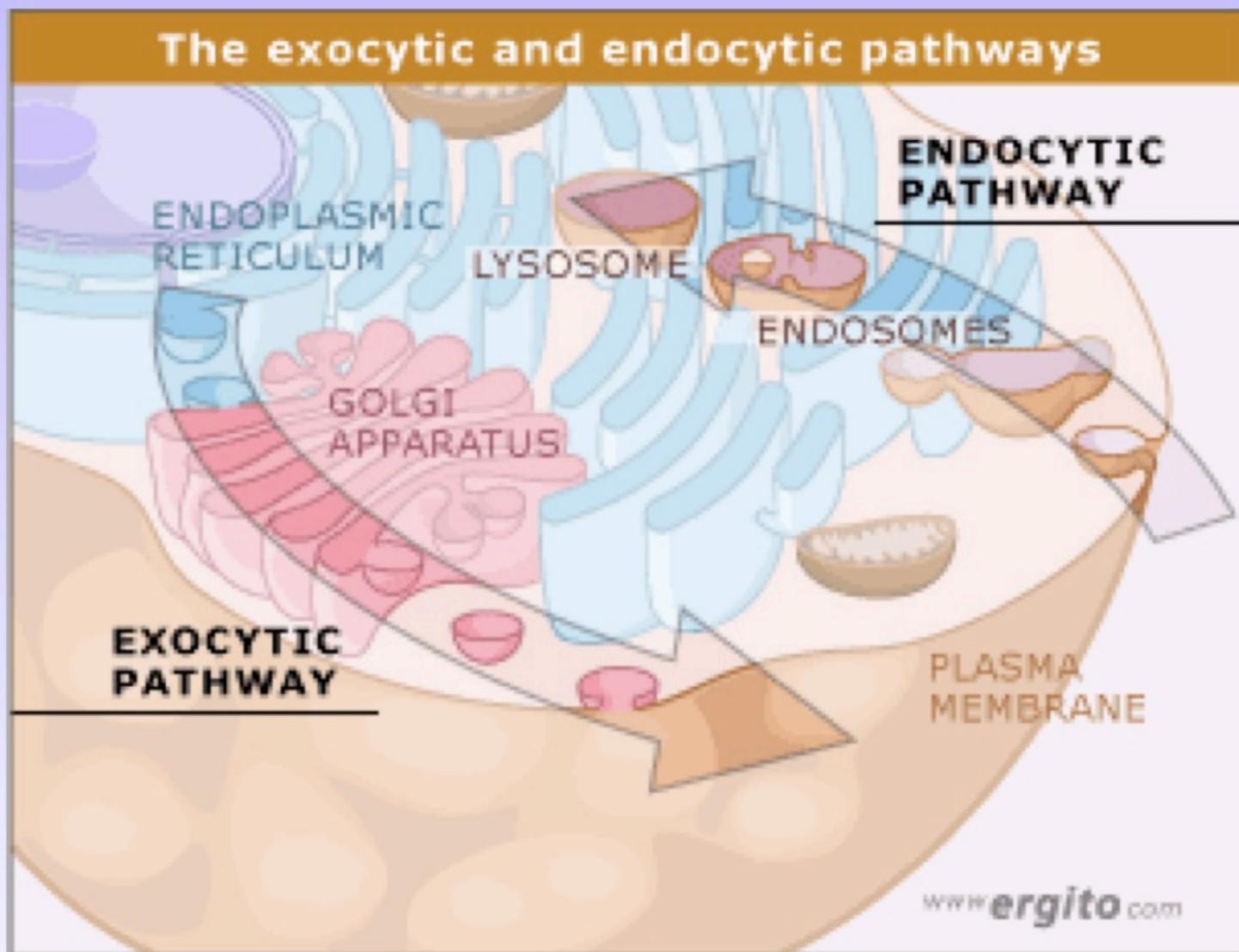
Utilisation des anti-synaptotagmine
 Sondes fluorescentes de type FM

6. Modèles physiopathologiques

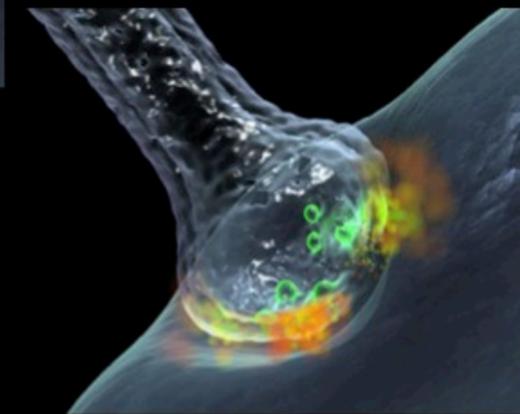
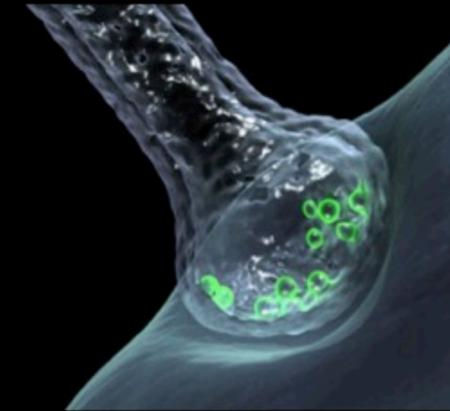
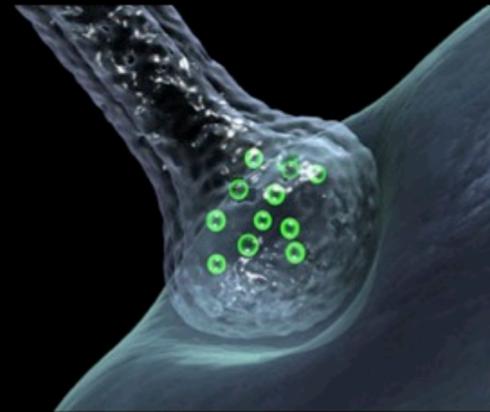
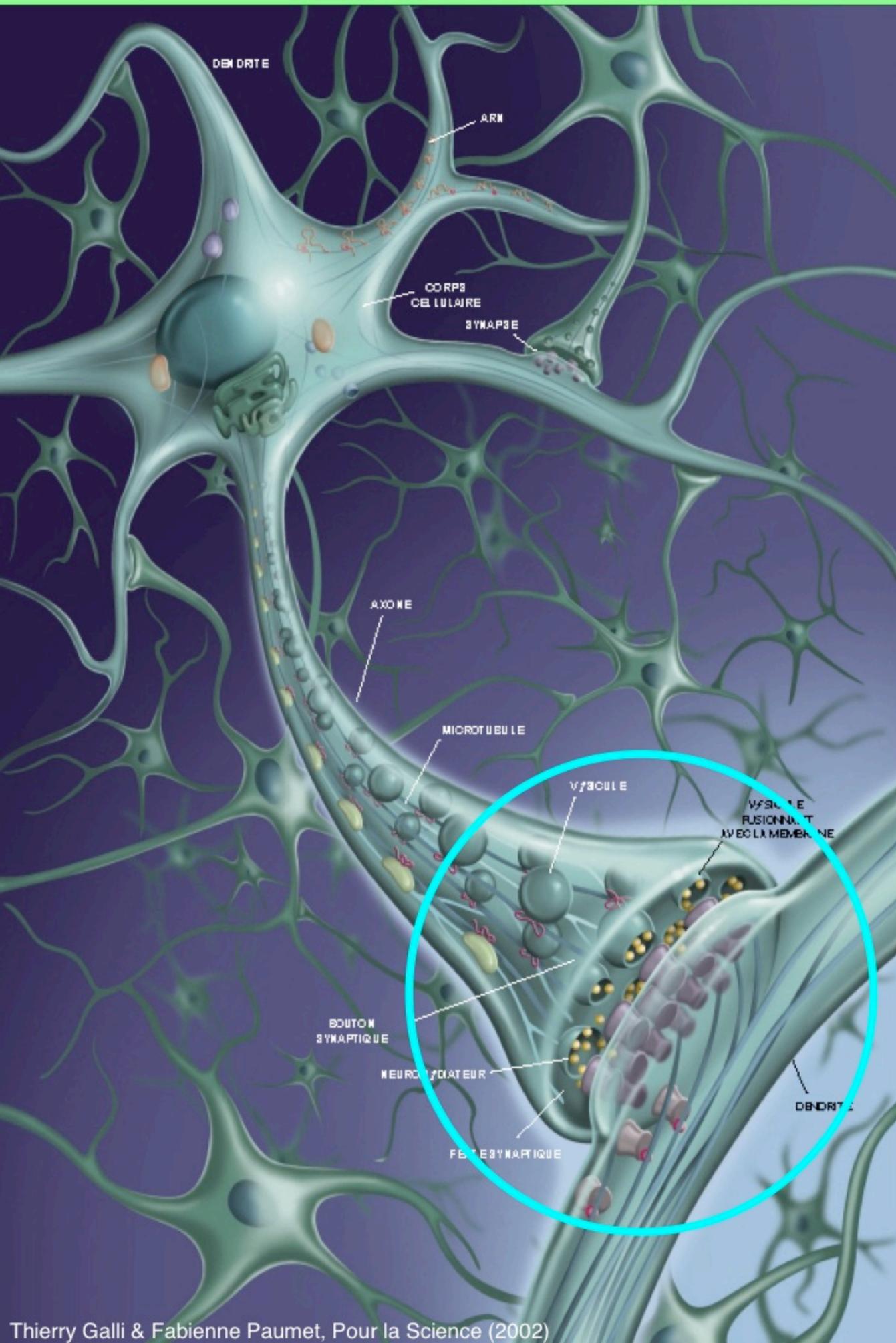
Rôle de la Cellubrevine dans la migration des cellules épithéliales
 Rôle de la synaptobrevine dans la libération des NT
 Rôles de Stx3 et SytVII dans la croissance neuritique
 Mutants de l'adaptateur AP-3: les mocha
 KO TI-VAMP
 Rôles de TI-VAMP dans l'exocytose et la diffusion des récepteurs



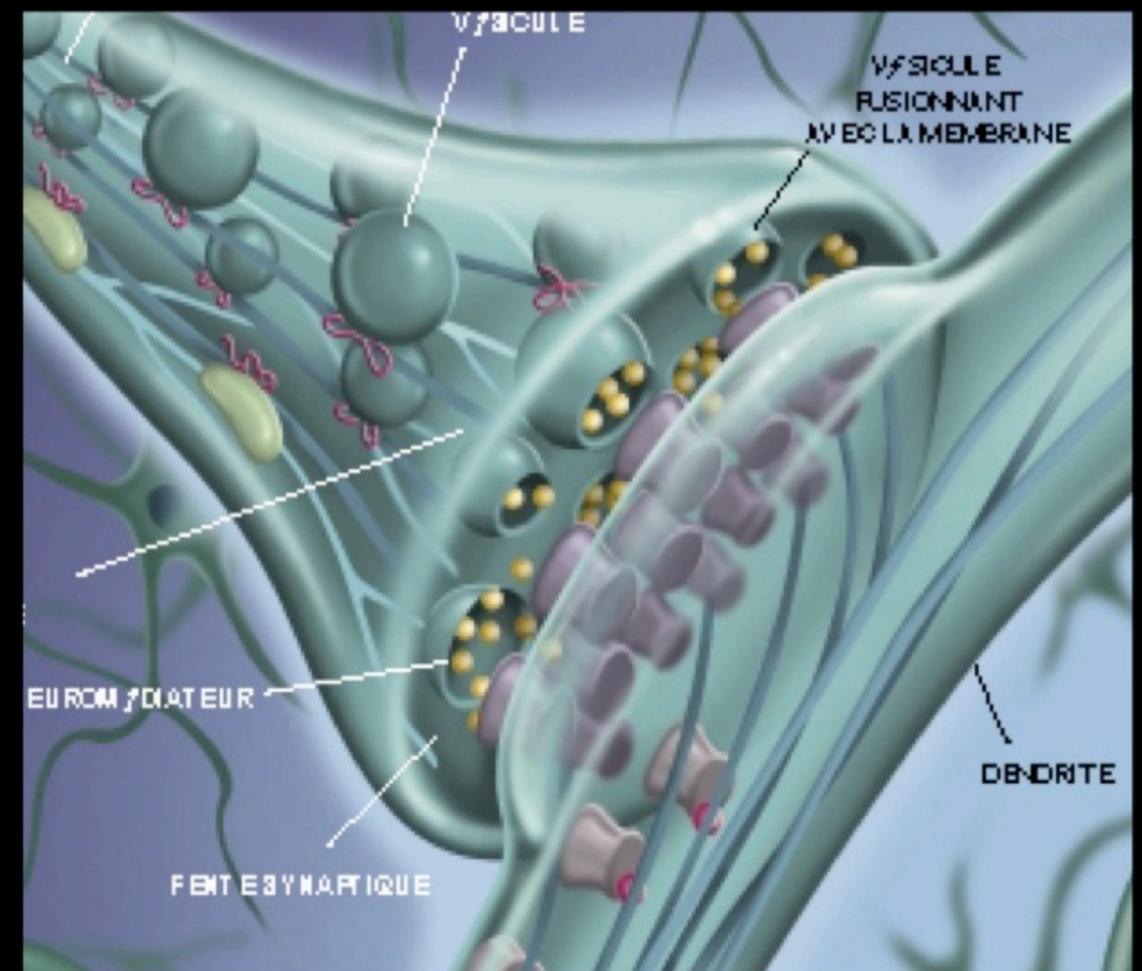
Exo-endocytose



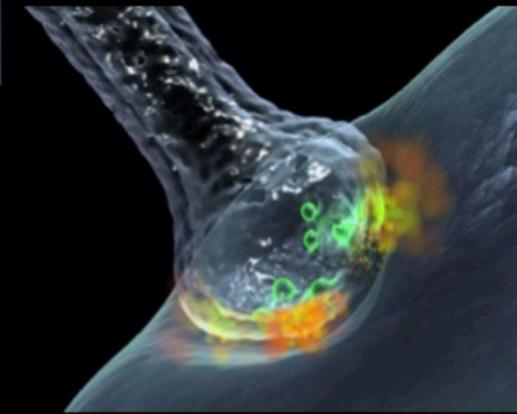
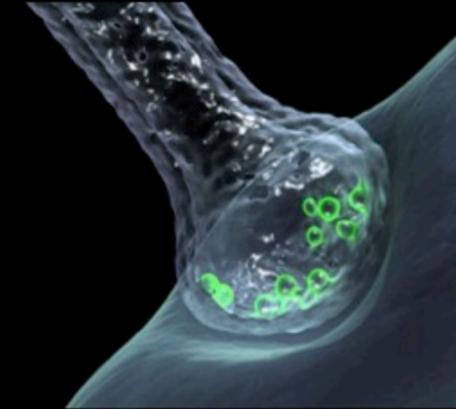
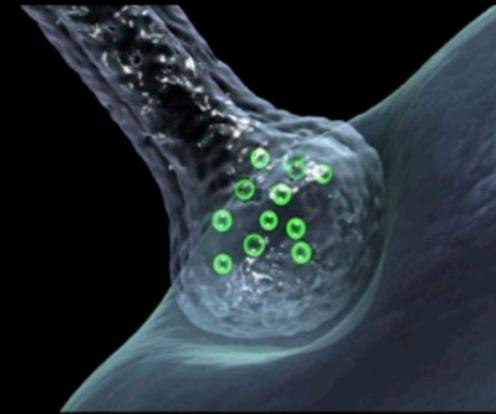
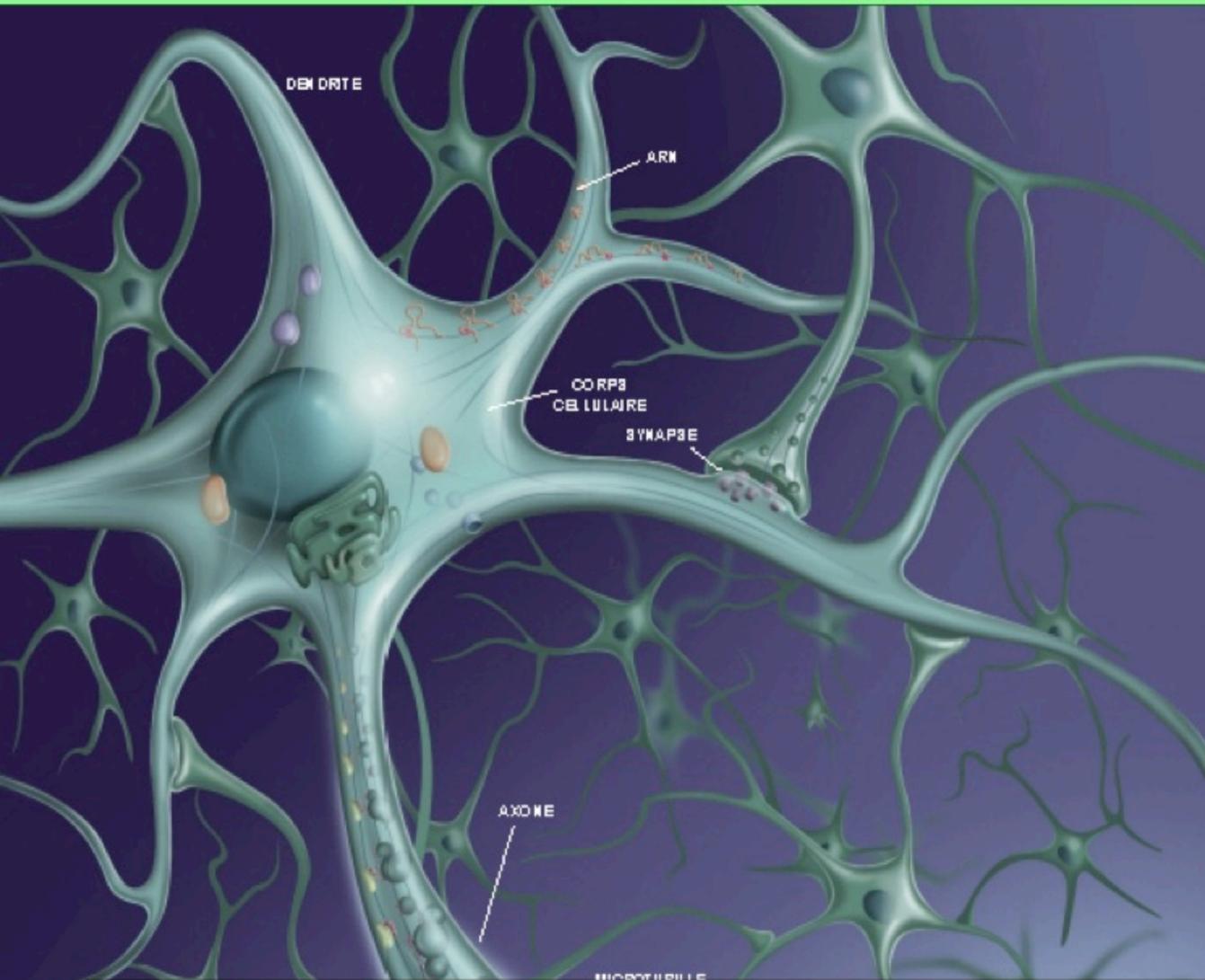
Membrane traffic : role of the vesicular SNARE at the synapse



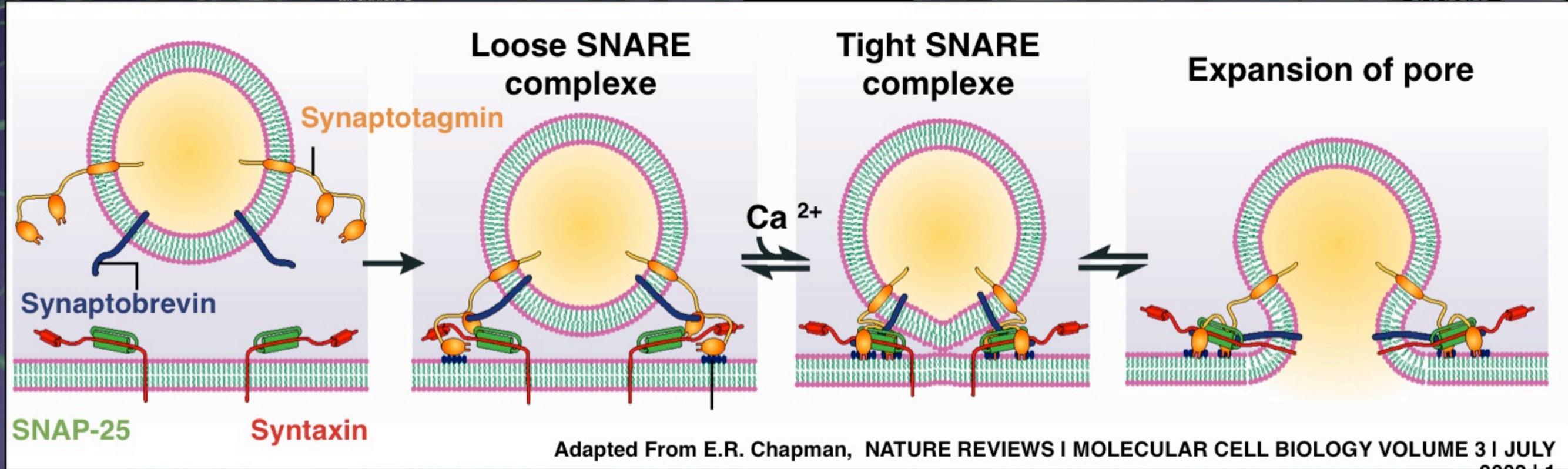
Exocytosis



Membrane traffic : role of the vesicular SNARE at the synapse

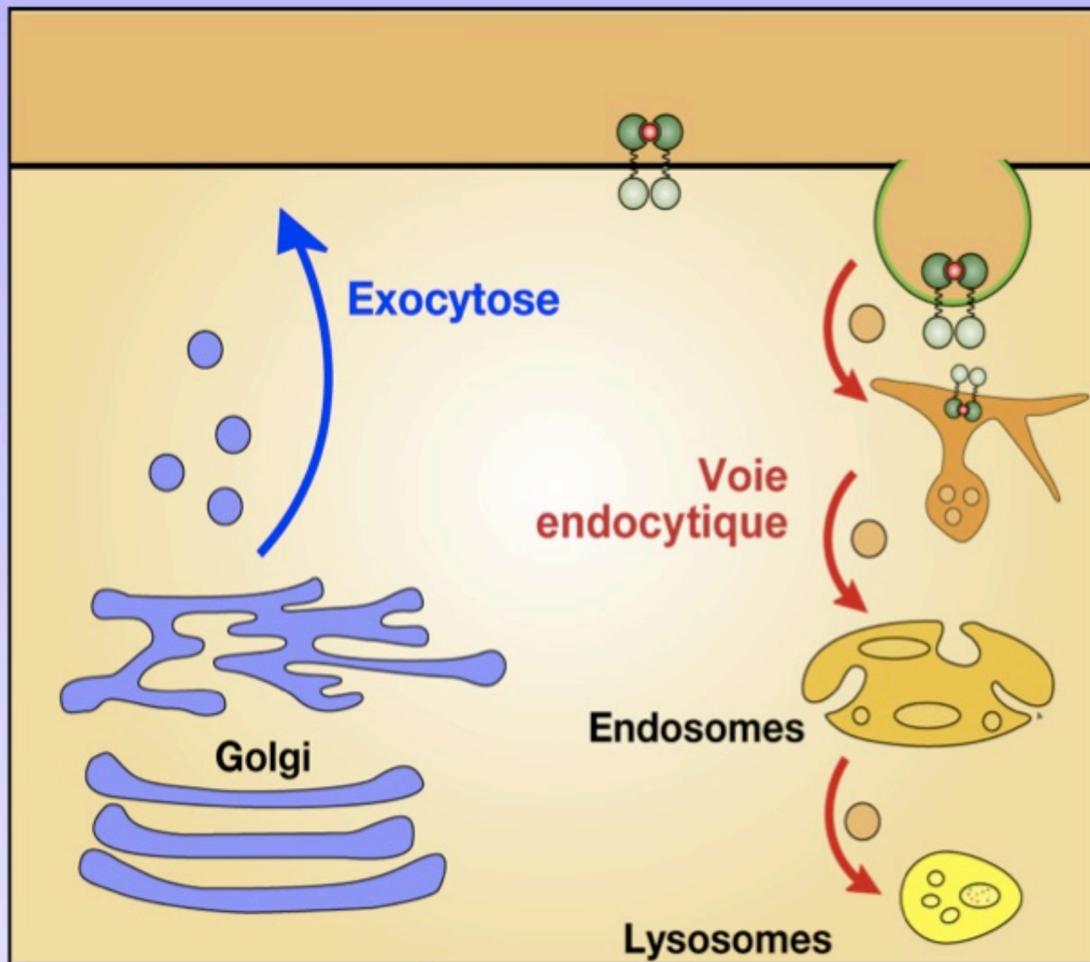


Exocytosis

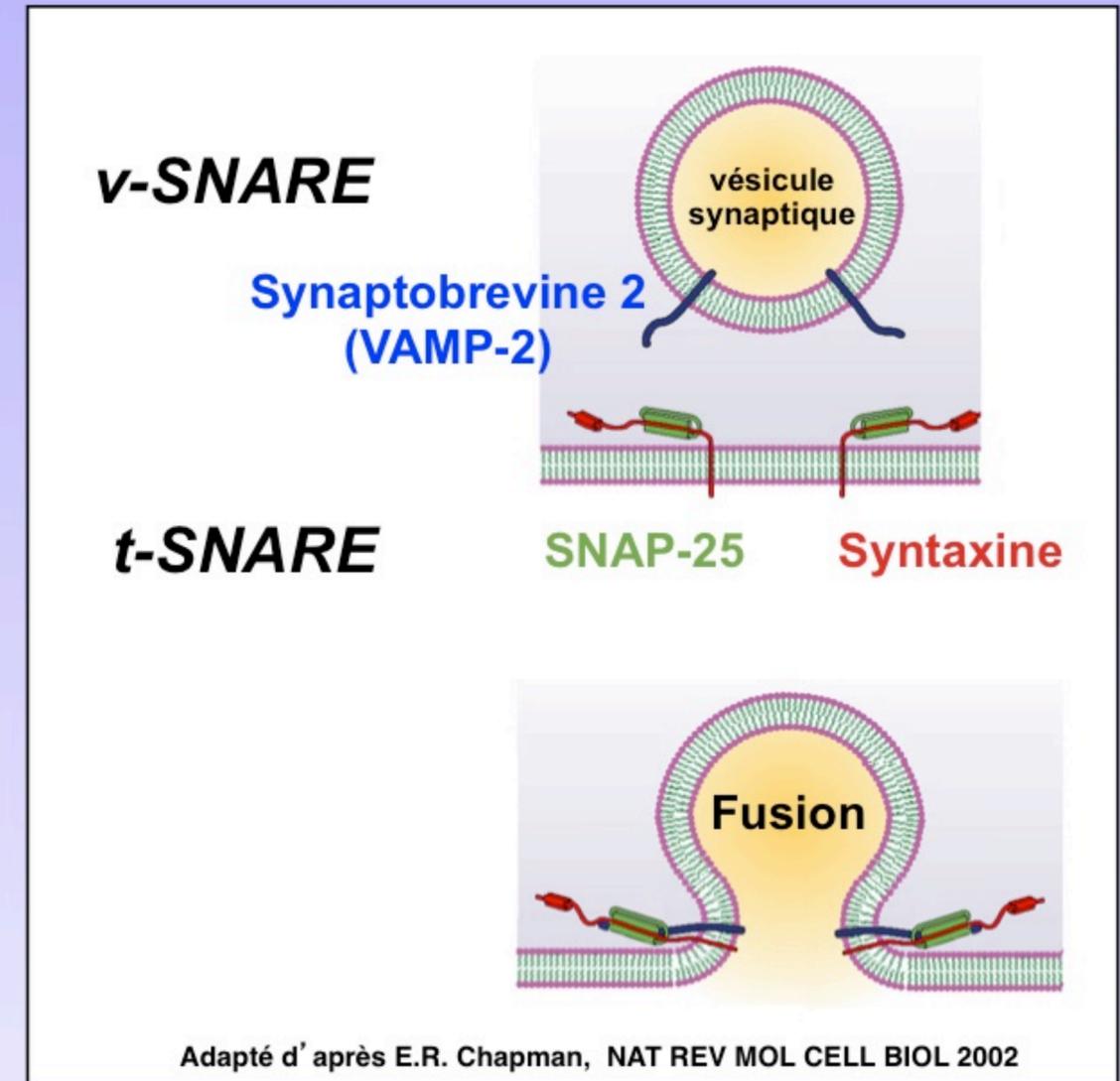


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Rôle central des protéines SNARE dans la fusion membranaire



Transport intracellulaire:
Processus d'Exocytose et
endocytose



Machinerie de fusion:
Les protéines SNARE

Role centrale de l'exocytose dans la croissance membranaire et le remodelage

- **Exocytose au pôle apical pole des cellules épithéliales**

(Galli et al., 1998, Lafont et al., 1999, Pocard et al. 2007)

- **Sécrétion des lysosomes (réparation, migration)**

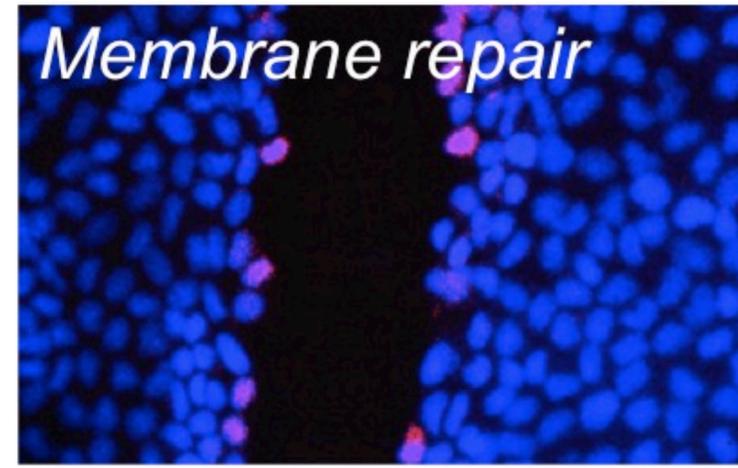
(Pryor et al., 2004; Proux-Gillardeaux 2007)

- **Croissance neuritique**

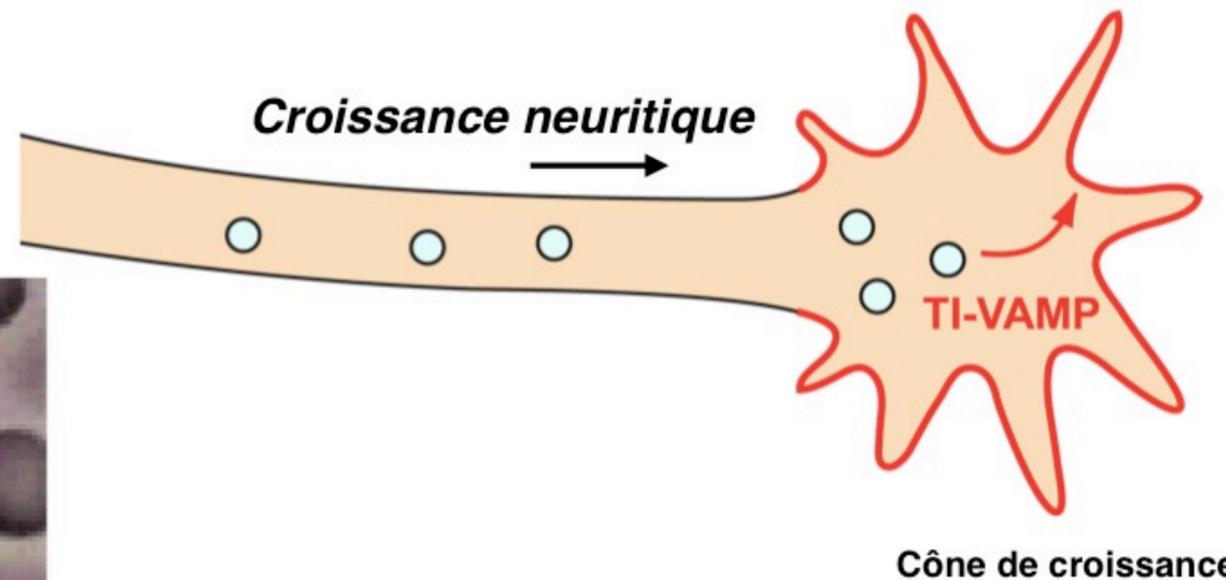
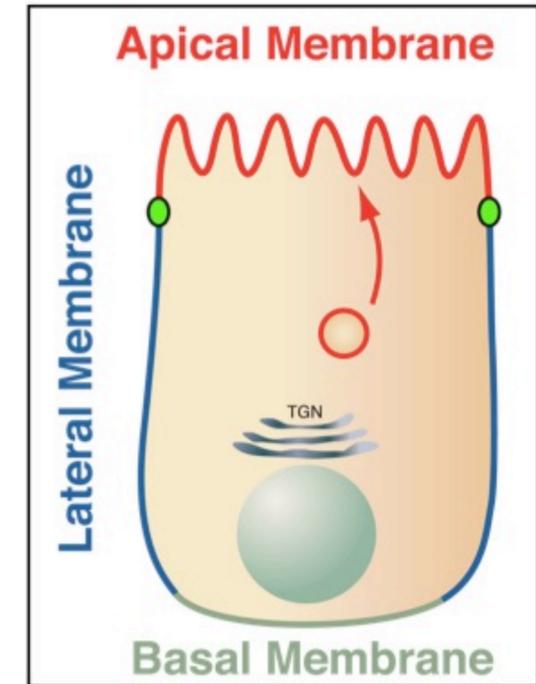
(Martinez-Arca et al., 2003)

- **Exocytose synaptique : libération de NT**

(Danglot et al. PNAS 2006)

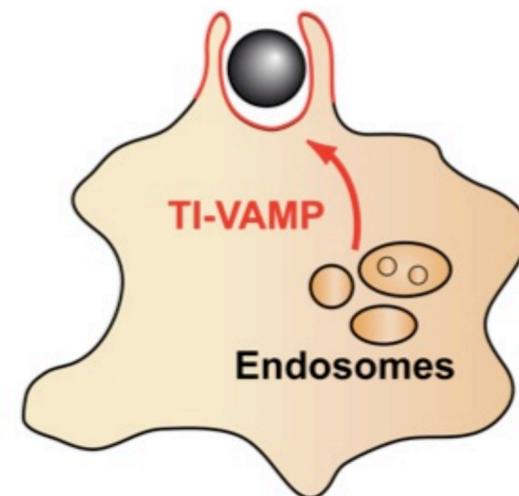


(Proux-Gillardeaux, 2007)



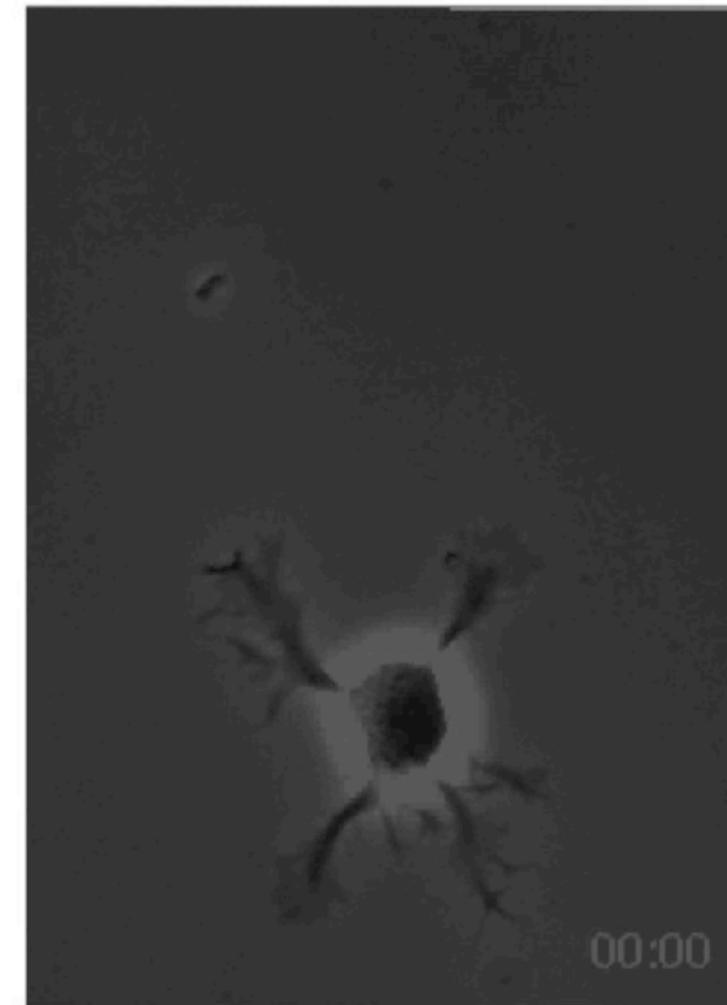
- **Phagocytose**

(Braun and Niedergang., EMBO J, 2004)



- **Degradation de la matrice dans les metastases (invadopodia)**

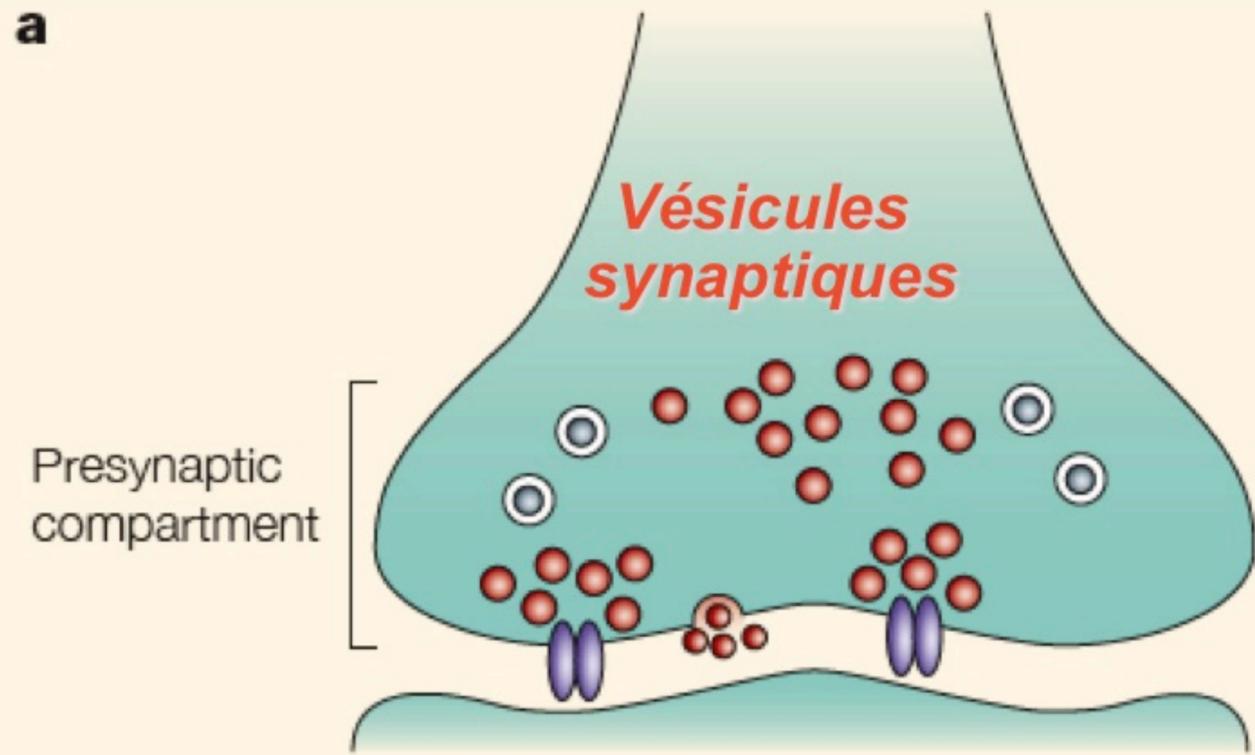
(Steffen and Chavrier, Curr Biol, 2008)



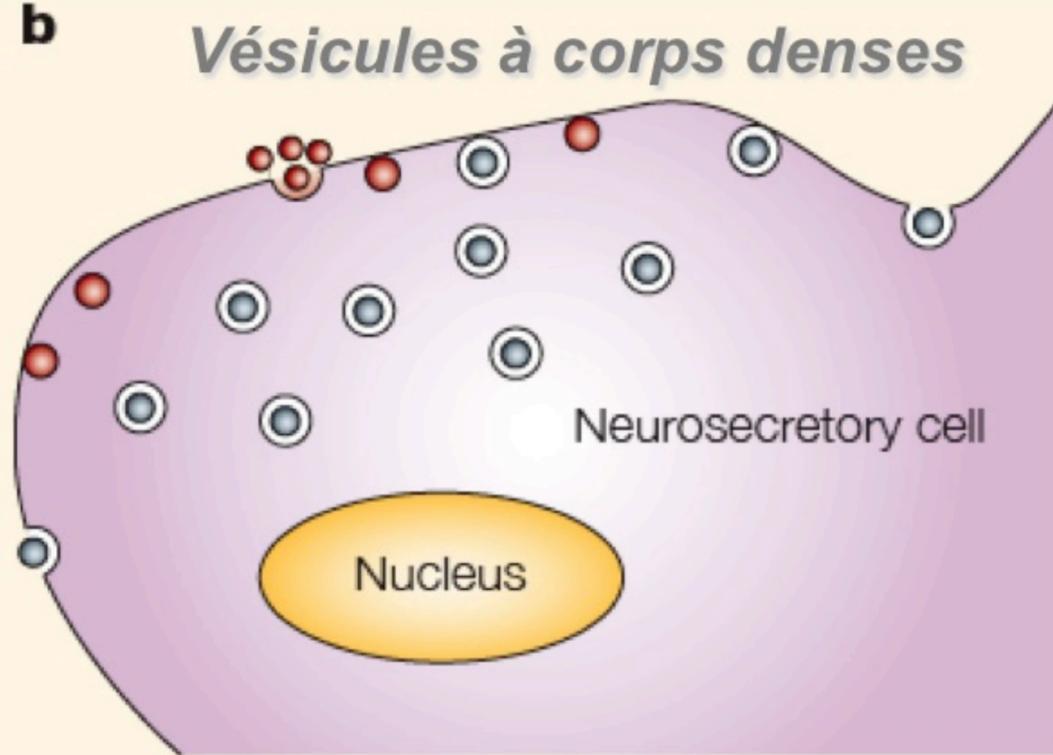
Croissance neuritique

<http://www.silvermanlab.org/>

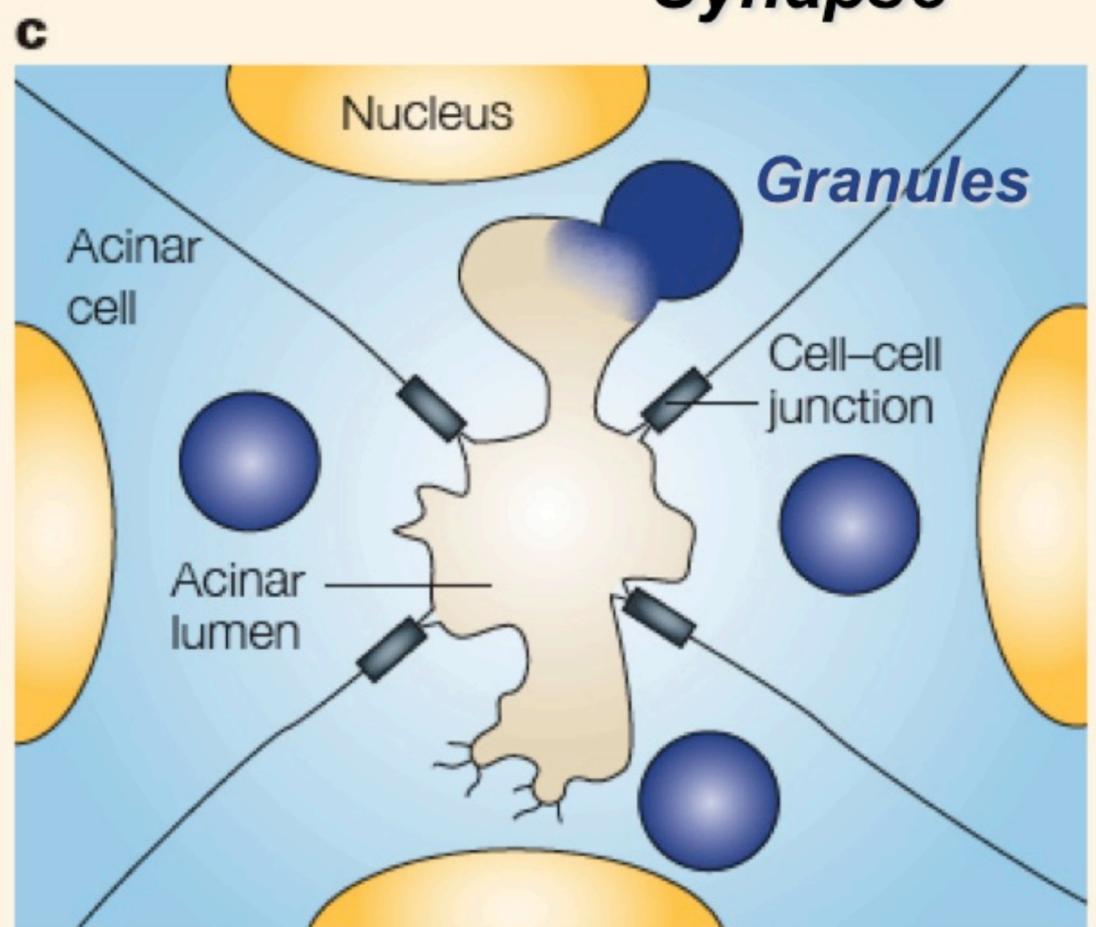
Exocytose régulée



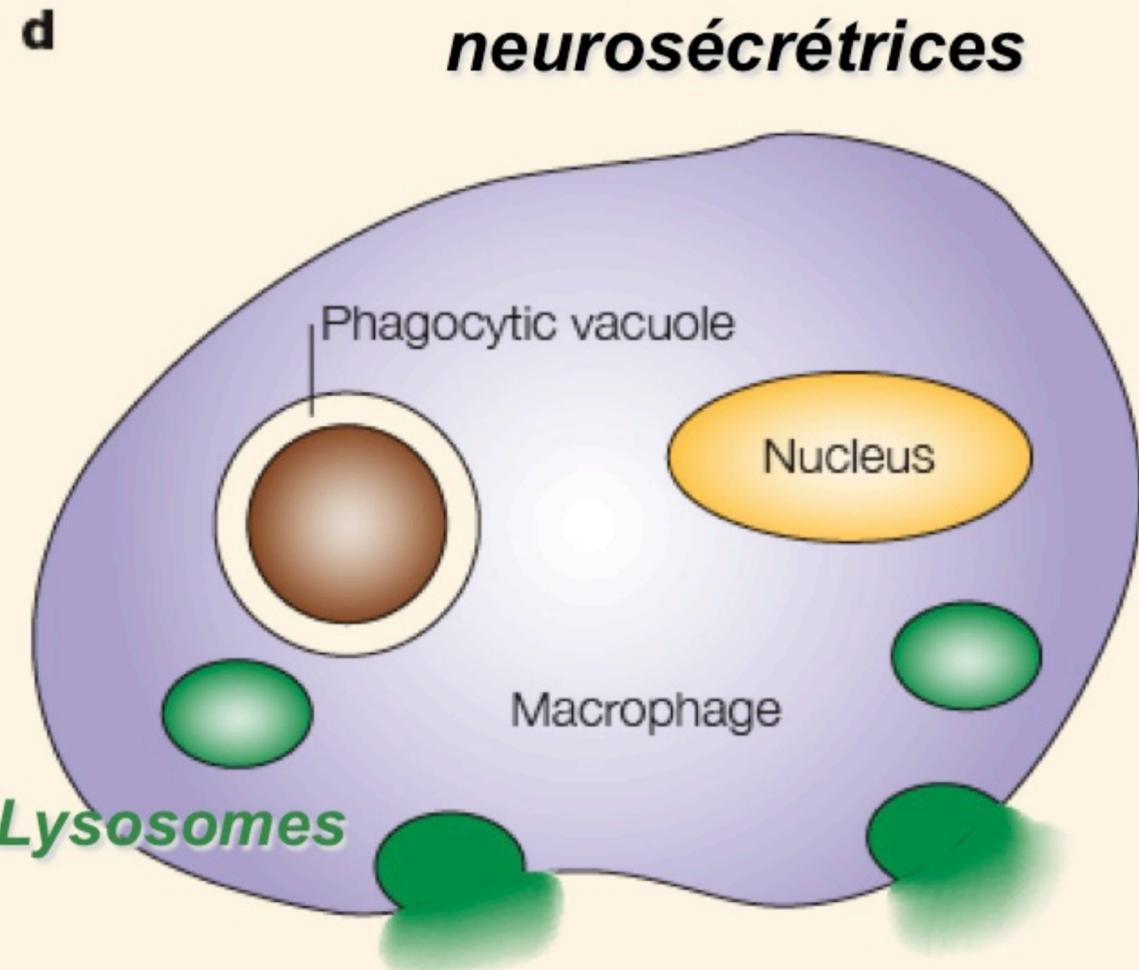
Synapse



Cellules neurosécrétrices

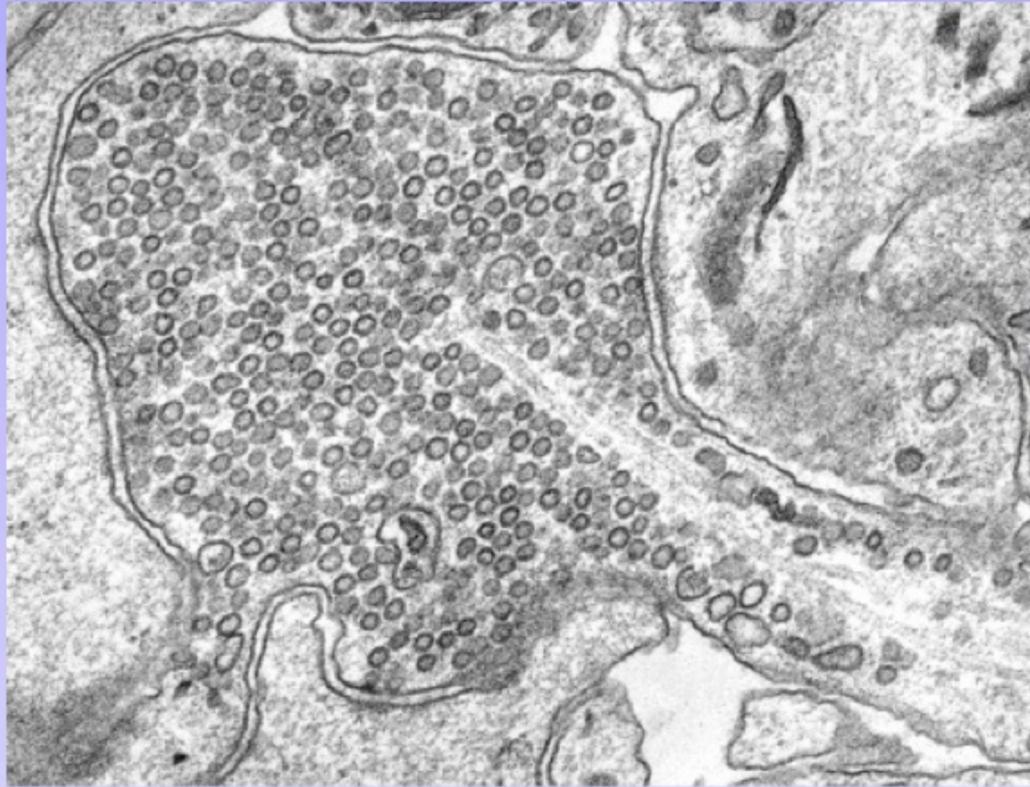


Glandes exocrines

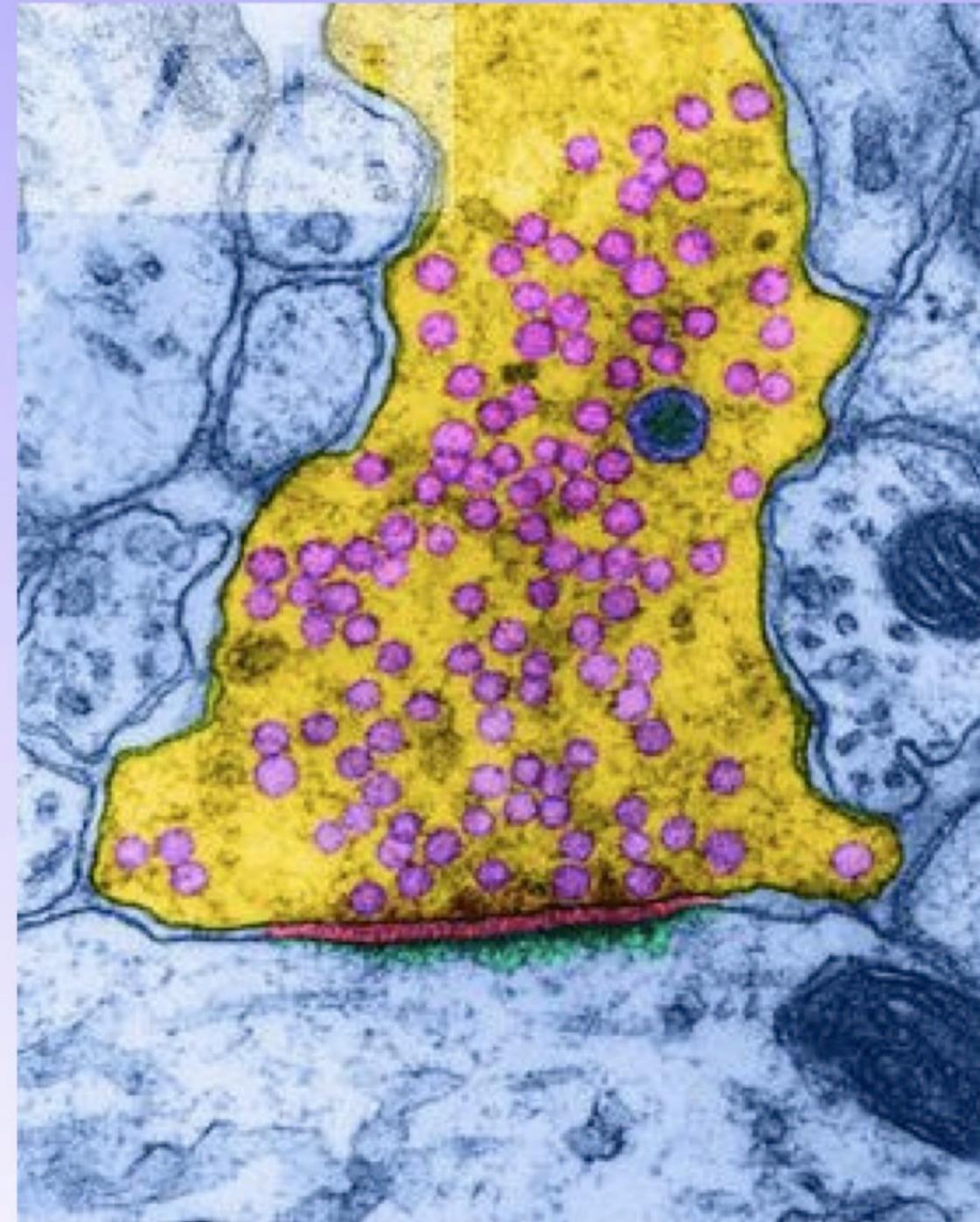


Macrophages

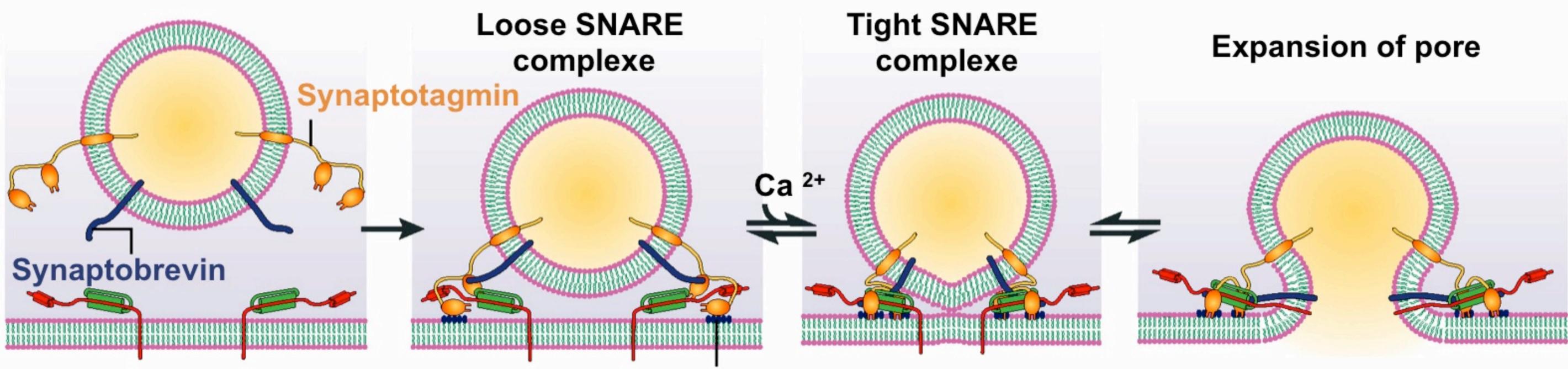
Les vésicules synaptiques



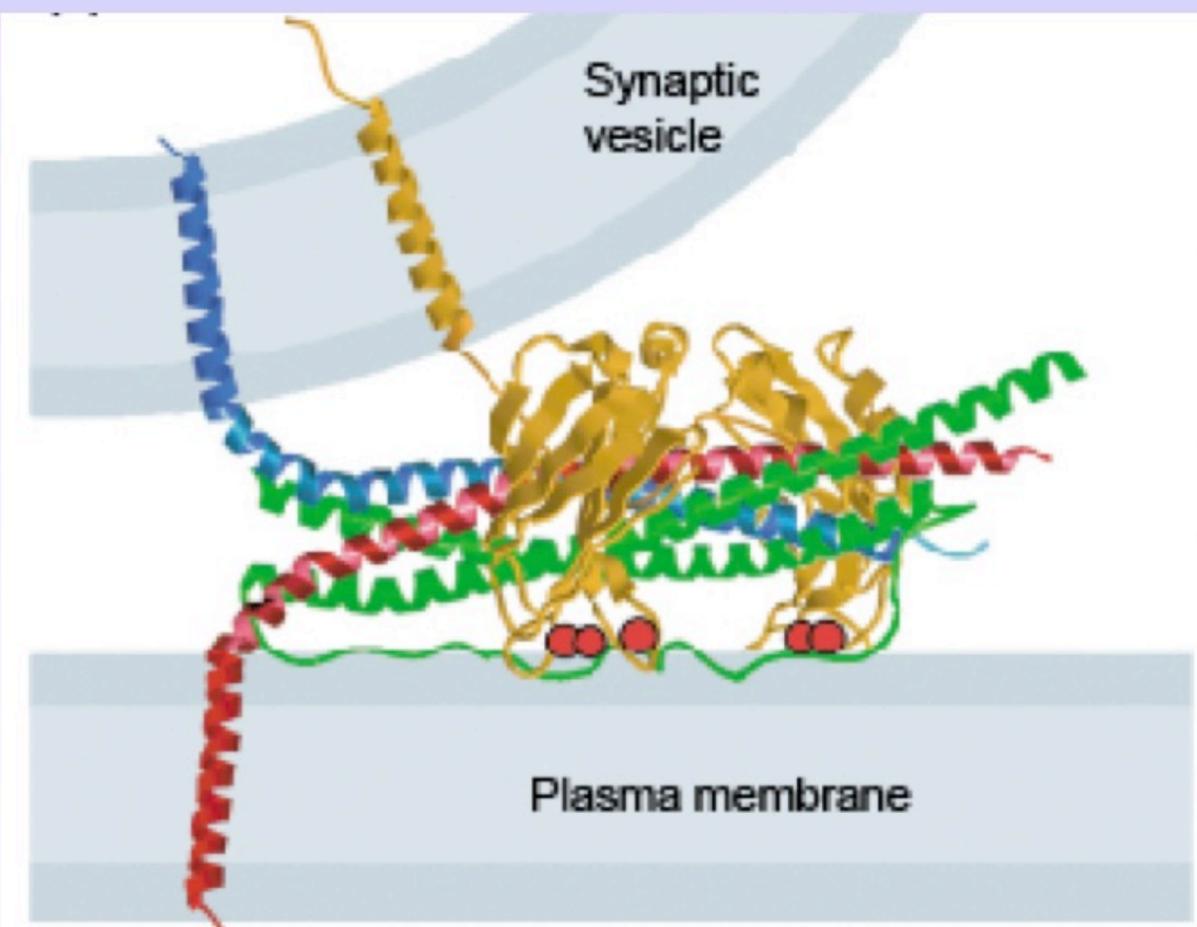
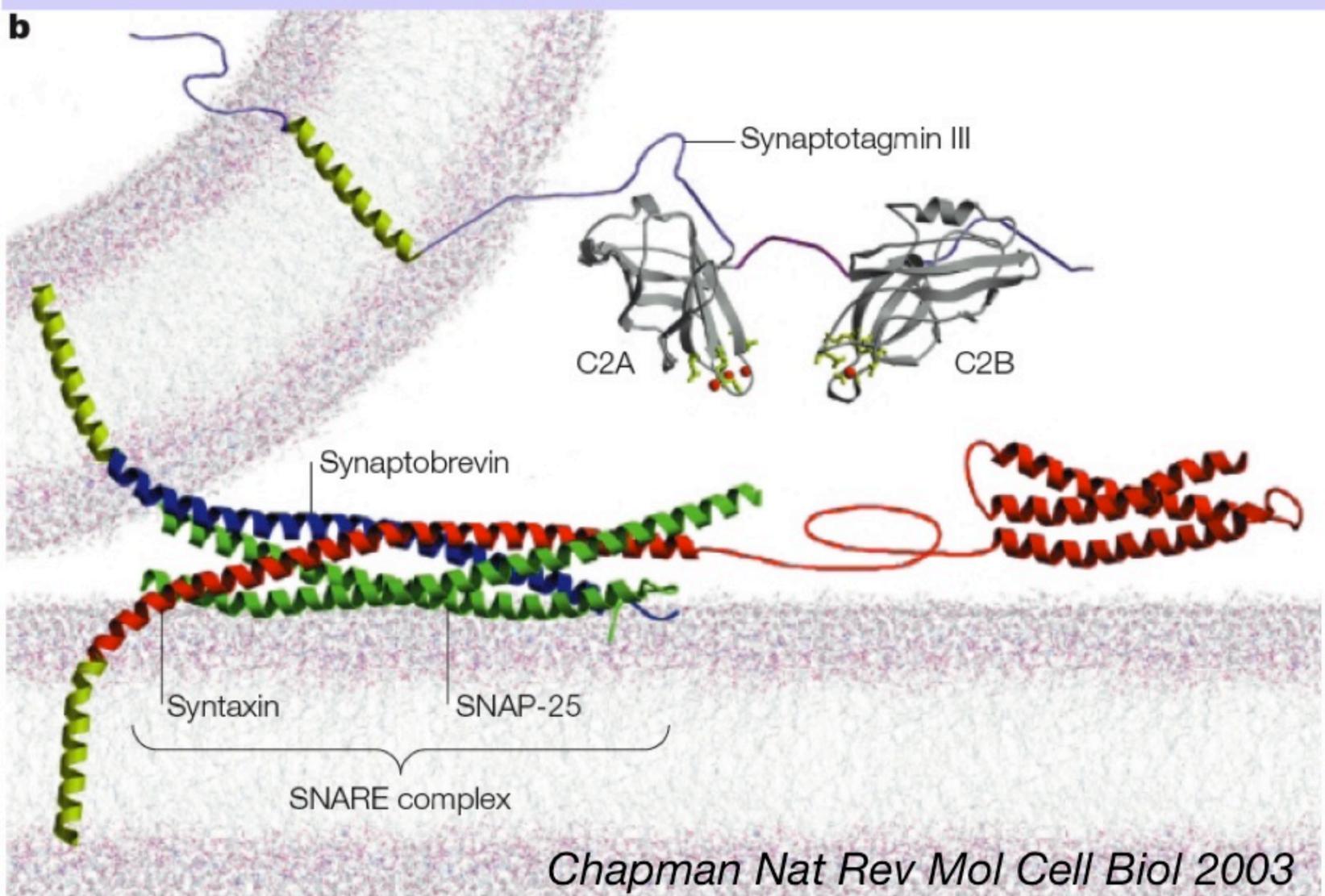
- 50 nm de diamètre
- 500 VS/terminaison
 - x10 000 contacts = 10^6 à 10^7 VS/neurone
 - x 10^{11} neurones = 10^{17} VS/SNC
- cholestérol:phospholipides = 1:2
- 12000 molécules de phospholipides / VS
- phospholipides:protéines = 1:1
- 20 à 40 protéines différentes / VS
- synaptophysine= 7% protéine VS = 0,3% protéines totales



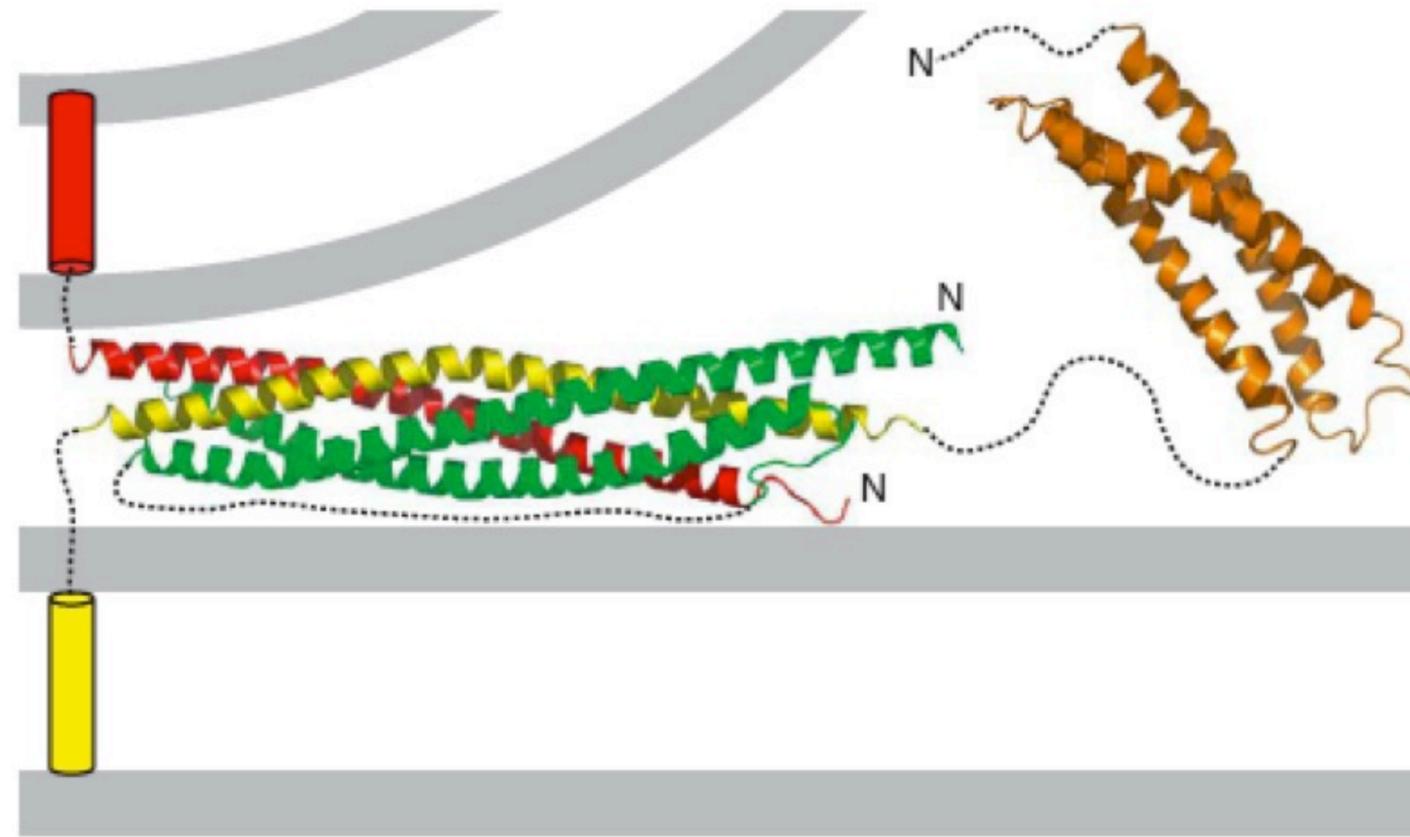
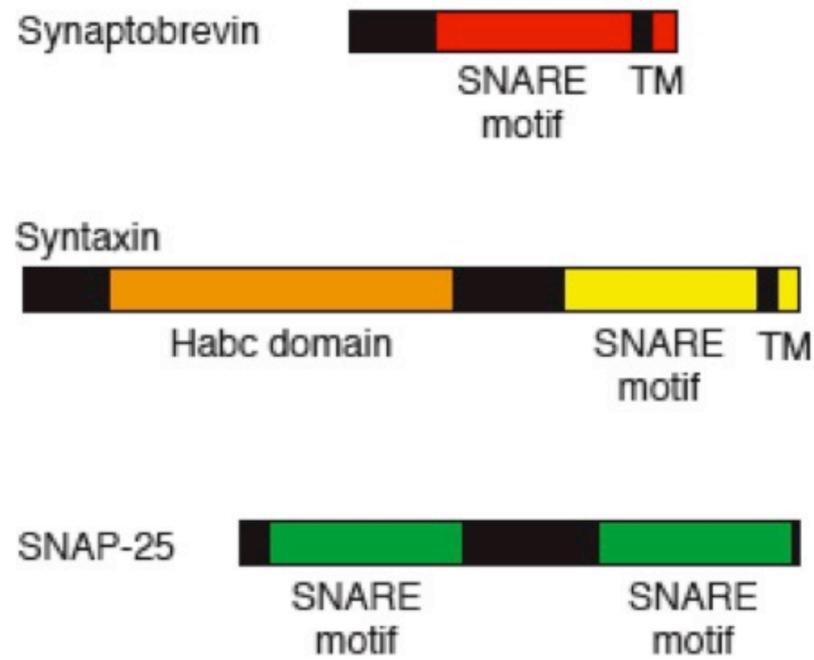
Les protéines SNARE et l'exocytose



Adapted From E.R. Chapman, NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 3 | JULY 2002 | 1

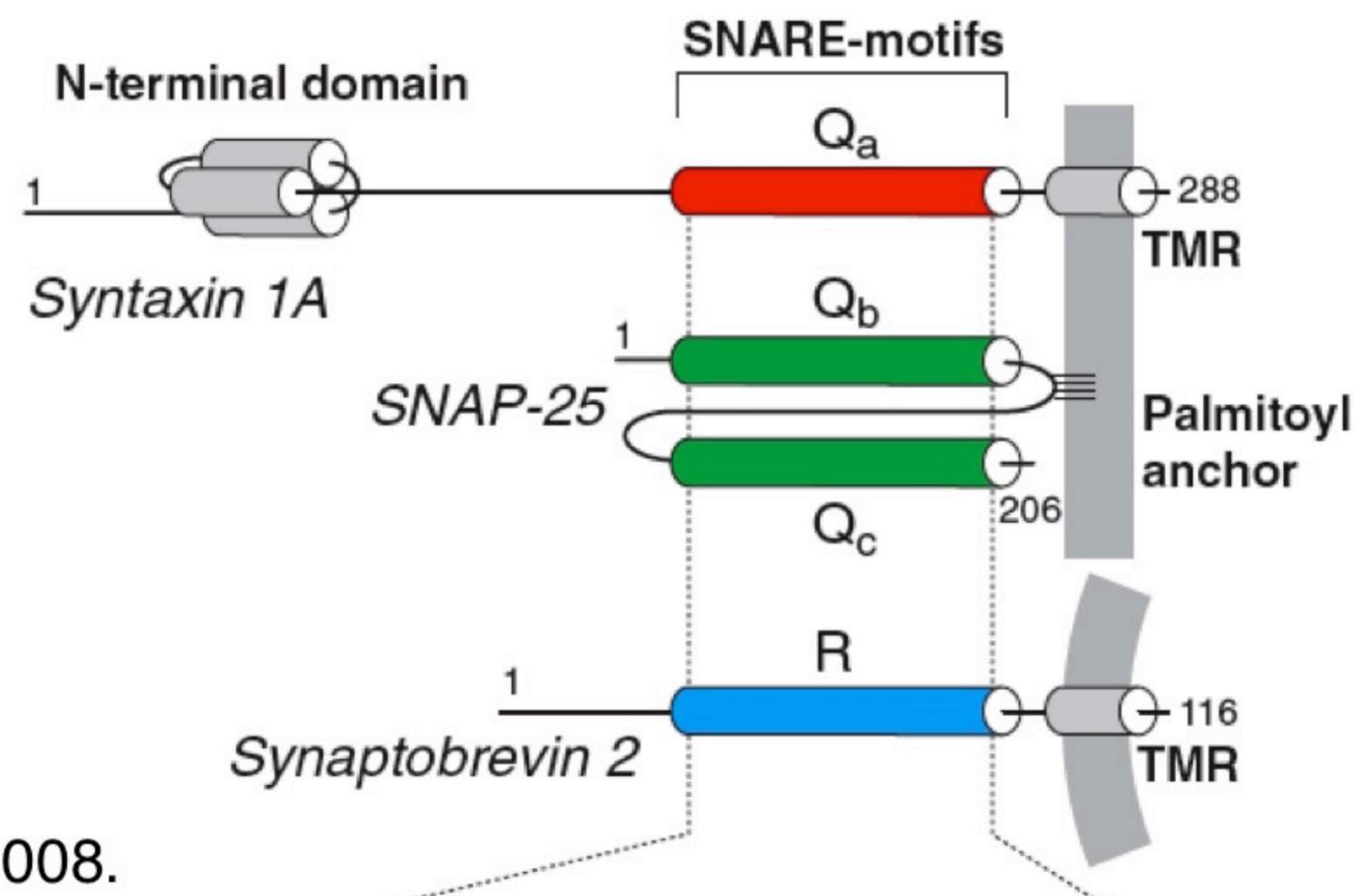


Le complexe SNARE



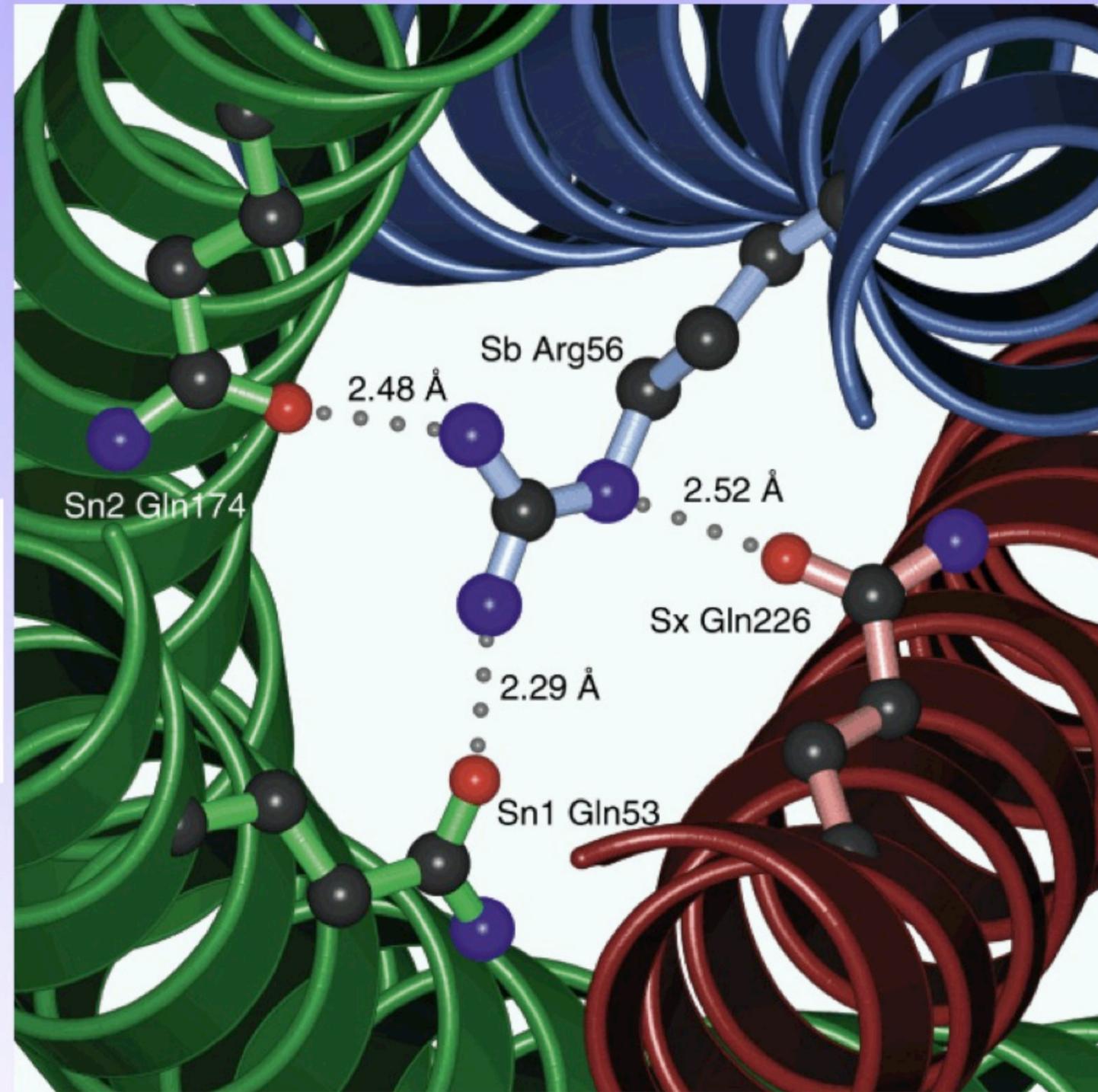
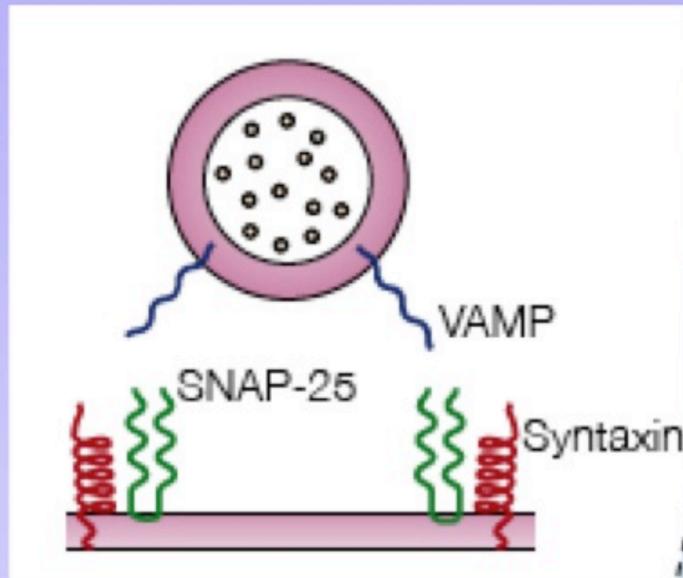
TRENDS in Cell Biology

Rizo, Chen et d'Arcq, Tins 2006.

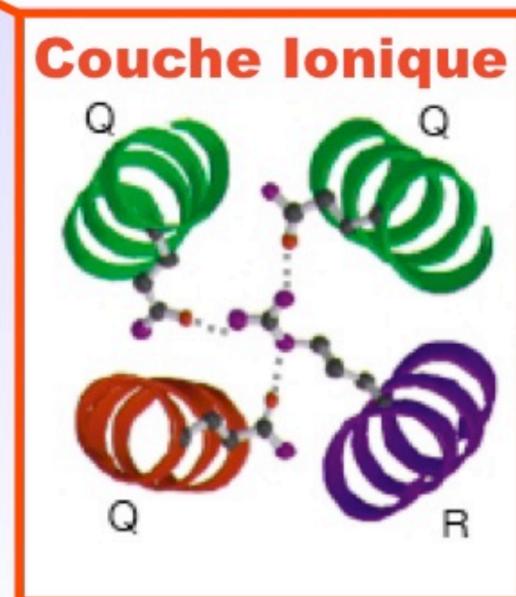
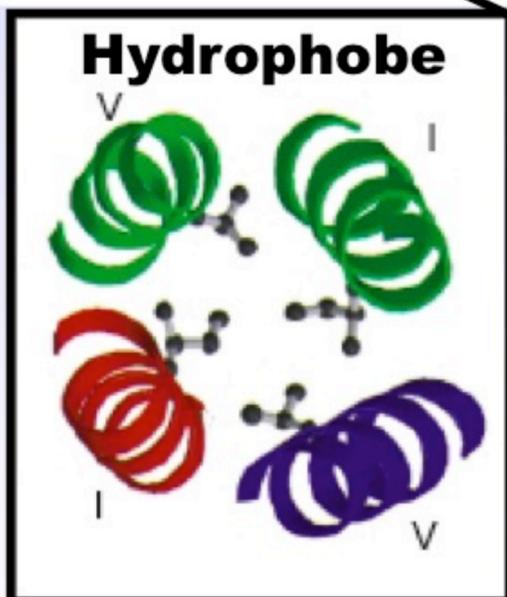
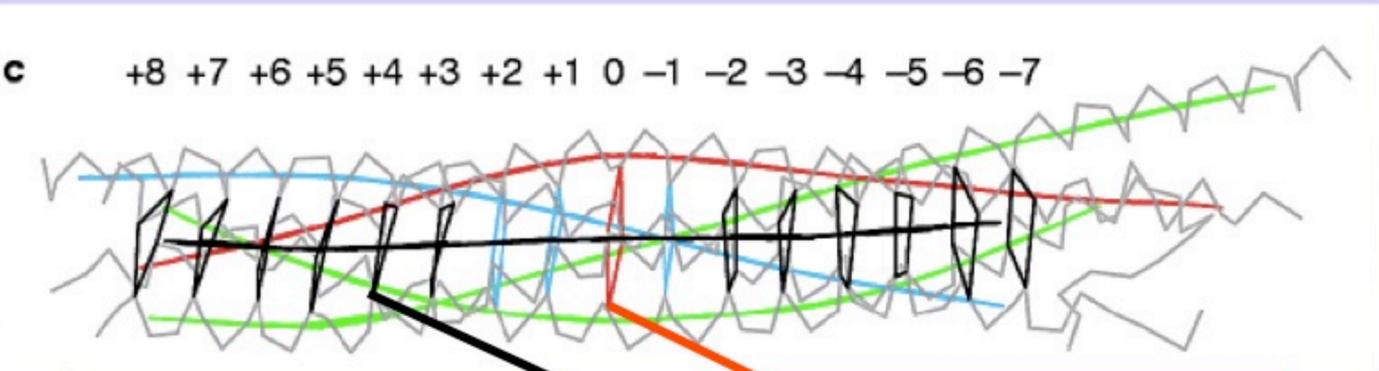


Core Proteins of the Secretory
 Lang and Jahn, Pharmacology of NT release, 2008.

Le complexe SNARE : Q-SNARE & R-SNARE



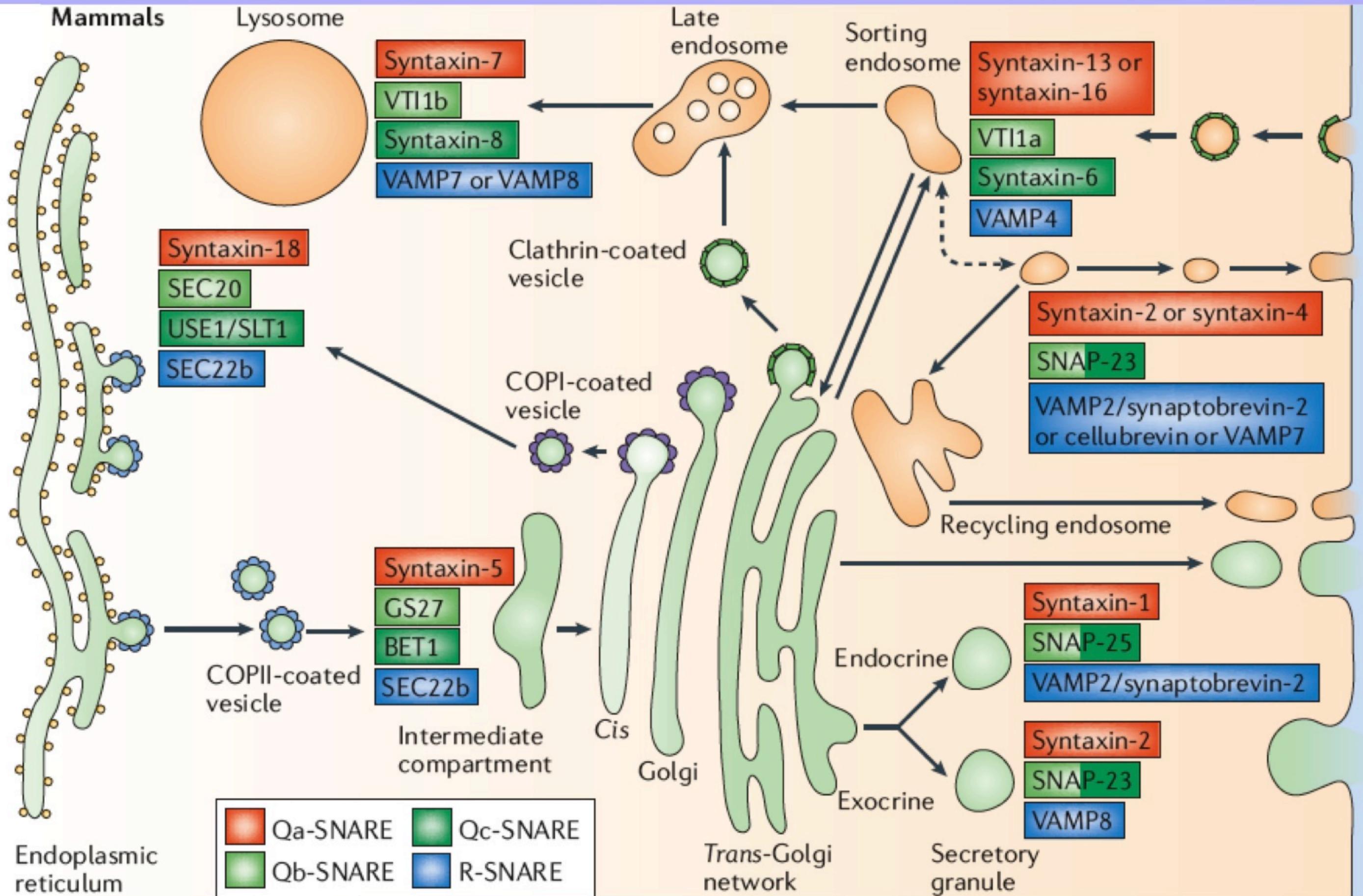
(Sutton et al., Nature 1998)



SNARE & compartiments

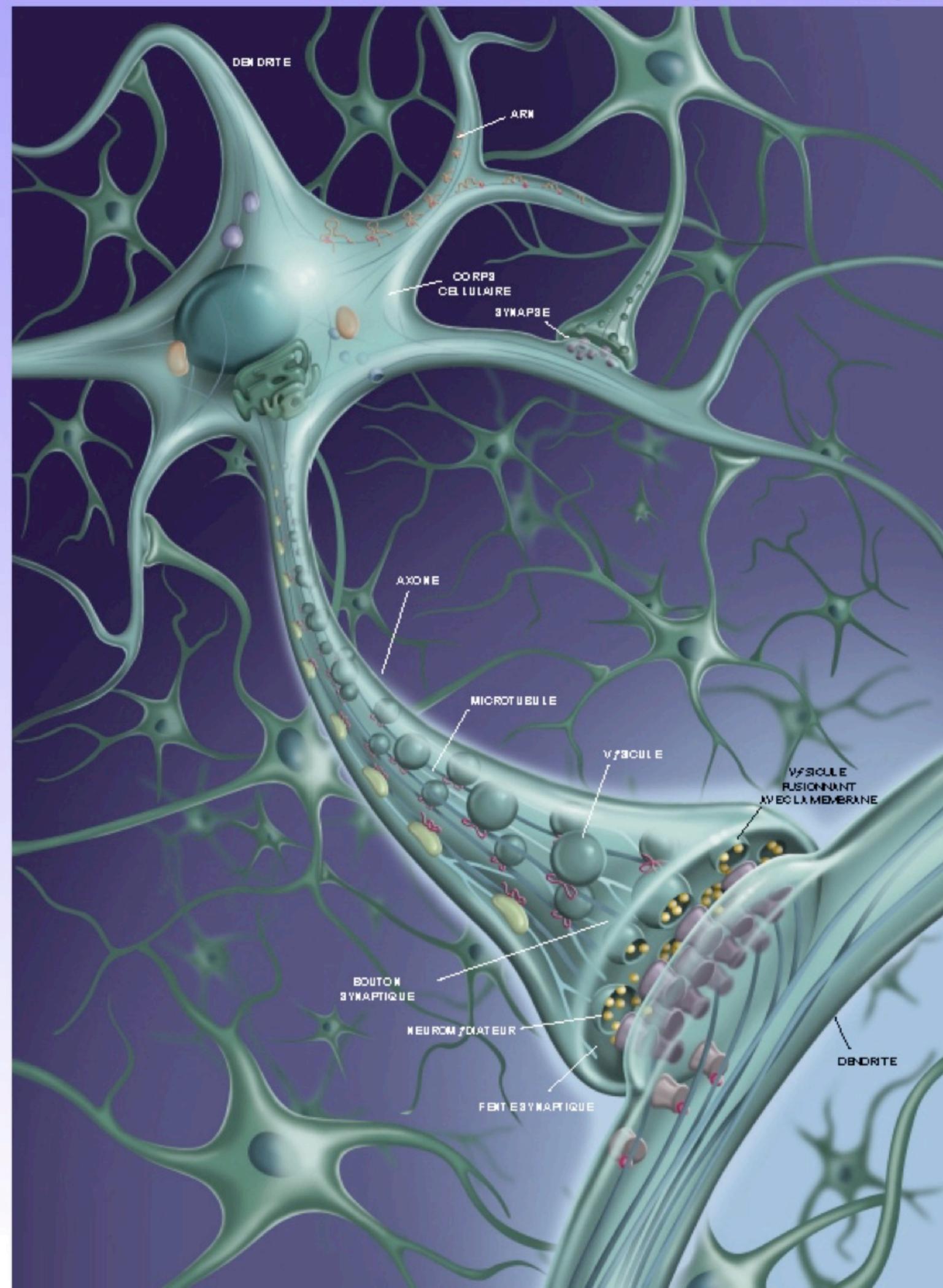
Chaîne	Levure	Nématode	Drosophile	Mammifères
SNAREs	21	23	20	35
Qa Syntaxines	7	9	7	12
Qb Nter SNAP25	5	7	5	9
Qc Cter SNAP25	6	4	5	8
R V-SNARE	5	6	5	9
Sec1	4	6	5	7
Rab	11	29	26	60

Les protéines SNARE et le trafic membranaire dans la cellule



Machinerie d'exocytose: le modele neuronal : l'exocytose synaptique

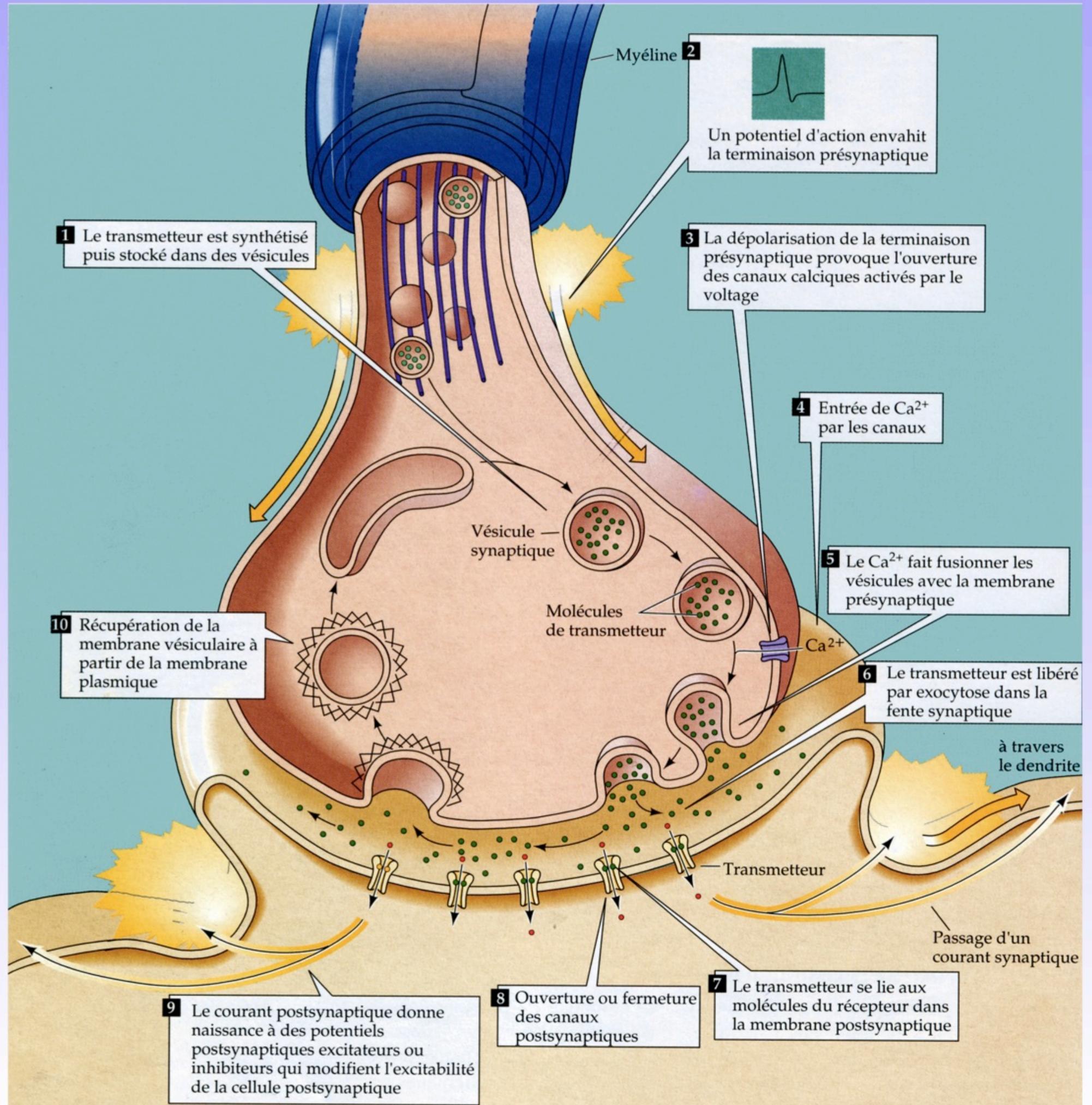
La fusion vésiculaire entre les vésicules synaptiques et la membrane présynaptique permet la libération des neurotransmetteur.



Thierry Galli & fabienne Paumet

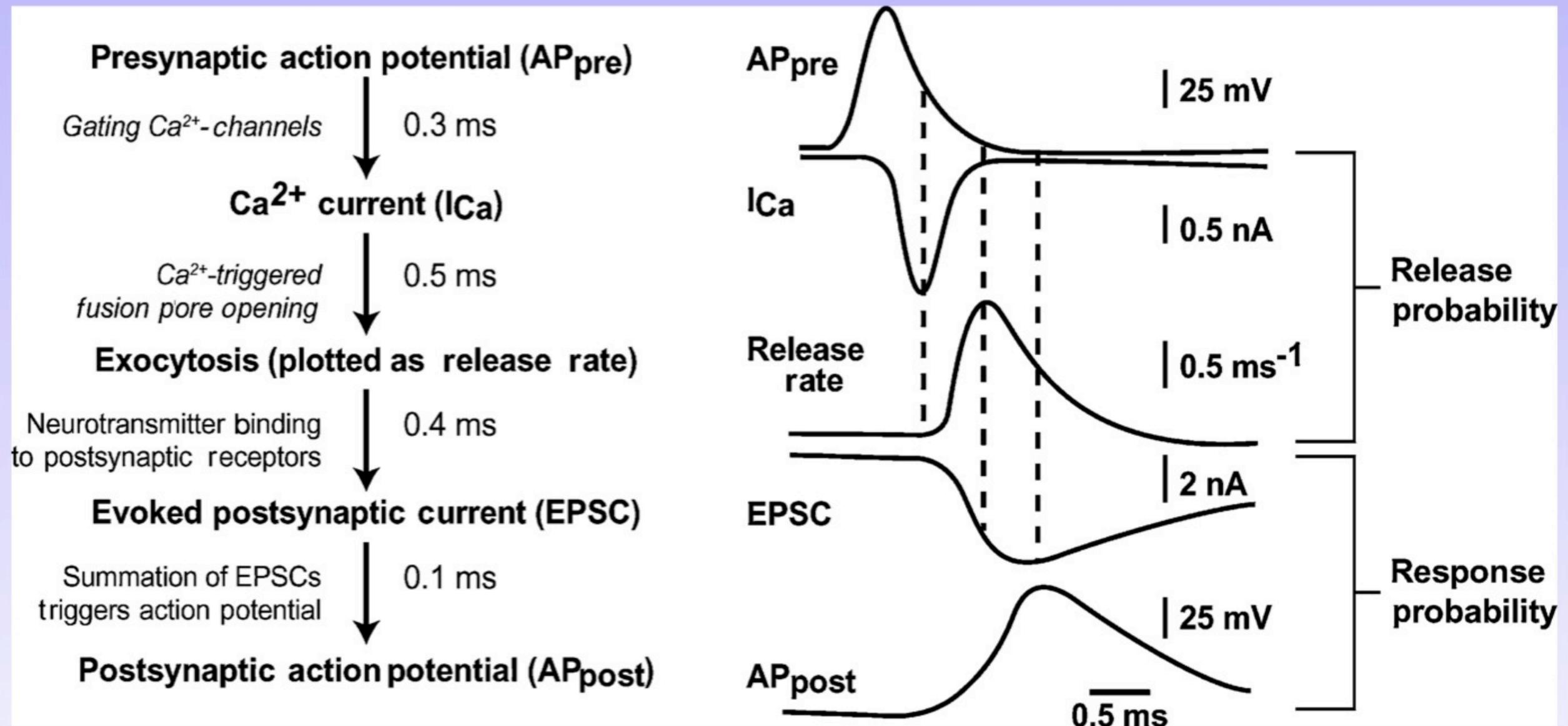
© POUR LA SCIENCE - N° 302 DÉCEMBRE 2002

L'exocytose synaptique



D'après Neurosciences,
à la découverte du cerveau
M. F. Bear

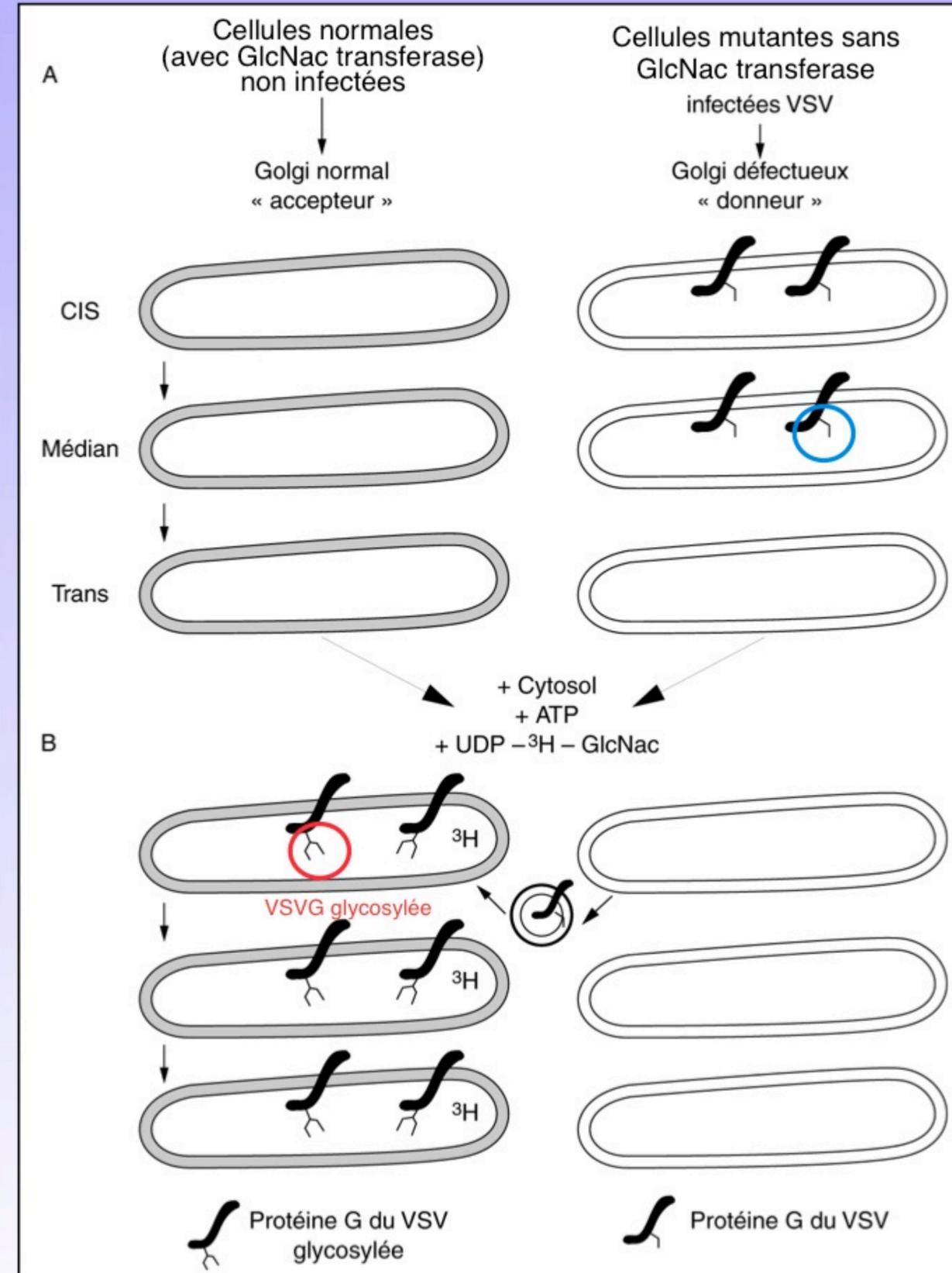
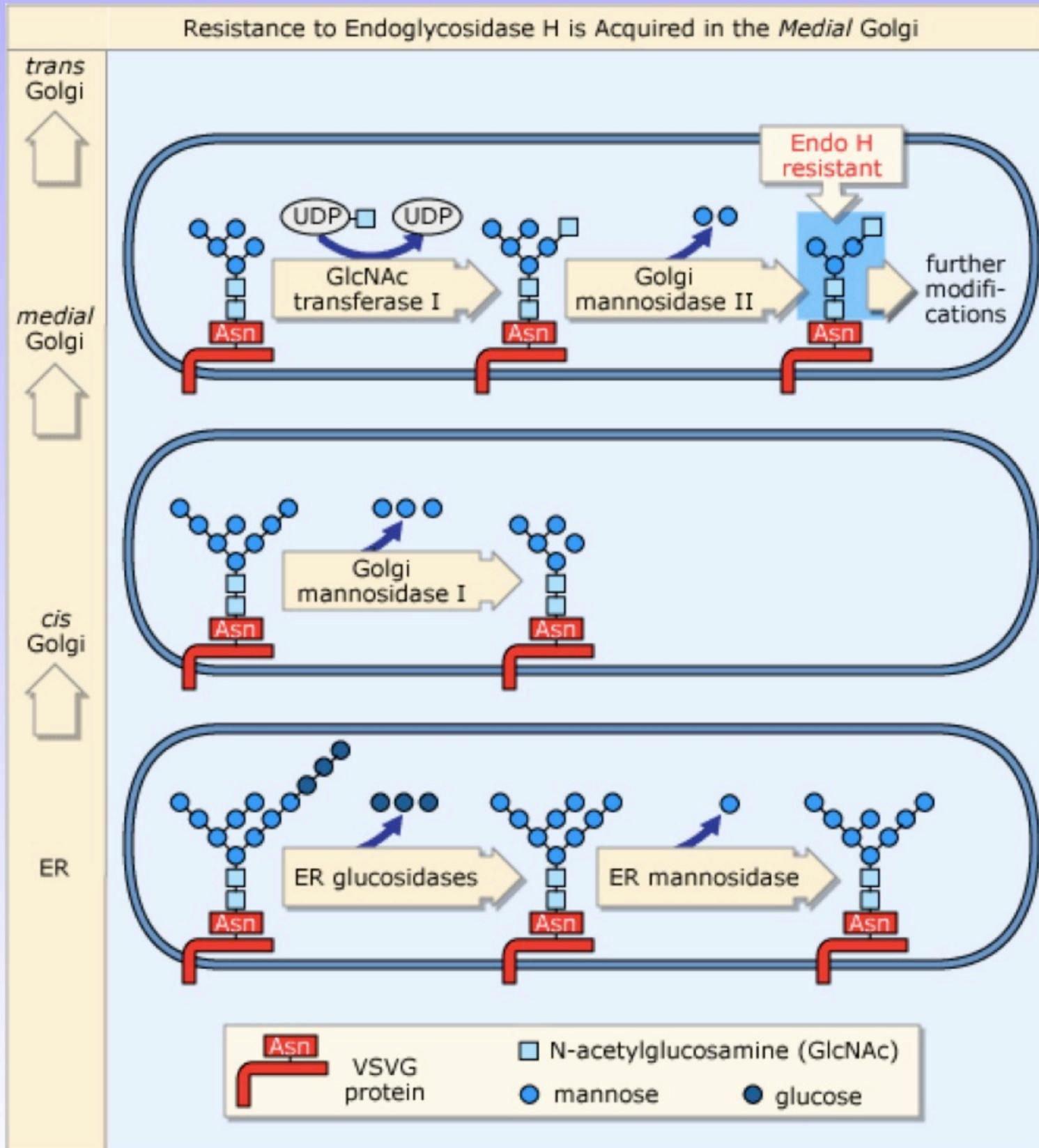
Chronologie: rapidité! Et rôle du calcium



Test in vitro de transport membranaire

(Balch et Rothman, Cell (1984))

Jim Rothman

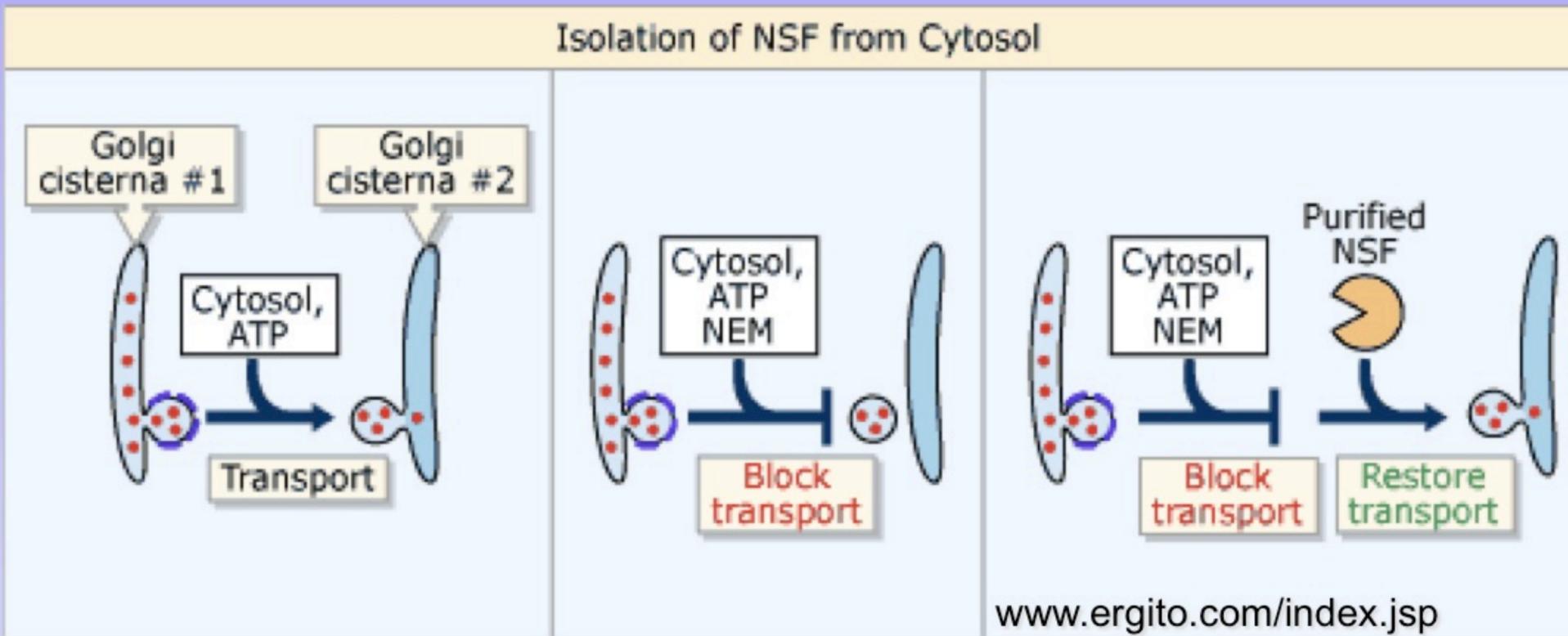


Les citernes restent distinctes, VSVG a été transportée : les auteurs formulent l'hypothèse du **transport vésiculaire**.



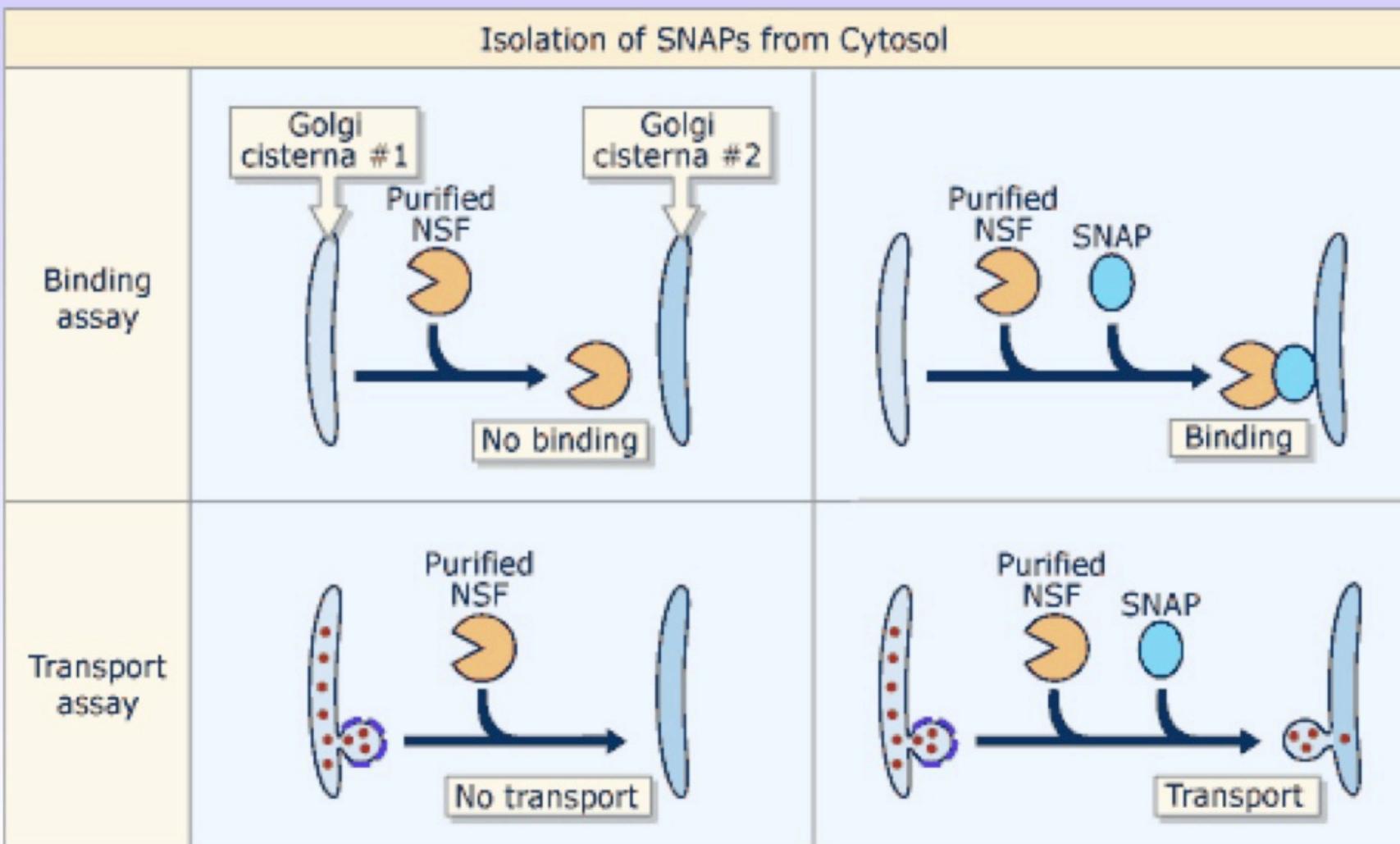
Jim Rothman

Découverte historique de NSF & SNAPs



NEM: N-Ethyl-Maleimide
NSF: NEM sensitive factor

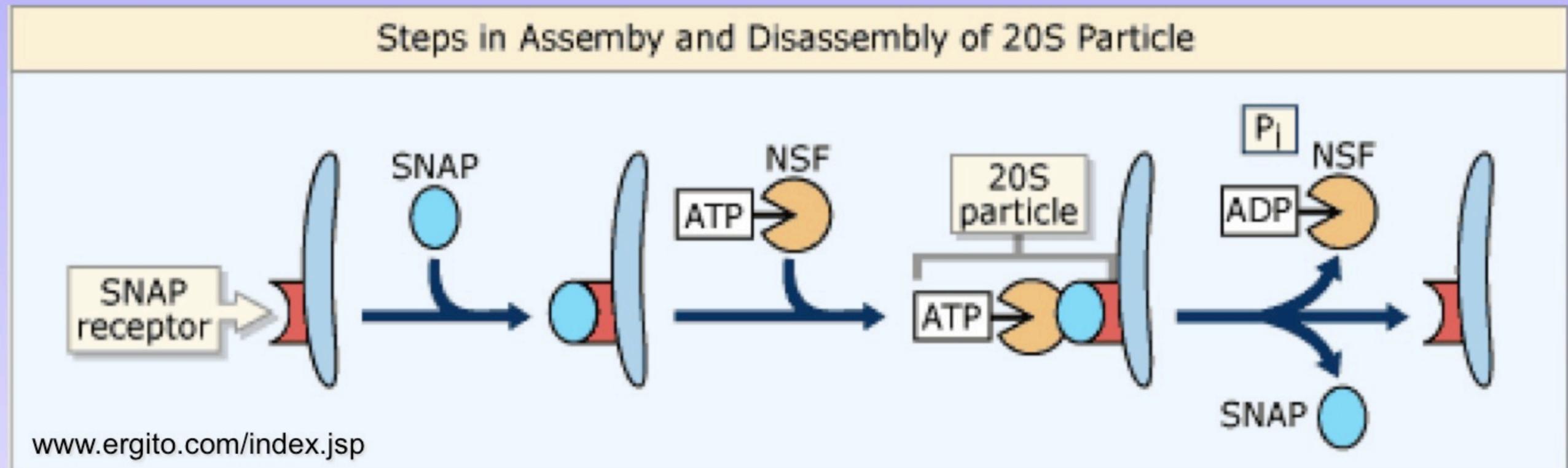
NSF est nécessaire à la fusion.
NSF est inhibée par le NEM.



SNAP:
Soluble NSF Attachment Factor

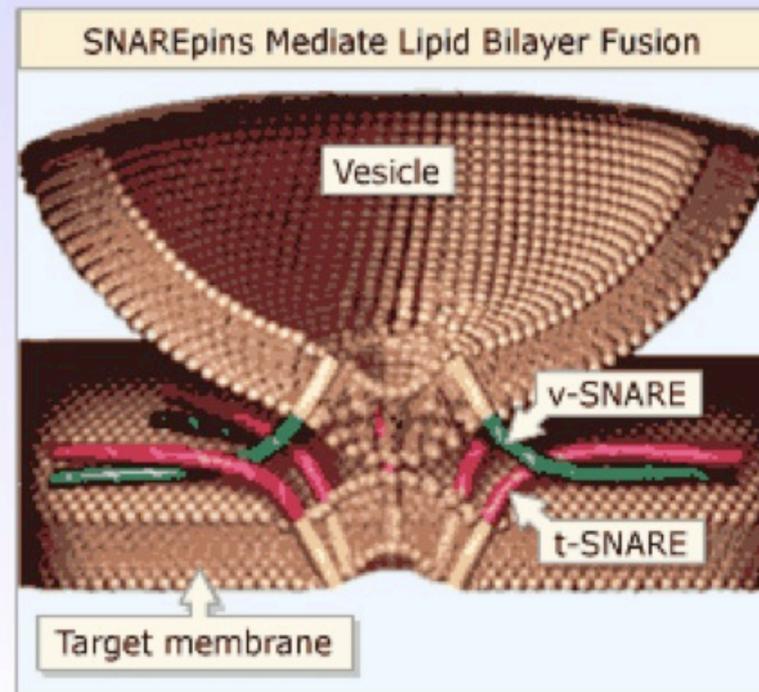
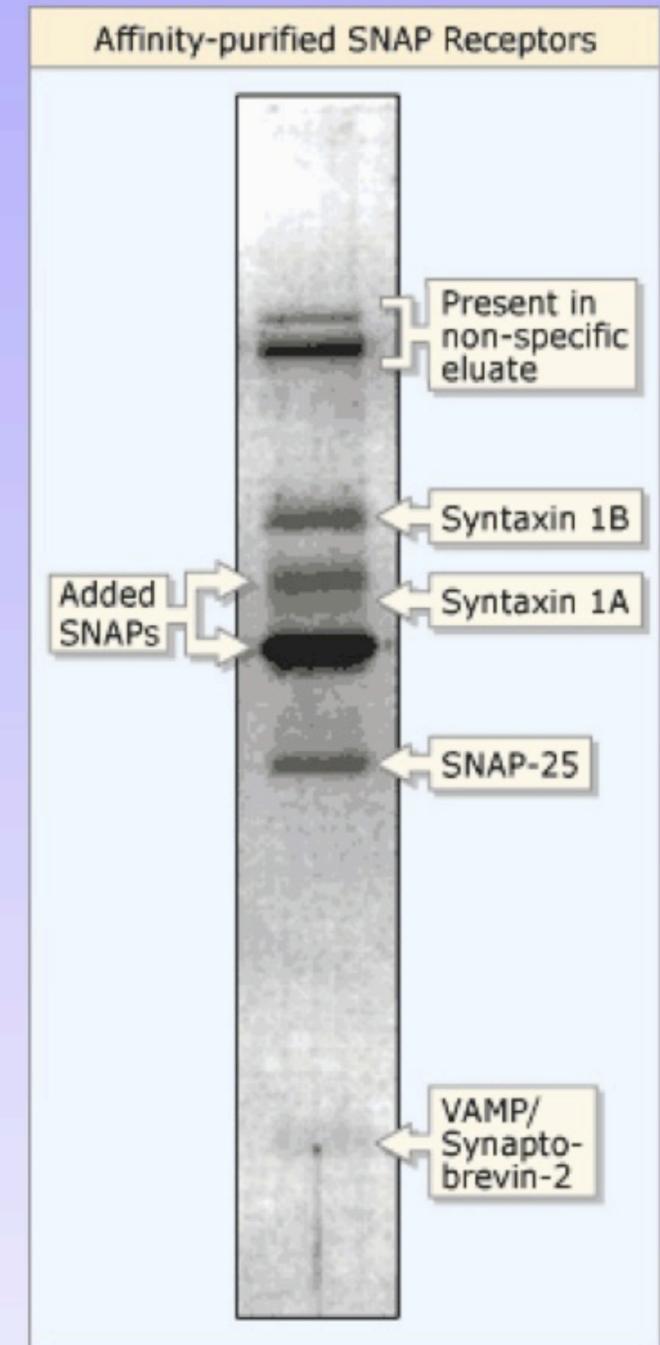
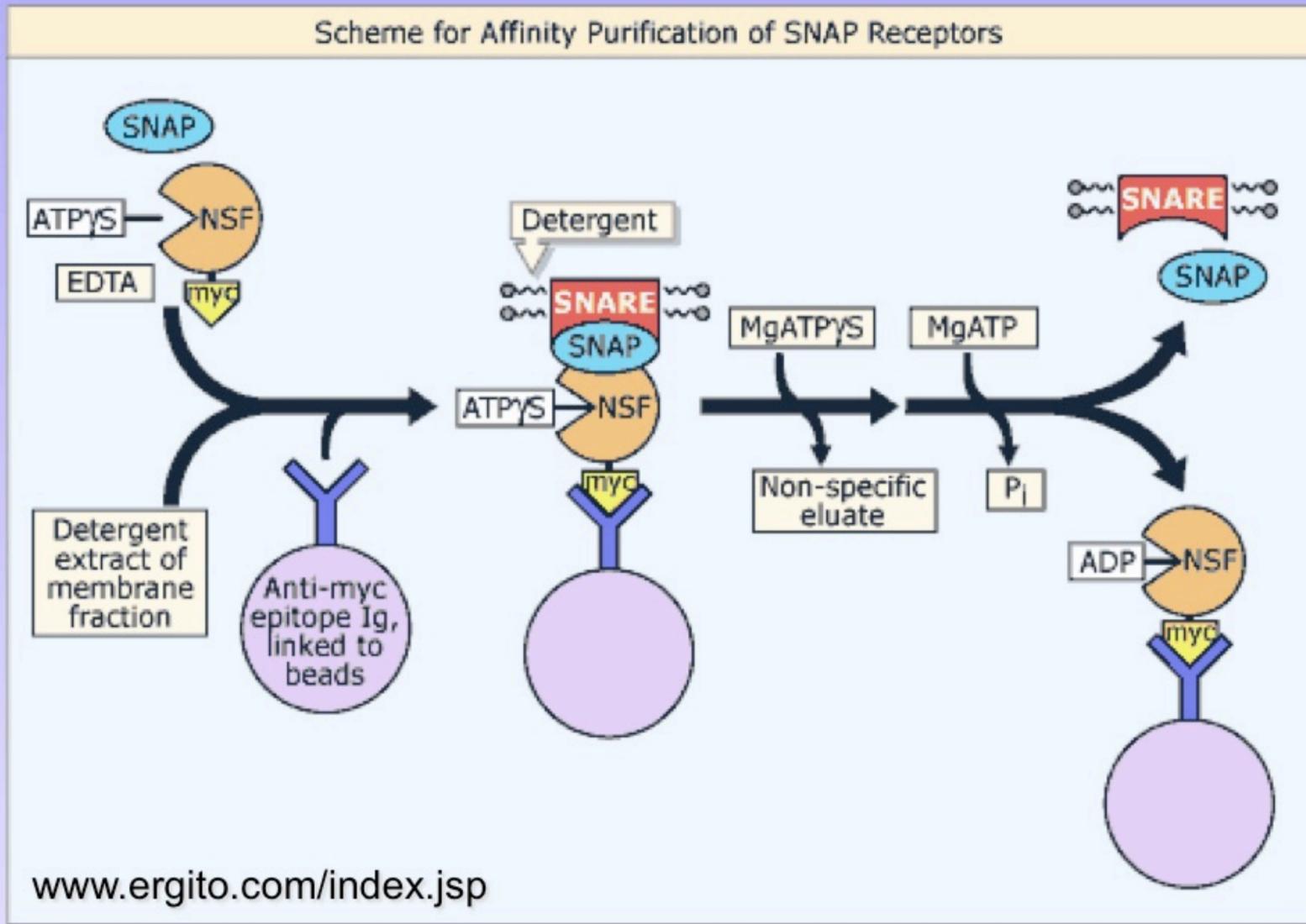
NSF est une protéine soluble qui ne peut se lier aux membranes que grâce aux SNAPs (3 isoformes α, β, γ).

Découverte historique des récepteurs de SNAP: les SNARE

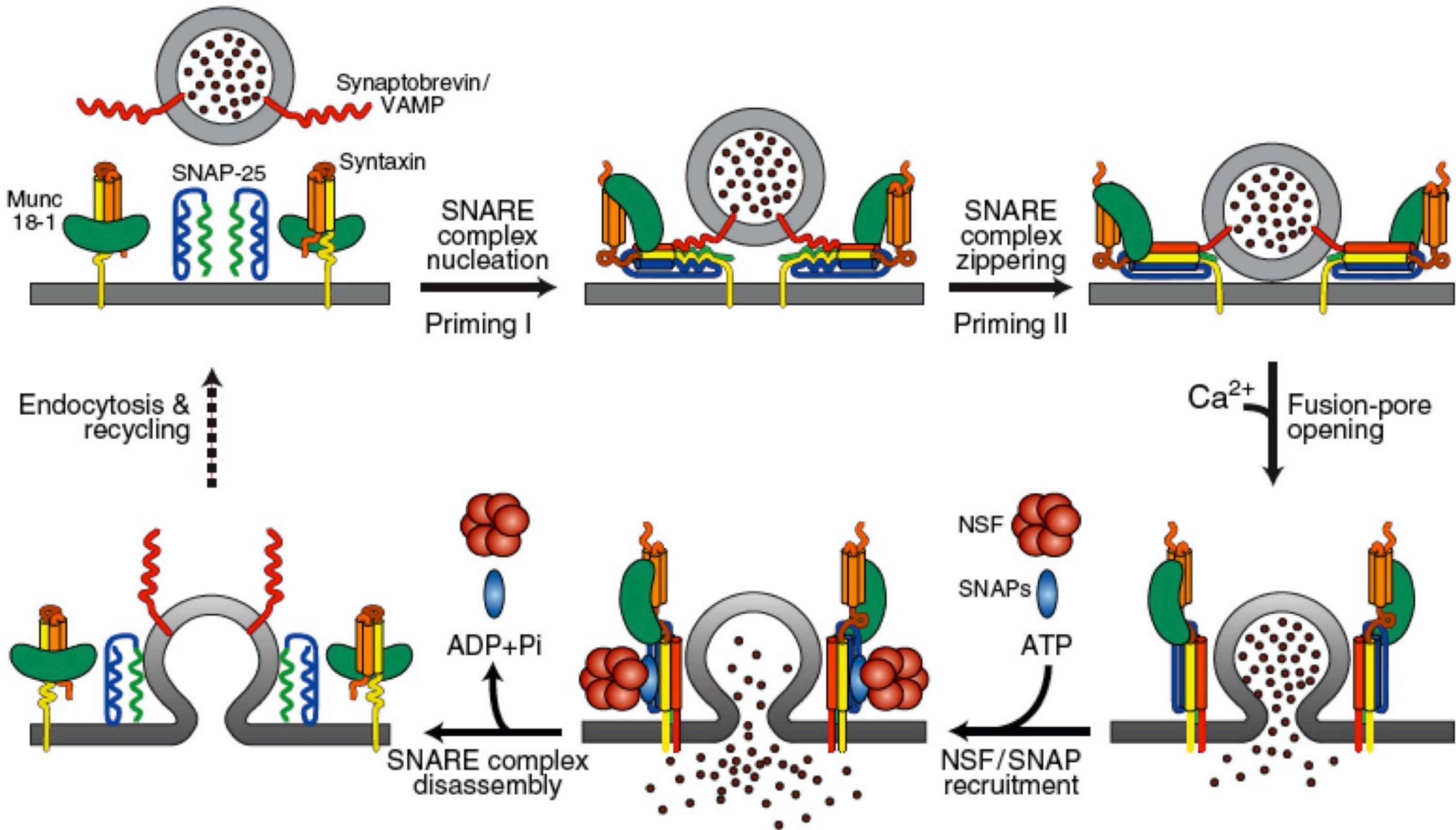


Comment isoler les récepteurs de SNAPs: les SNARE?

Isolement des SNAREs



Dissociation des SNARE par NSF

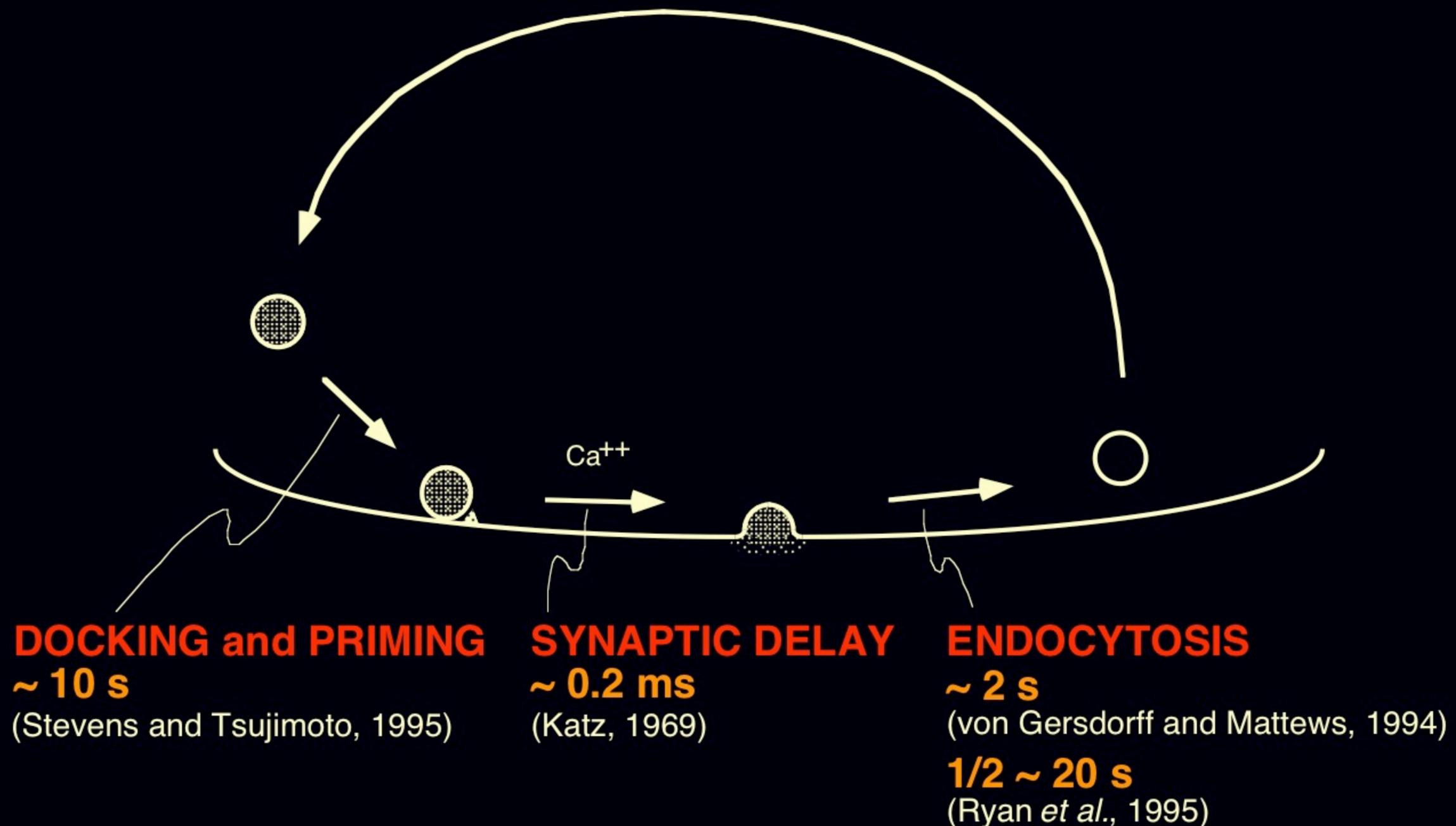


Recyclage des vésicules synaptiques

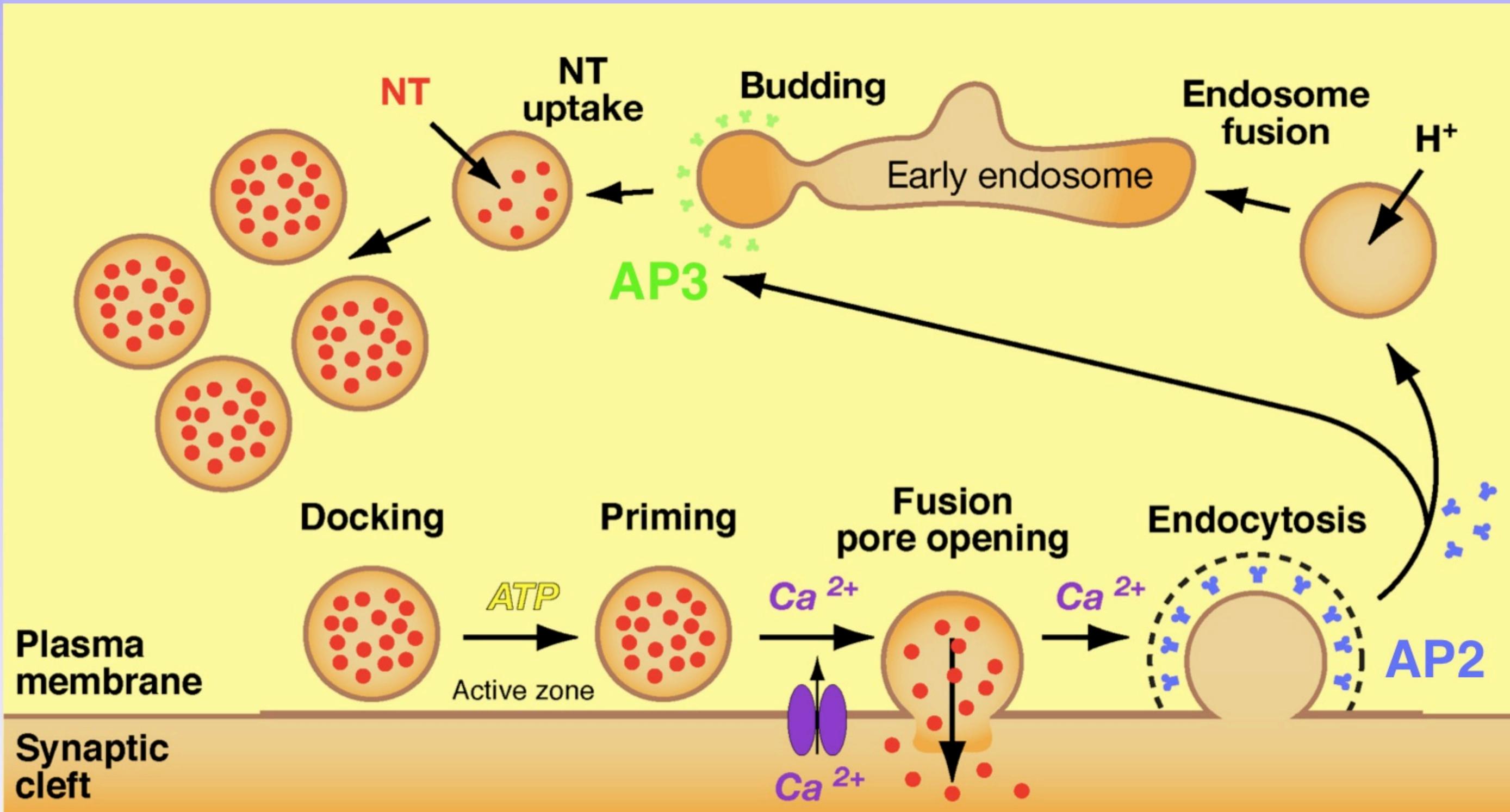
RECYCLING TIME

less than 60 s

(Betz and Bewick, 1993; Ryan *et al.*, 1995)

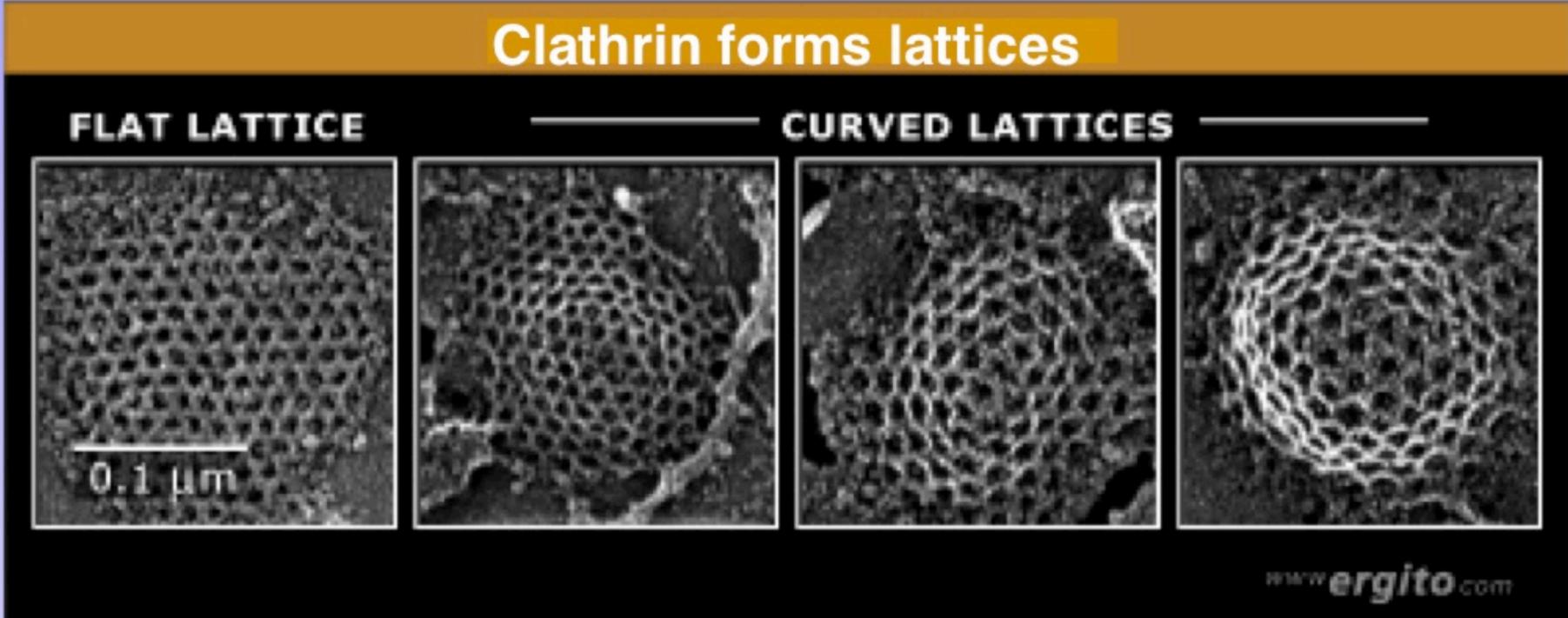


Recyclage des vésicules synaptiques

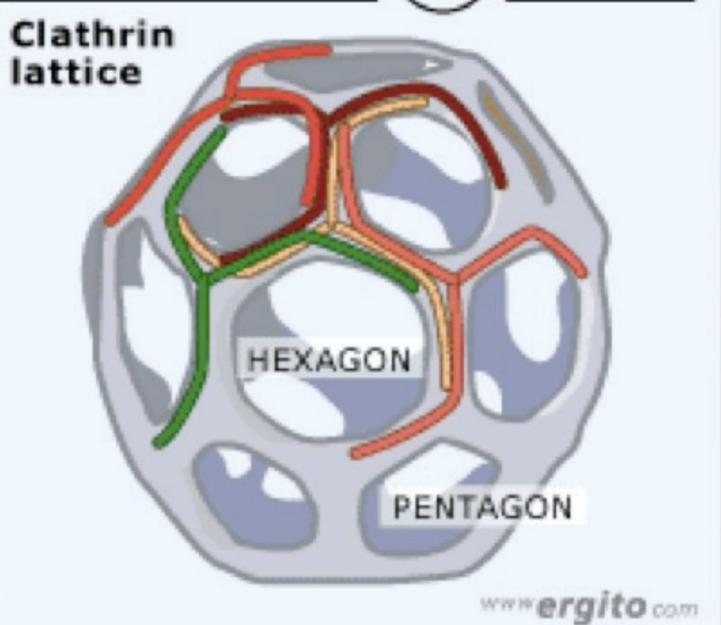
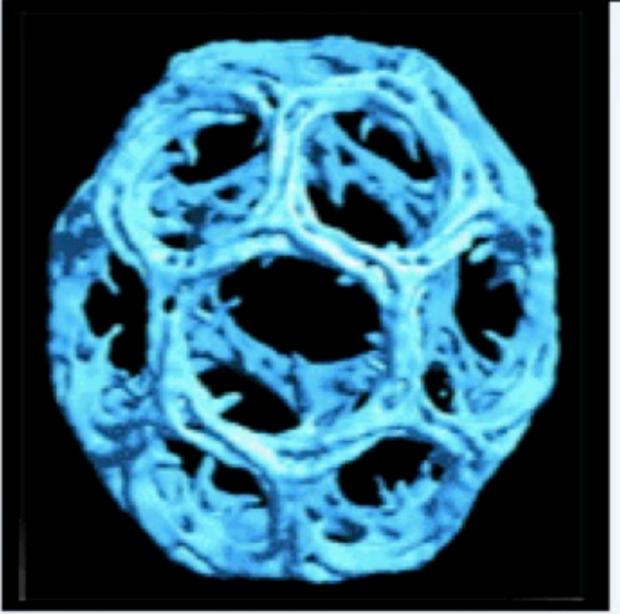
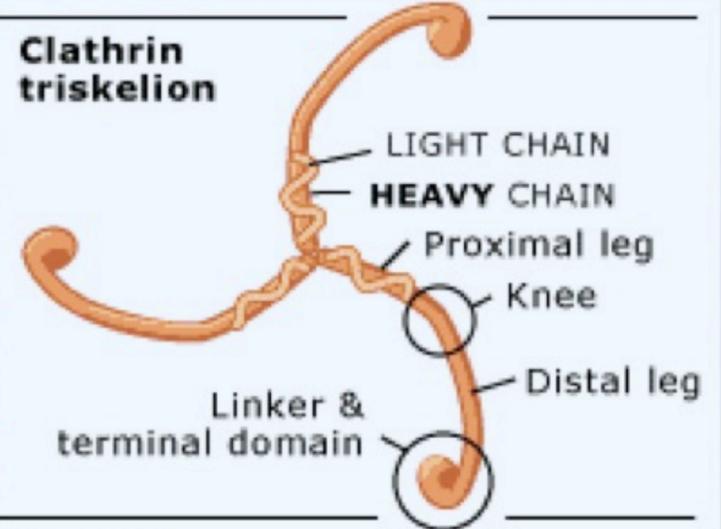
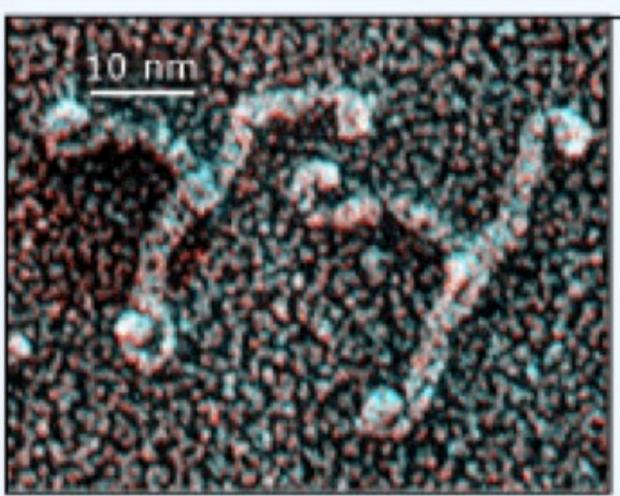


Danglot & Galli, *Biology of the cell*, 2003.

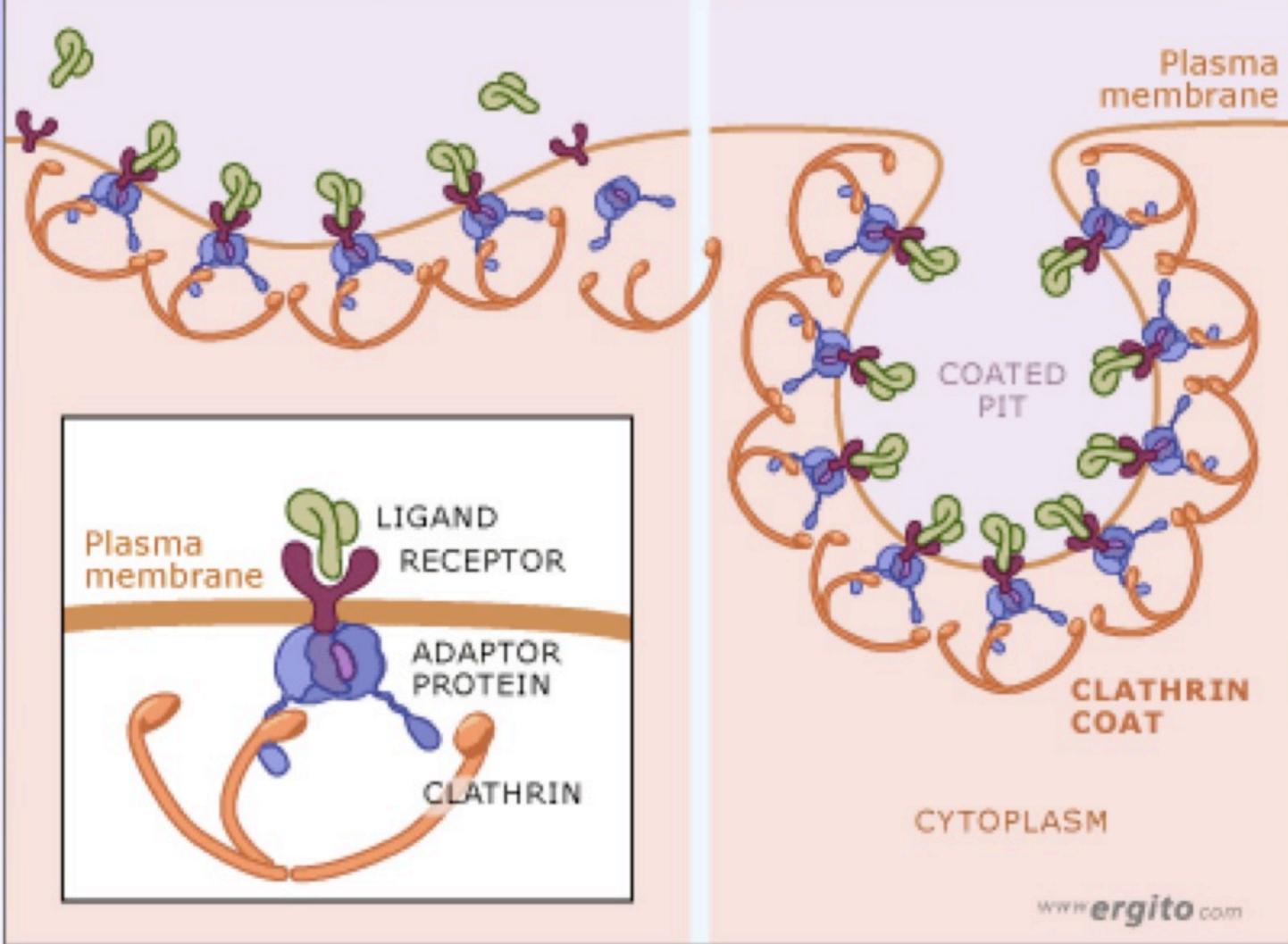
Clathrine



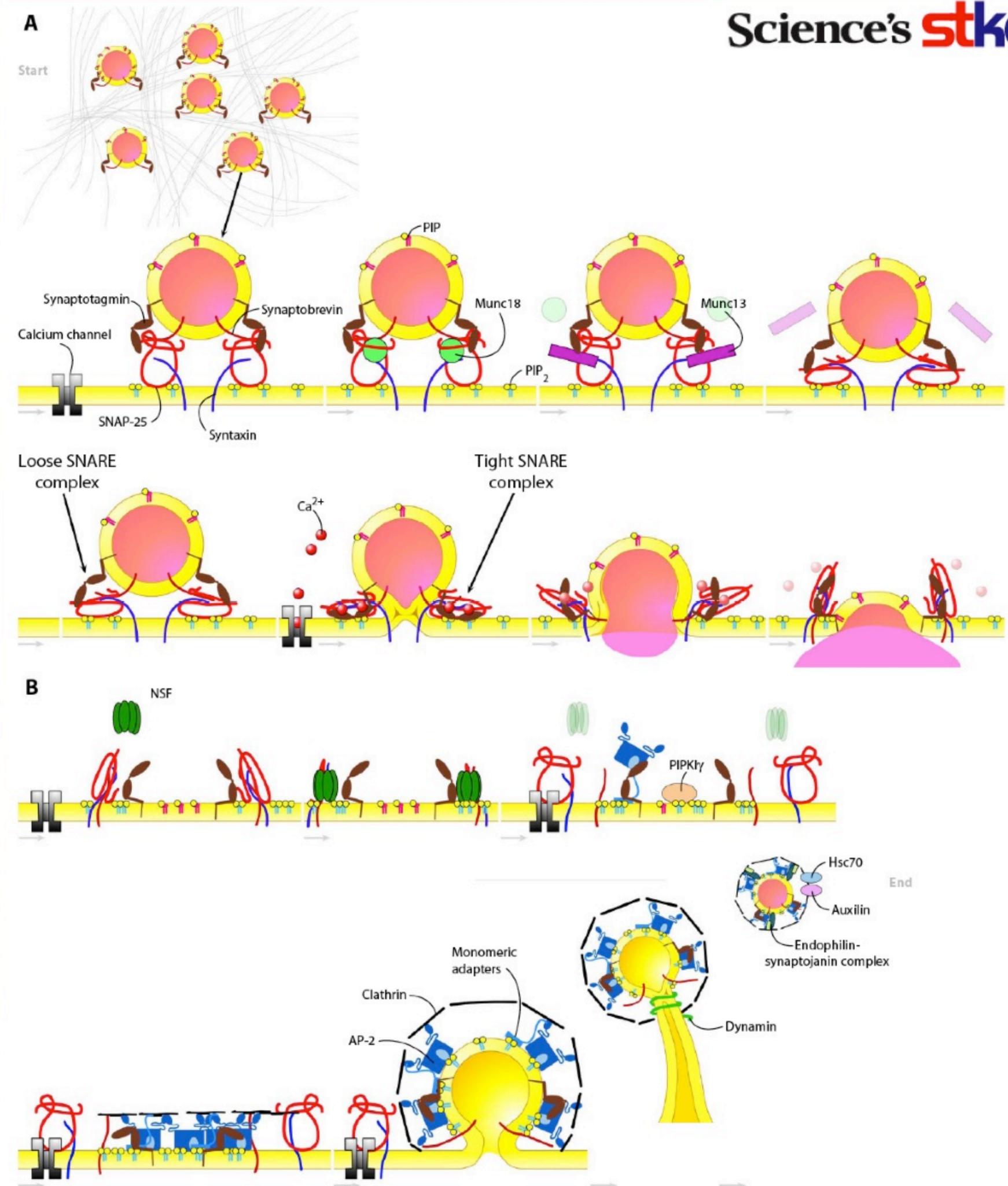
Structure of clathrin



Adapters links cargo & clathrin



Recyclage des Vésicules synaptiques:
endocytose médiée par la clathrine
(la voie lente)





A Slow Track for Synaptic Vesicle Retrieval by Clathrin-Mediated Endocytosis

T. Galli, V. Haucke,

Cycling of synaptic vesicles: How far? How fast? *Sci. STKE* **2004**, re19 (2004).

Animation by Cameron Slayden

Click the screen to begin

Kiss and Run ?



A Fast Track for Synaptic Vesicle Cycling.

T. Galli, V. Haucke,

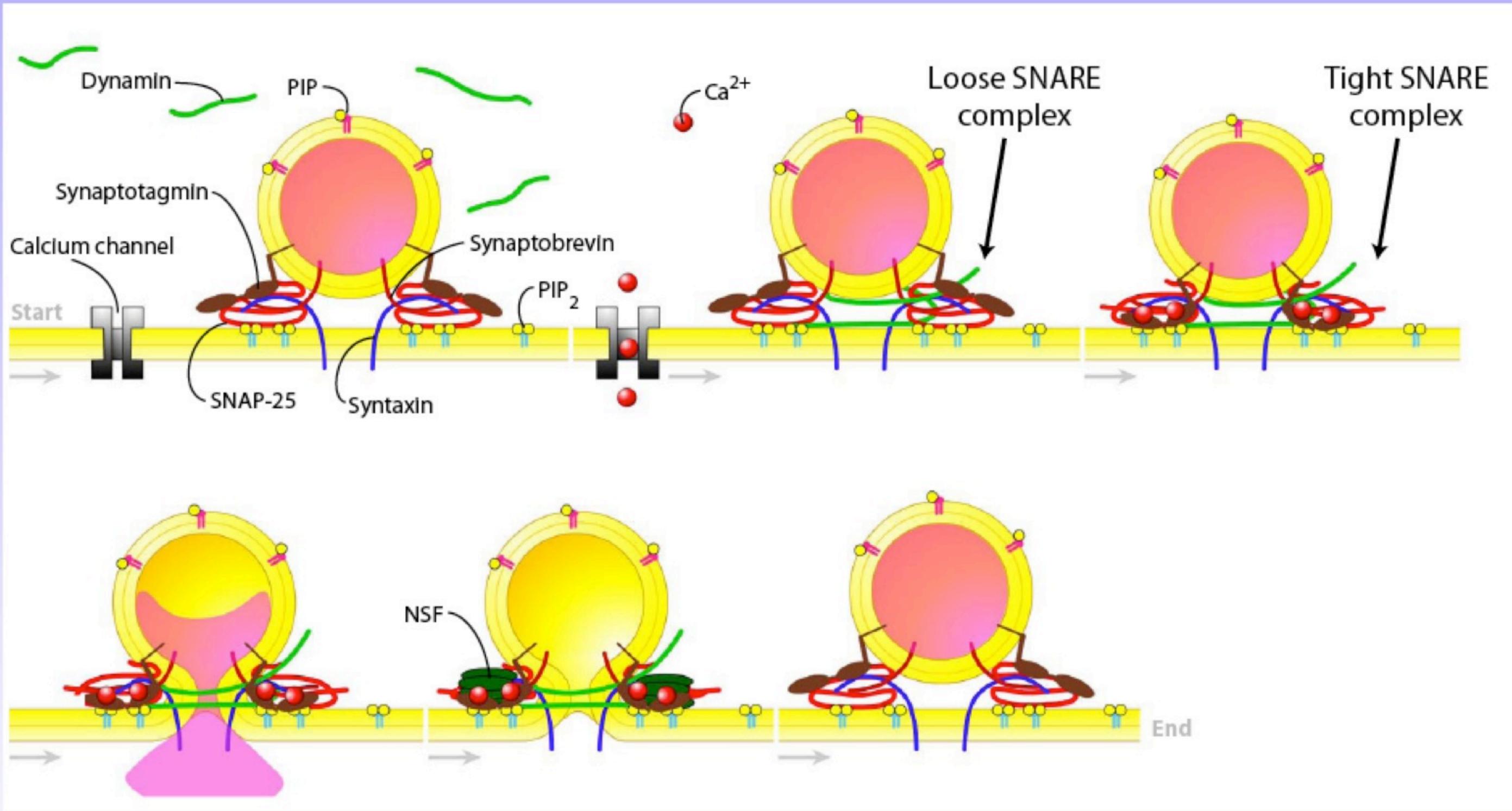
Cycling of synaptic vesicles: How far? How fast? *Sci. STKE* **2004**, re19 (2004).

Animation by Cameron Slayden

Click the screen to begin



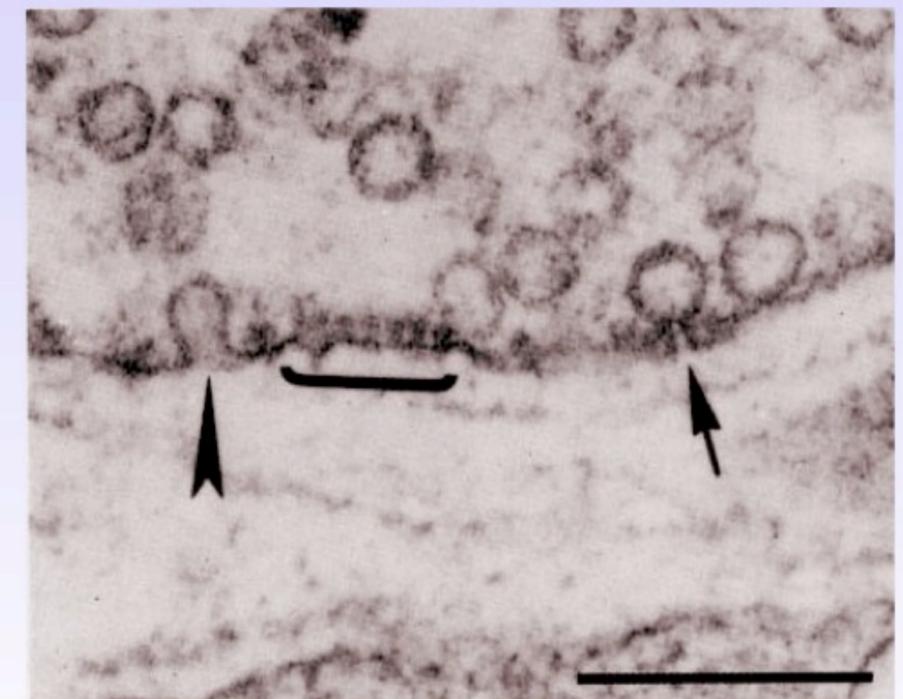
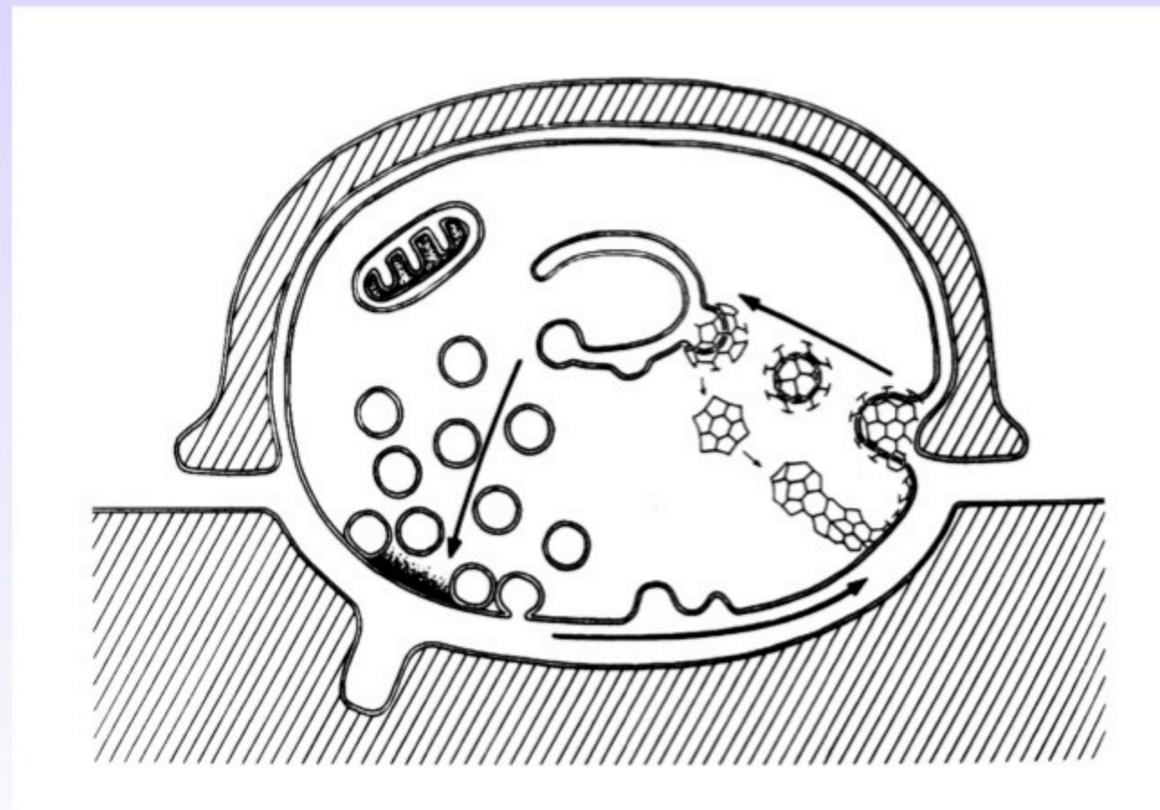
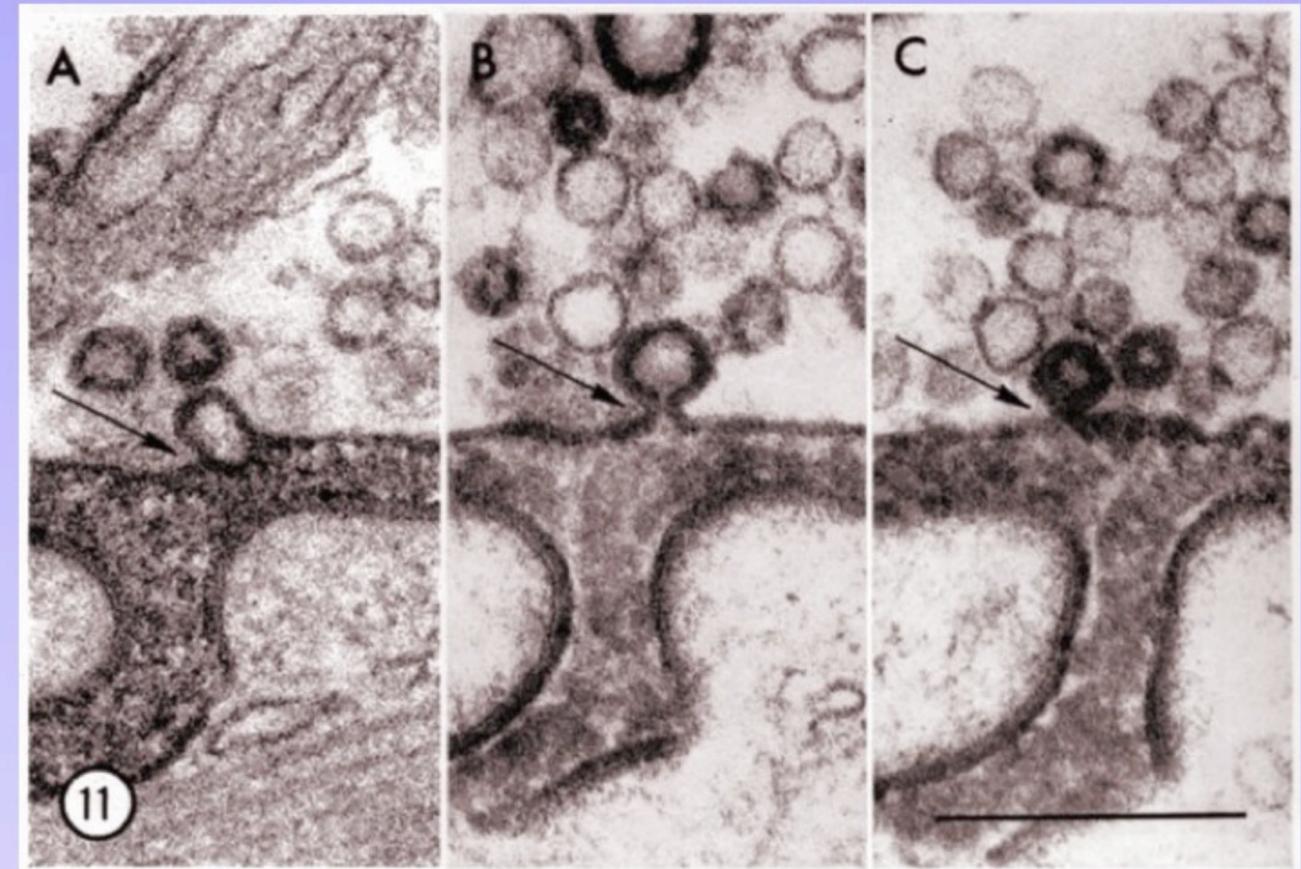
Kiss and Run ?



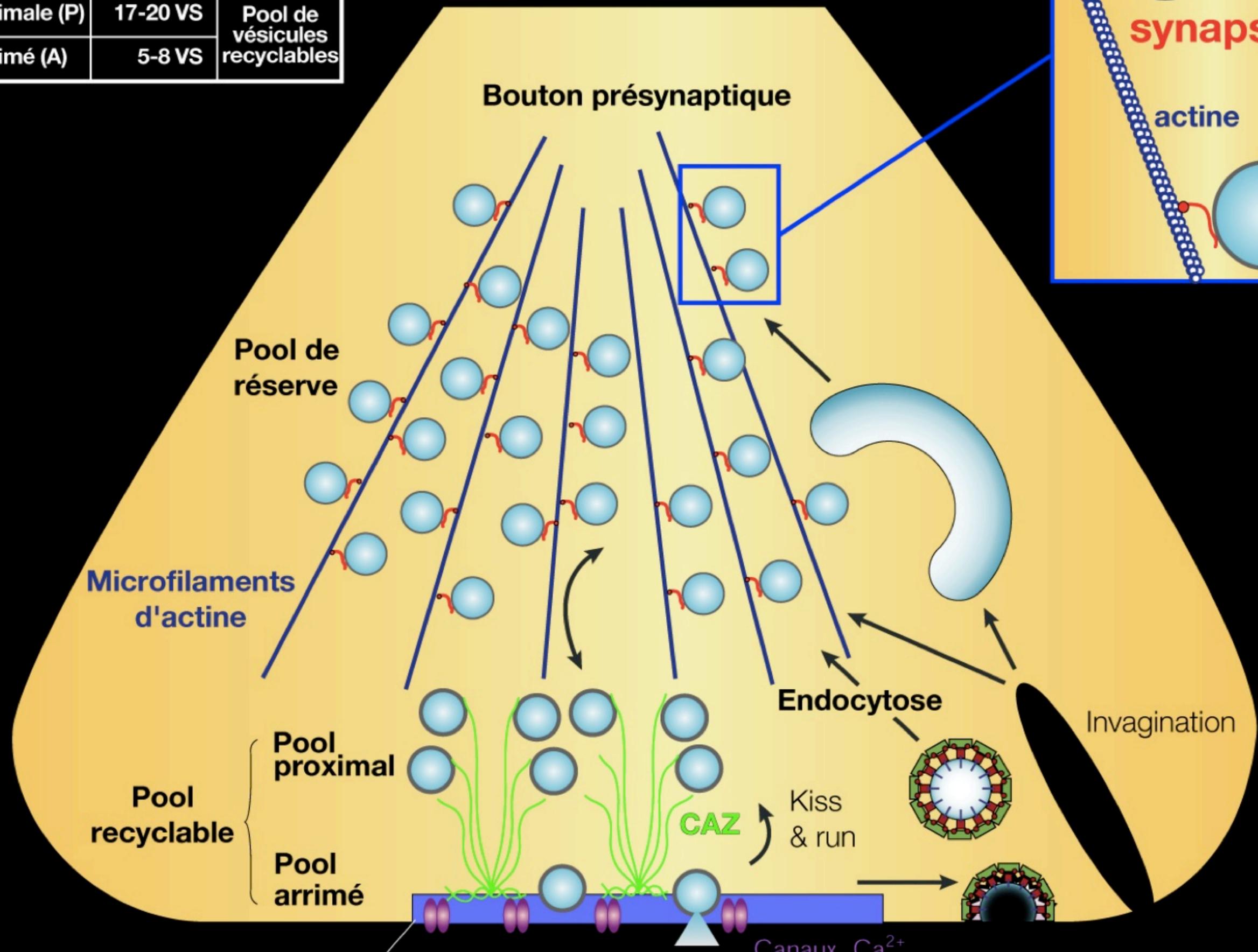
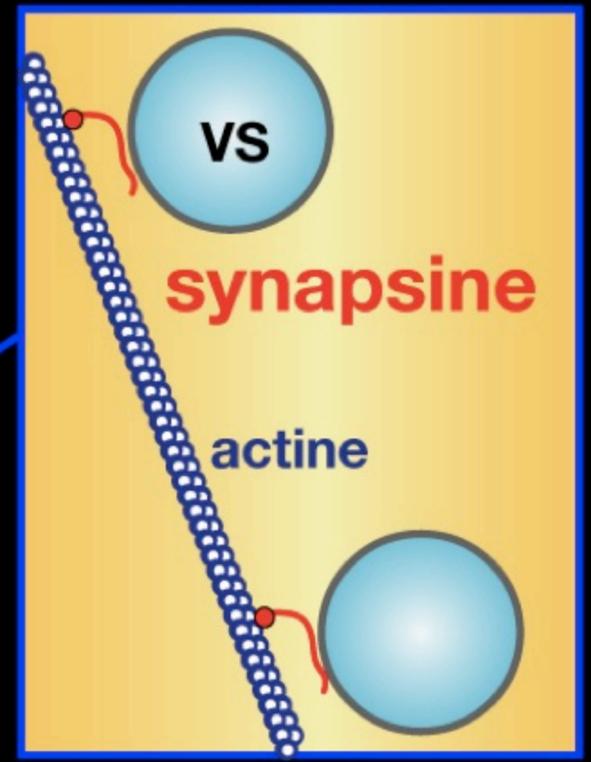
Thierry Galli¹ and Volker Haucke

www.stke.org/cgi/content/full/sigtrans;2004/264/re19

***Et en microscopie
électronique ?***



Type de pool	Nombre de vésicules	
de réserve (R)	180 VS	
proximale (P)	17-20 VS	Pool de vésicules recyclables
arrimé (A)	5-8 VS	



Zone active

Comment mesurer l'exocytose ?

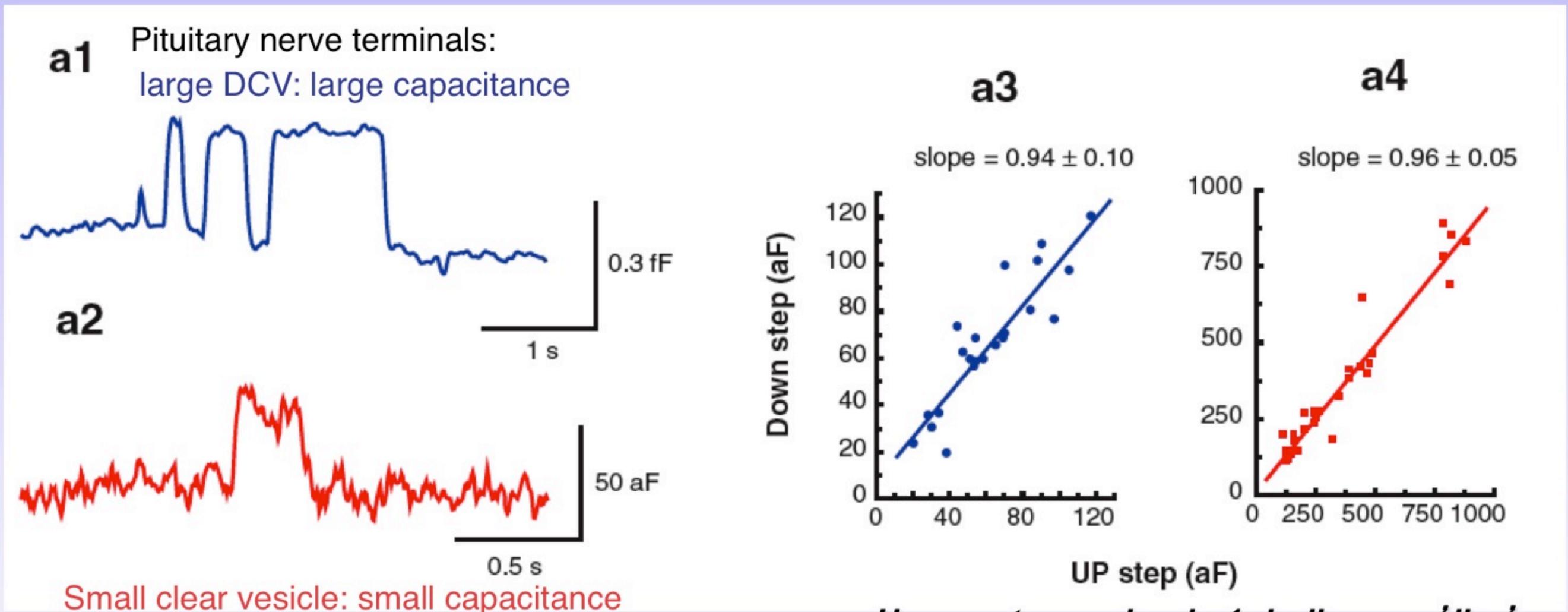
1) Capacitance:

La mesure de la capacitance de la cellule est proportionnelle à la surface de la membrane.

L'enregistrement de la capacitance permet de mesurer l'addition de membrane provoquée lors d'un évènement de fusion membranaire.

Cellules chromaffines (medullosurrénales): la fusion d'une vésicules produit une augmentation de la capacitance de 1fF (Neher, PNAS 1982).

Mastocytes : ont des vésicules + larges: augmentation de 16fF

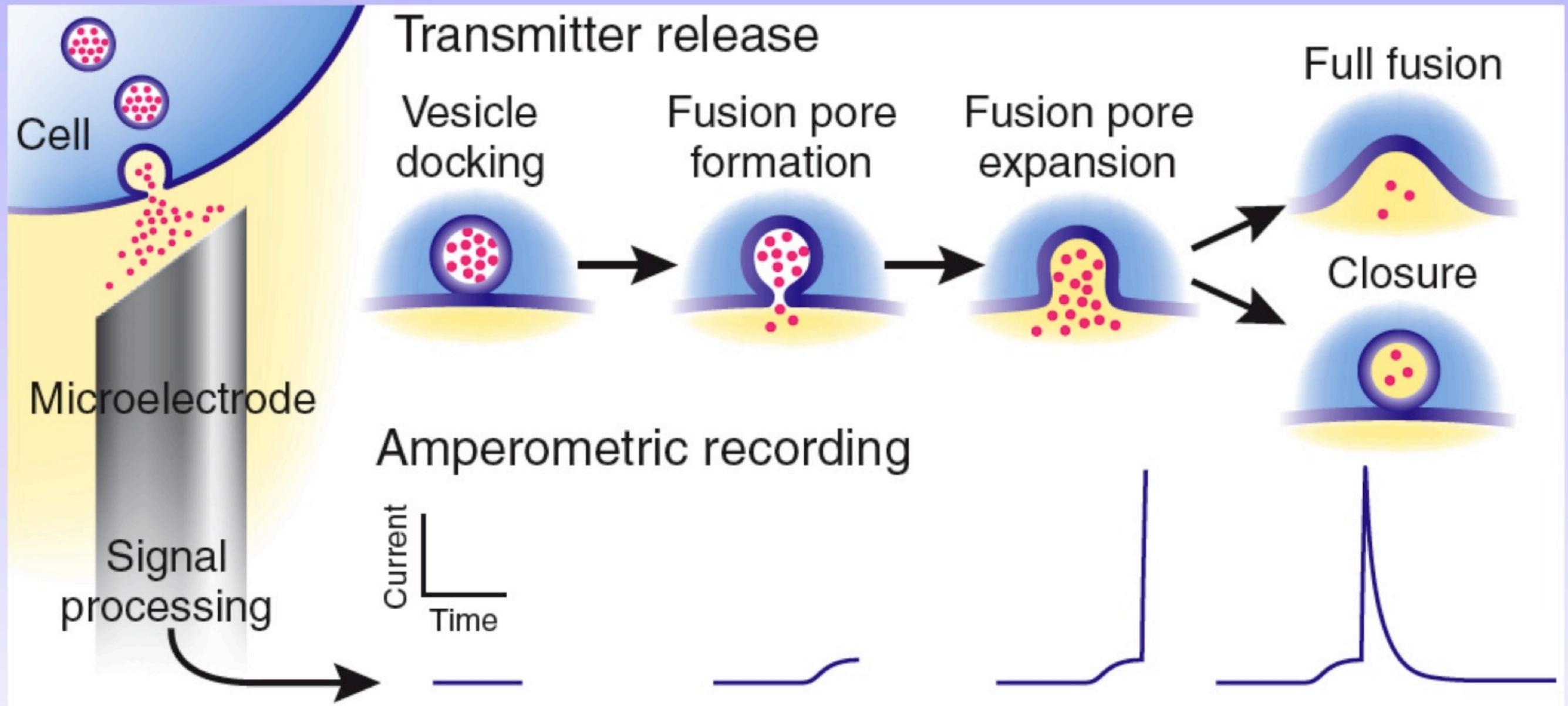


Une pente proche de 1, indique qu'il n'y a pas de transfert de membrane et indique plutôt un événement de type kiss-and-run.

Comment mesurer l'exocytose ?

2) L'ampérométrie à fibre de carbone (5-10 microns de diamètre):

On stimule les cellules par une dépolarisation. La cellule sécrète alors des molécules. En présence d'un potentiel approprié, les molécules (catécholamines, indolamines) sécrétées s'oxydent et libèrent des électrons. La mesure du courant d'oxydation donne accès à la quantité de molécules sécrétées par événement unitaire d'exocytose.



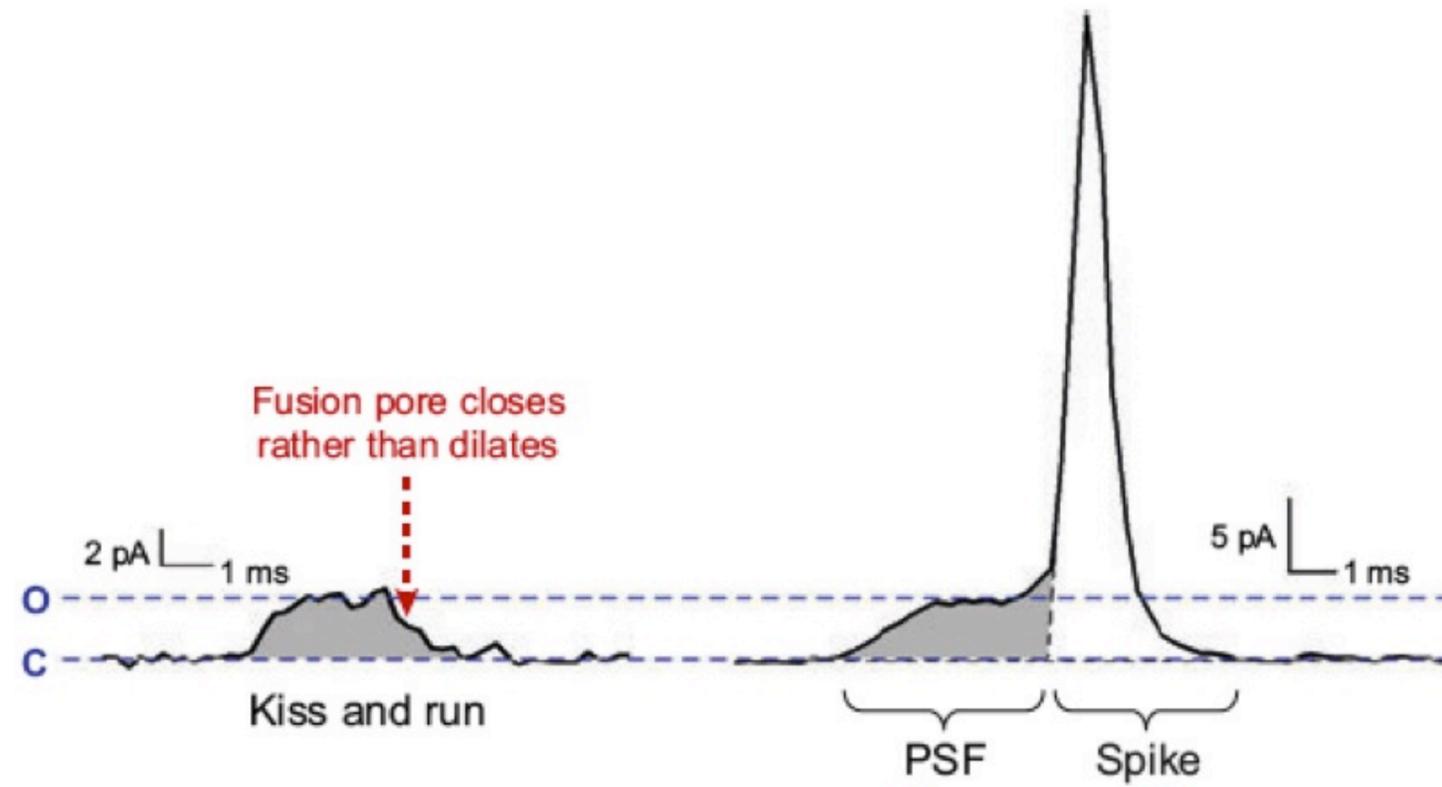
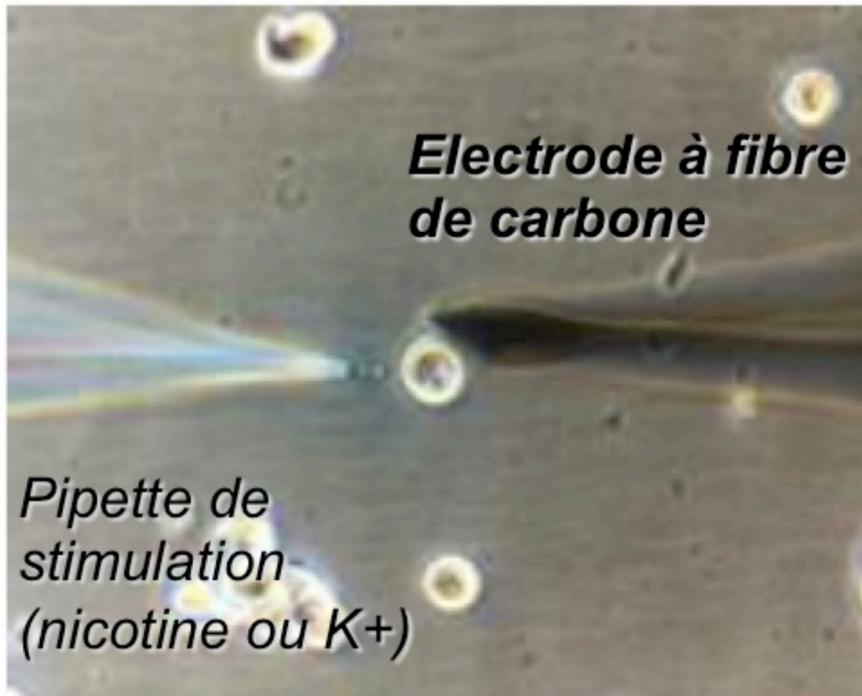
Cellules mesurées: cellules chromaffines (noradrénaline, adrénaline), mastocytes (histamines, serotoninines), et cellules β du pancreas (insuline).

Résolution temporelle: $<1\text{ms}$

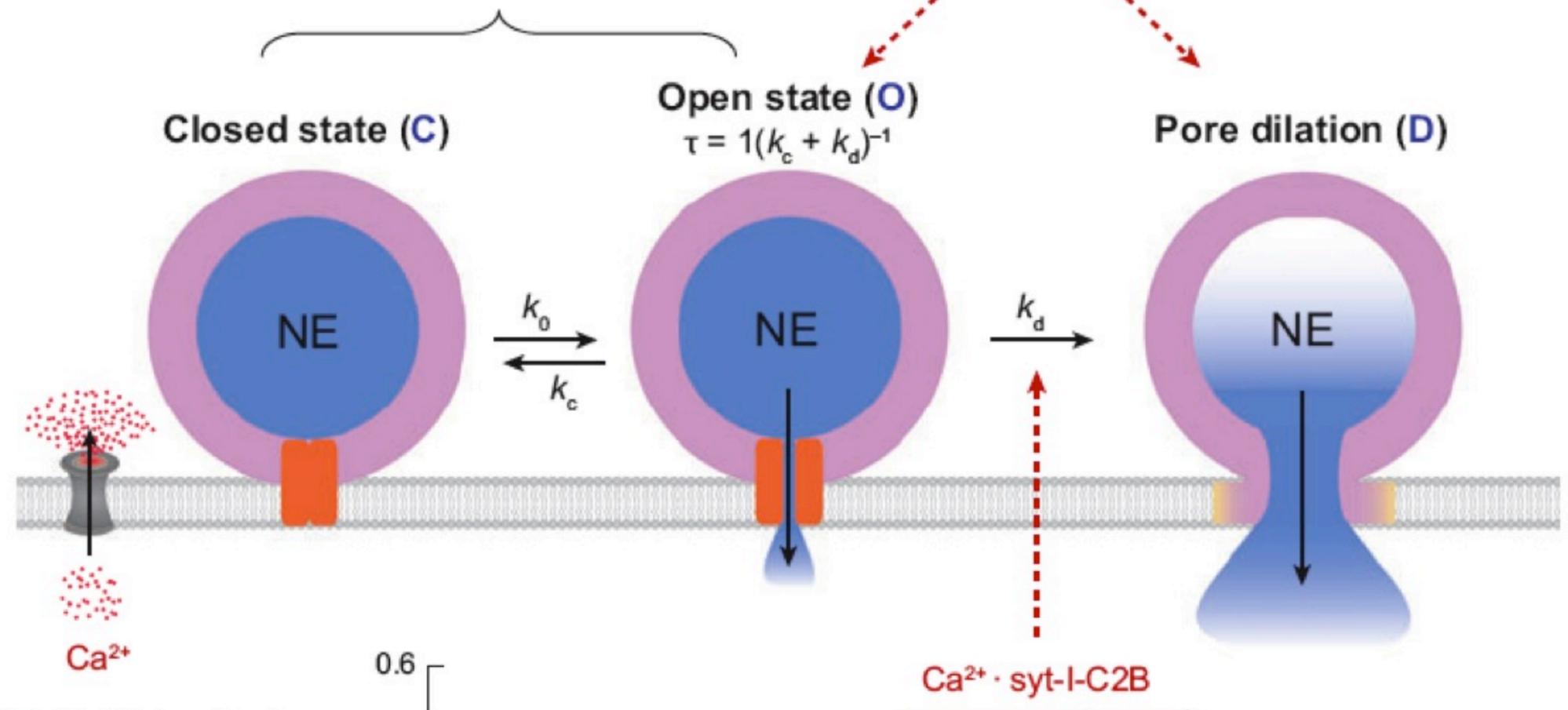
Sensibilité de détection: quelques milliers de molécules (Chen et al., 1994)

Comment mesurer l'exocytose ?

2) L'ampérométrie à fibre de carbone:

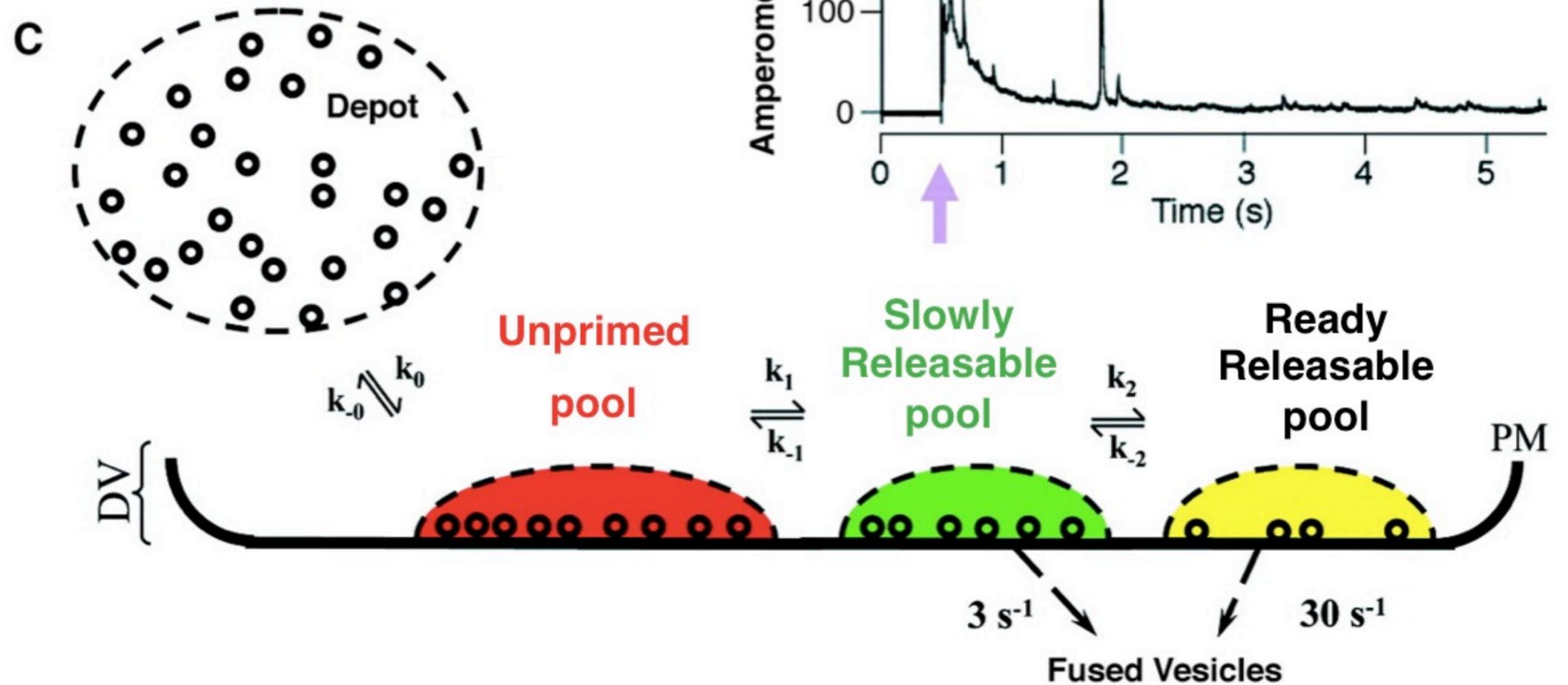
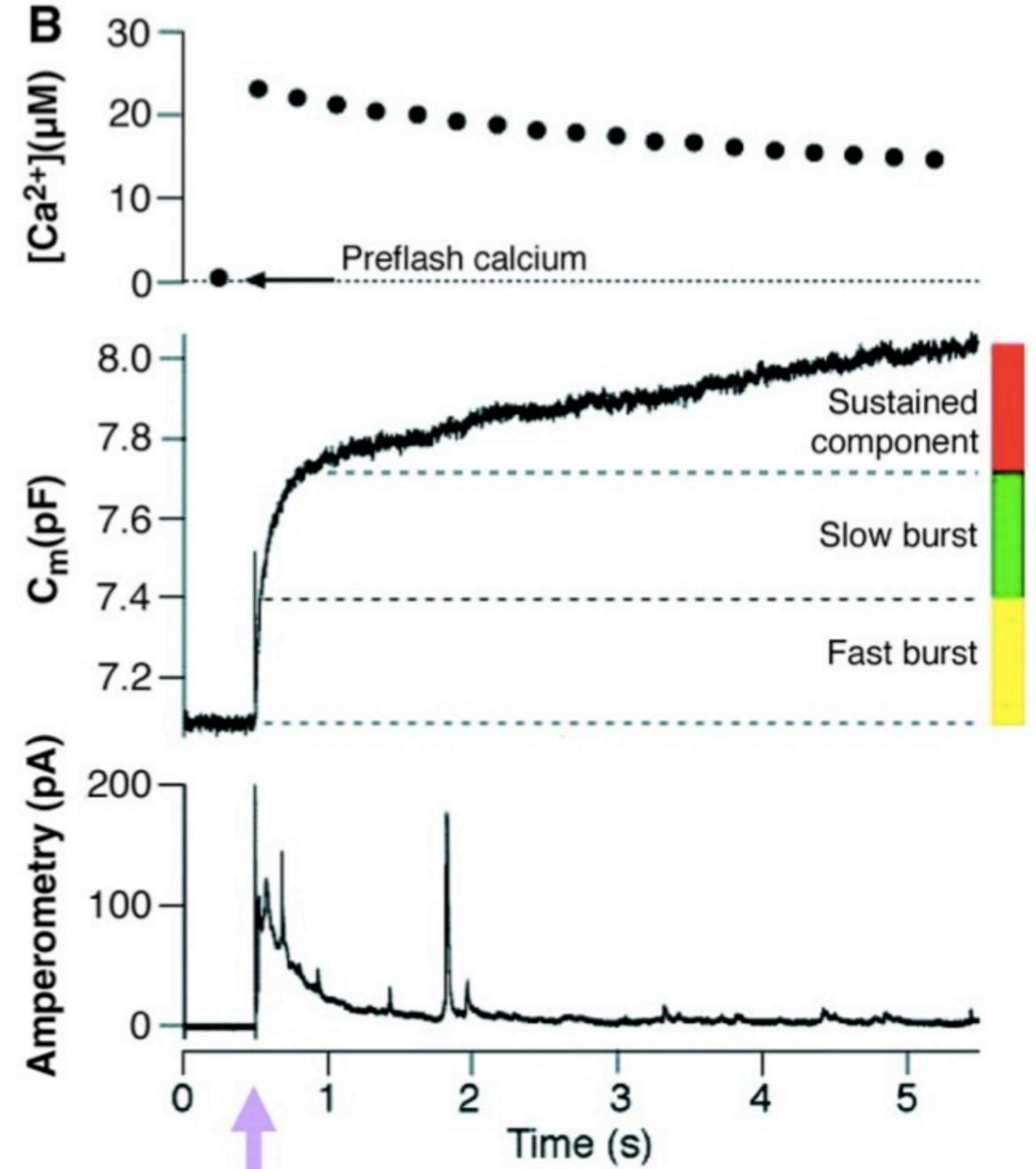
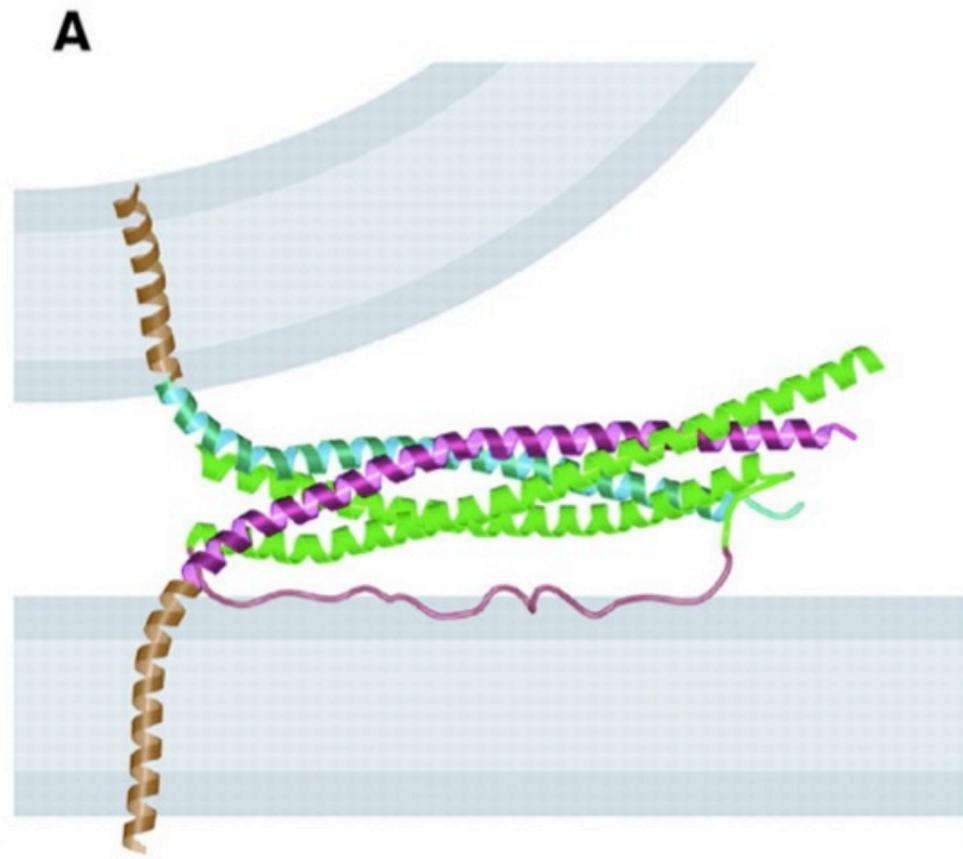


F. Darchen (UPR1929)



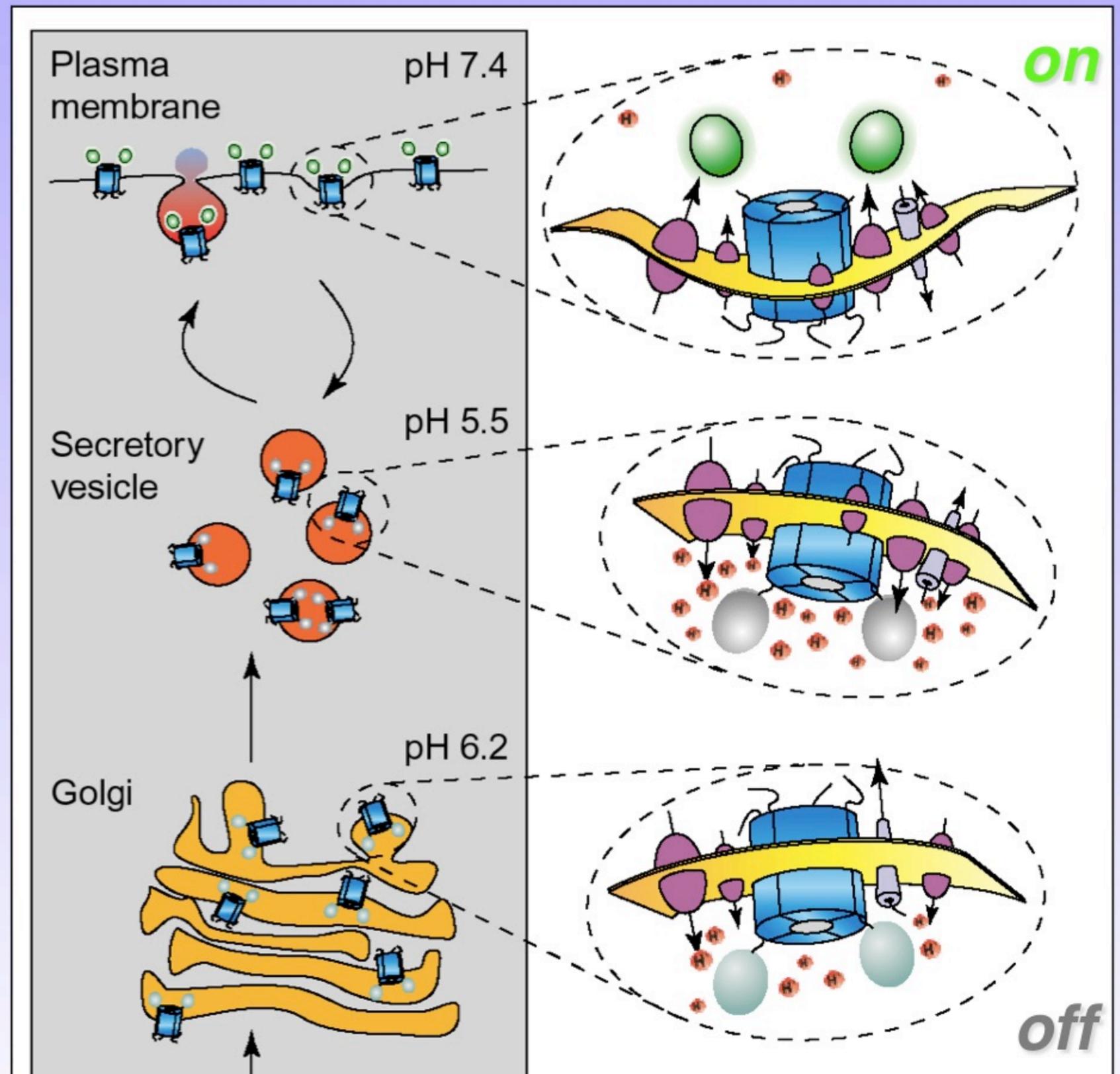
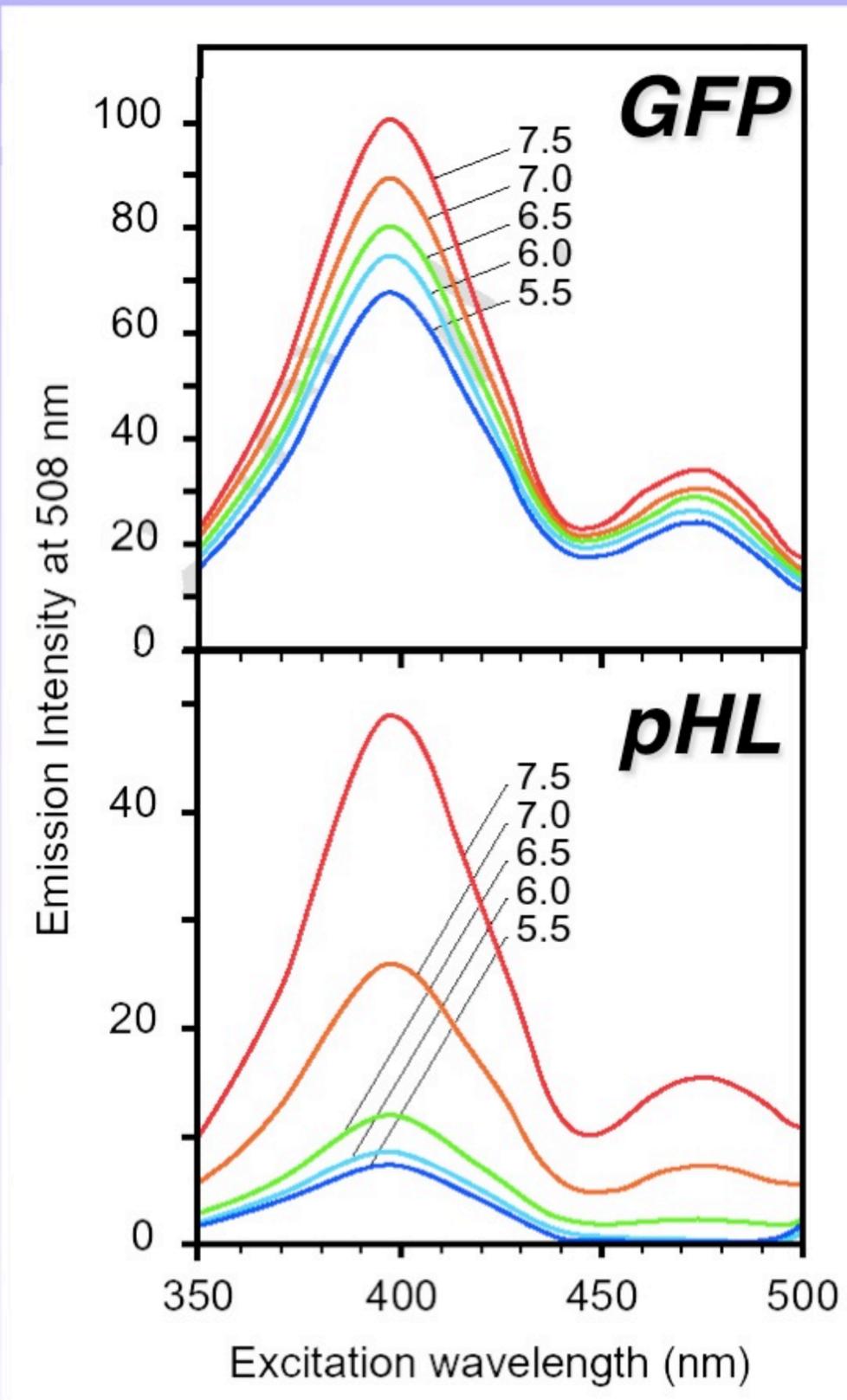
Ex: Syt1 prolonge le PSF, Syt4 le diminue.

Réserve, Amorçage, Fusion



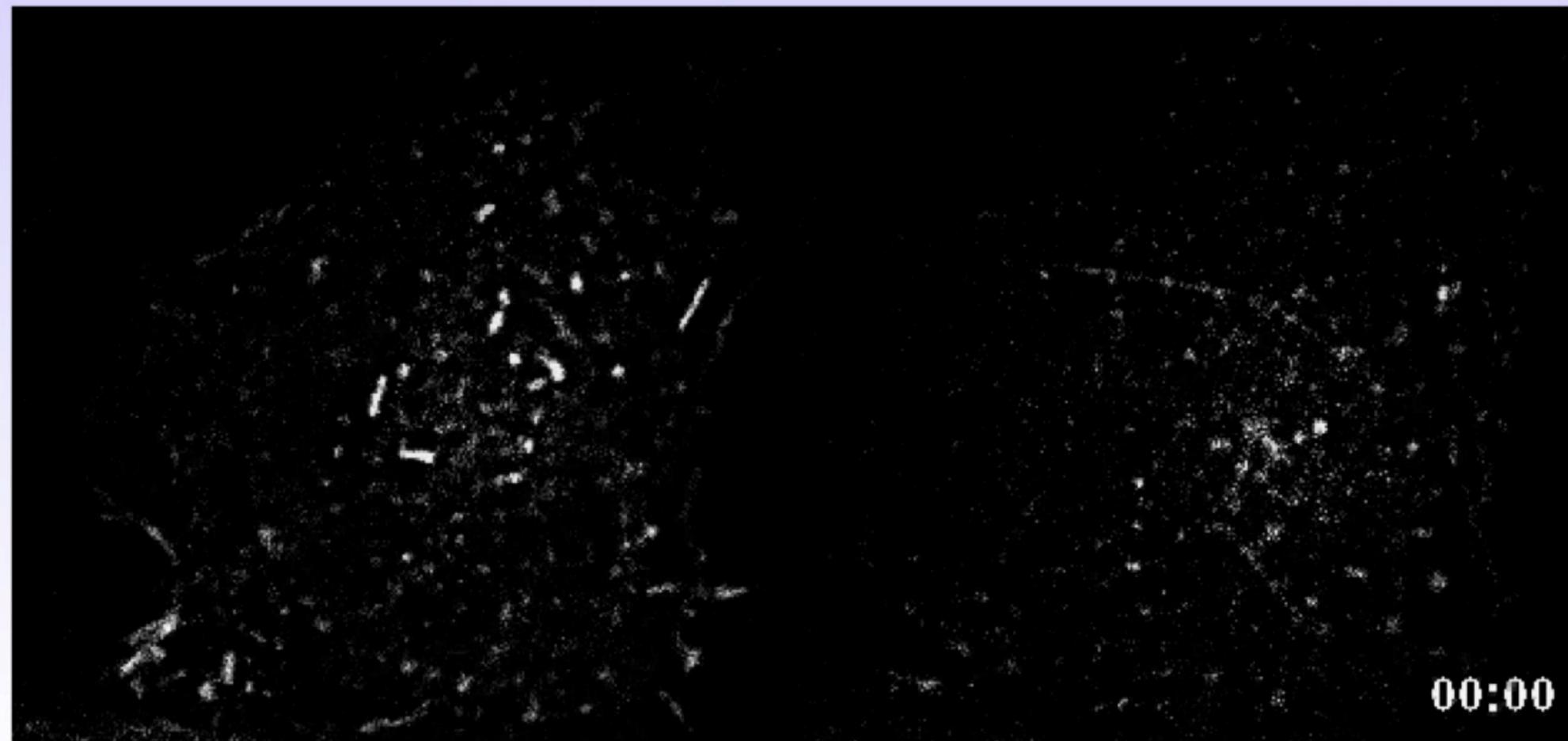
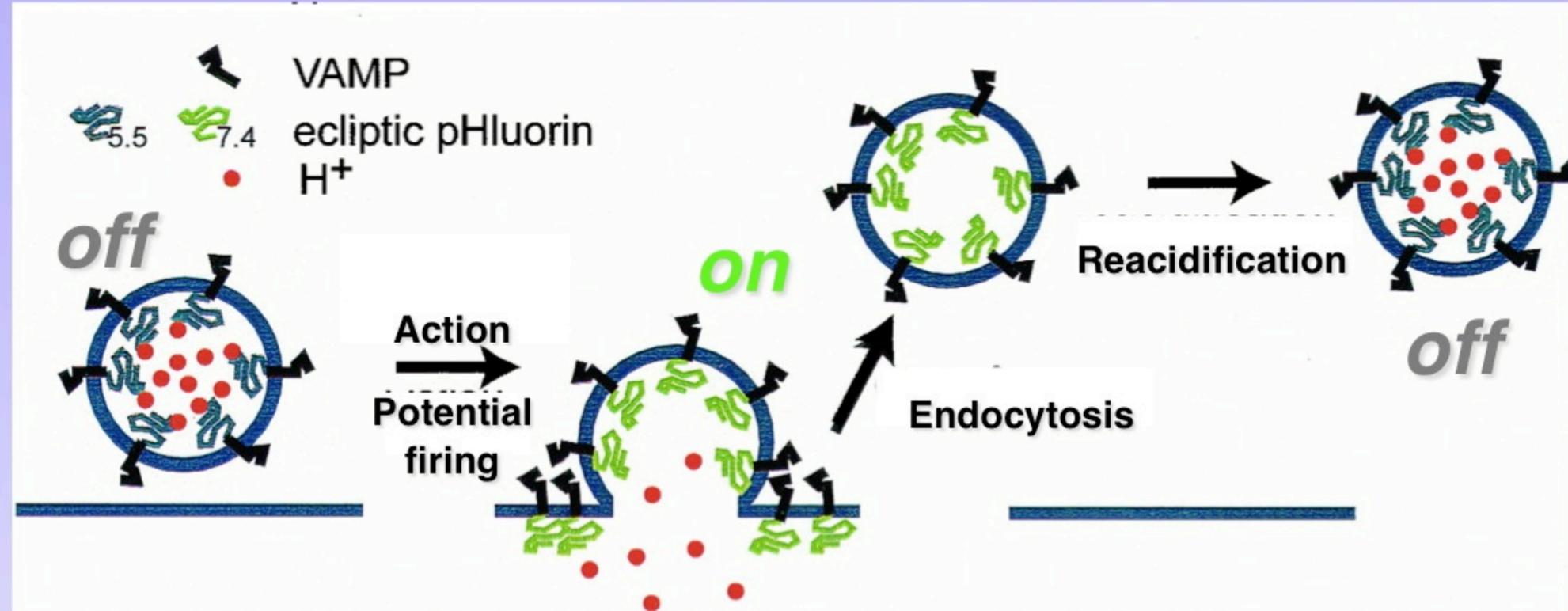
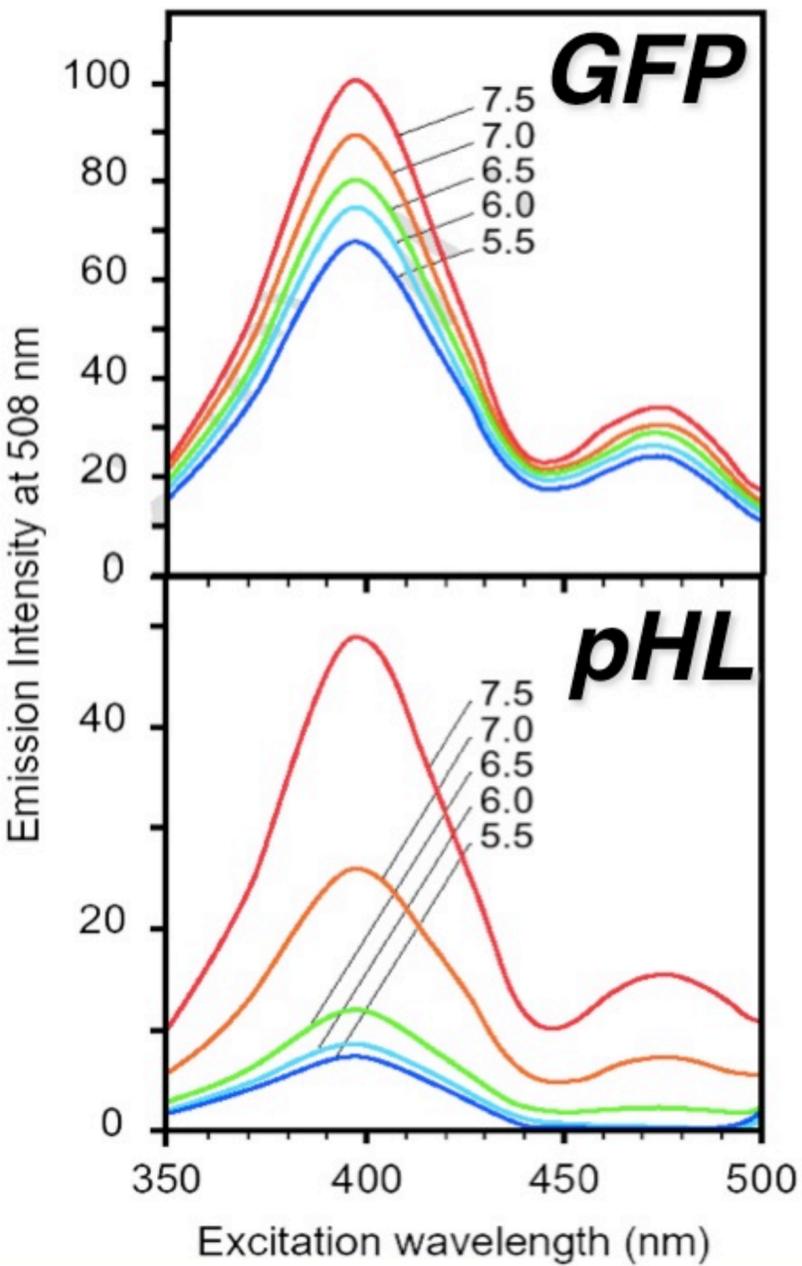
Comment mesurer l'exocytose ?

3) Mesure de fluorescence avec la GFP sensible au pH : la pHluorin



Comment mesurer l'exocytose ?

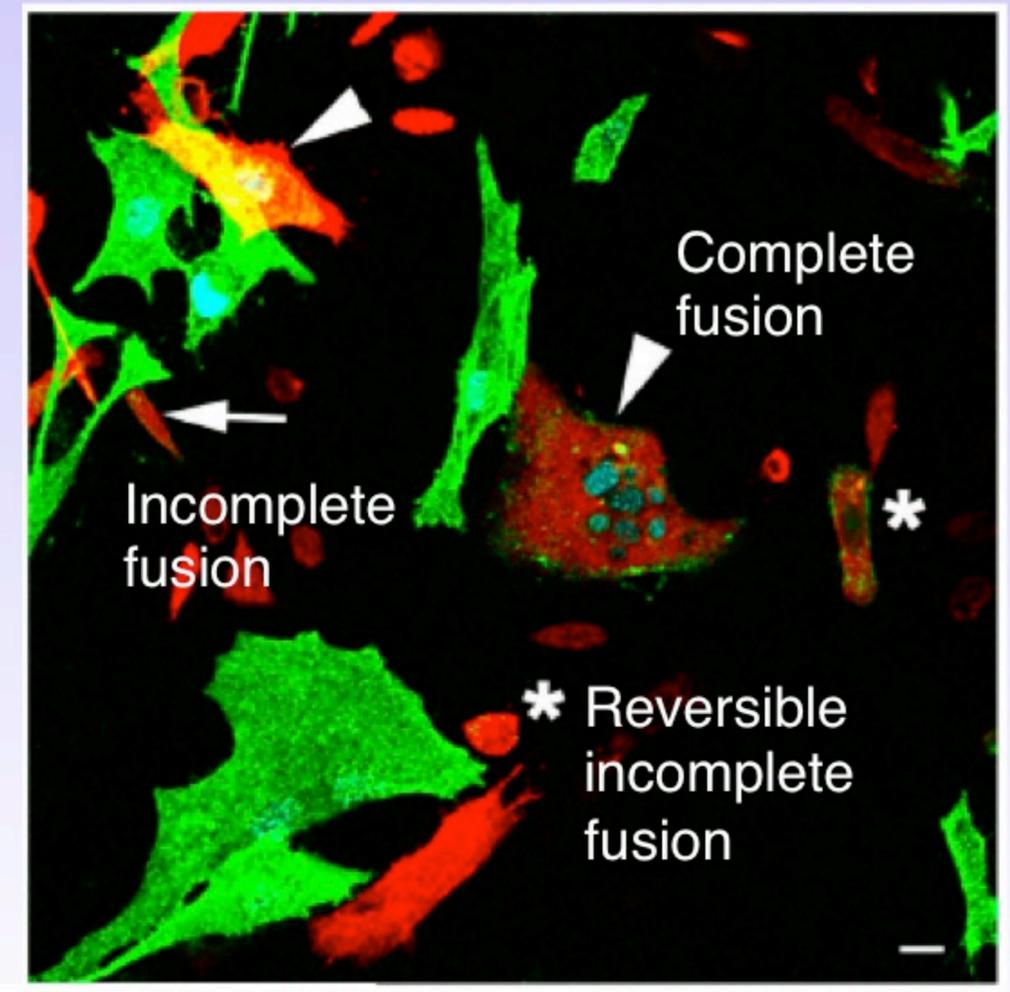
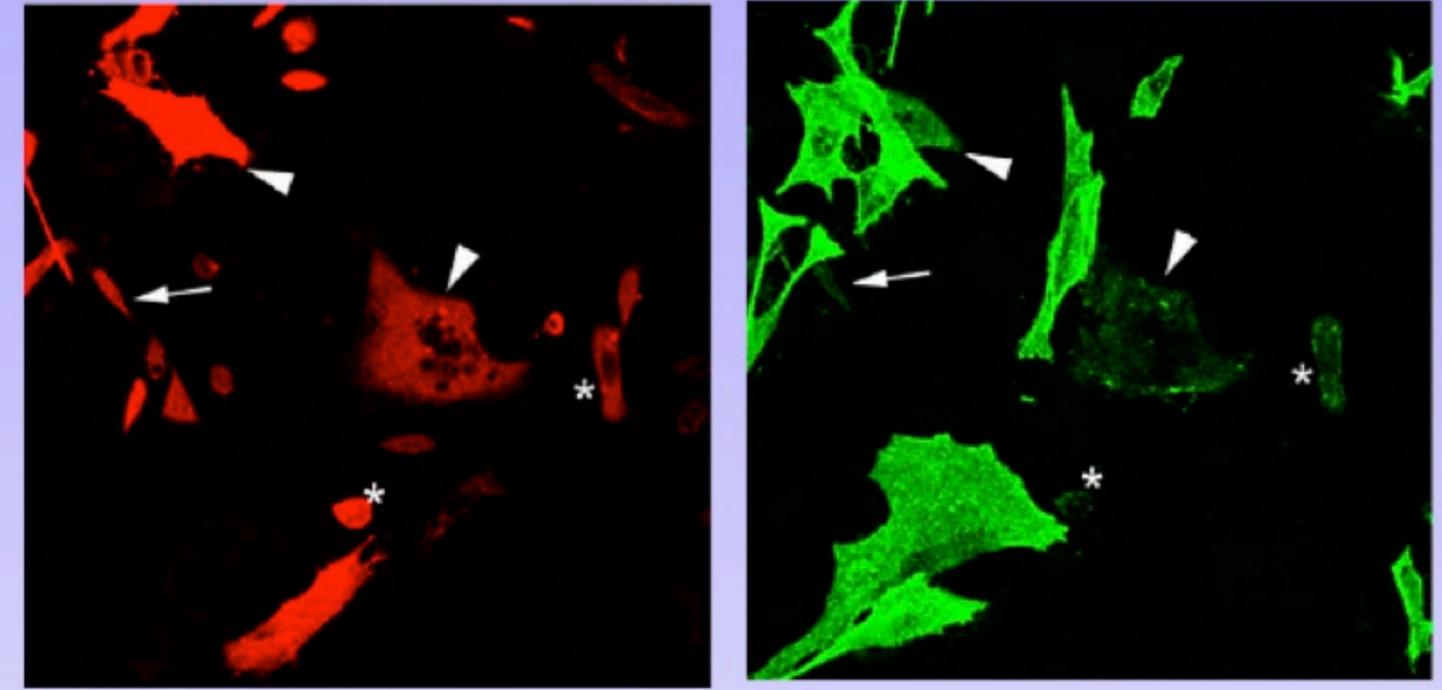
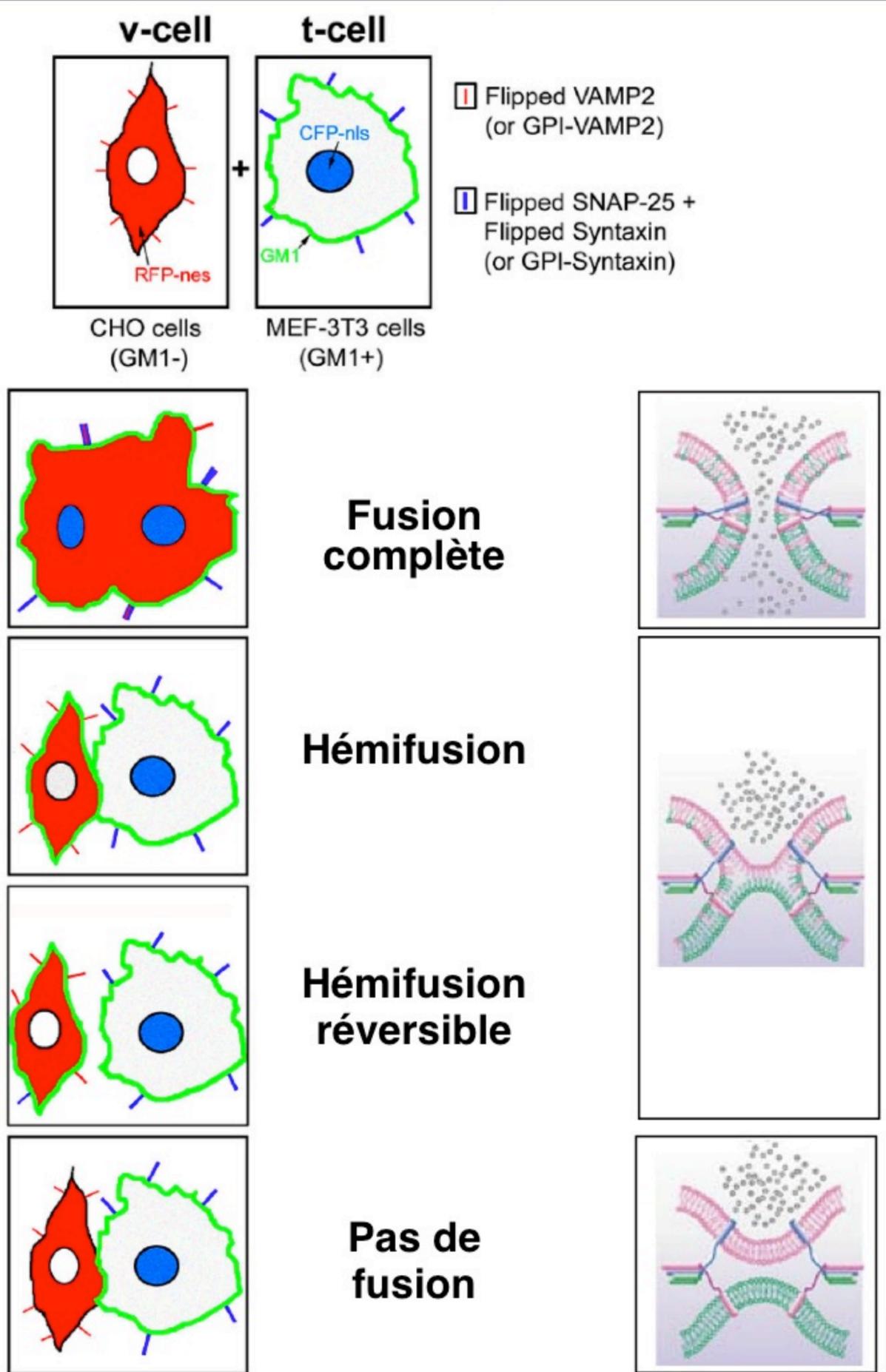
3) Mesure de fluorescence avec la GFP sensible au pH : la pHluorin





Comment mesurer l'exocytose ?

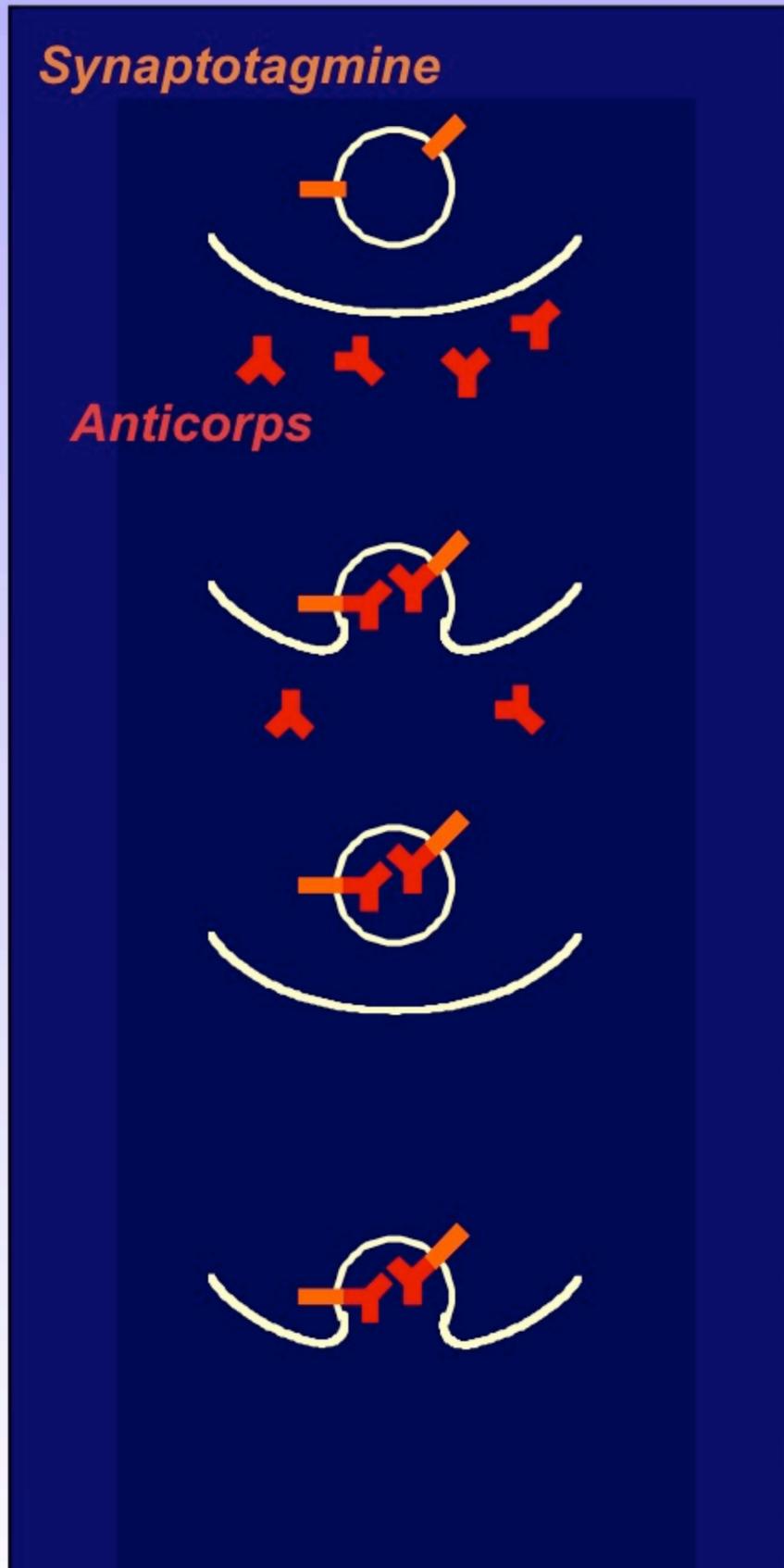
4) Evaluer la fusion par la technique des SNARE inversées (flipped SNARE fusion assay)



Jackson MB, Chapman ER. 2006. Annu. Rev. Biophys. Biomol. Struct. 35:135-60

Comment mesurer le recyclage ?

1) L'utilisation d'anticorps anti-synaptotagmine



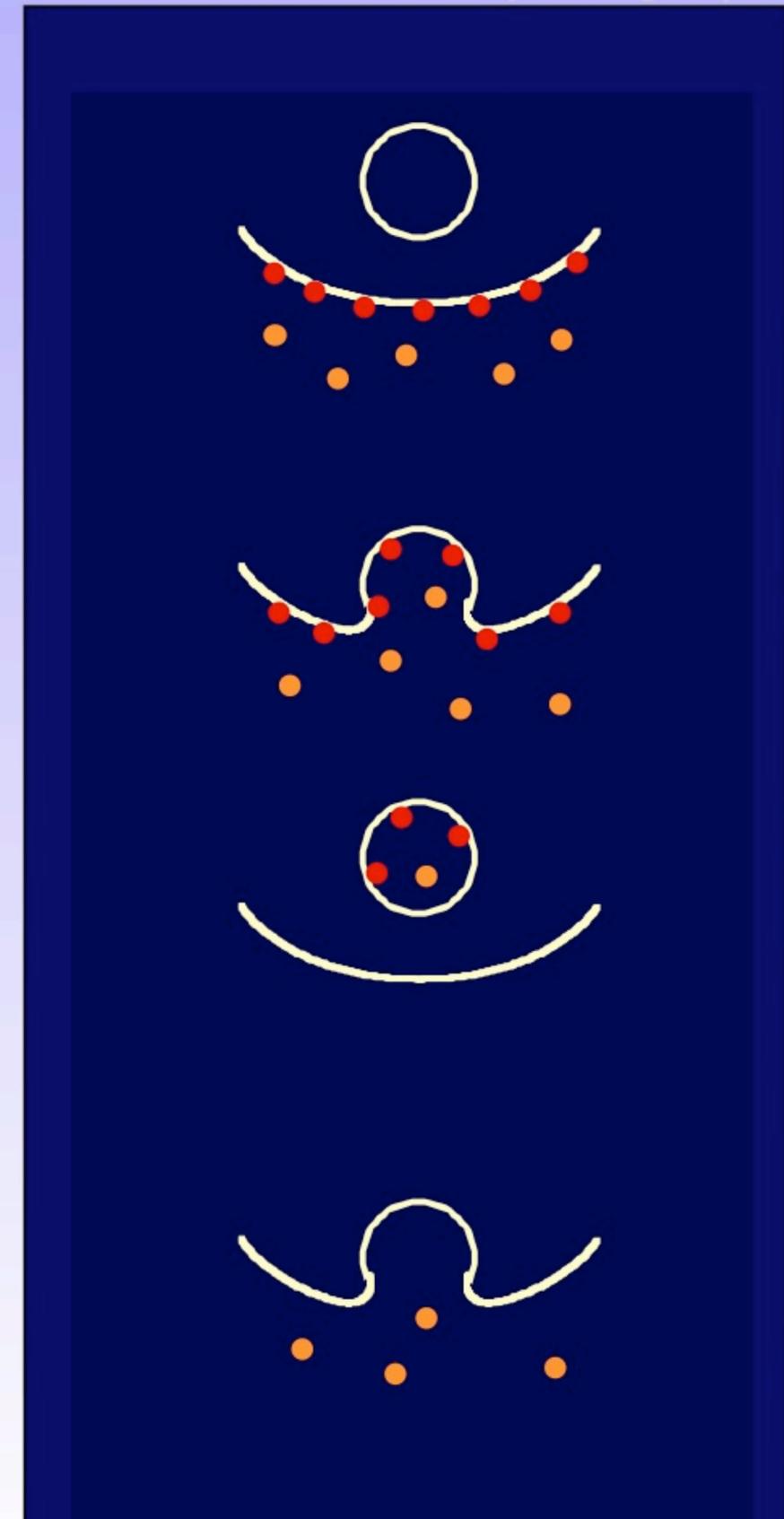
**Incubation
avec anticorps
ou sonde**

Endocytose

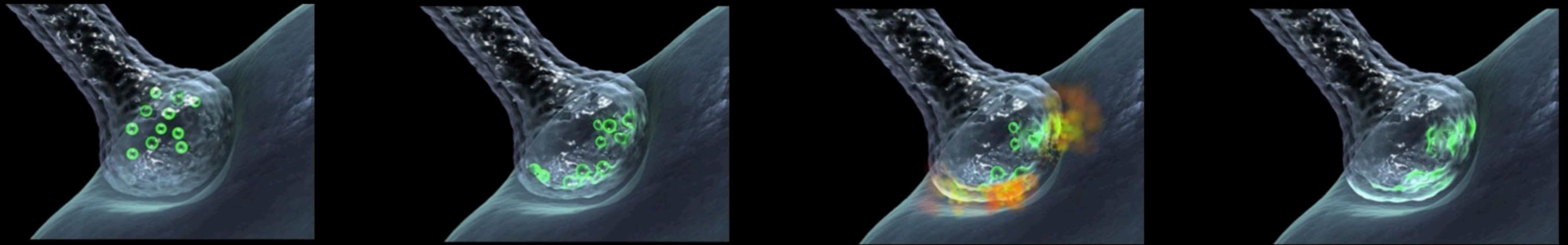
**Vésicules
chargées**

Exocytose

2) L'utilisation de sondes
fluorescentes (FM Dyes)

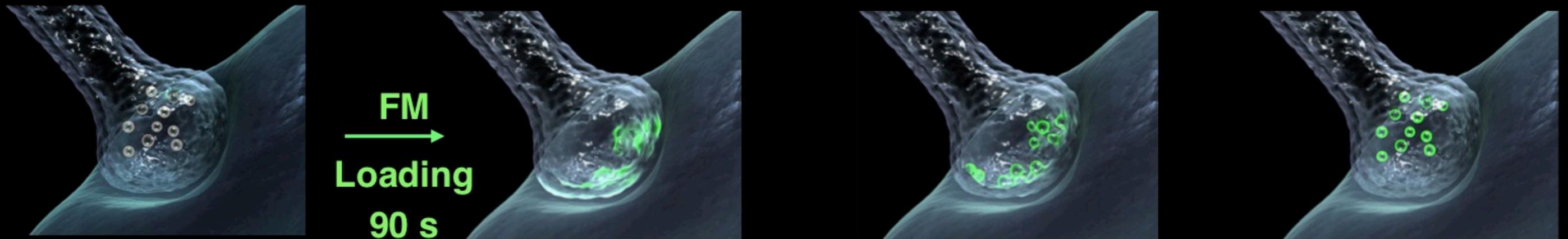


Neuronal transmission: exocytosis



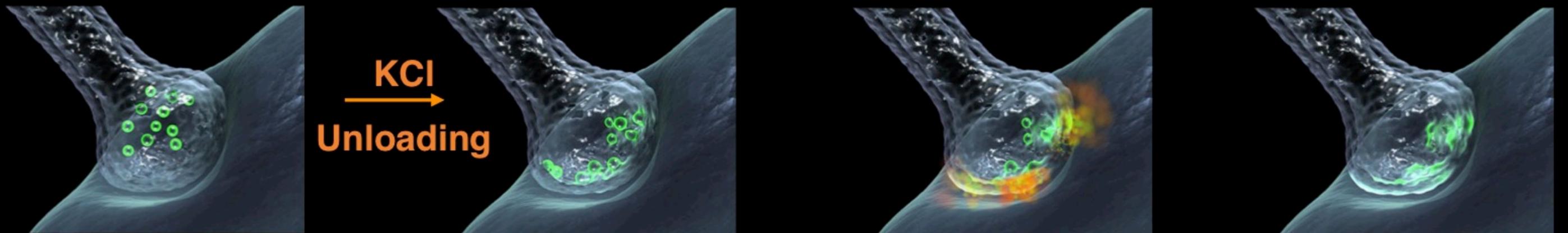
FM experiments

1. Loading of fluorescent dyes



Zero Ca^{2+} : block ExoC

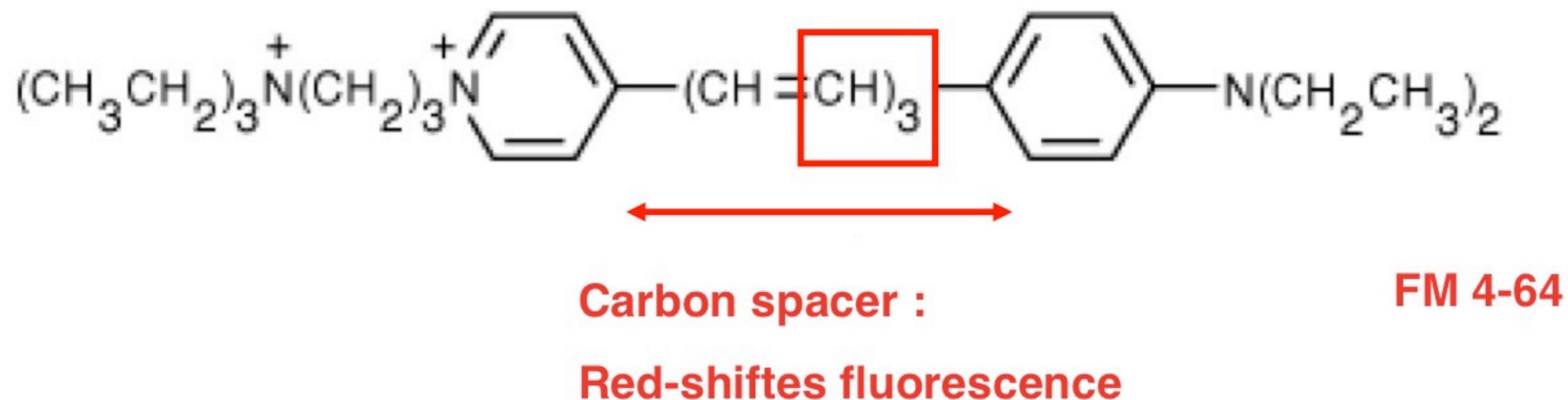
2. Measurement of the unloading of FM dyes



Basic properties of the FM dyes



FIG. 1. Chemical structures of FM 2-10, FM 1-43, and FM 1-84.



Basic properties of the FM dyes

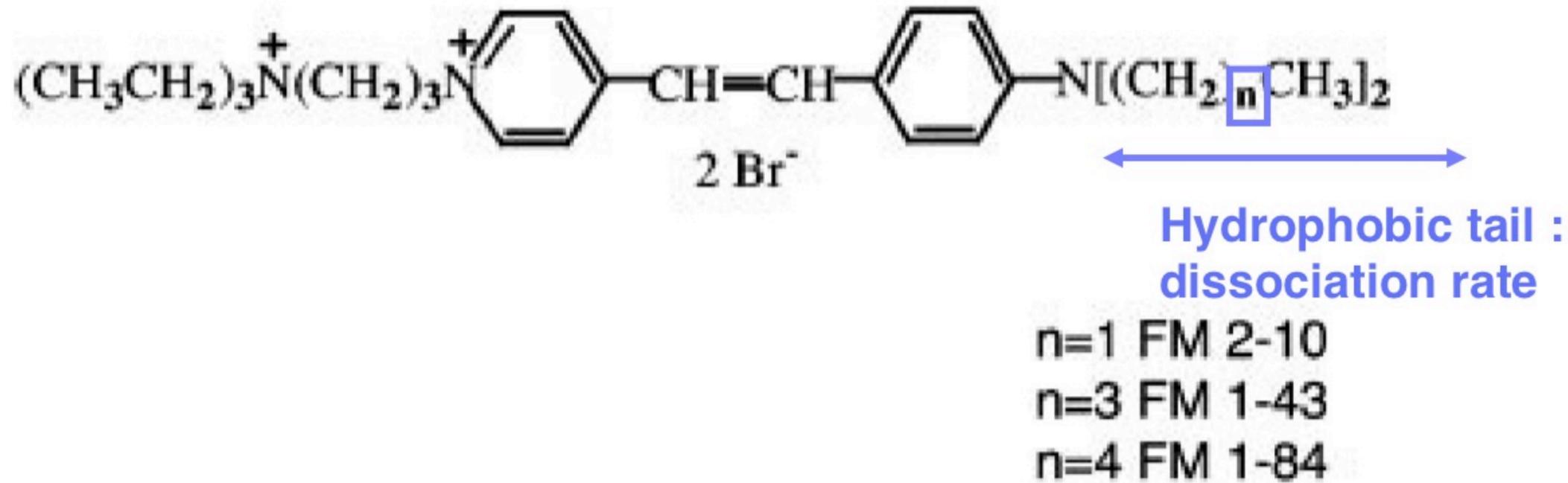
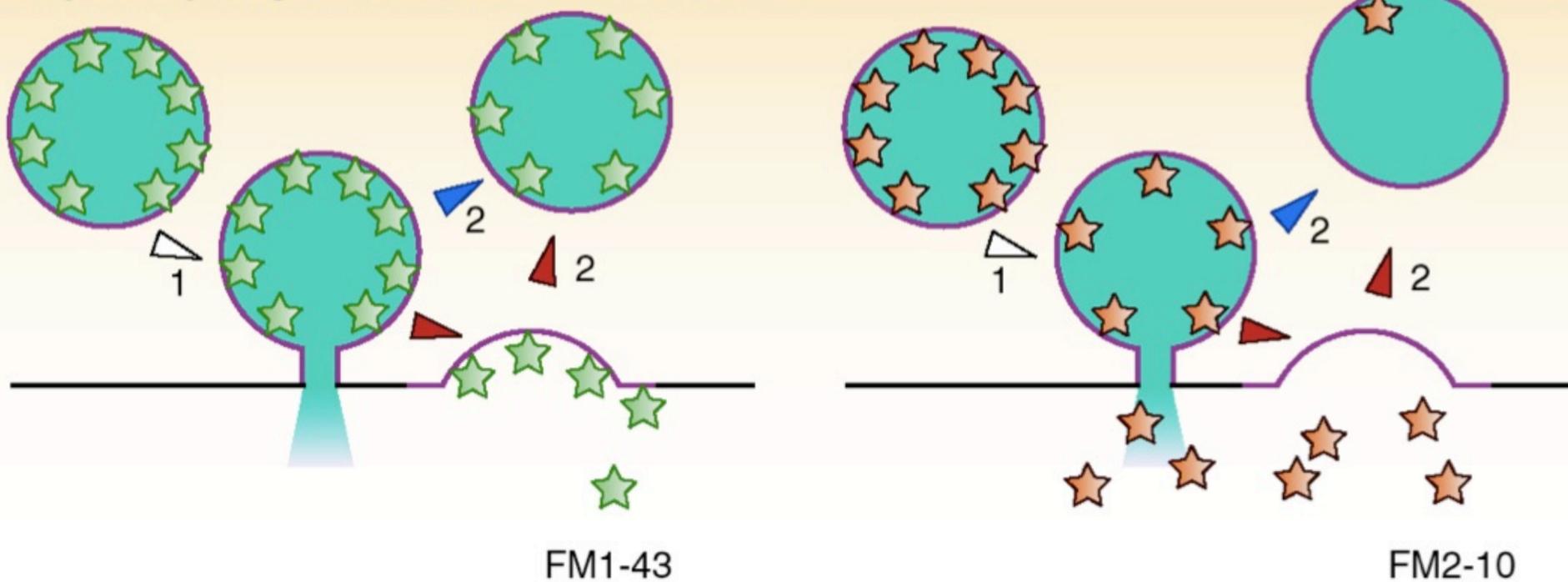


FIG. 1. Chemical structures of FM 2-10, FM 1-43, and FM 1-84.

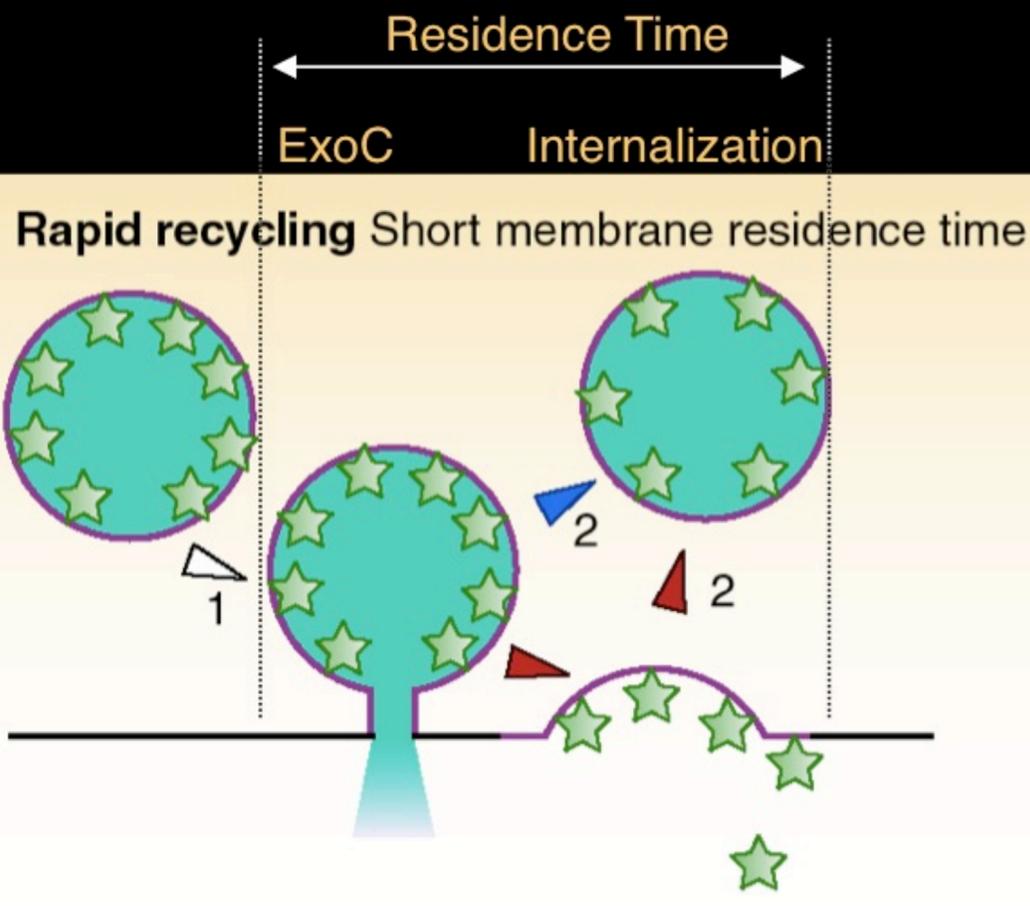
NATURE CELL BIOLOGY | VOL 4 | NOVEMBER 2002 |

Rapid recycling Short membrane residence time



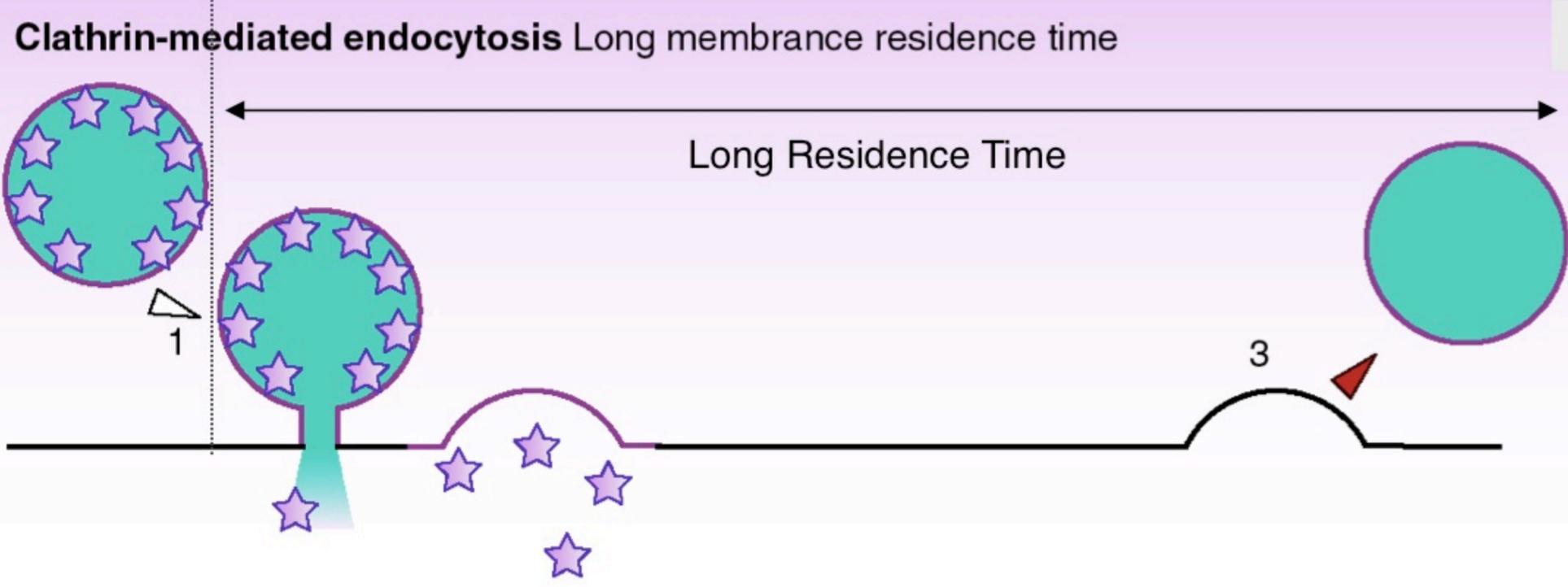
**FM2-10 is faster
than FM1-43**

- ★ Dye with slow 'off time'
- ★ Dye with fast 'off time'
- Vesicle membrane
- Pre-synaptic membrane
- Neurotransmitter



How to discriminate between Kiss & run and Endocytosis

Kiss and run :
Short Residence time (Res.T)

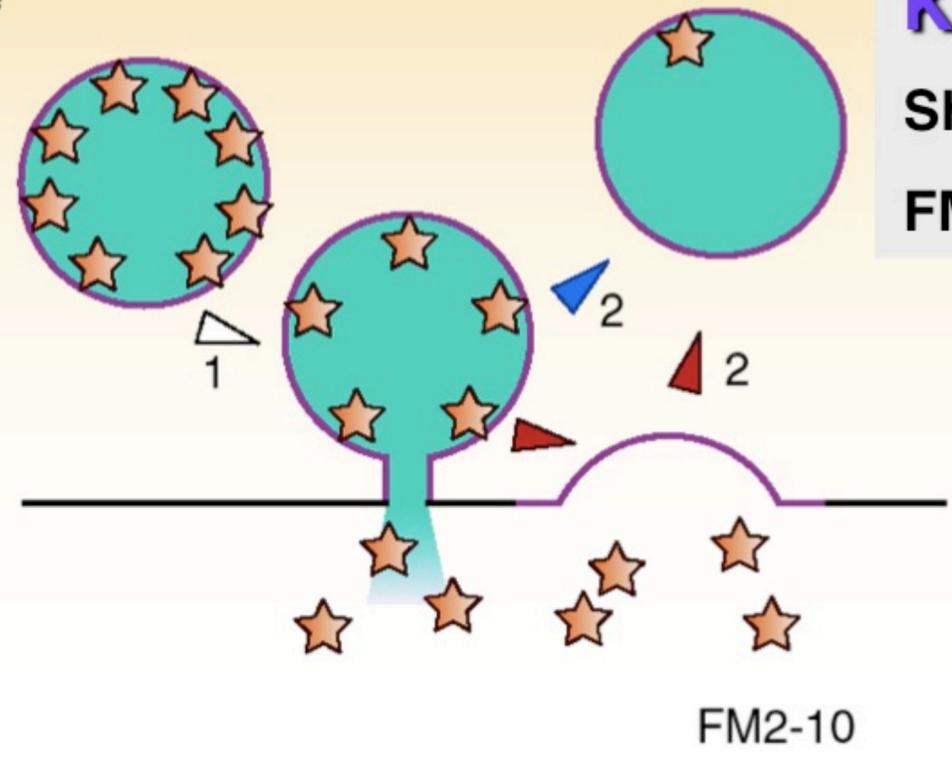
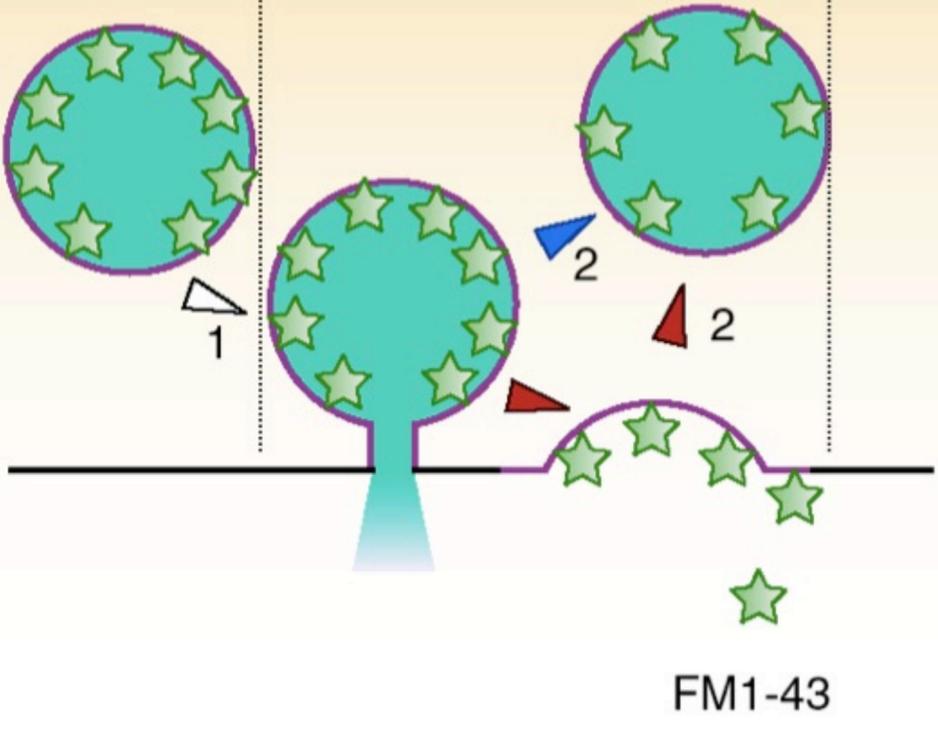


Clathrin mediated endocytosis:
Long Residence time

- ★ Dye with slow 'off time'
- ★ Dye with fast 'off time'
- Vesicle membrane
- Pre-synaptic membrane
- Neurotransmitter

Residence Time
ExoC Internalization

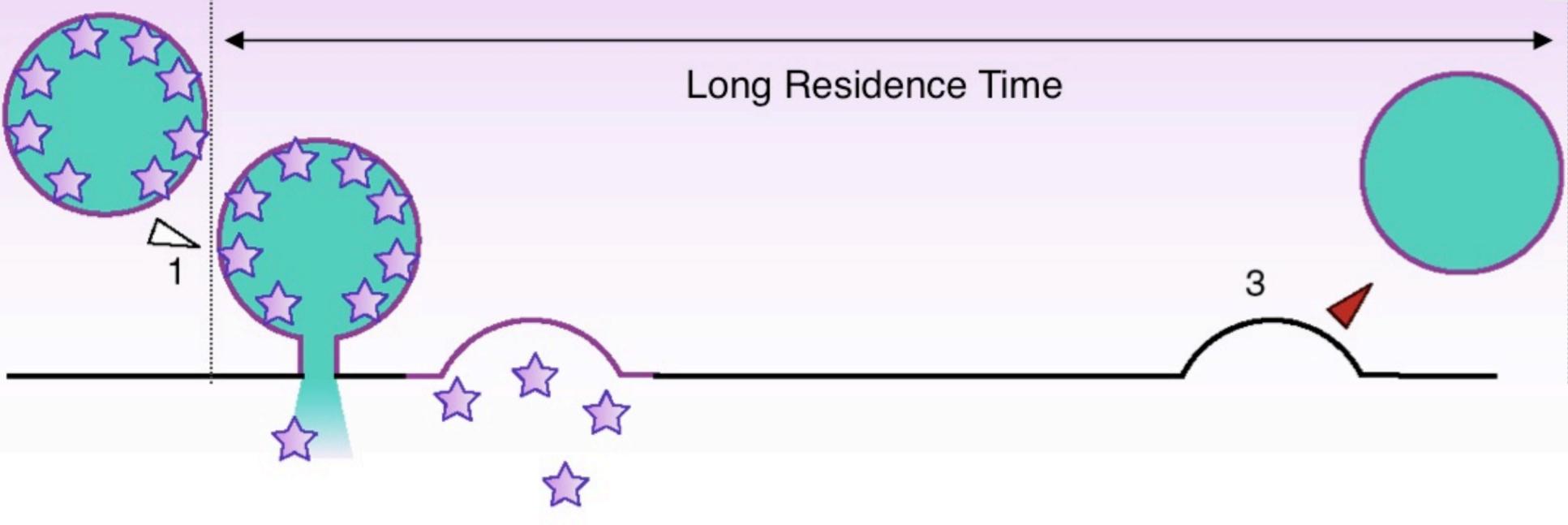
Rapid recycling Short membrane residence time



Kiss and run :
Short Residence time (Res.T)
FM2-10 < Res. T < FM 1-43

FM2-10 is faster than FM1-43 :
« Off time »
FM2-10 < FM 1-43

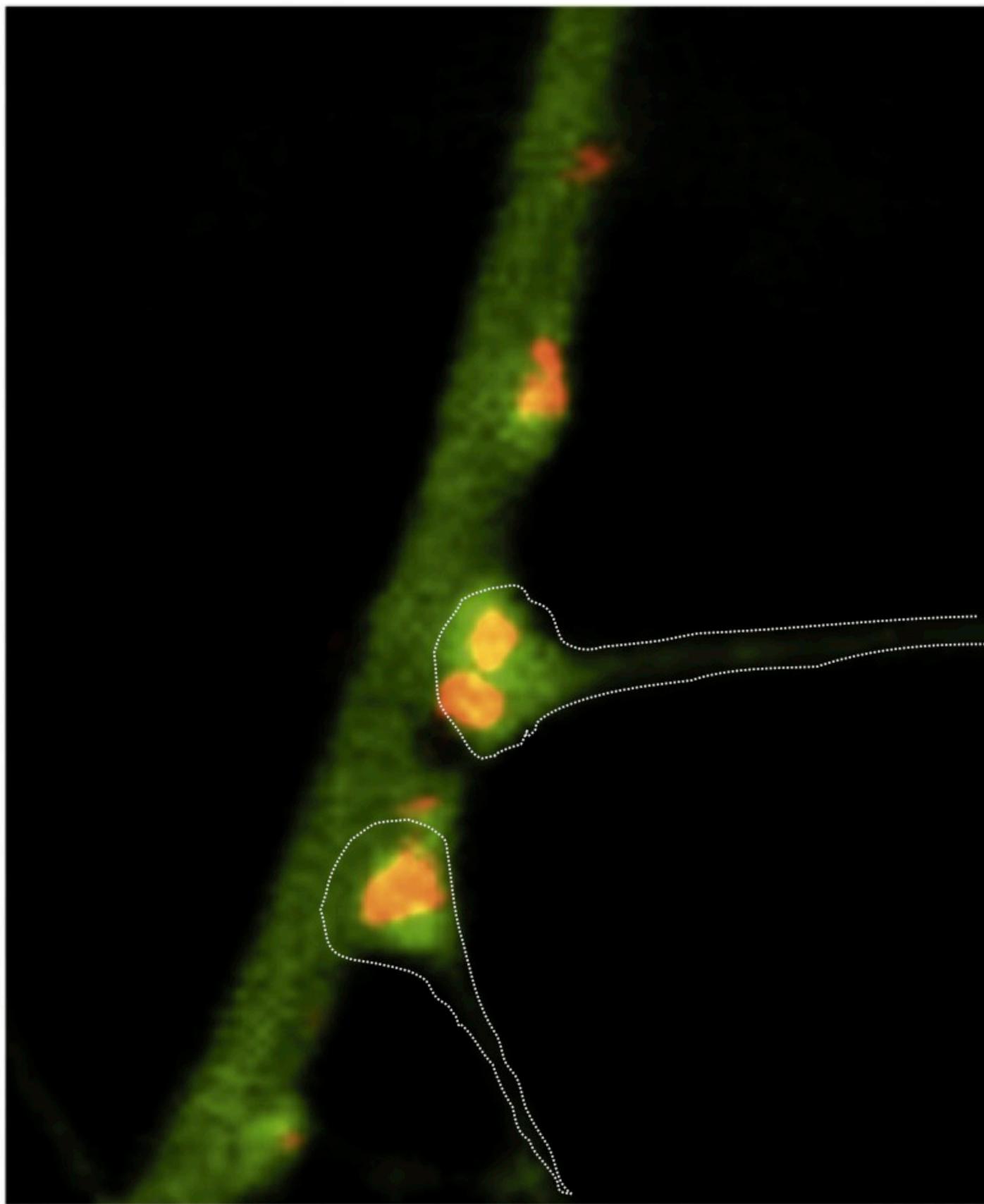
Clathrin-mediated endocytosis Long membrane residence time



Clathrin mediated endocytosis:
Long Residence time
Residence time >> FM off time
Res. T >> FM 1-43 > FM2-10

There is no difference between FM2-10 and FM1-43
Both can dissociate before internalization

- ★ Dye with slow 'off time'
- ★ Dye with fast 'off time'
- Vesicle membrane
- Pre-synaptic membrane
- Neurotransmitter



Current Opinion in Neurobiology

FM 4-64 labeling of synaptic vesicle clusters in hippocampal neurons. FM 4-64, which is a red-shifted variant of FM 1-43, was applied during AP firing to hippocampal neurons in cell culture. Two GFP-expressing cells that form an axo-dendritic contact containing two clusters of recycling vesicles labeled by FM 4-64 are shown.

Evidence for a Role of Dendritic Filopodia in Synaptogenesis and Spine Formation

Noam E. Ziv and Stephen J Smith Neuron, Vol. 17, 91–102, July, 1996.

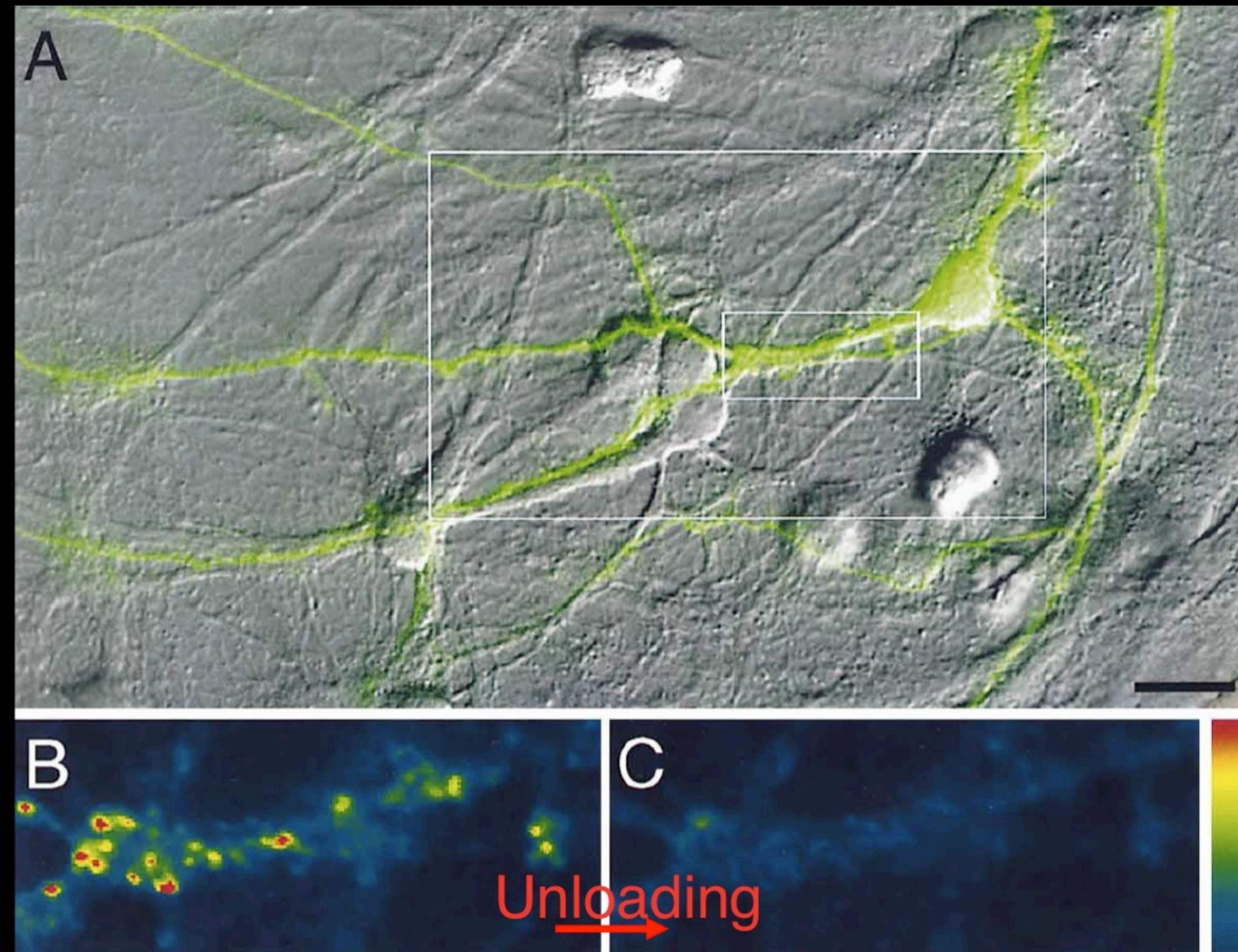


Figure 1. Imaging of Dendritic Structure and Presynaptic Boutons in Live Cultured Hippocampal Neurons

(A) A fluorescence image of a single pyramidal neuron labeled with FAST DiO, digitally overlaid on a DIC image of the same field. The neurons shown in this figure were grown for 13 DIV prior to the experiment.

(B) A pseudocolor fluorescence image of presynaptic boutons loaded with FM 4-64. The area shown corresponds to the inner rectangle in (A). Fluorescence intensity is coded according to color bar on far right.

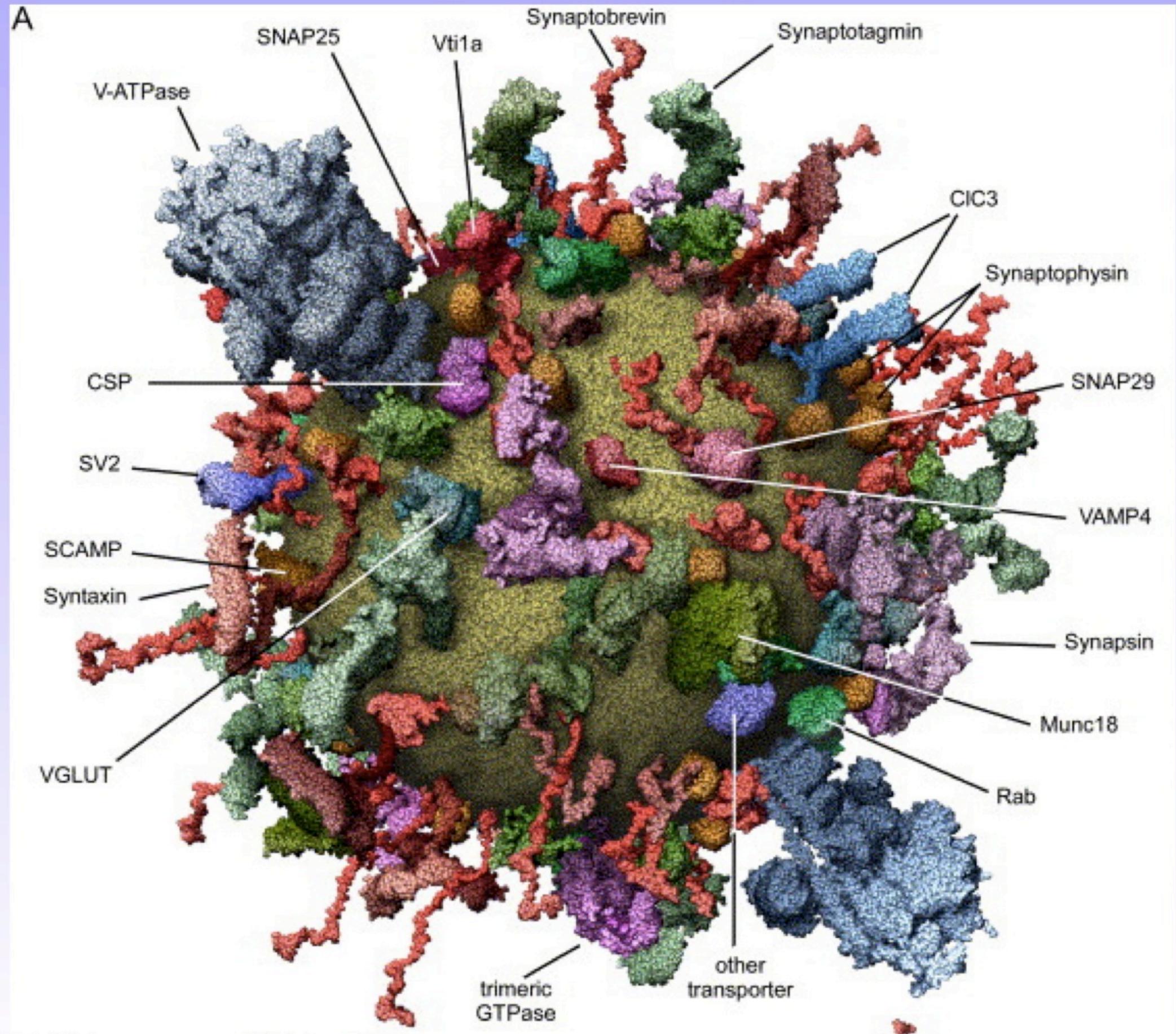
(C) The same field shown in (B) after the dye was unloaded by stimulating the neurons to fire action potentials for 60 s at 10 Hz.

(D) Digital superposition of the FM 4-64 difference image (red), created by subtracting the image in (C) from that in (B), onto the fluorescence image of the FAST DiO-labeled neuron (green). Area shown corresponds to outer rectangle in (A). Scale bars, 20 μm (A) and 10 μm (D).

Vésicules Synaptiques

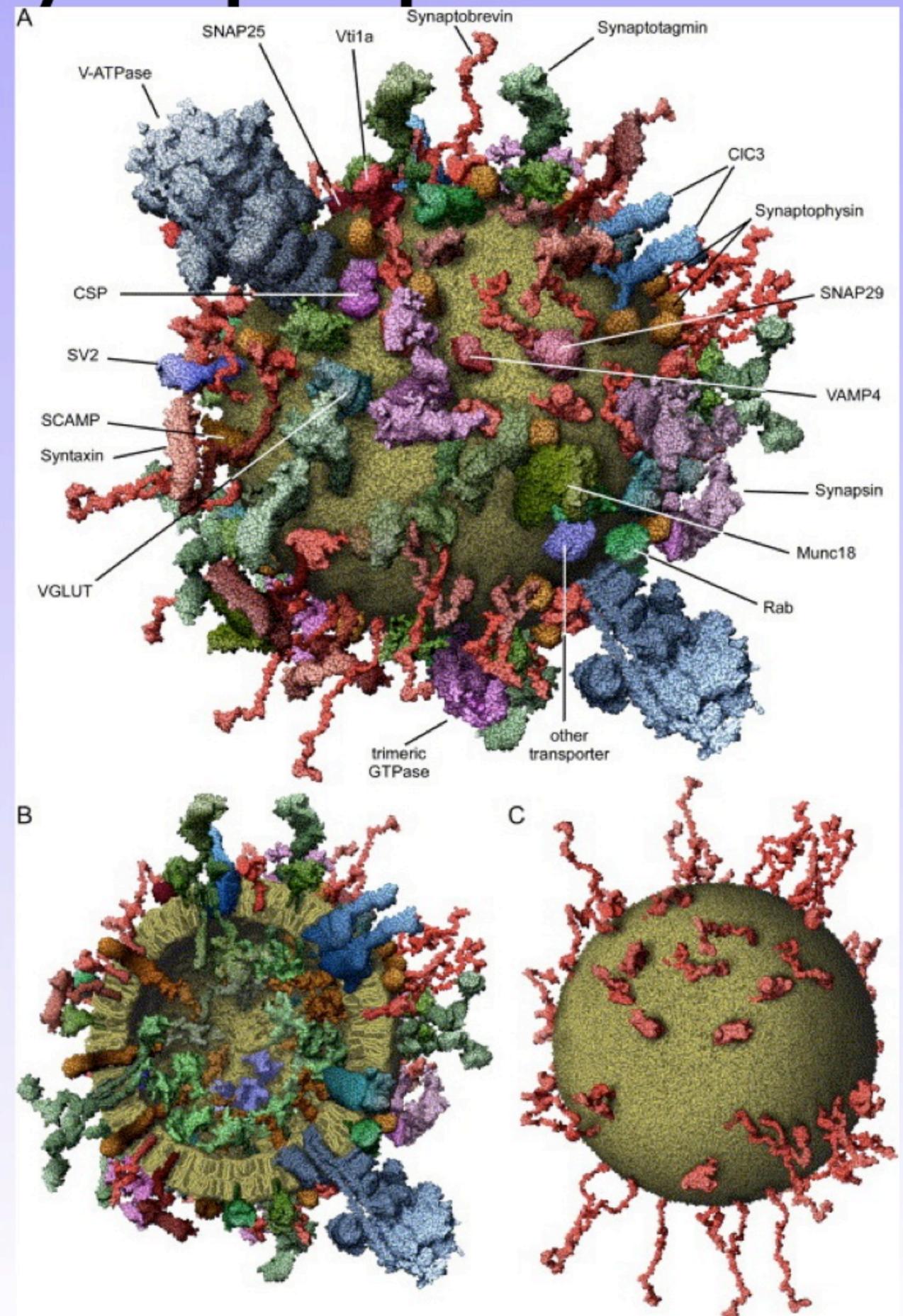


R. Jahn

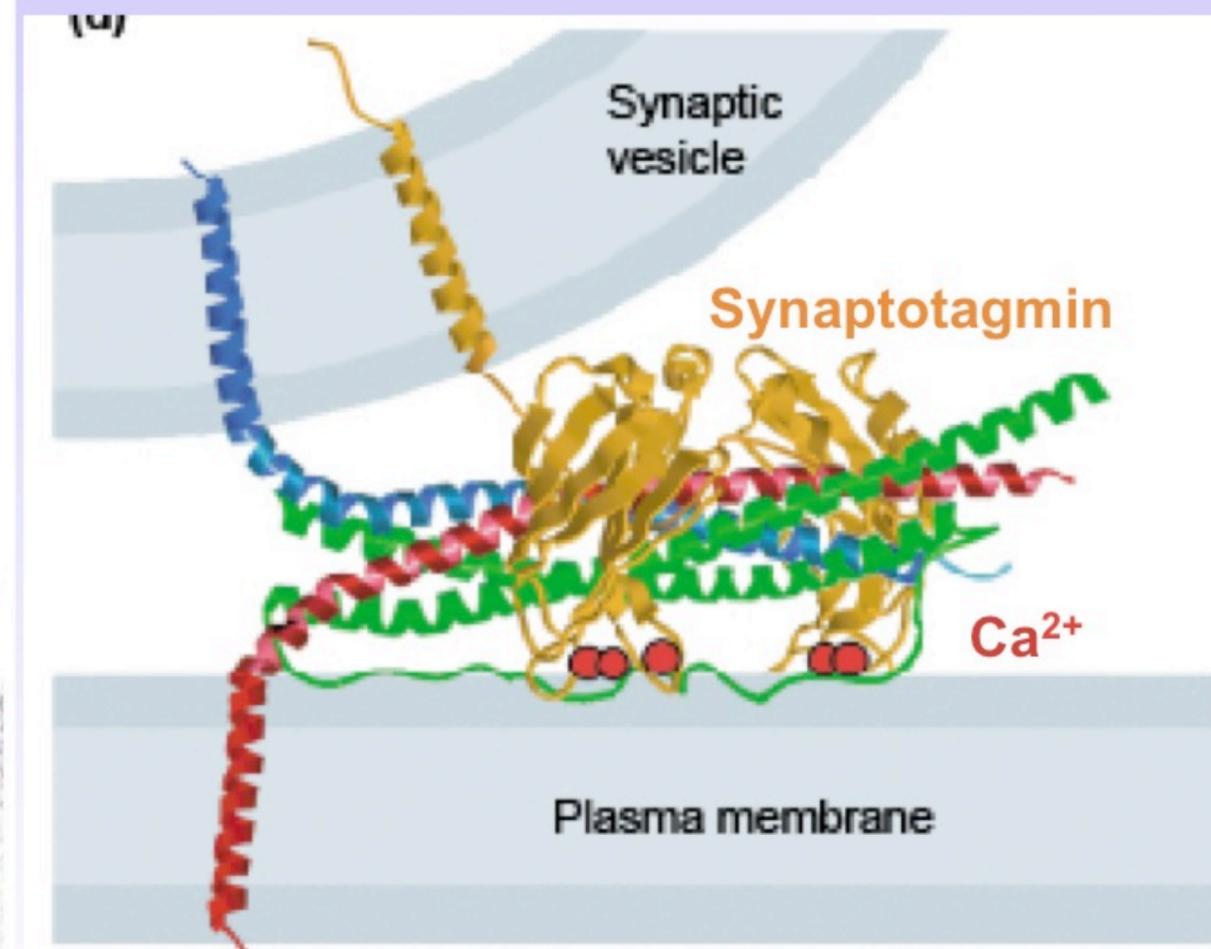
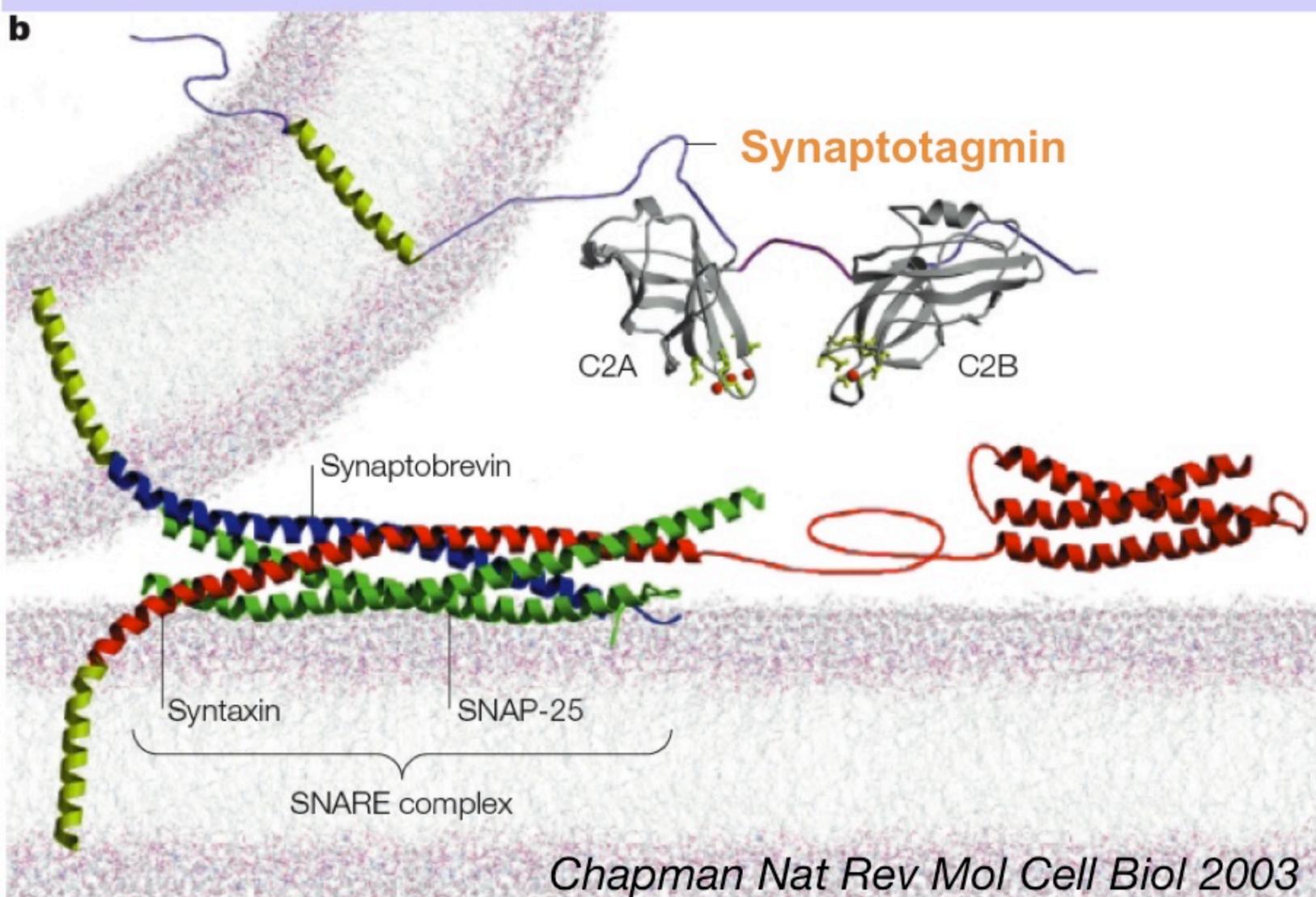
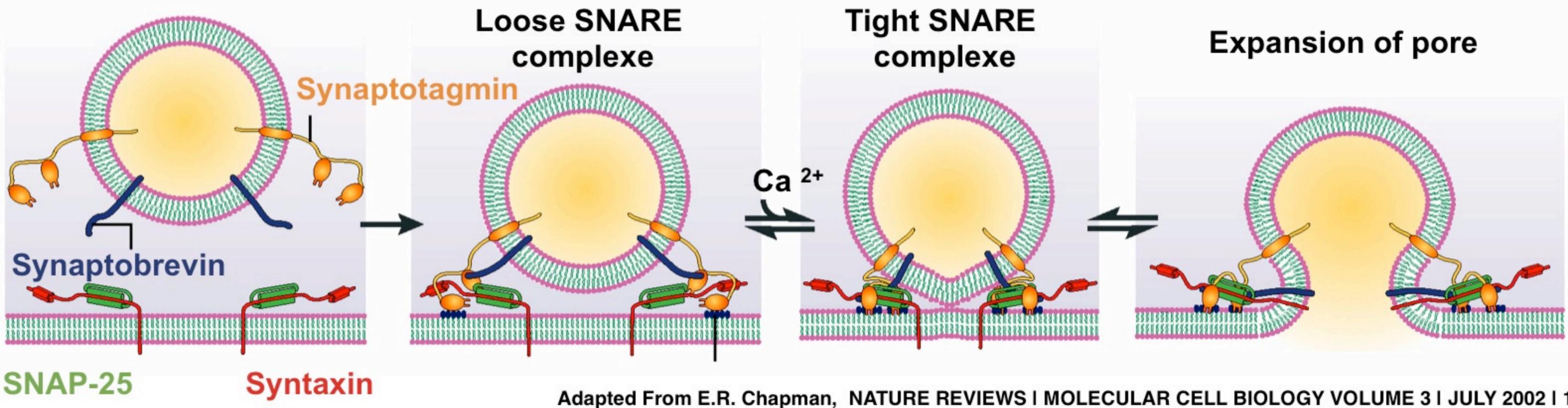


Vésicules Synaptiques

Protein	% of total SV proteins
Synaptophysin	10.20 ± 1.54 ^(1,4)
Synaptobrevin 2	8.60 ± 1.55 ⁽¹⁾
Syntaxin 1	2.00 ± 0.27 ^(1,5)
SNAP25	0.40 ± 0.06 ^(1,6)
Synapsins	6 ^(2,3)
Rab3A	2.5 ⁽²⁾
Synaptotagmin 1	7 ⁽³⁾
Synaptogyrin 1	0.5 ⁽²⁾
SV2	1.4 ⁽²⁾
SCAMP	0.3 ⁽²⁾
CSP	0.6 ⁽²⁾
VGLUT1	5.36 ± 1.11 ^(1,7)
VGLUT2	9.01 ± 2.31 ^(1,7)
V-ATPase V1-B subunit	1.15 ± 0.21 ⁽¹⁾



Régulation de l'exocytose par la synaptotagmine



Régulation de l'exocytose

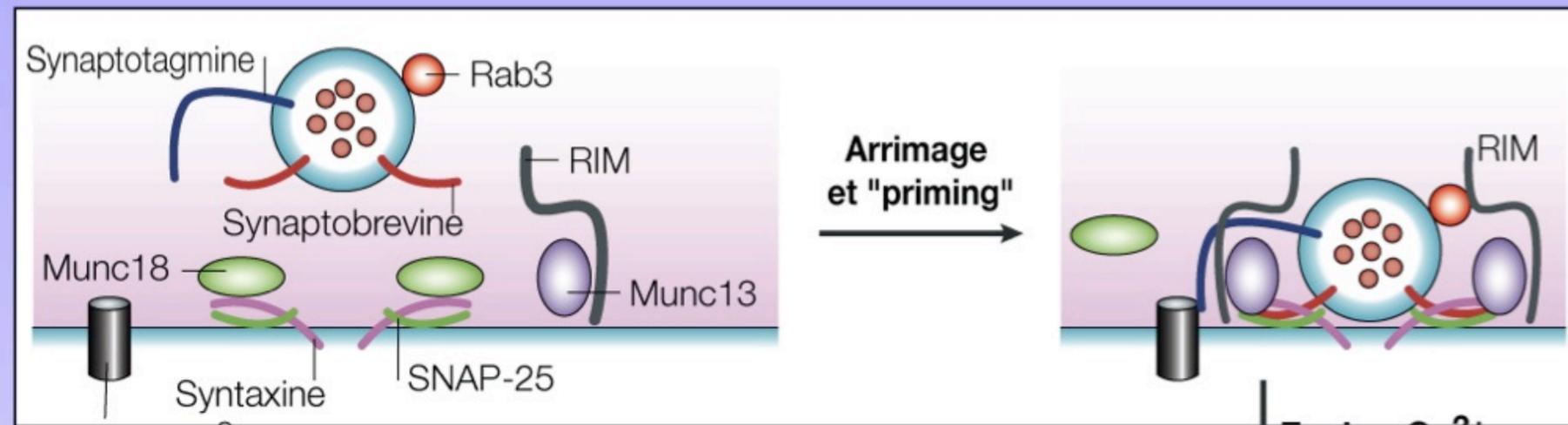
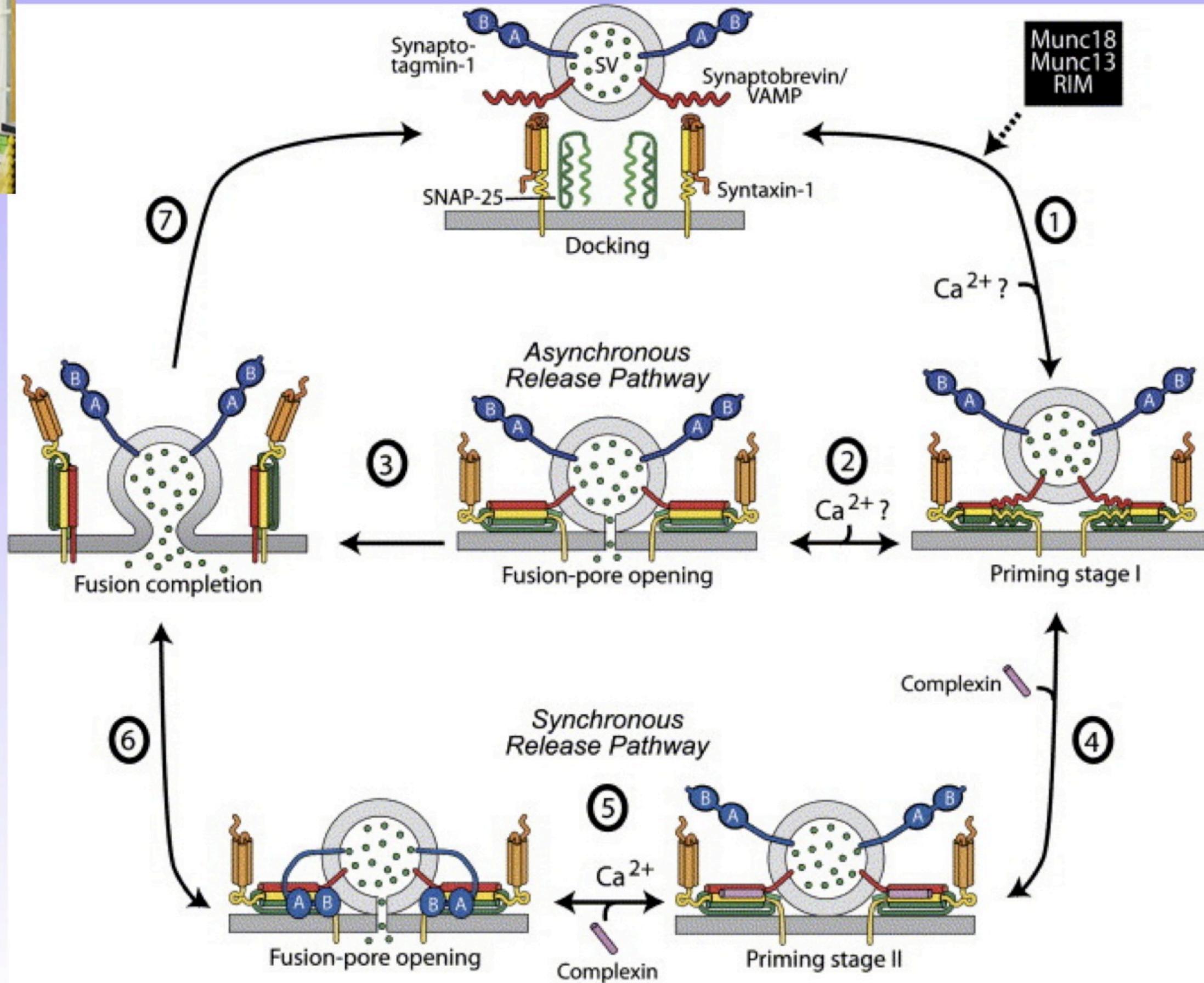


Figure 2. Overview of the steps in the secretory vesicle cycle that are affected by deletion of the respective genes. Deletion of *munc18-1* affects all steps in the cascade. Deletion of the SNARE genes (reviewed in [1]) results in priming defects with syntaxin-1 sharing a more upstream (docking) phenotype with Munc18-1 [25]. Deletion of *munc13-1* and *munc13-2* [68] and *synaptotagmin-1* [72] does not affect vesicle harboring at the membrane, but results in priming and fusion triggering defects, respectively.

Régulation par la complexine



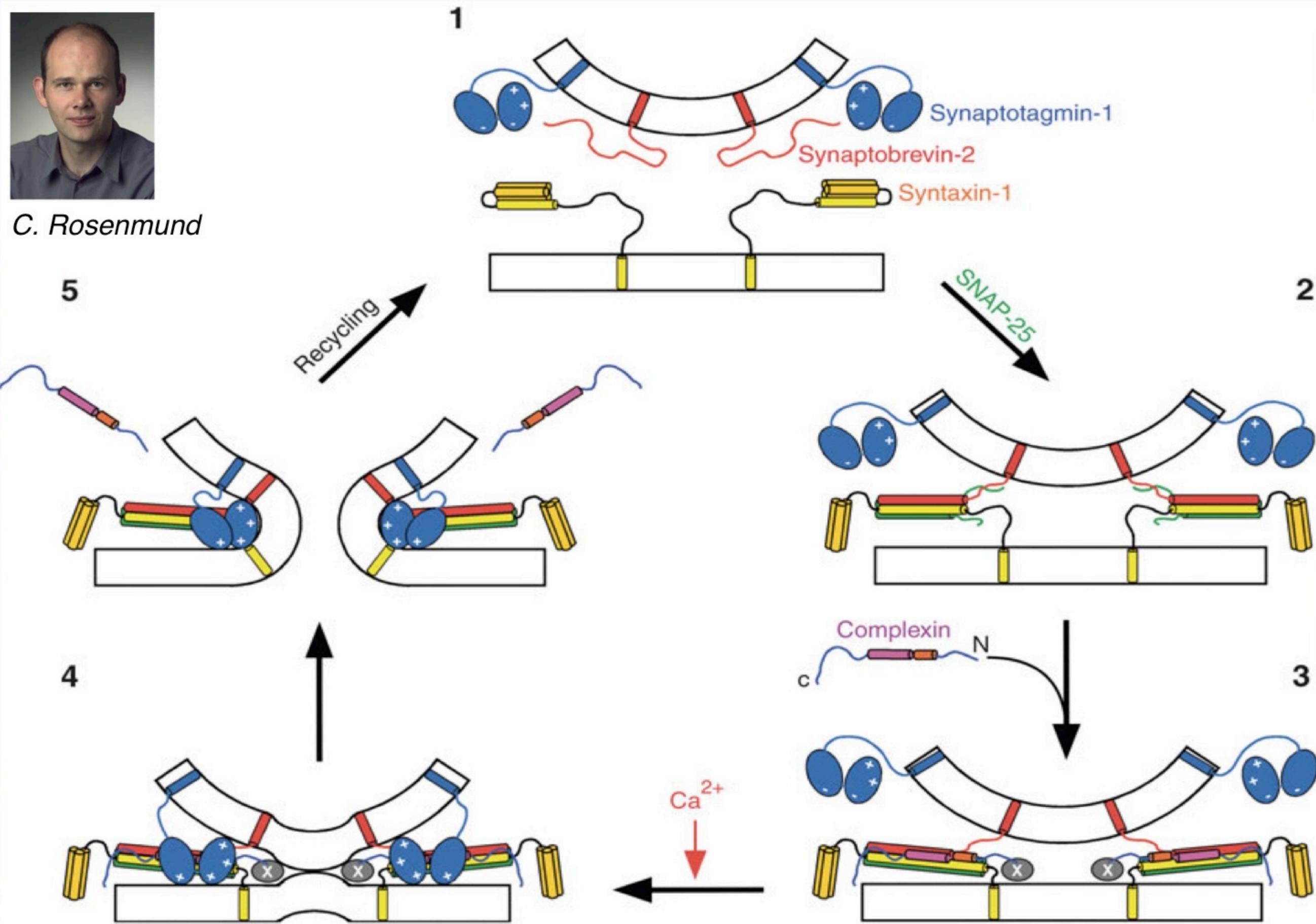
T. Sudhof



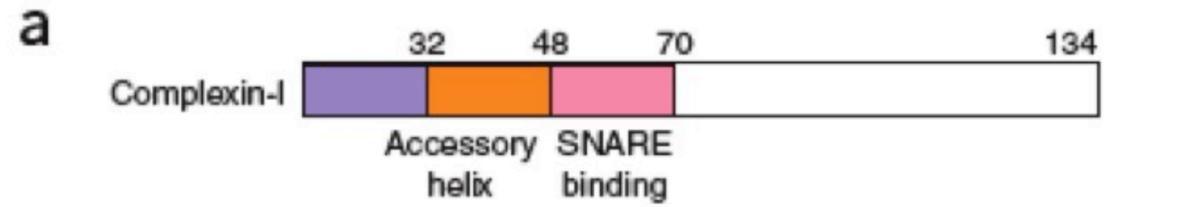
Régulation par la complexine



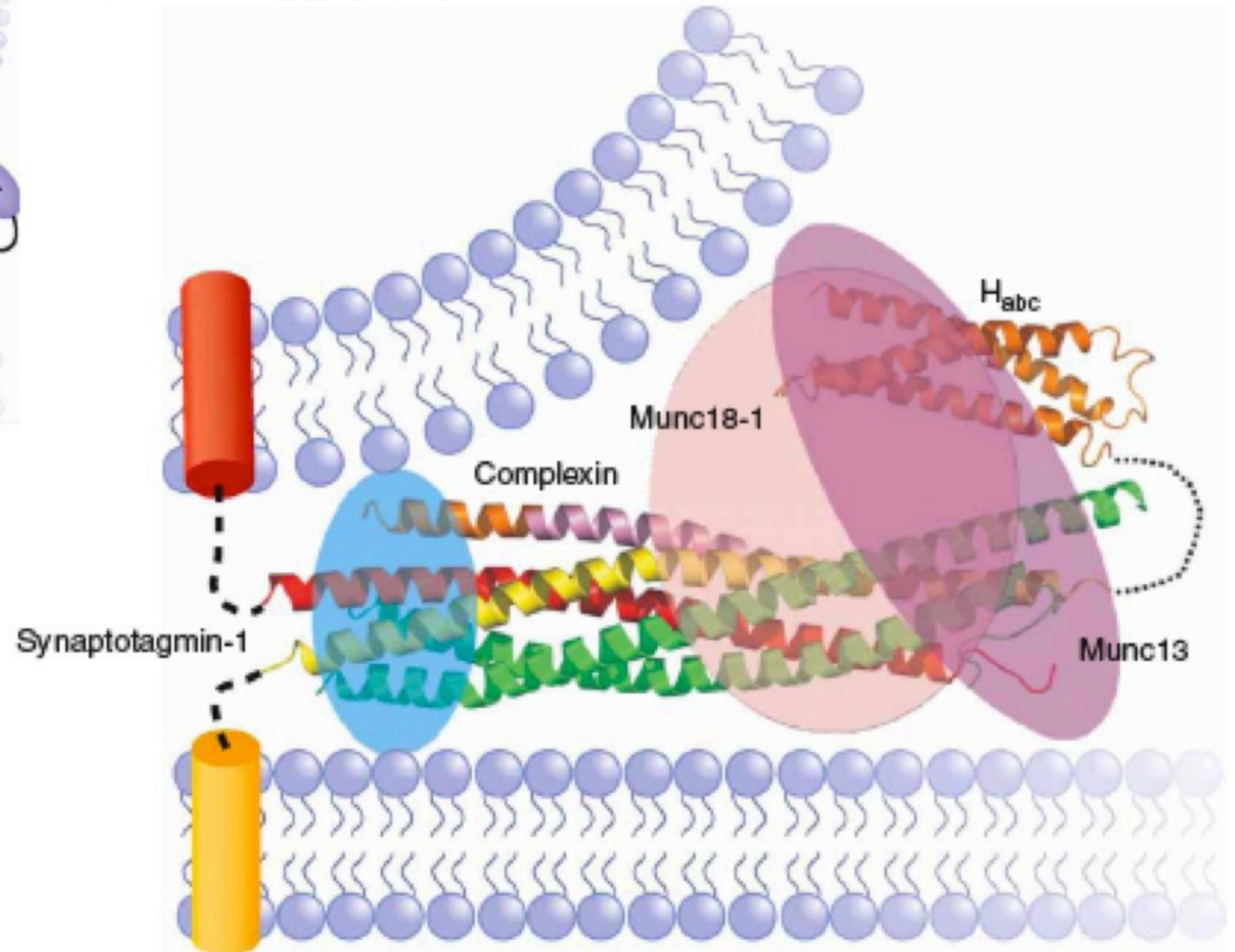
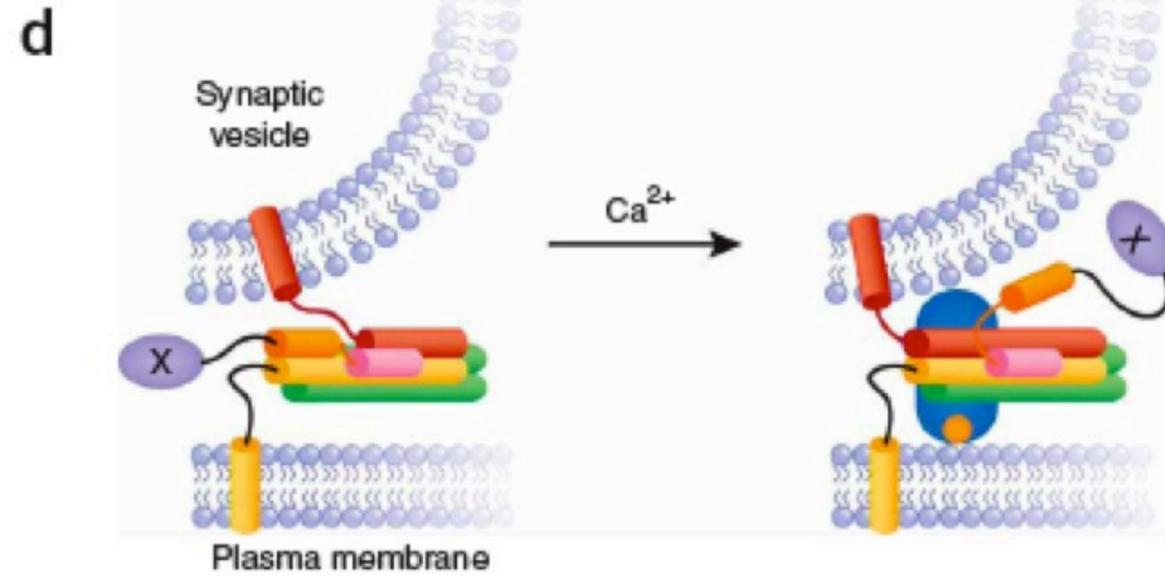
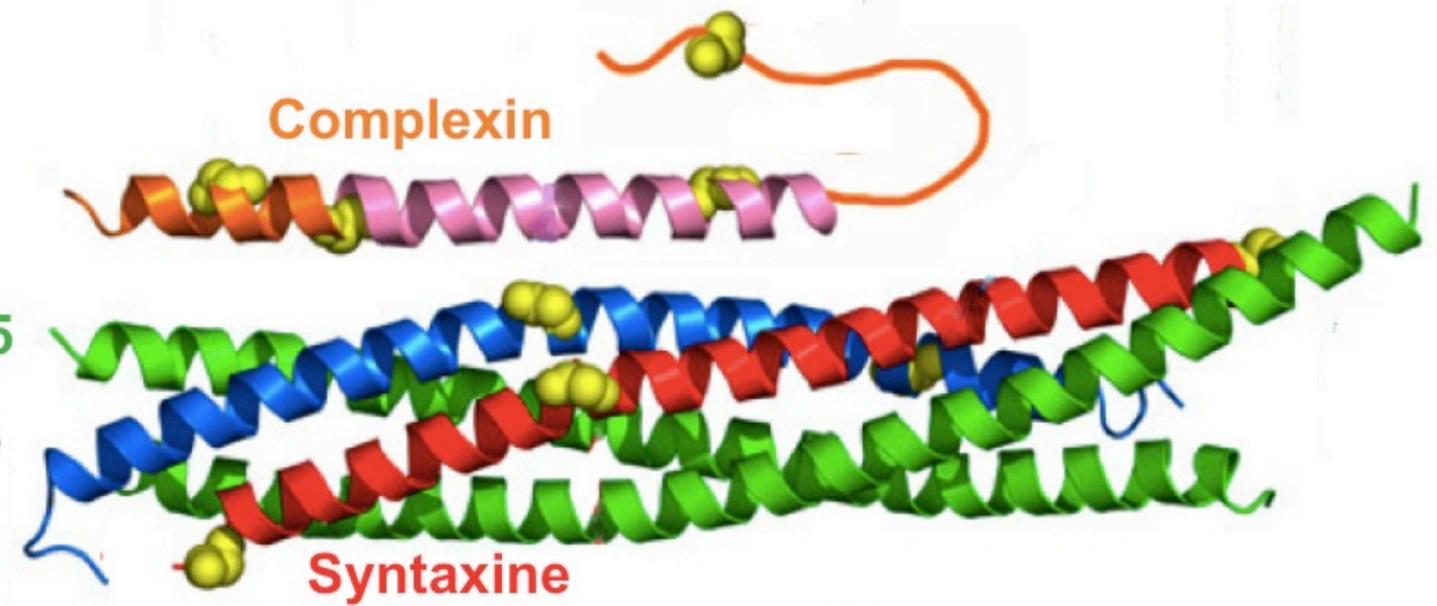
C. Rosenmund



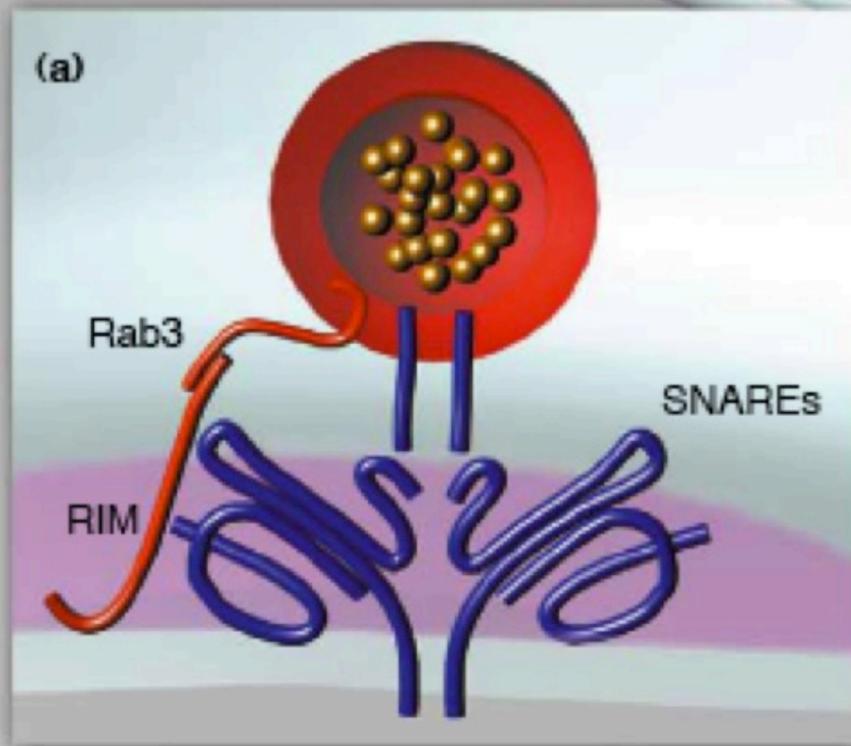
Régulation par la complexine



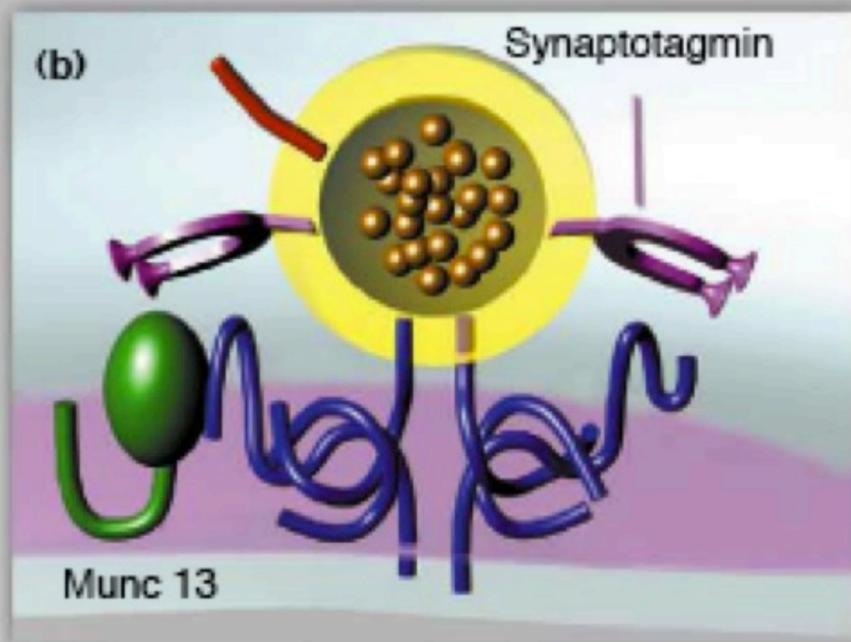
SNAP-25
Synaptobrevine



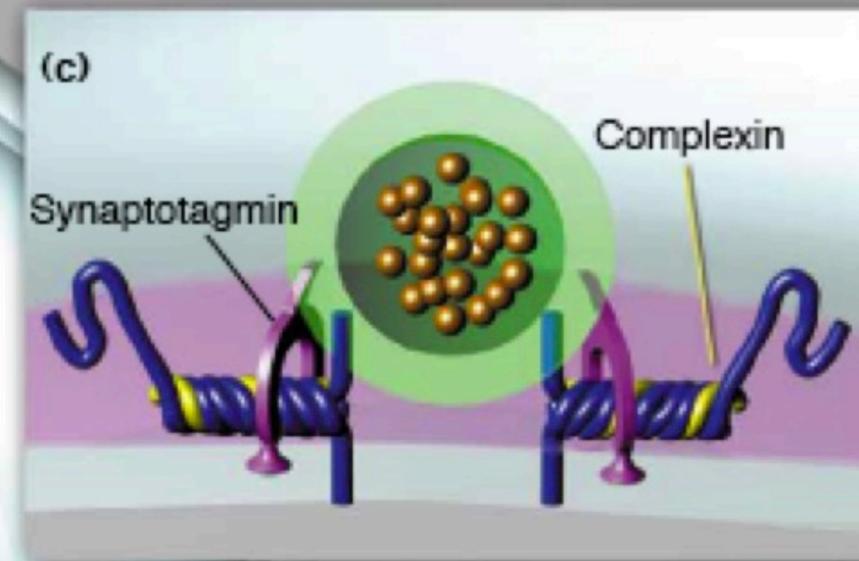
Régulation de l'exocytose



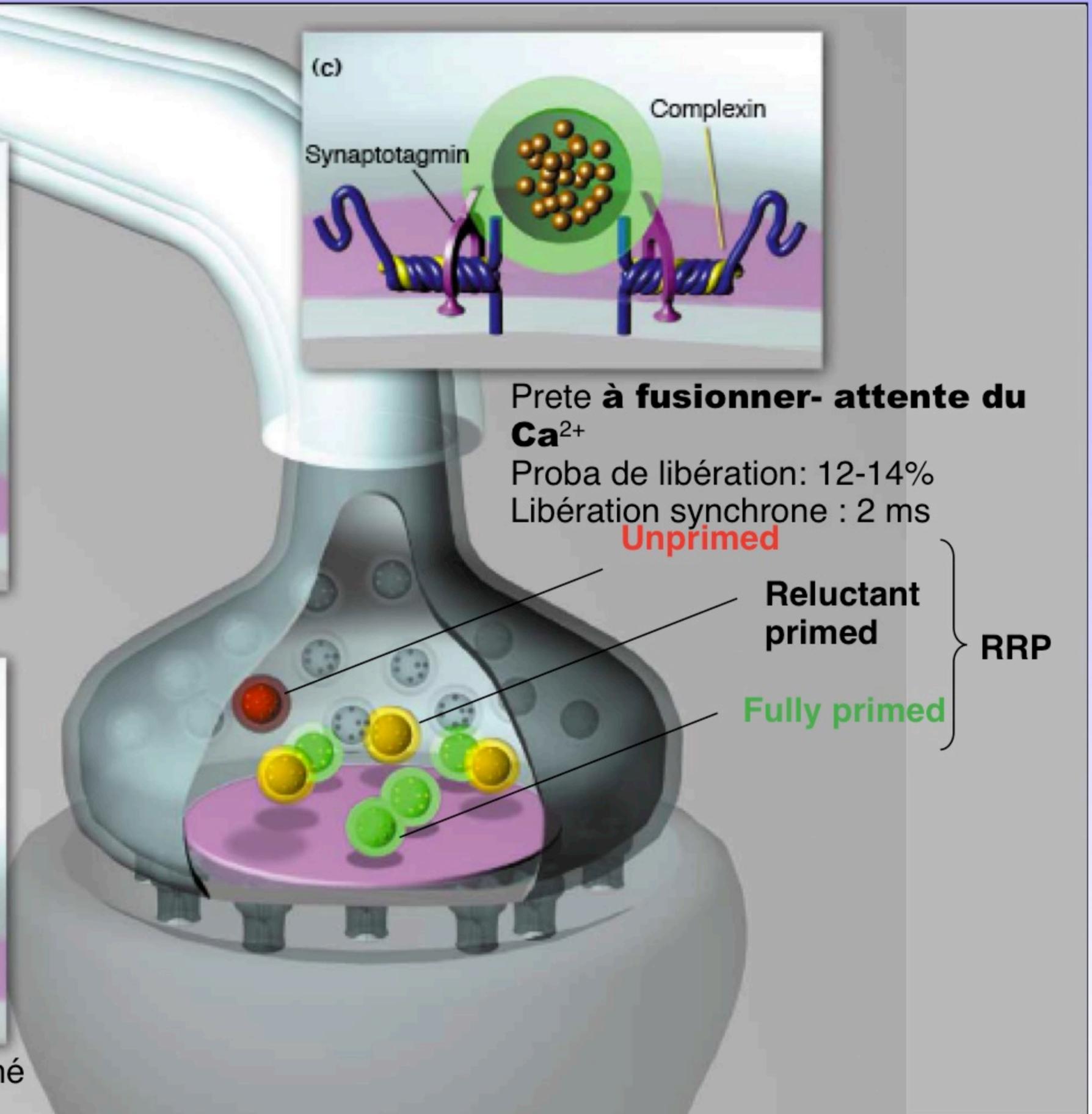
Docking-Initiation du priming



Priming- complexe SNARE relaché
 Proba de libération: 1-2%
 Libération asynchrone : 20 ms



Prete à fusionner- attente du Ca^{2+}
 Proba de libération: 12-14%
 Libération synchrone : 2 ms
Unprimed



Reluctant primed
 Fully primed
 } RRP

Trafic, exocytose et modèles physiopathologiques

1. Exocytose et complexe SNARE

Les voies d'exocytose régulée
Définition du complexe SNARE
Nomenclature v/t-SNARE et R/Q-SNARE

2. Exemple de la synapse

Découverte de NSF et SNAP
Isolement des SNARE
Rôle de NSF & SNAP dans la fusion

3. Le cycle des vésicules synaptiques

Voie lente: endocytose médiée par la clathrine
Voie courte: kiss and run
Les différents « pool » vésiculaires

4. Comment mesurer l'exocytose ?

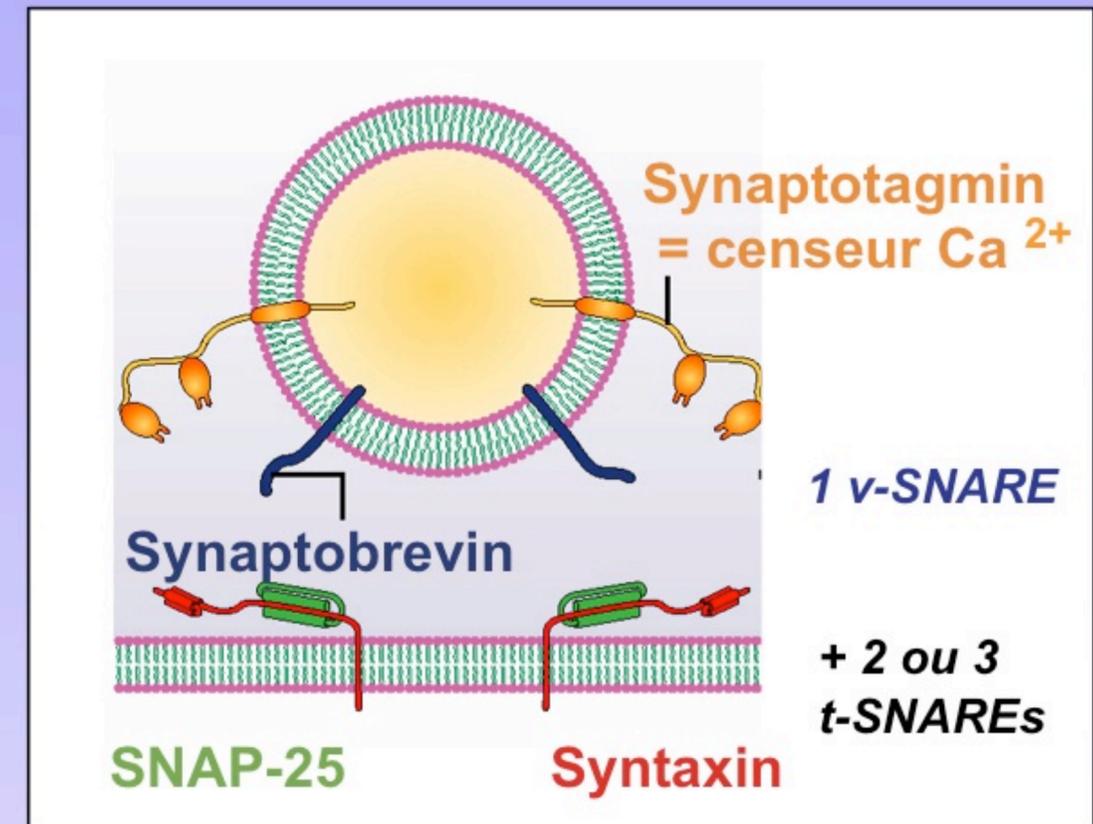
Capacitance
Ampérométrie
GFP pH sensible: la Phluorin
Utilisation des SNARE inversée

5. Comment mesurer le recyclage ?

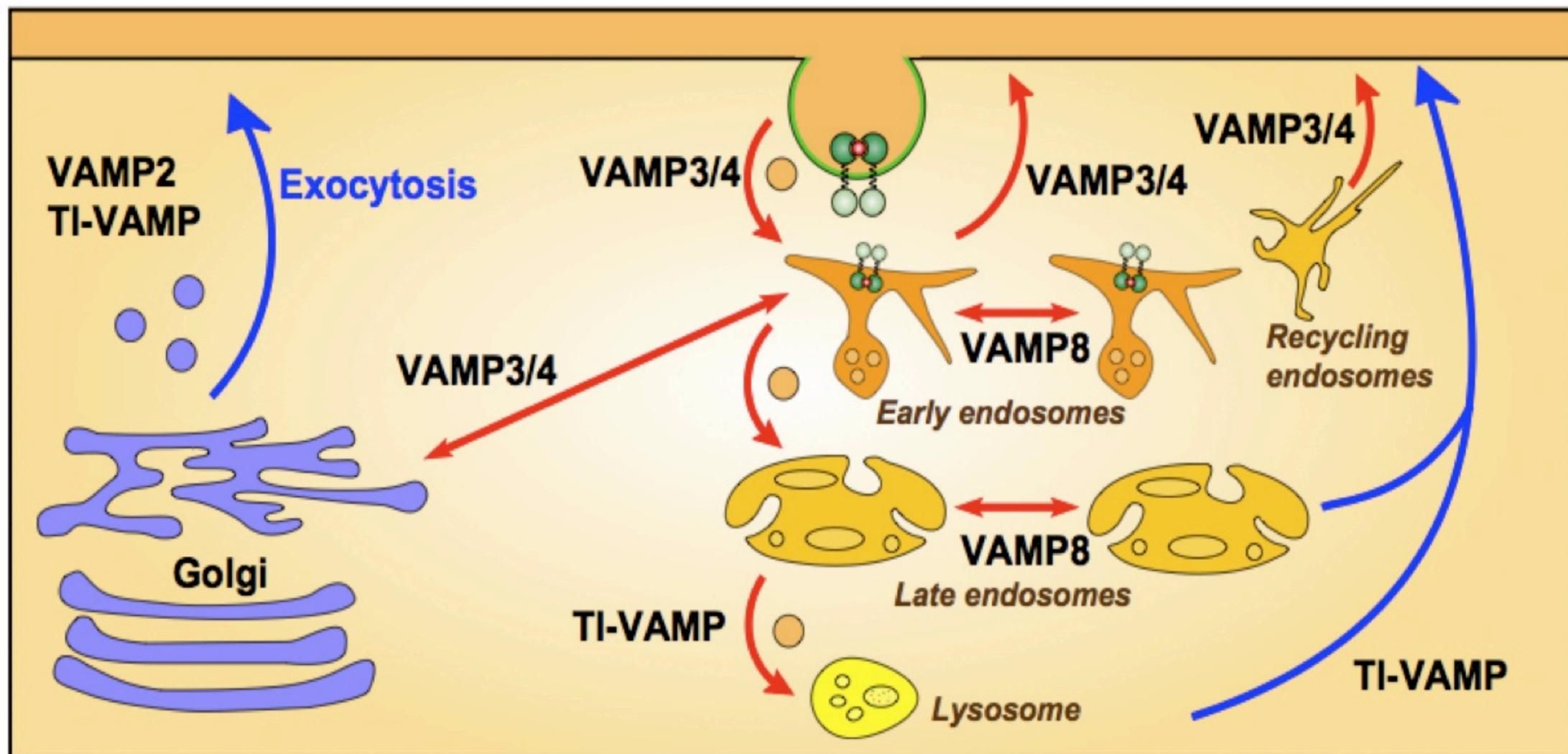
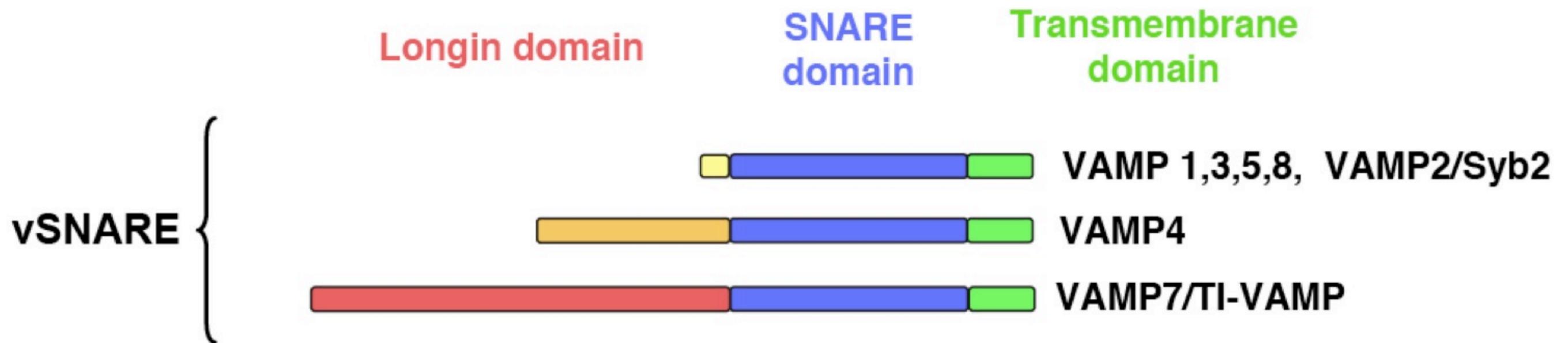
Utilisation des anti-synaptotagmine
Sondes fluorescentes de type FM

6. Modèles physiopathologiques

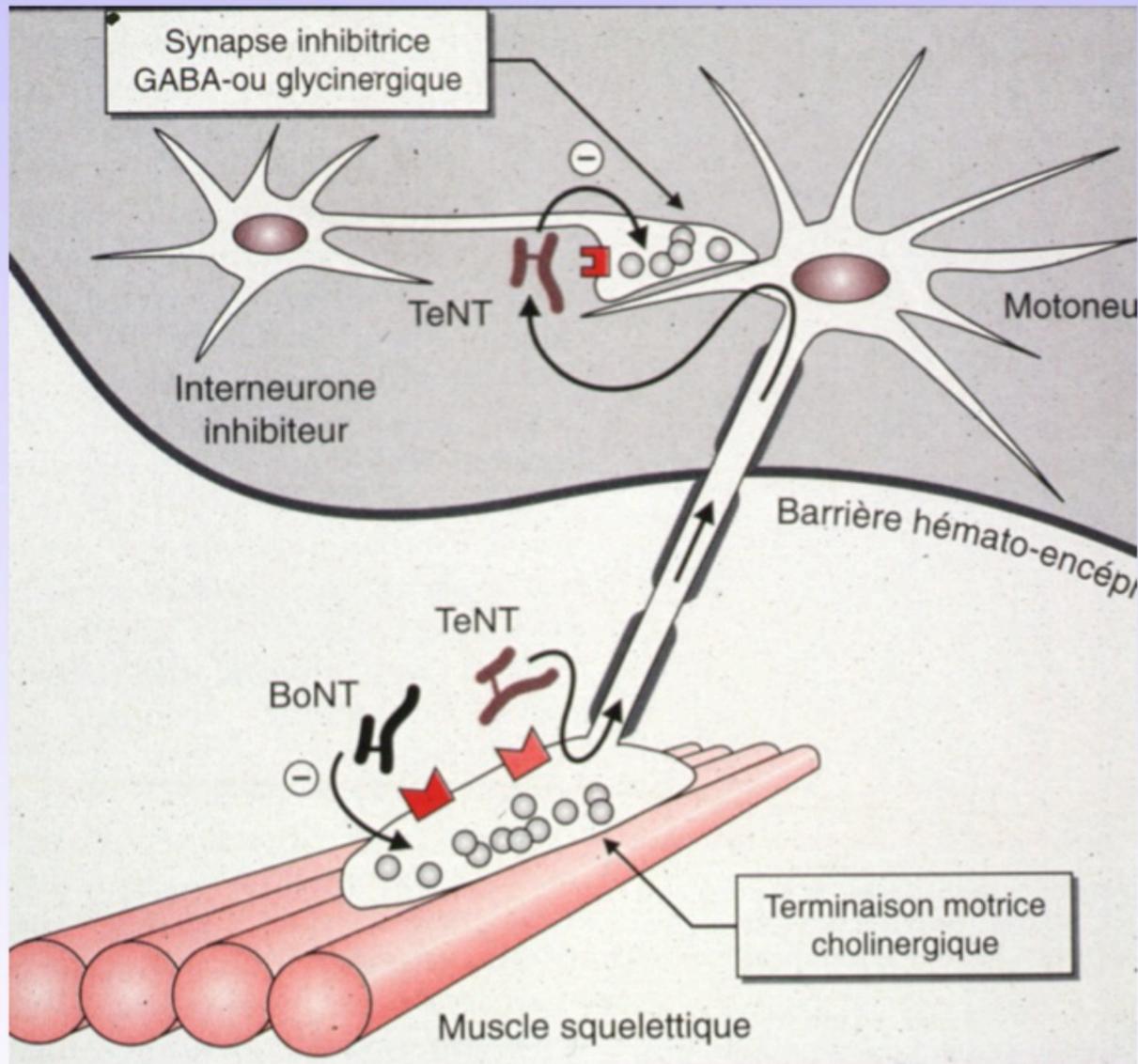
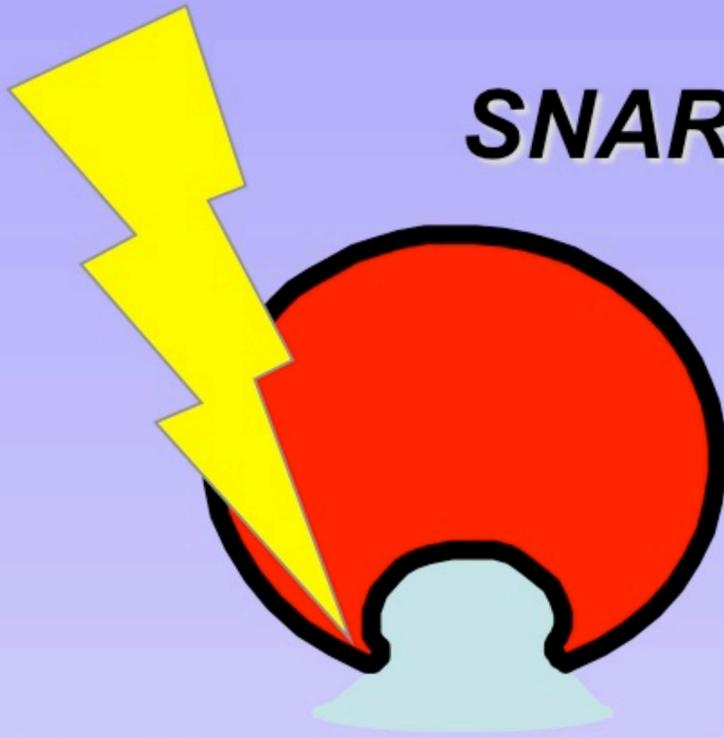
Rôle de la Cellubrevine dans la migration des cellules épithéliales
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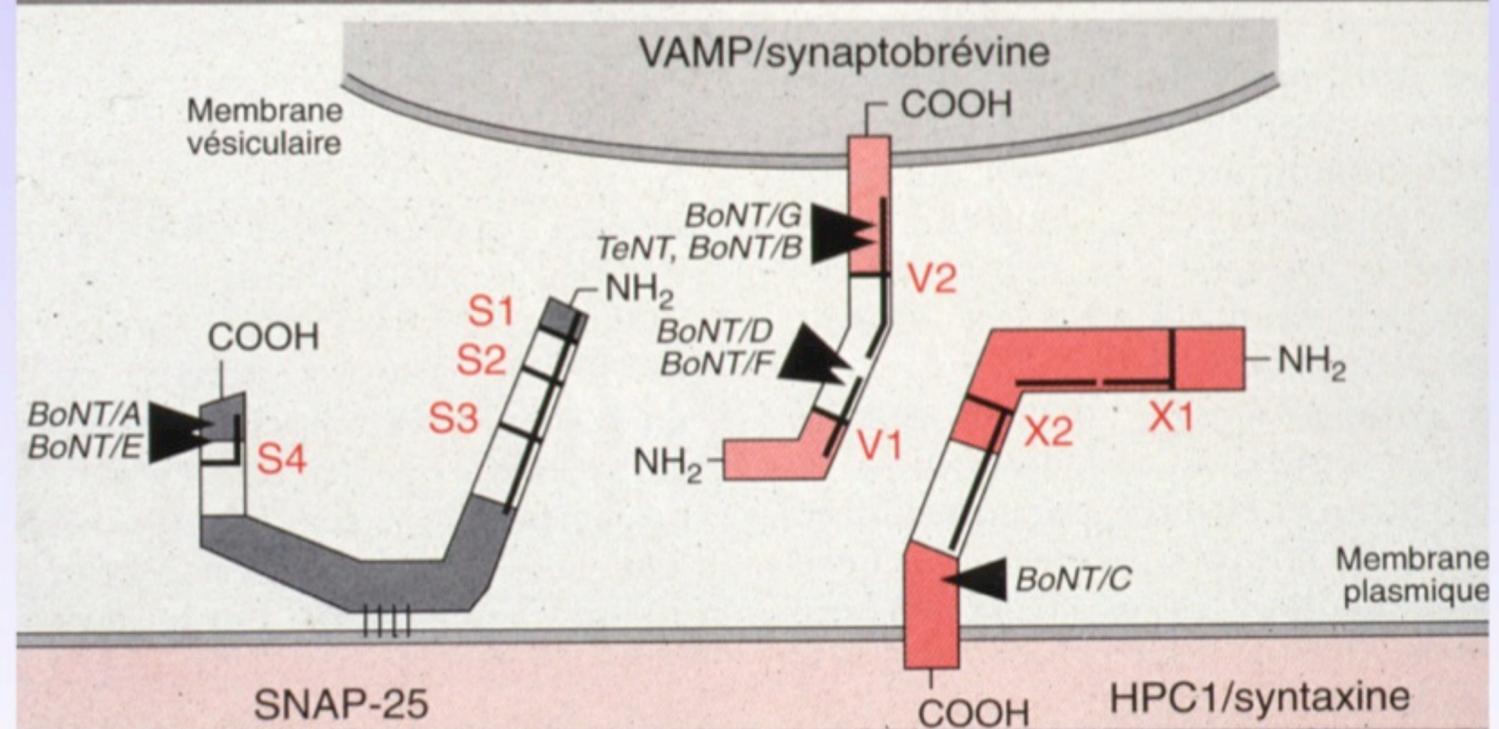
Membrane traffic : role of the vesicular SNARE in the cell



SNAREs: cibles des neurotoxines clostridiales



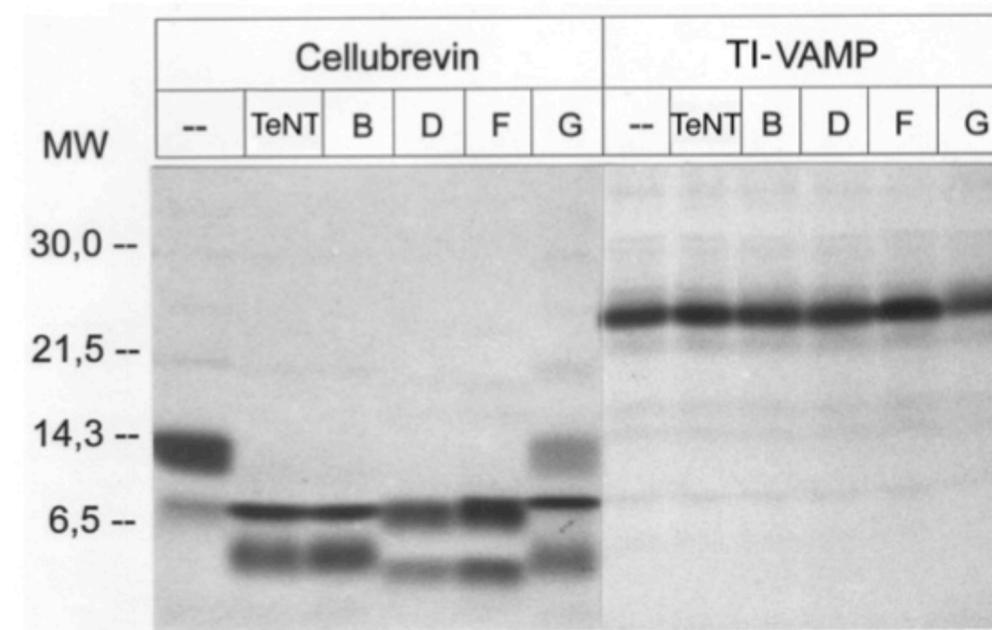
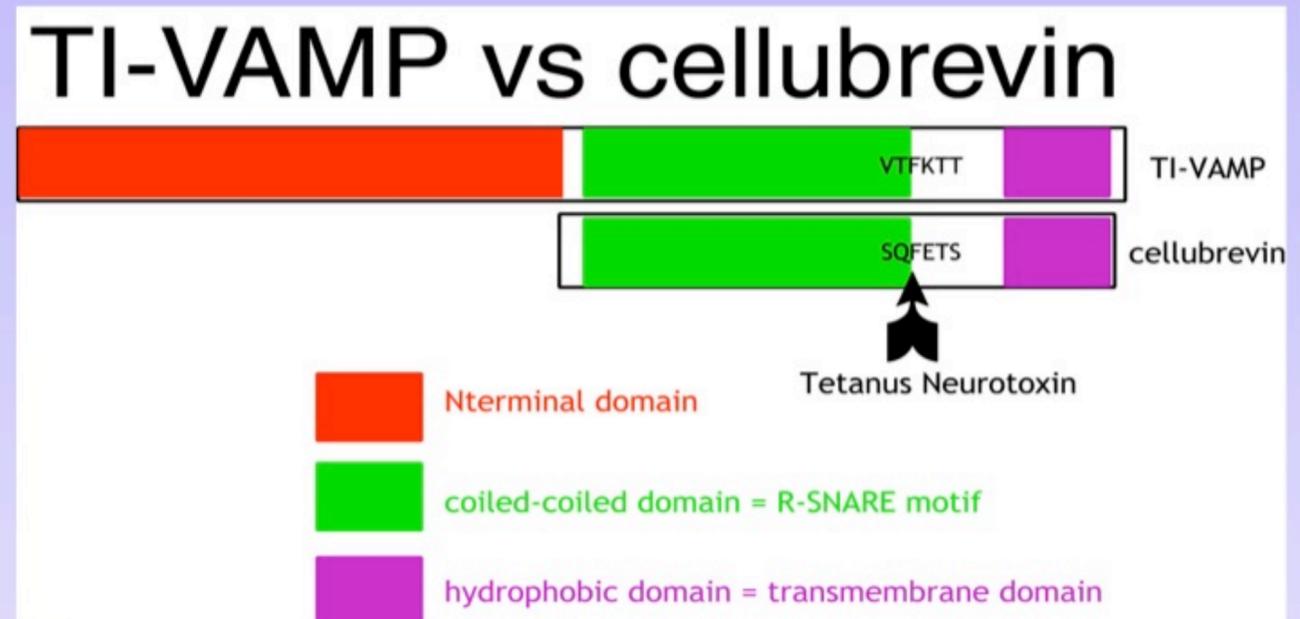
Toxine	Cible	Site de reconnaissance	Site de clivage
TeNT	VAMP	V2 : ELDDRADALQ	ASQFETS
BoNT/B	VAMP	V2 : ELDDRADALQ	ASQFETS
BoNT/D	VAMP	V1 : QVDEVVDIMR	DQKLSEL
BoNT/F	VAMP	V1 : QVDEVVDIMR	RDQKLSE
BoNT/G	VAMP	V2 : ELDDRADALQ	ESAAKLK
BoNT/A	SNAP-25	S4 : EMDENLEQVSG	ANQRATK
BoNT/E	SNAP-25	S4 : EMDENLEQVSG	KTRIDEA
BoNT/C	Syntaxine	X2 : ELEDMLESGN	TKKAVKY
motif consensus		xh--xh-xhp	



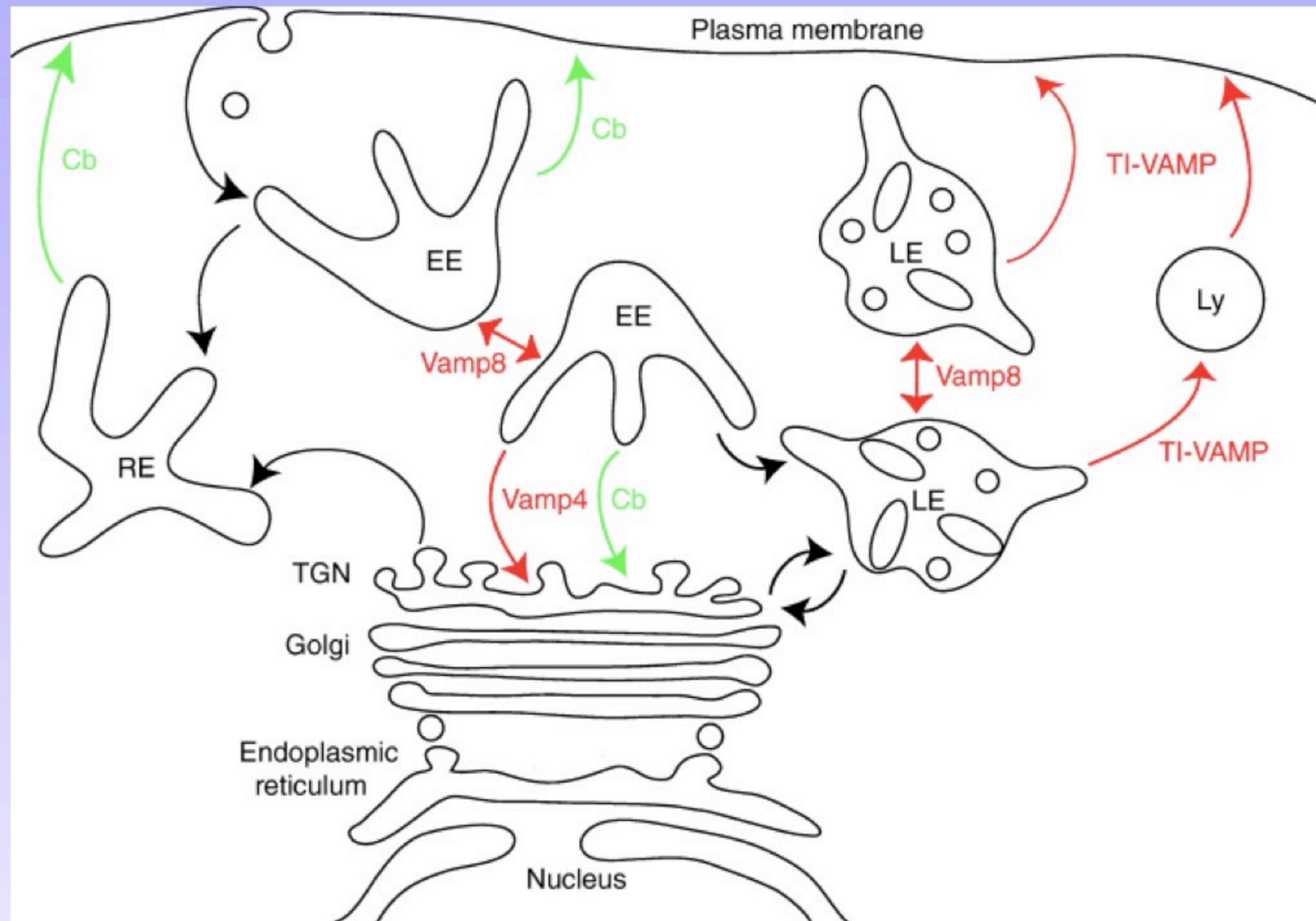
Tetanus Neurotoxin-Insensitive Vesicle-Associated Membrane Protein (TI-VAMP)

TI-VAMP/VAMP7

- X-linked gene
- 25kD v-SNARE
- ubiquitous
- Insensitive to NTs
- N-terminal extension of 100aa called **Longin domain**



Tetanus neurotoxin **sensitive** and **insensitive** exocytosis



V-SNARE	Sensitive to TeNT	Expressed in neurons
Synaptobrevin/ VAMP2	Yes	Yes
Cellubrevin/VAMP3	Yes	No
TI-VAMP/VAMP7	No	Yes

Applications des Neurotoxines ...

Dysport
TOXINE BOTULIQUE TYPE A • 500 UNITÉS SPEYWOOD

La maîtrise biopharmaceutique...

Une action focalisée sur la clef synaptique

... au service des Dystonies

Une cible spécifique : la jonction neuromusculaire

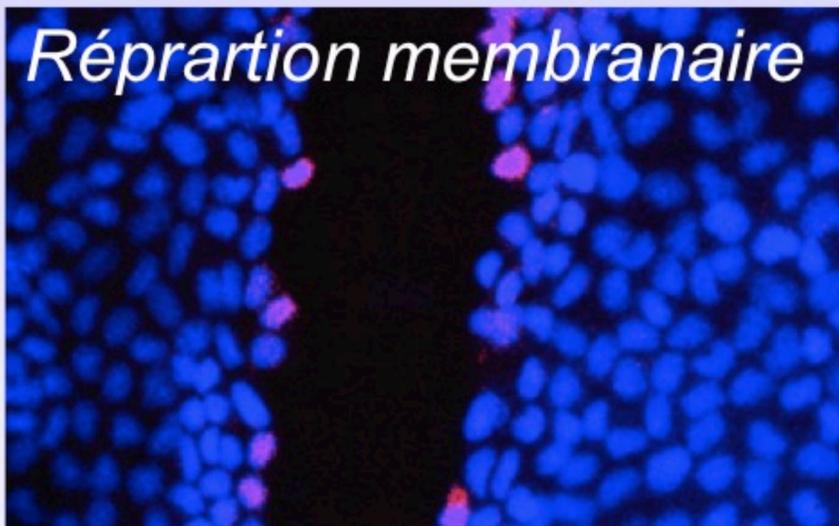
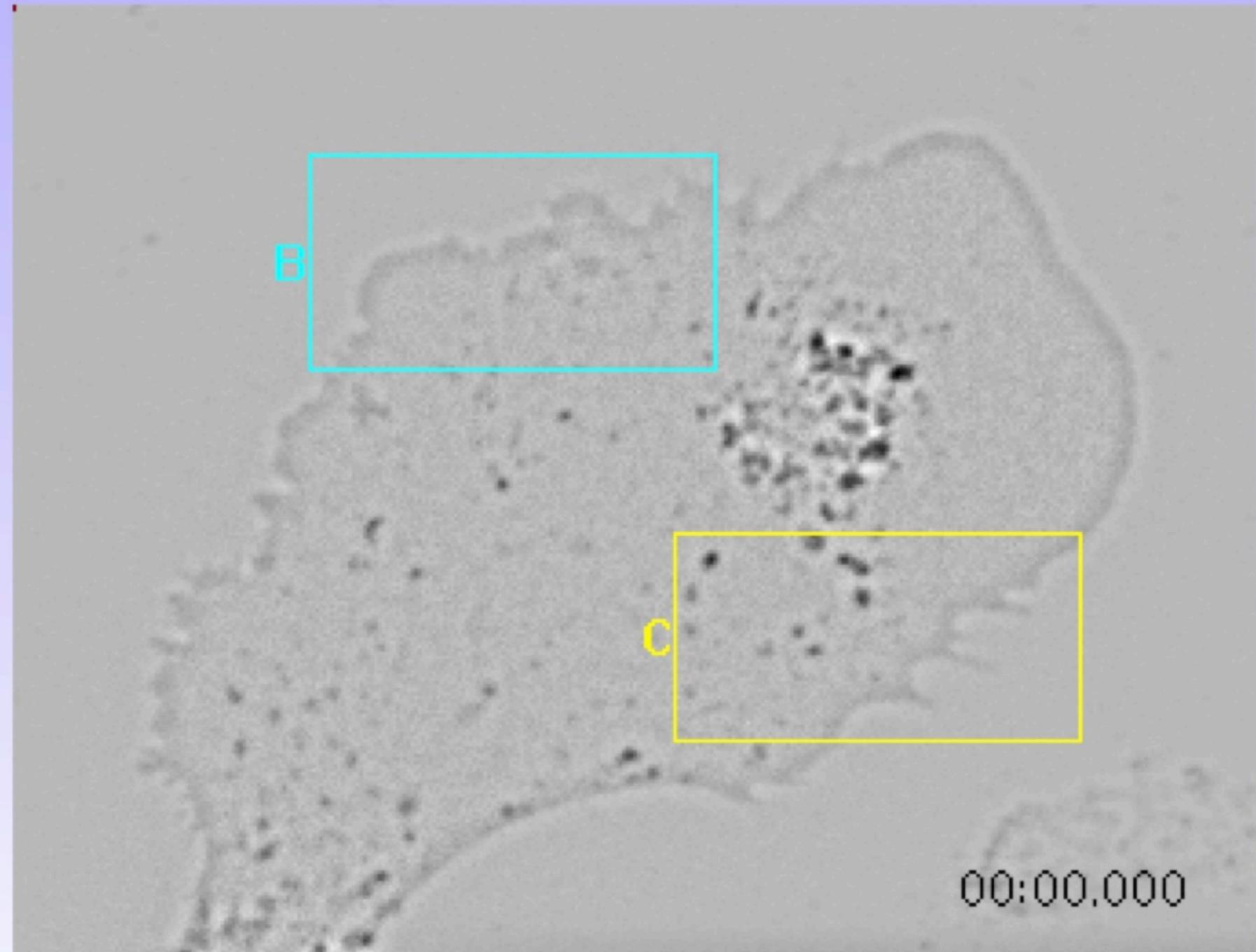
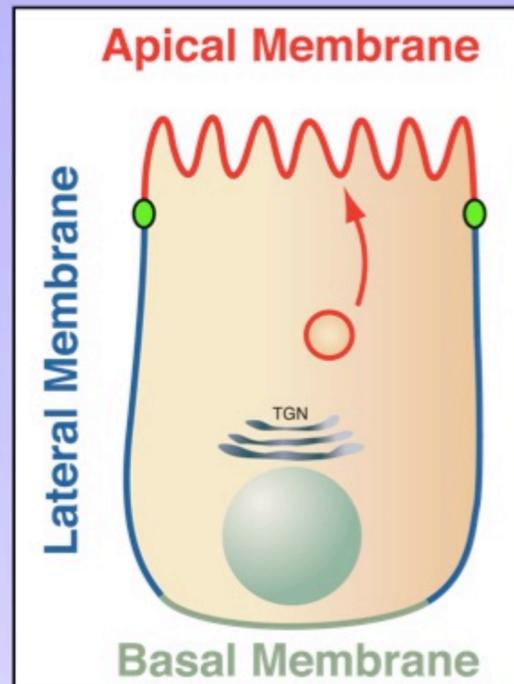
Vésicule d'acétylcholine
Fente synaptique
Fibre musculaire contractile



Traitement des dystonies (hypercontractions musculaires involontaires et douloureuses).

Esthétique: injection de « BOTOX »:
Paralysie musculaire pendant 5 à 6 mois.

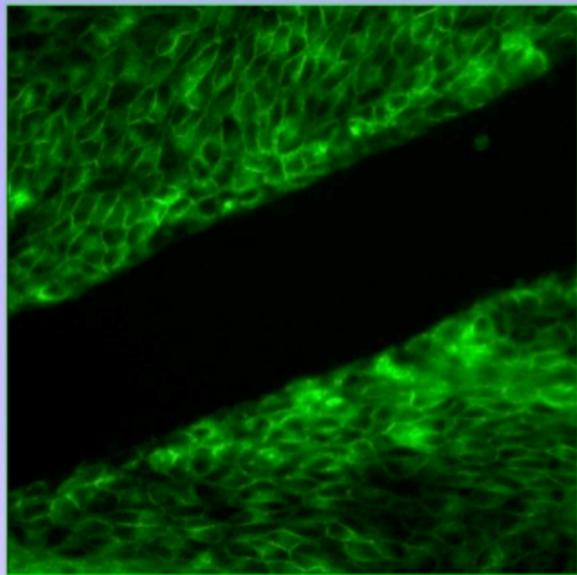
La cellubrevine est nécessaire à la réparation membranaire et la migration des cellules épithéliales



(Proux-Gillardeaux, 2007)

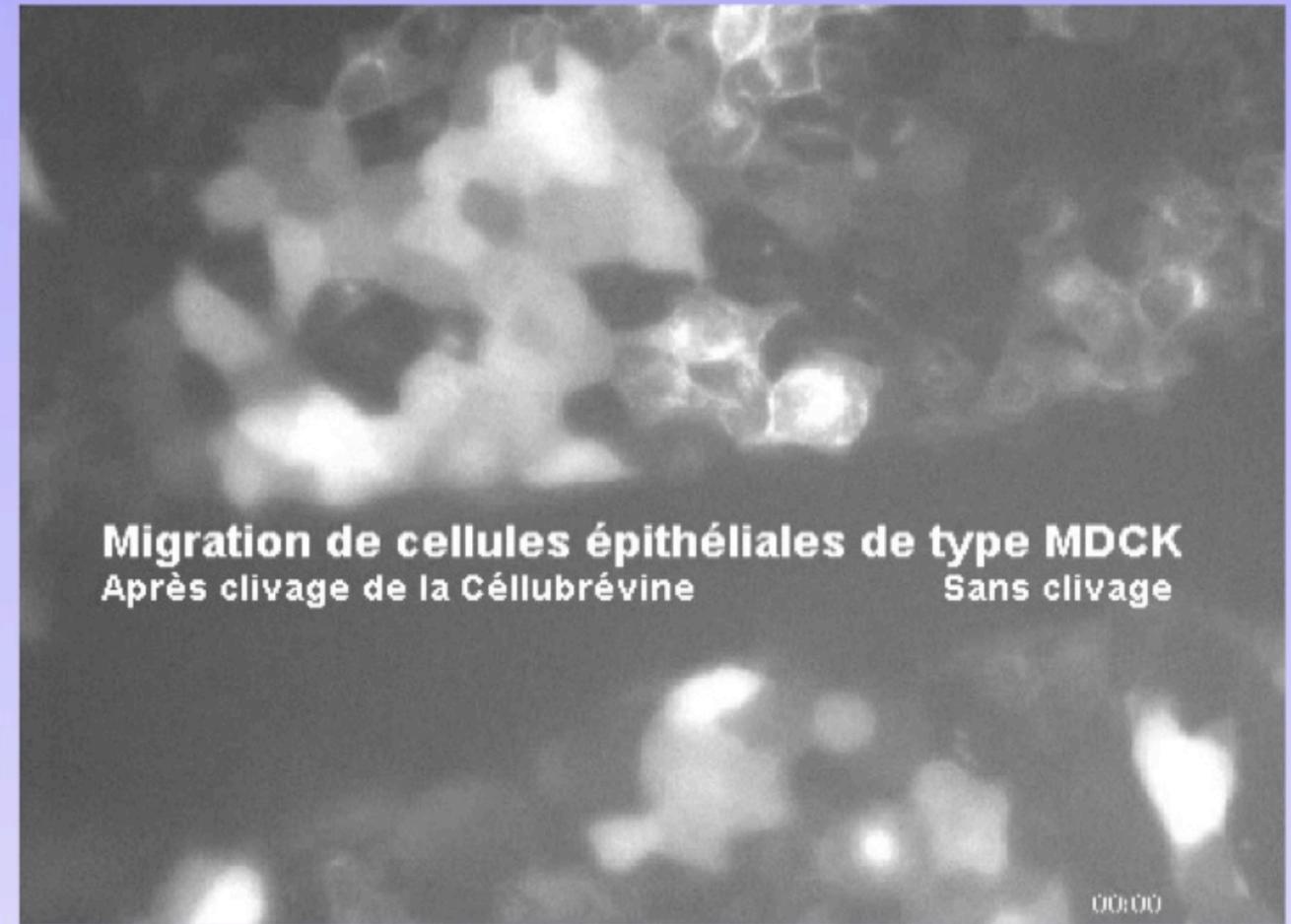
Proux-Gillardeaux & al, PNAS 2005

La toxine tétanique clive la cellubrévine et ralentit la migration des cellules épithéliales



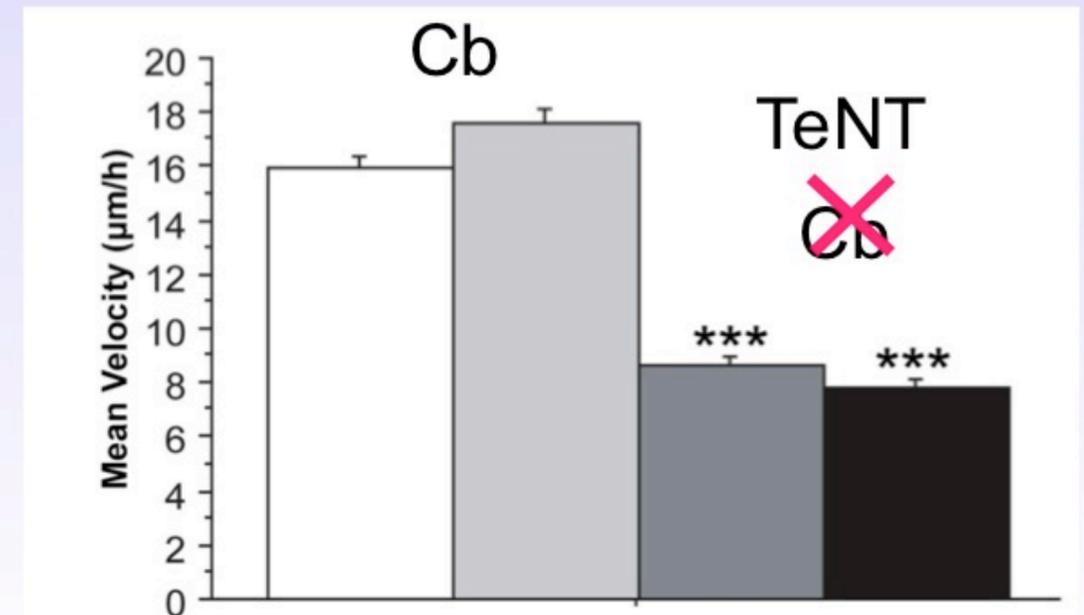
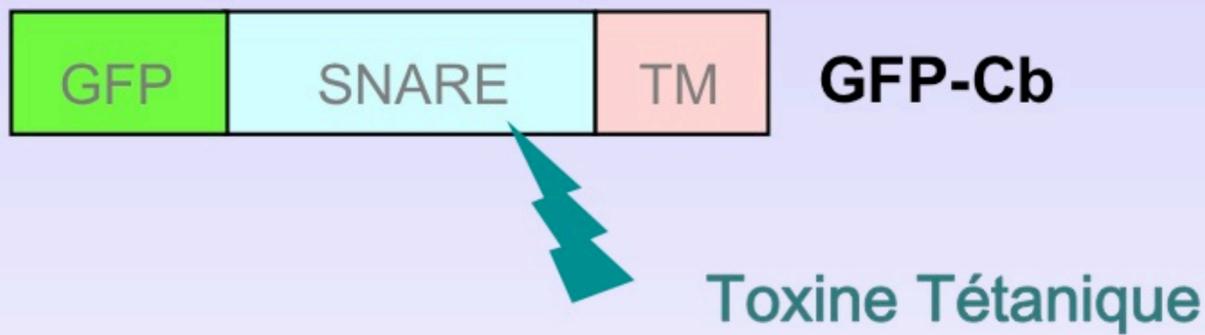
Blessure réalisée sur un tapis de cellule épithéliale :
-> favorise la migration

GFP-Cb



Migration de cellules épithéliales de type MDCK
Après clivage de la Célubrévine Sans clivage

Proux-Gillardeaux & al, PNAS 2005, BoC 2007



Proux-Gillardeaux & al, PNAS 2005, BoC 2007

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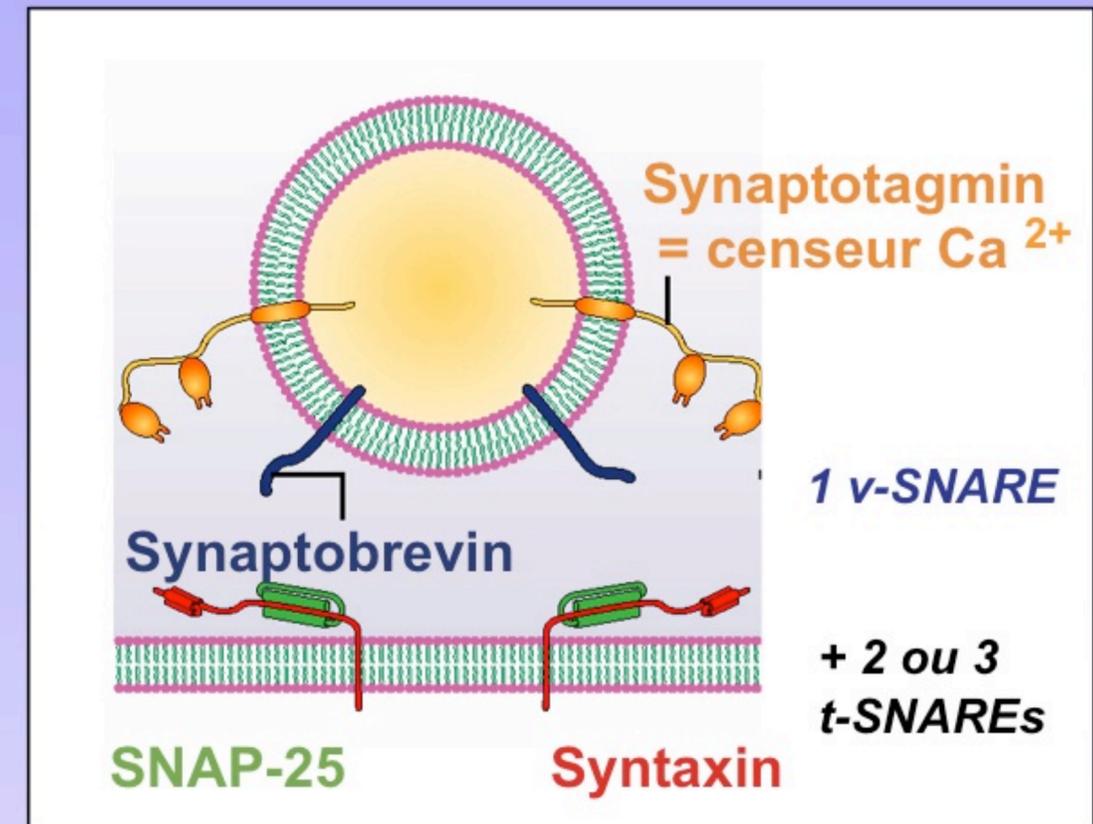
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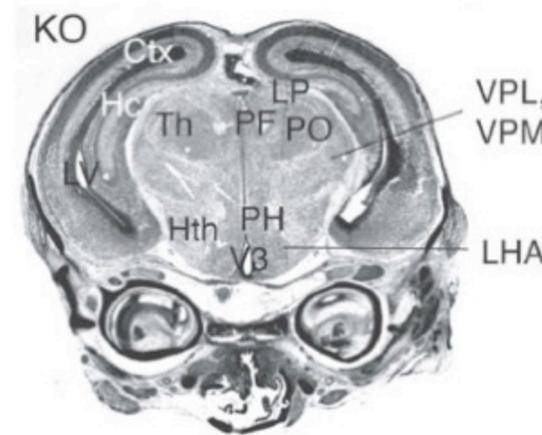
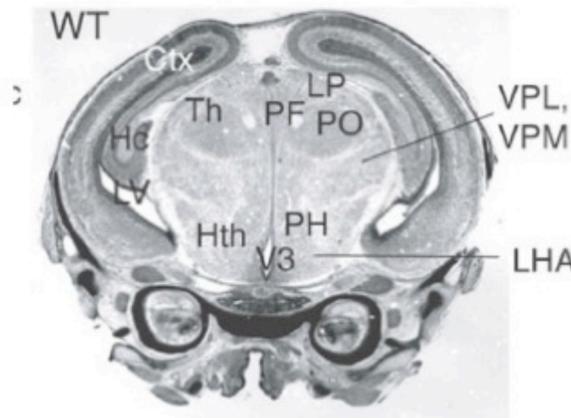
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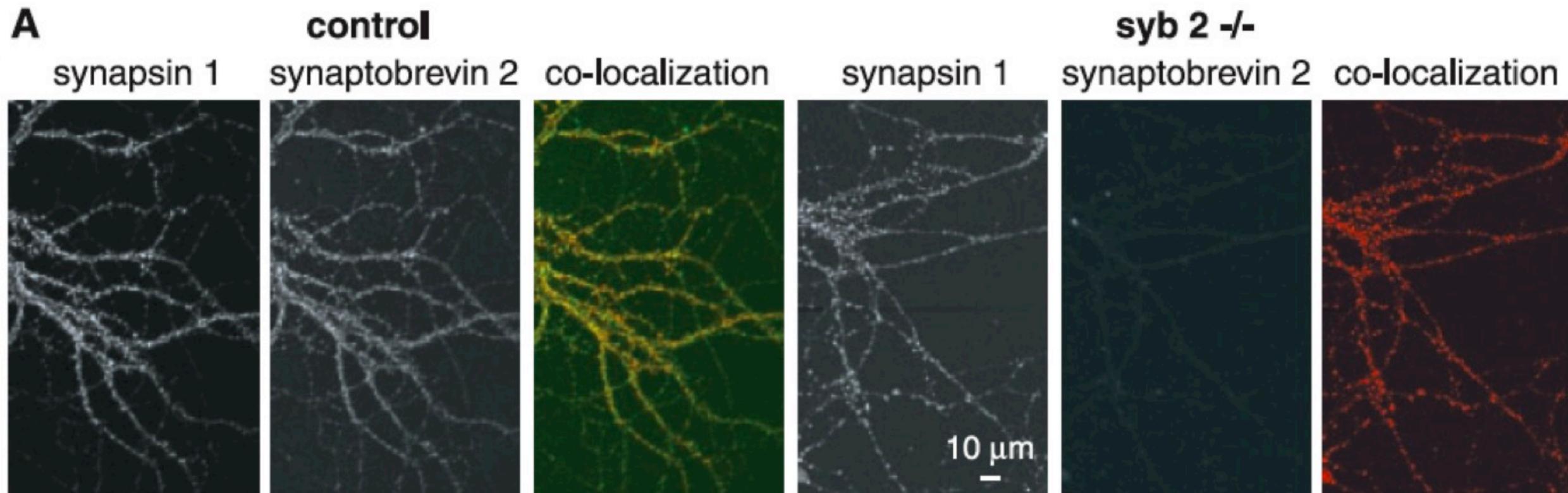
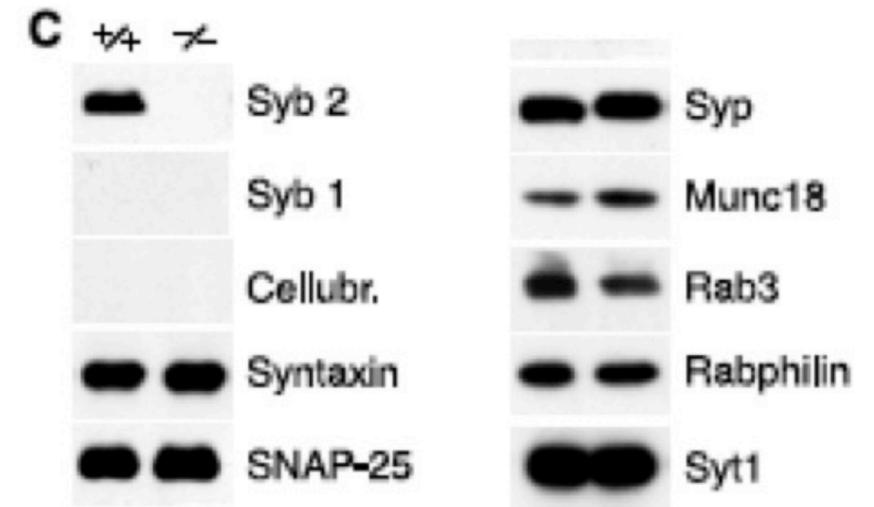
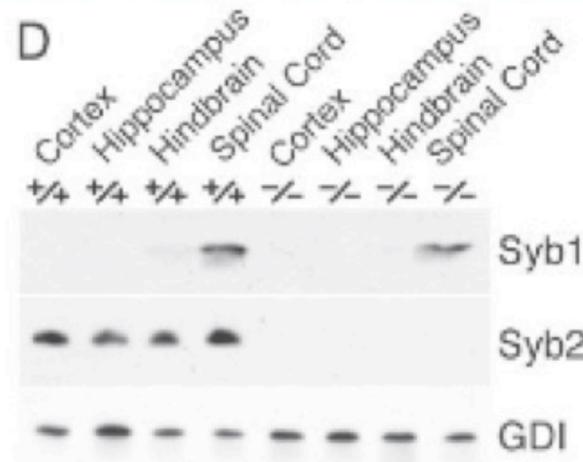


Synaptobrevin2 (Syb2) KO



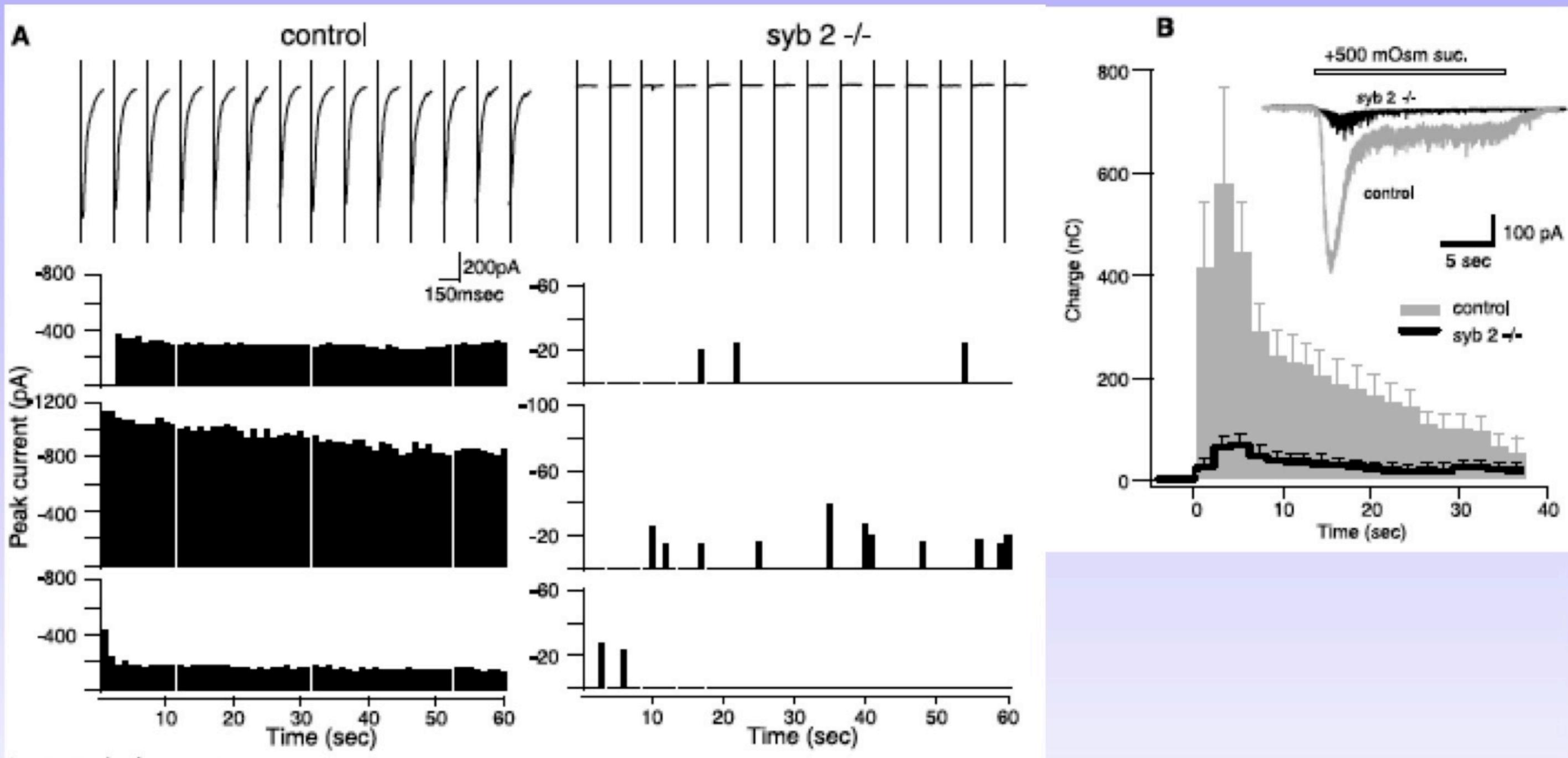
SNARE Function Analyzed in Synaptobrevin/VAMP Knockout Mice

Susanne Schoch,^{1,4*} Ferenc Deák,^{1,4*} Andreas Königstorfer,^{5*} Marina Mozhayeva,¹ Yildirim Sara,¹ Thomas C. Südhof,^{1,2,4†} Ege T. Kavalali^{1,3†}



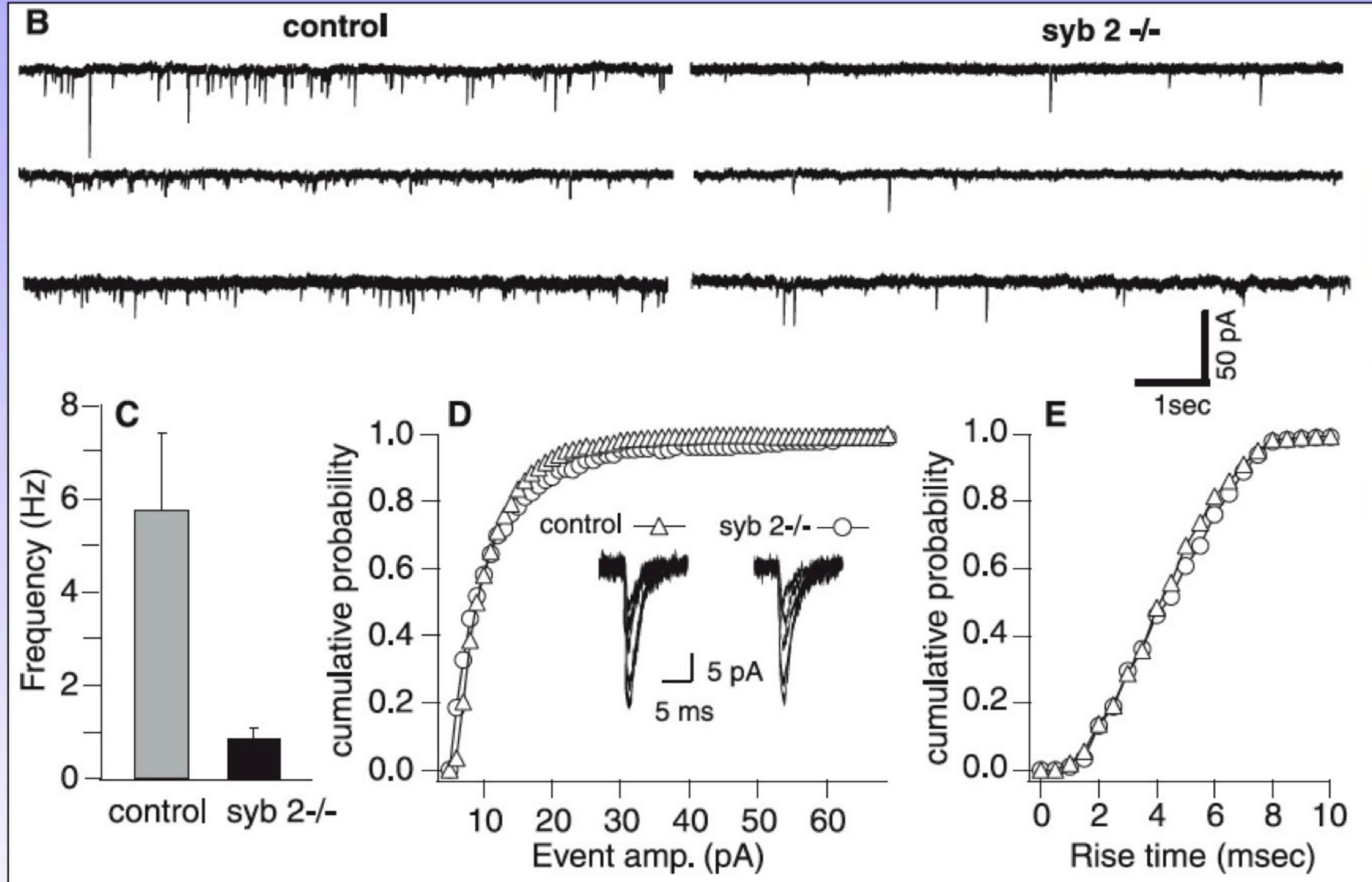
En l'absence de Syb2, les synapses sont toujours présentes, le cerveau se forme normalement mais les animaux meurent à la naissance.

Synaptobrevin2 (Syb2) KO



En l'absence de Syb2, la réponse évoquée est réduite d'un facteur 100.

Synaptobrevin2 (Syb2) KO



En l'absence de Syb2, la réponse spontanée est réduite d'un facteur 10.

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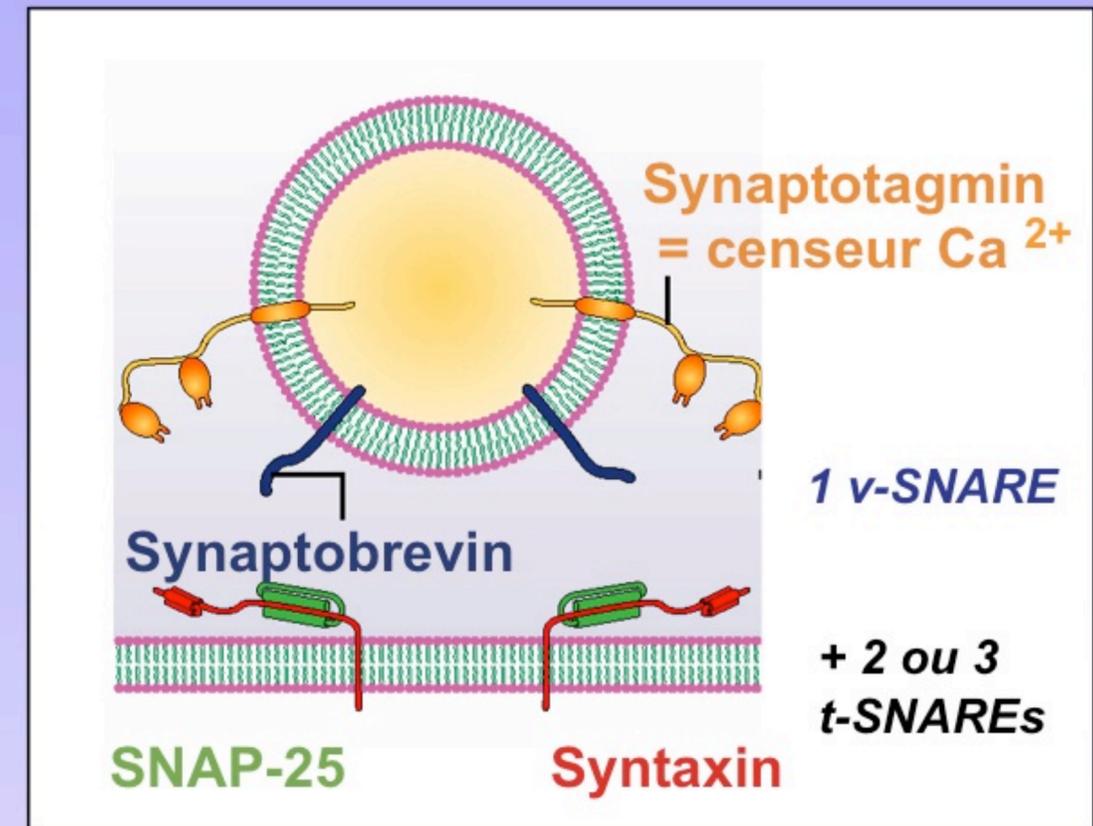
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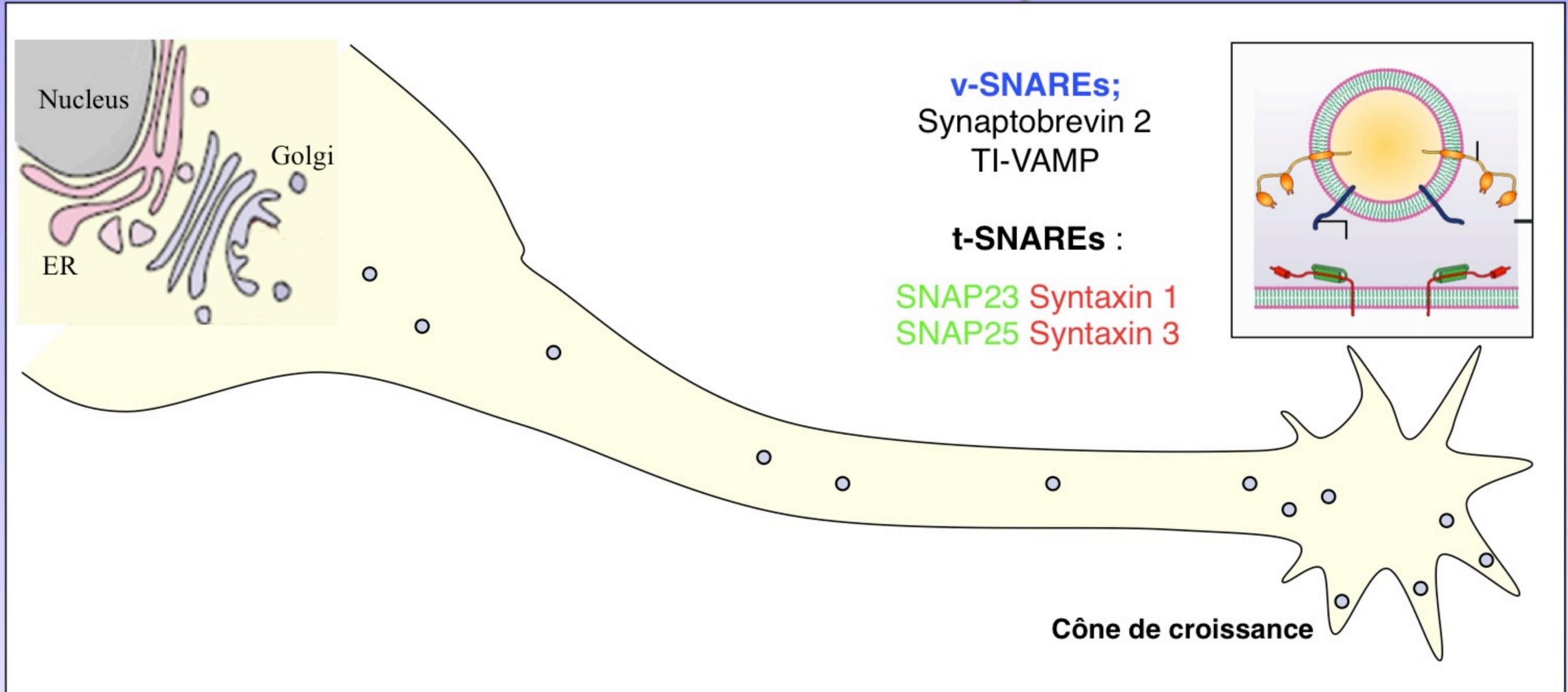
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SNAREs et croissance neuritique



SNAREs à la synapse

SNAREs au cône de croissance

v-SNAREs:

t-SNAREs :

v-SNAREs:

t-SNAREs :

Synaptobrevin 2

Syntaxin 1
SNAP25

Synaptobrevin 2
TI-VAMP

Syntaxin 1,3
SNAP23,25

Perte de Syb2 ou SNAP25:
Perte de sécrétion évoquée

Devpt cérébral normal, croissance neur normale

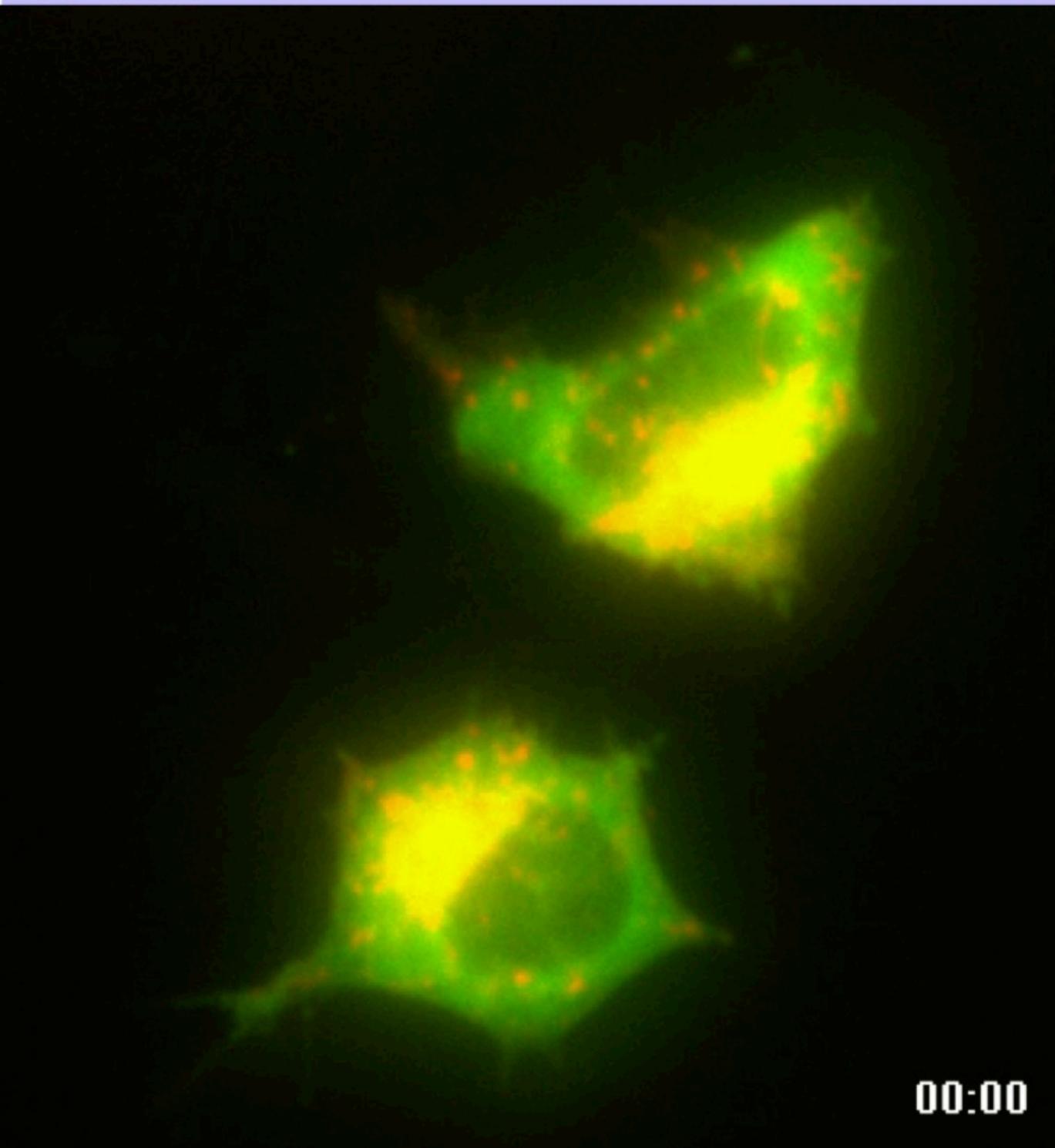
Croissance neuritique:

Résistante à la TeNT qui clive Syb2

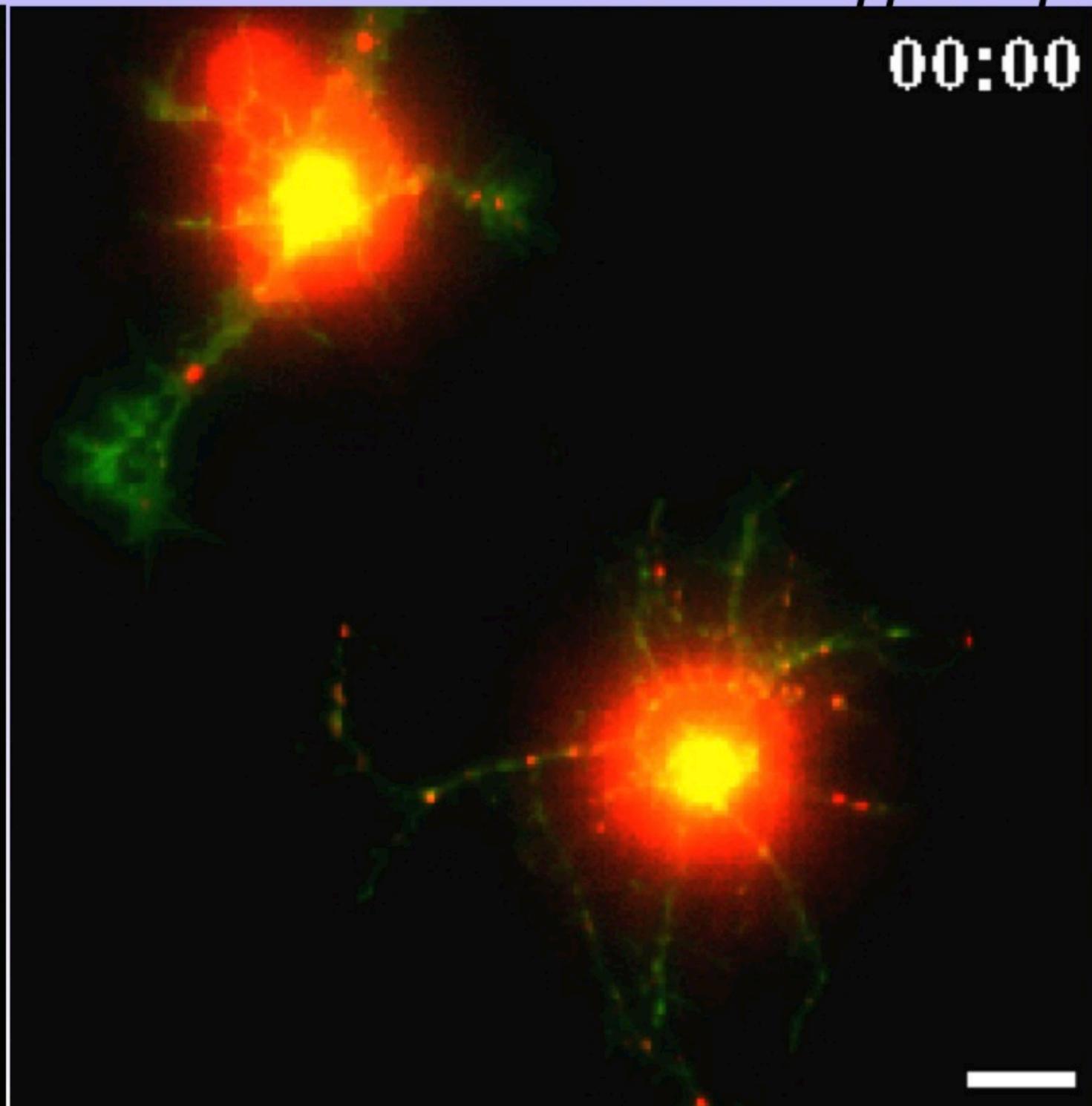
Nécessite TI-VAMP et stx3

TI-VAMP et la croissance neuritique

Cellules PC12



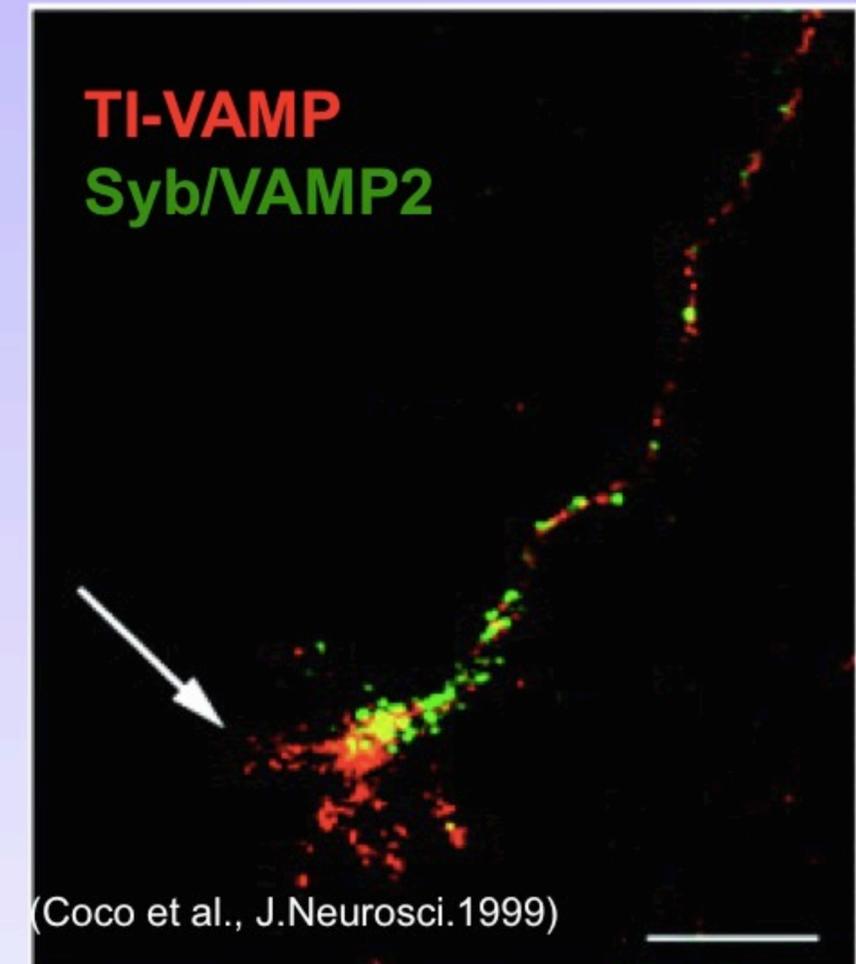
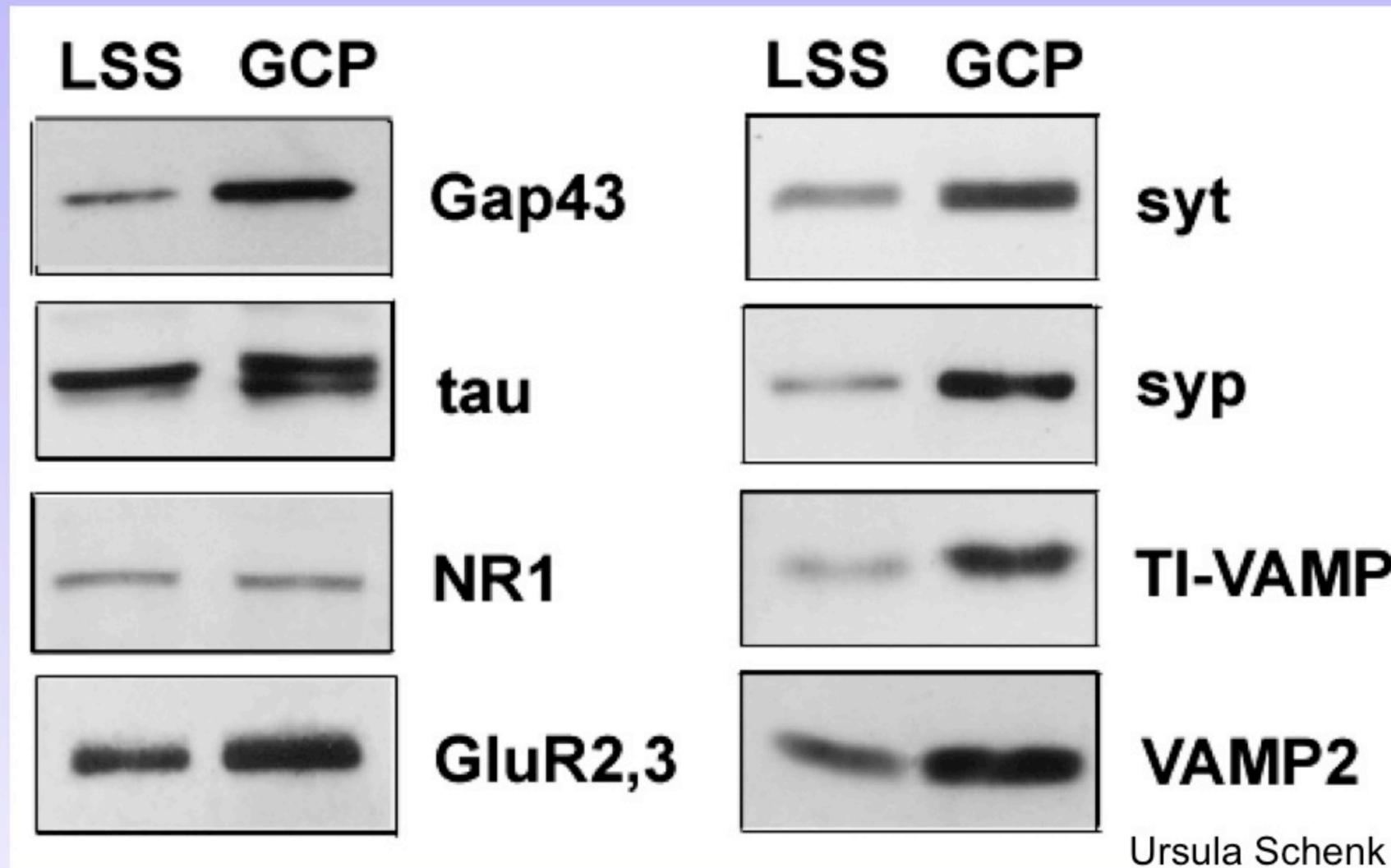
Neurones d'hippocampe



Tubulin **RFP-TI-VAMP**

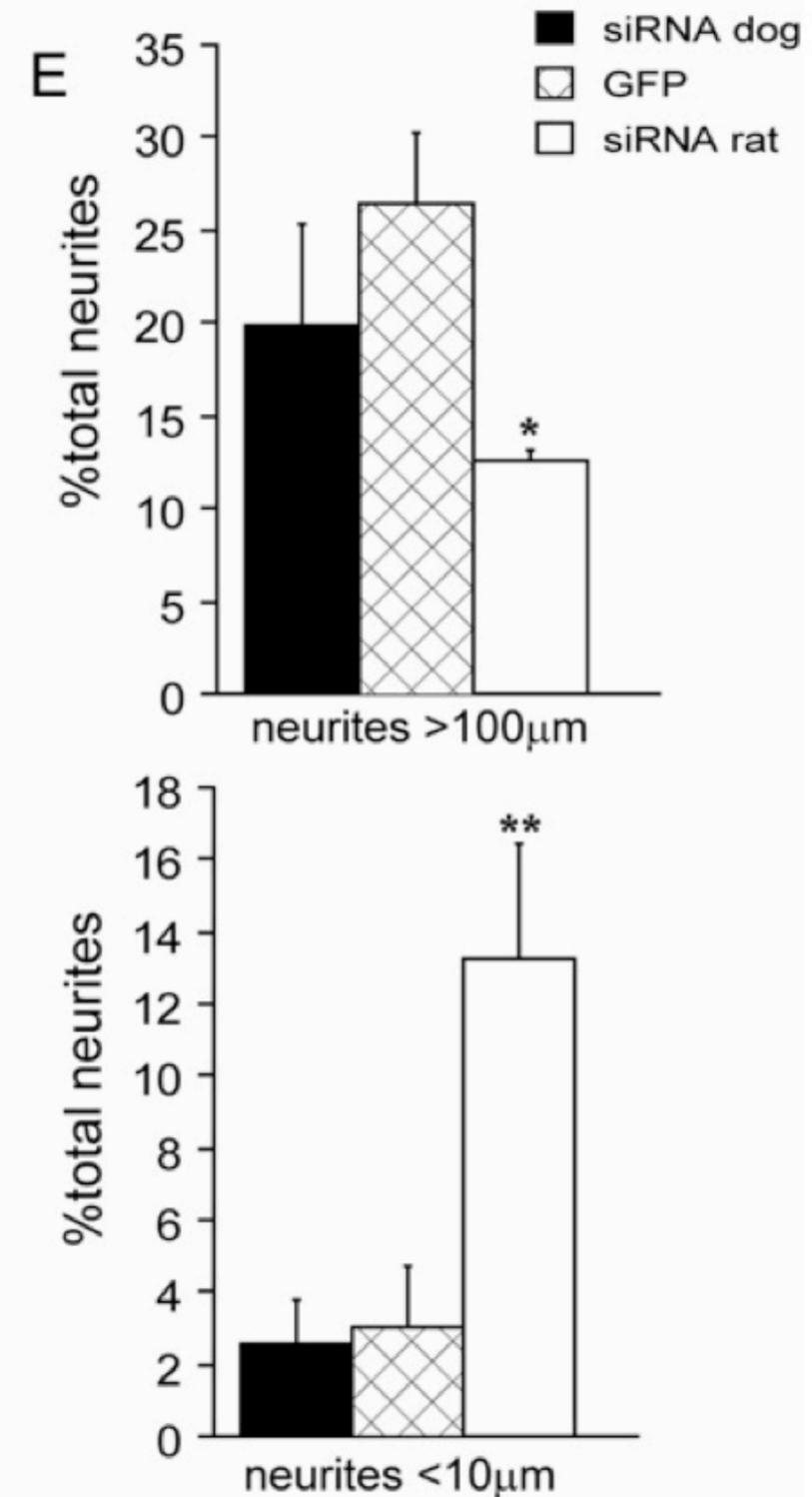
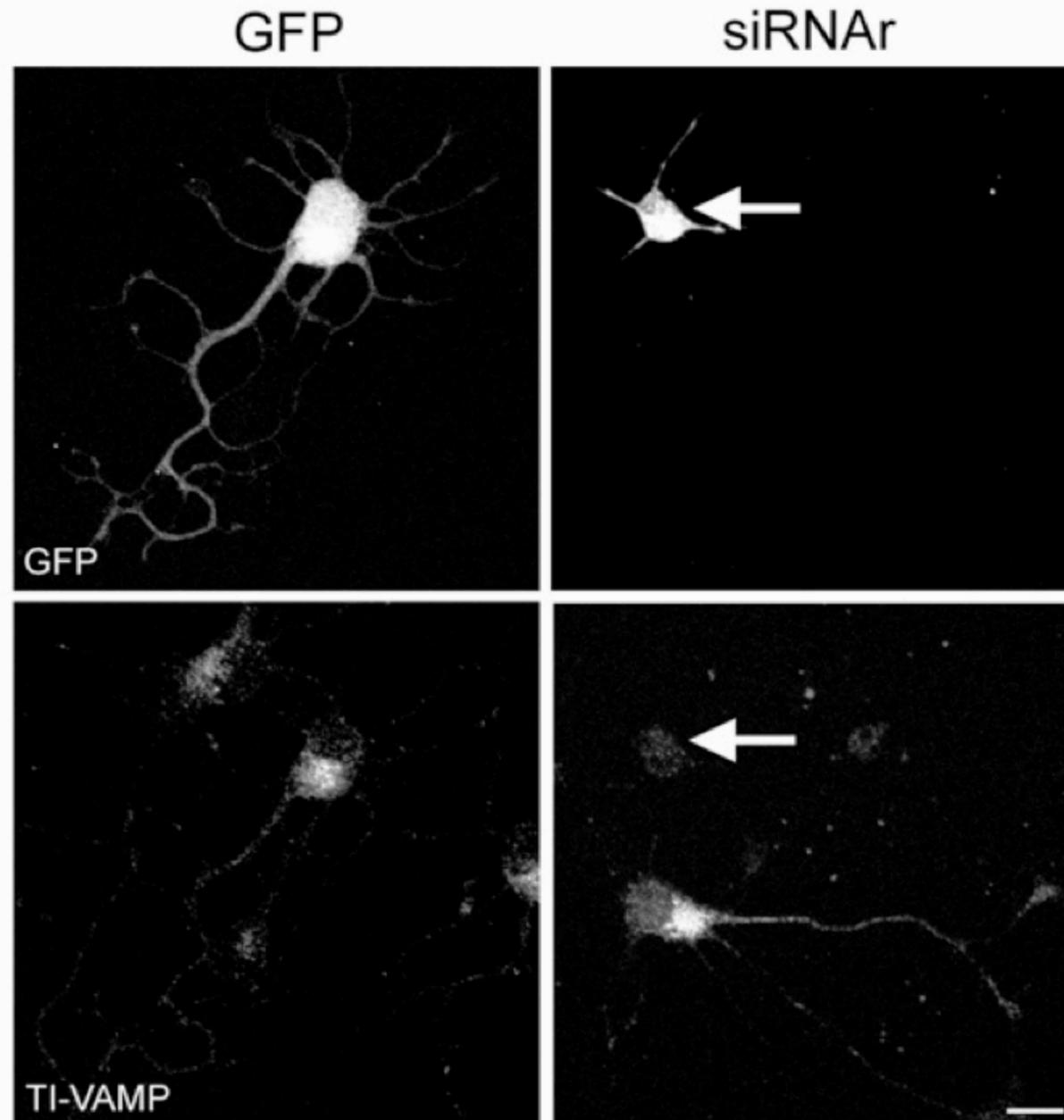
Courtesy of Andrea Burgo

TI-VAMP dans les cones de croissance

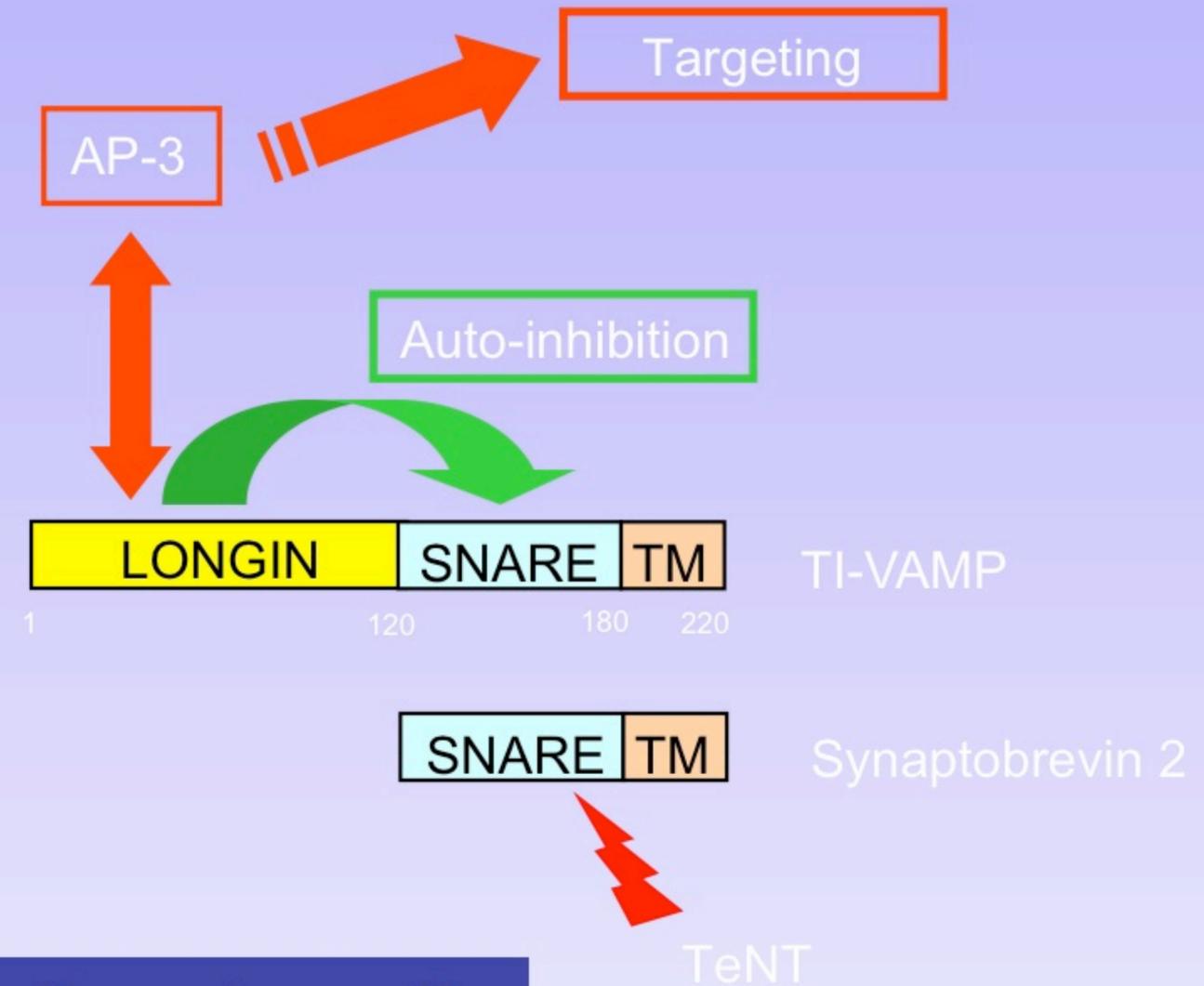
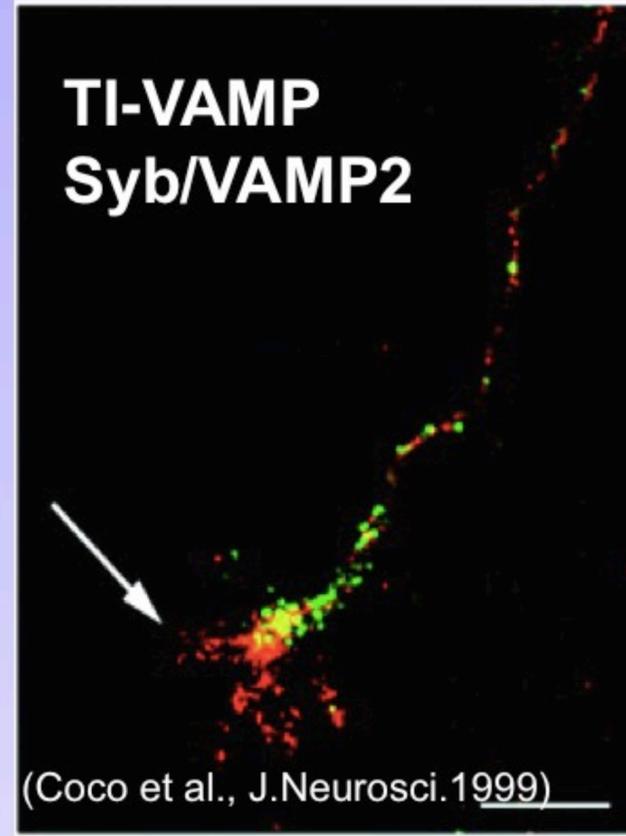
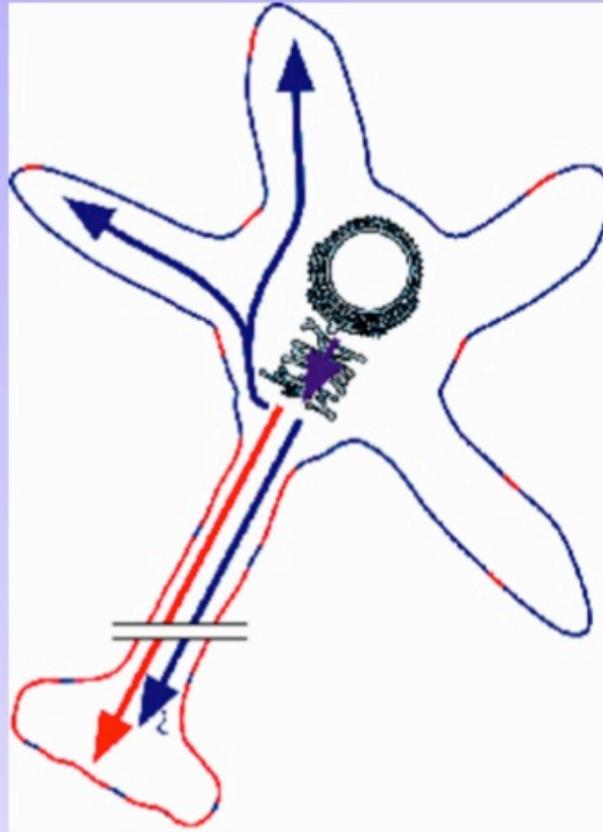


Les marqueurs présynaptiques sont enrichis dans les préparations de cones de croissance (GCP).

TI-VAMP est essentiel à la croissance neuritique

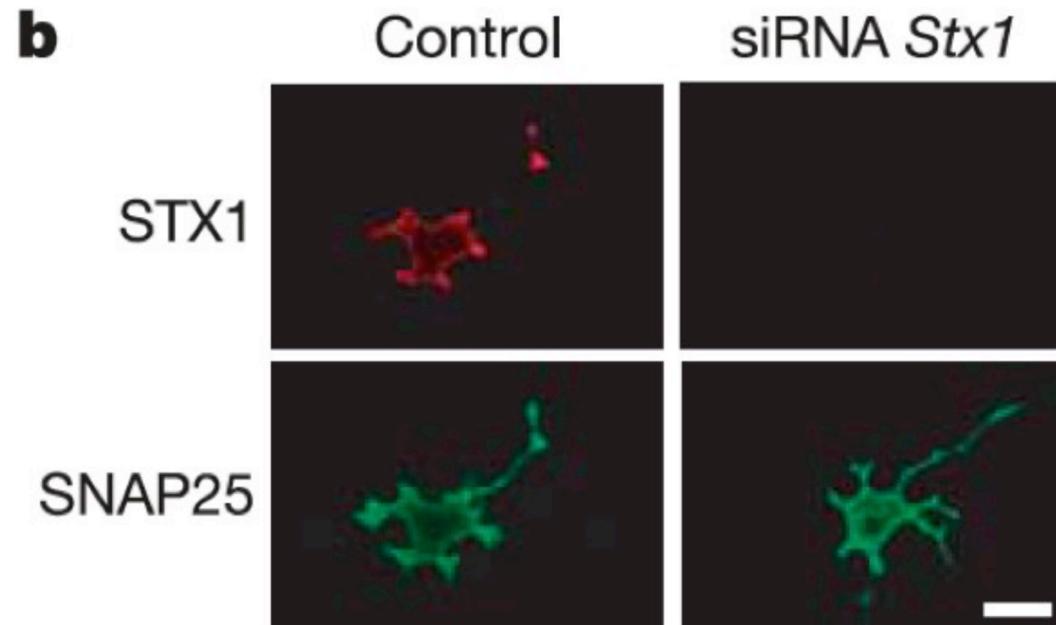


TI-VAMP: v-SNARE mediating neurite outgrowth

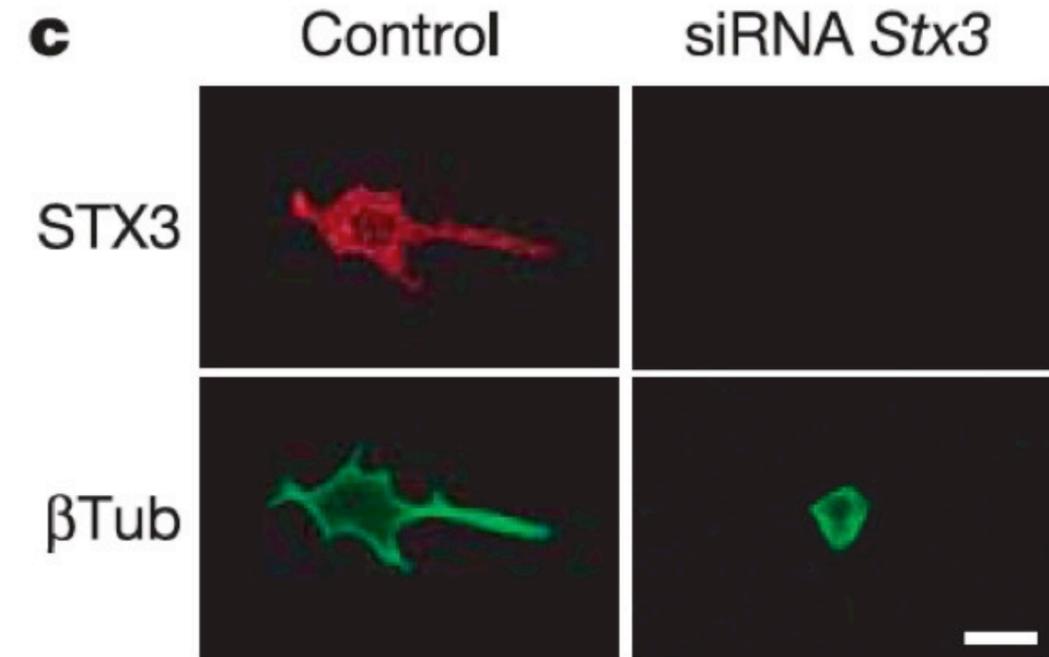


	Neurite outgrowth
Δ Longin-TIVAMP	↗
Longin-TIVAMP	↘
ARNi	↘

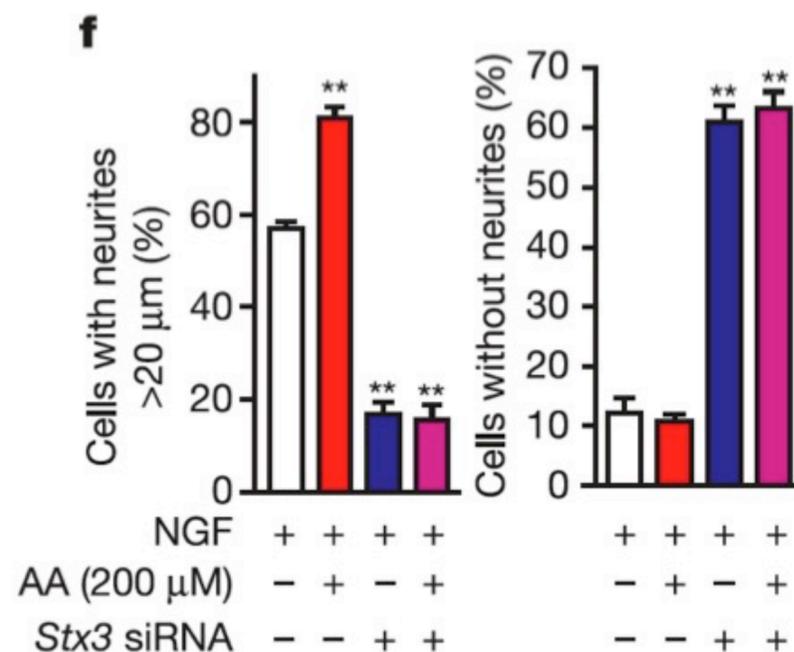
Implication de la syntaxine 3 dans la croissance neuritique



La depletion en *Stx1* n'interfère pas avec la croissance neuritique.

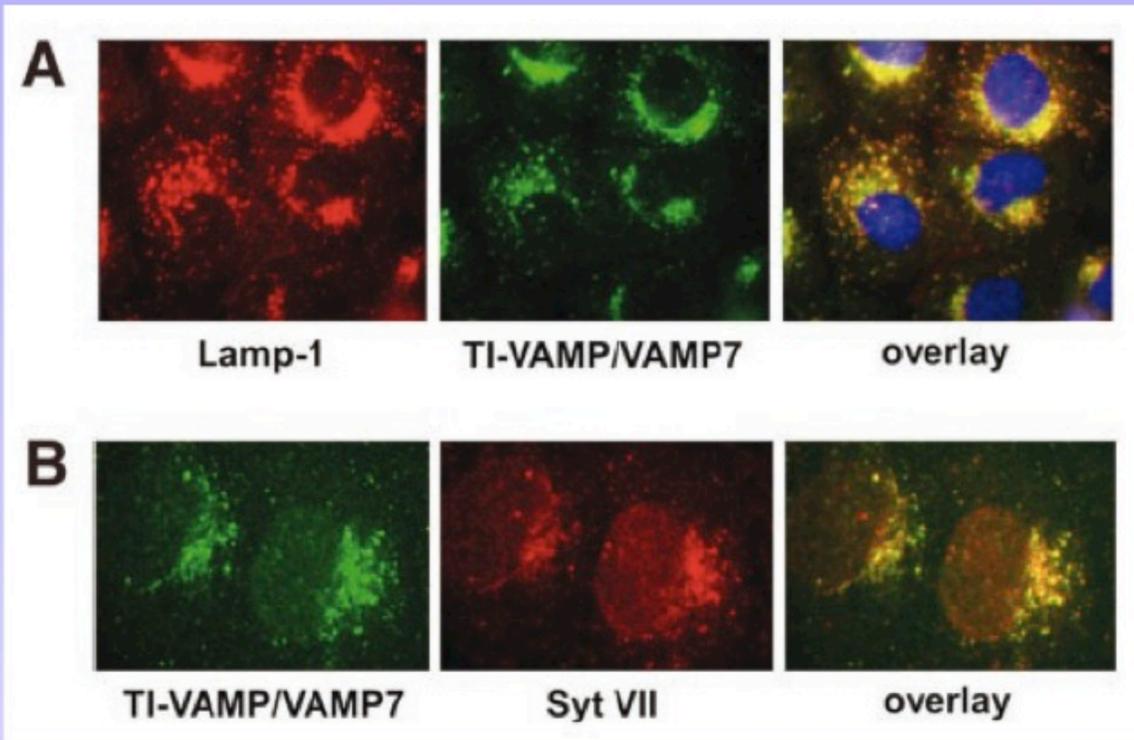


La depletion en **Stx3 bloque** la croissance neuritique.

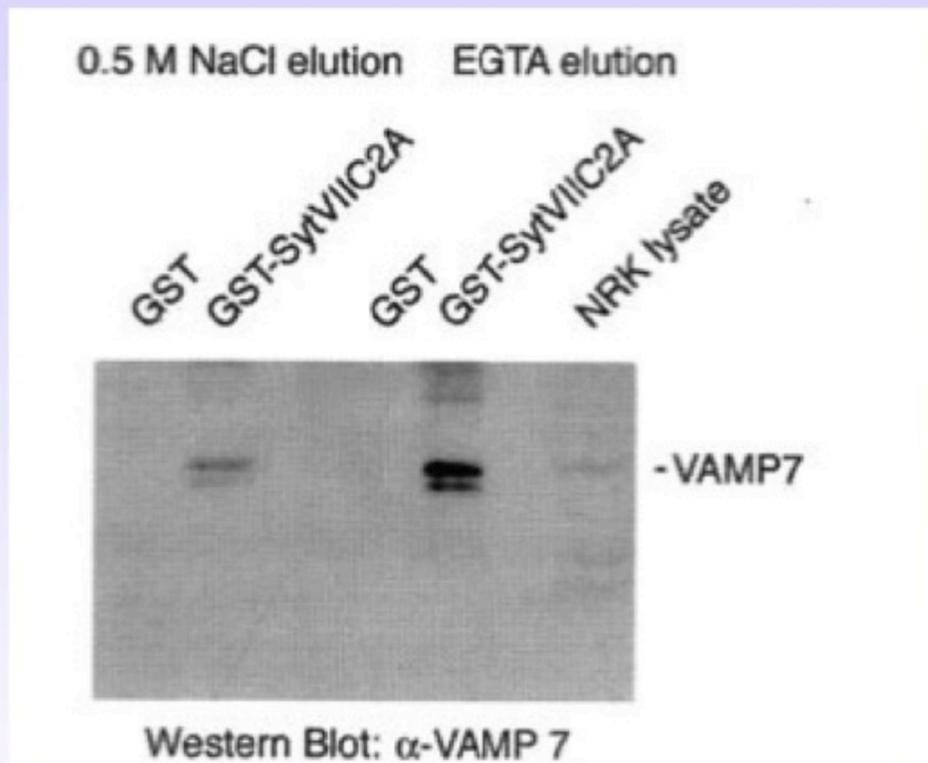


La depletion en **Stx3 bloque** augmente la proportion de cellules PC12 sans neurites.

La croissance neuritique est diminuée dans les explants de ganglions spinal de souris *SytVII* ^{-/-}

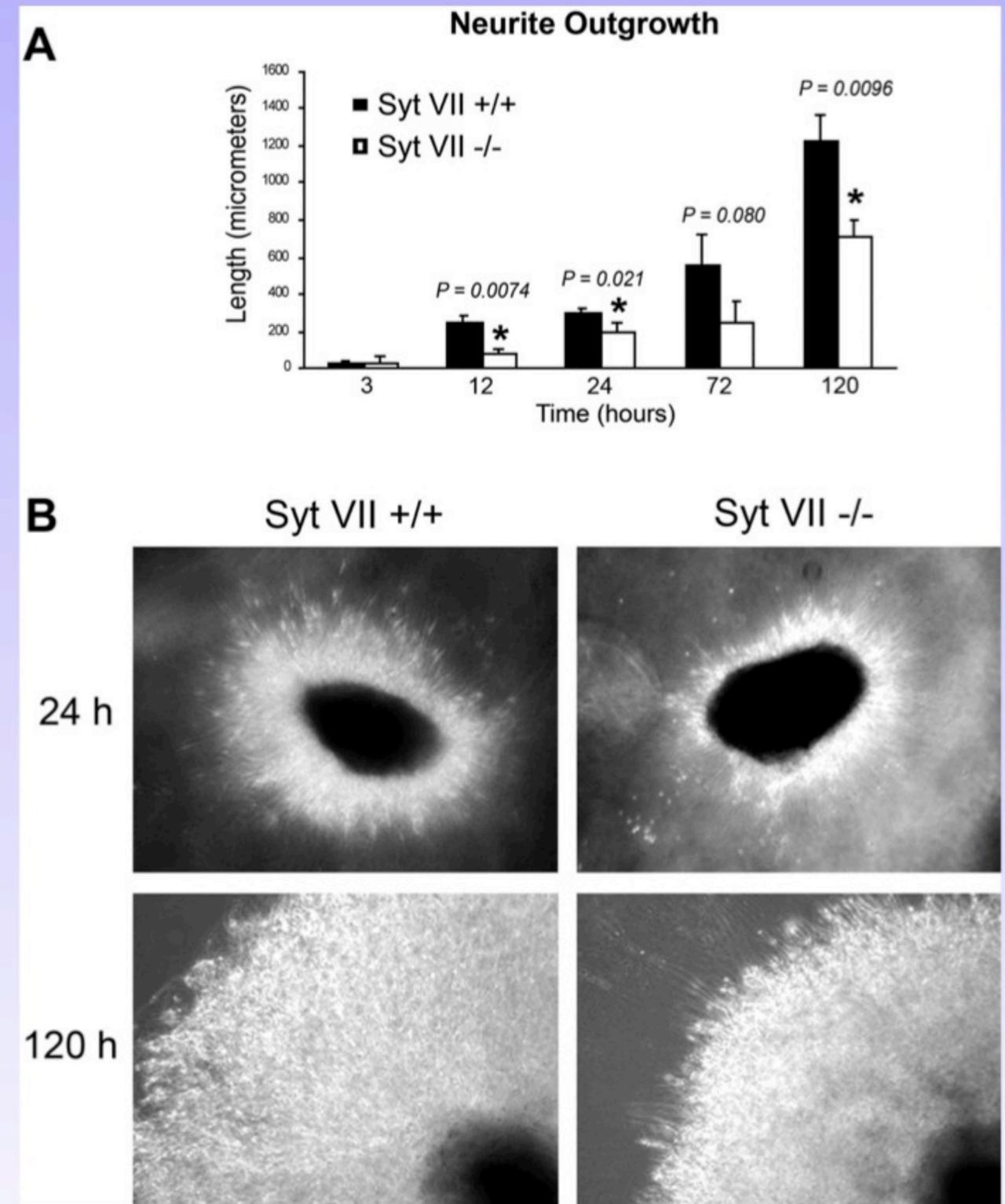


TI-VAMP colocalise avec SytVII dans les endosomes.



TI-VAMP interagit avec SytVII de manière Ca^{2+} dépendante.

Rao & al JBC 2004

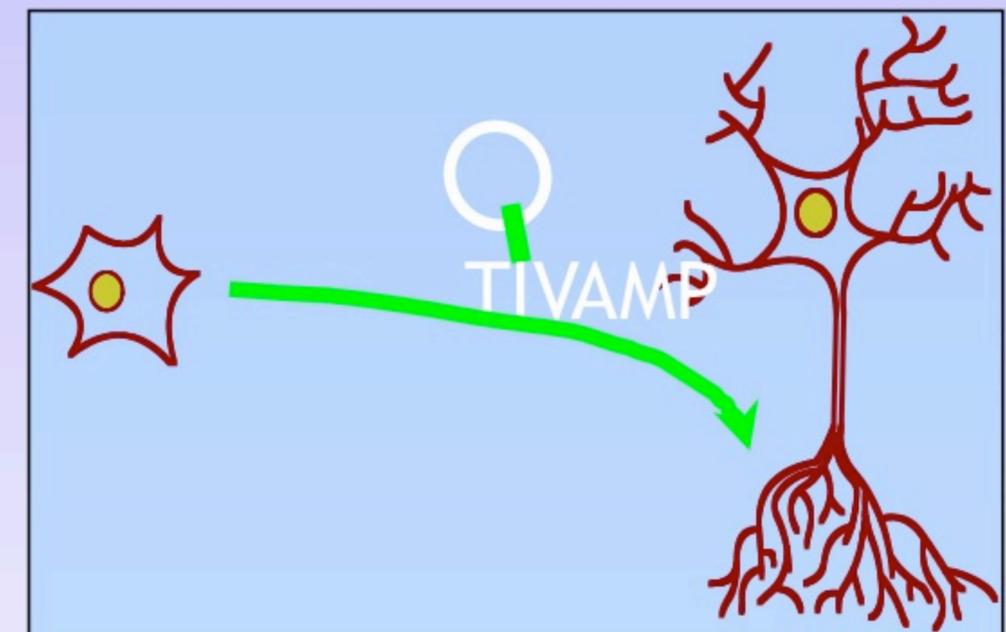
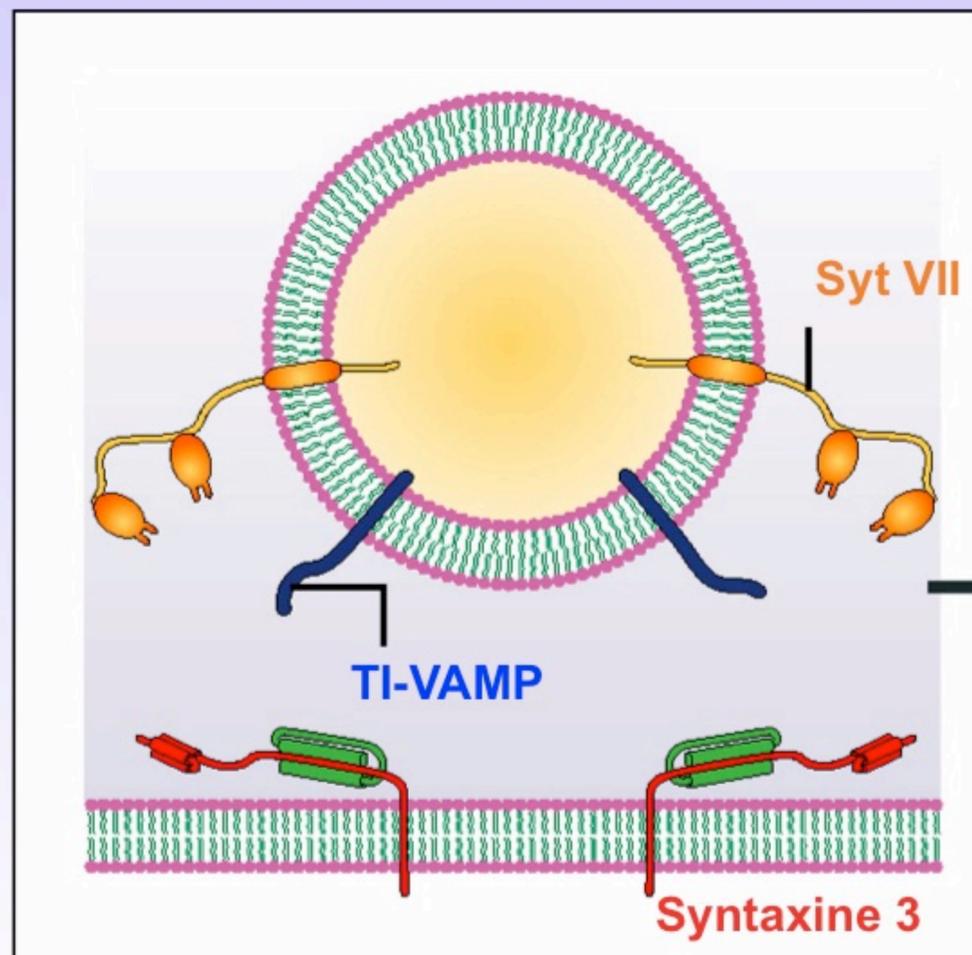


Arantes & al J. Neurosci. 2006

Role de l'exocytose dans la croissance neuritique

Mécanisme moléculaire impliquant:

- TIVAMP come v-SNARE
- Syntaxine 3 comme t-SNARE
- Synaptotagmin VII



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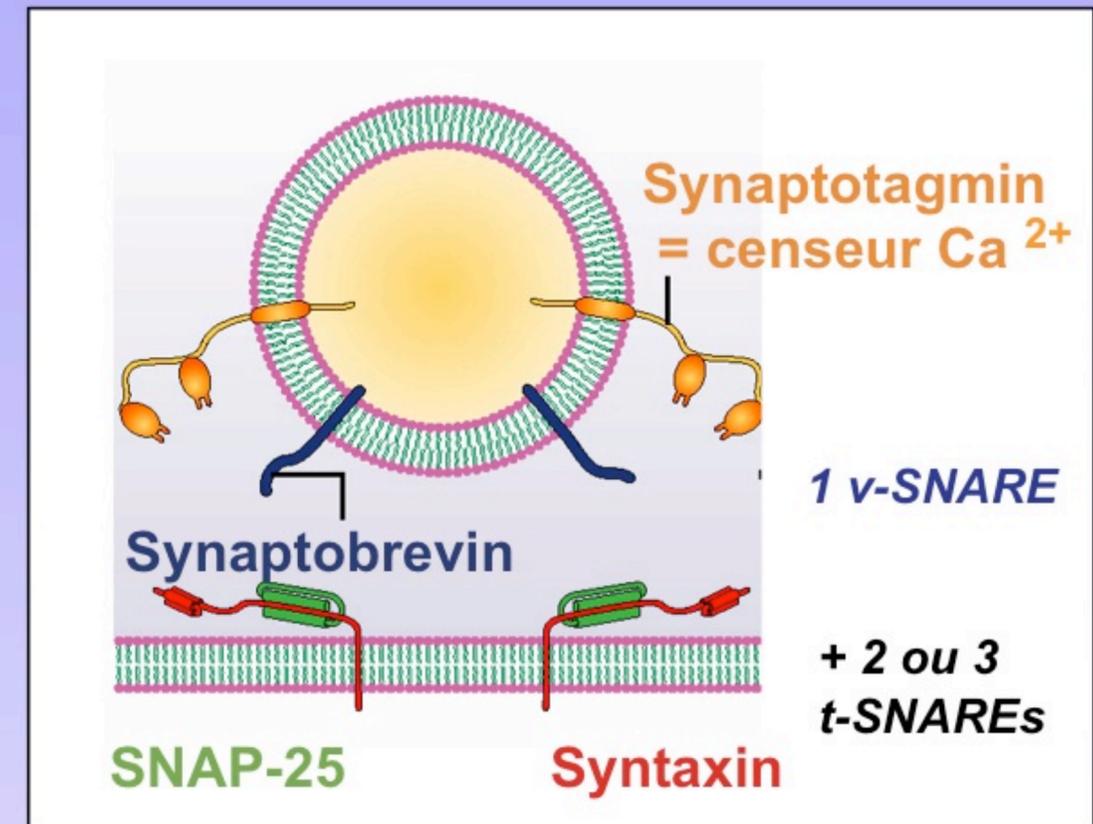
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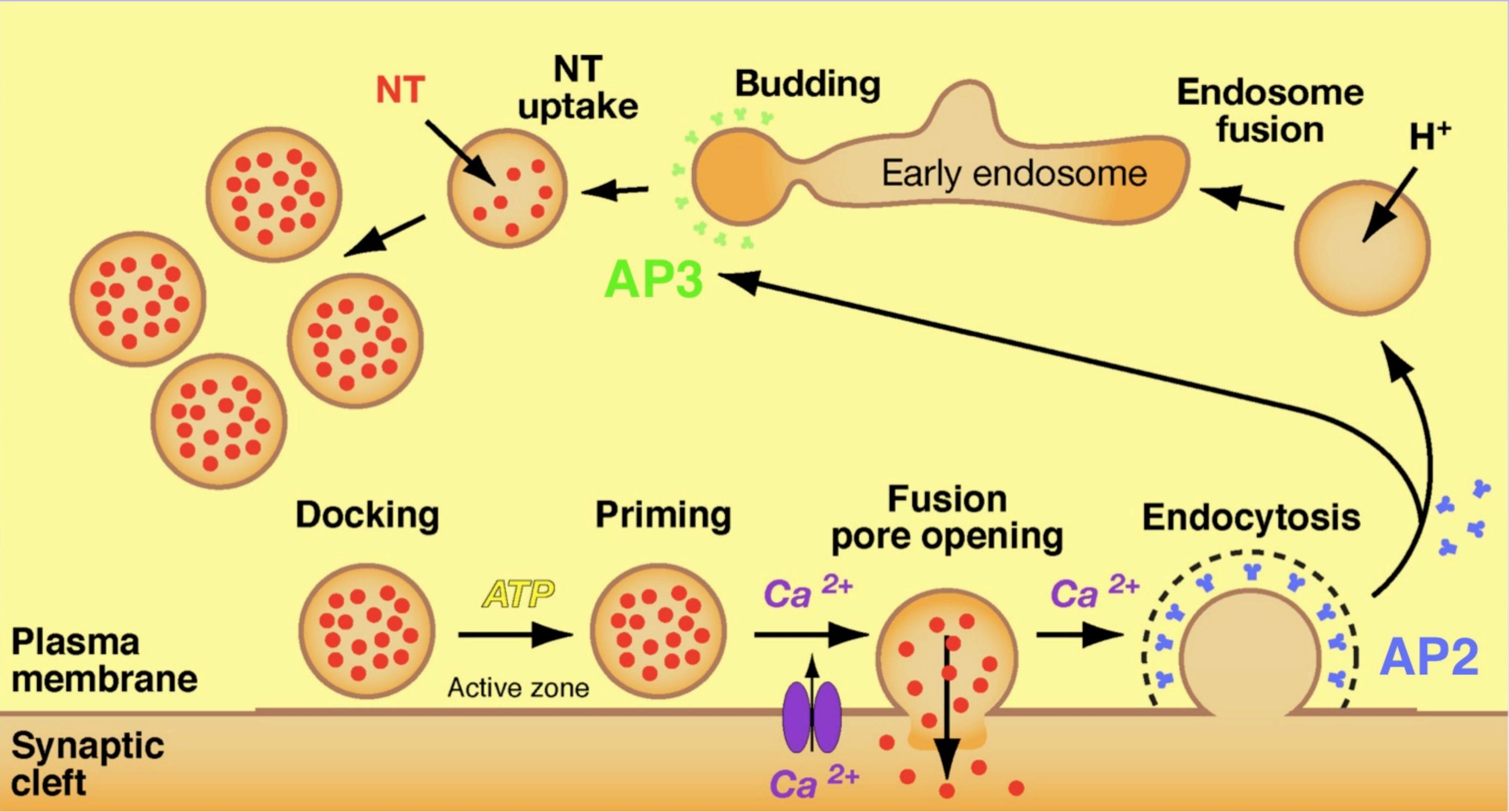
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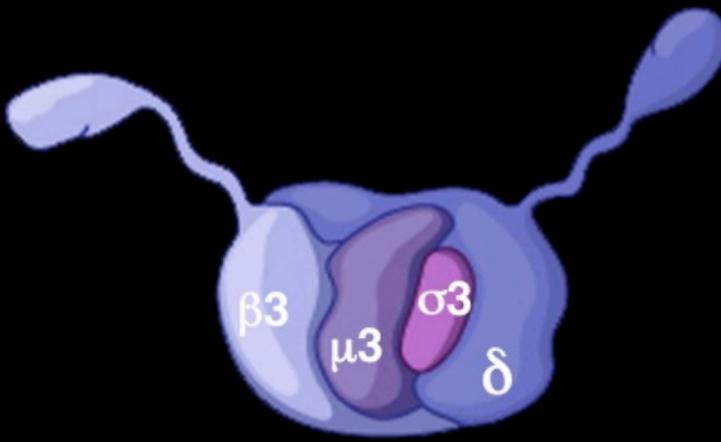
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SV recycling



AP3 est un complexe adaptateur heterotetramérique



Fonctions d' AP3:

Ciblage de certaines protéines vers les:

- melanosomes,
- granules contenant les plaquettes,
- vésicules synaptiques

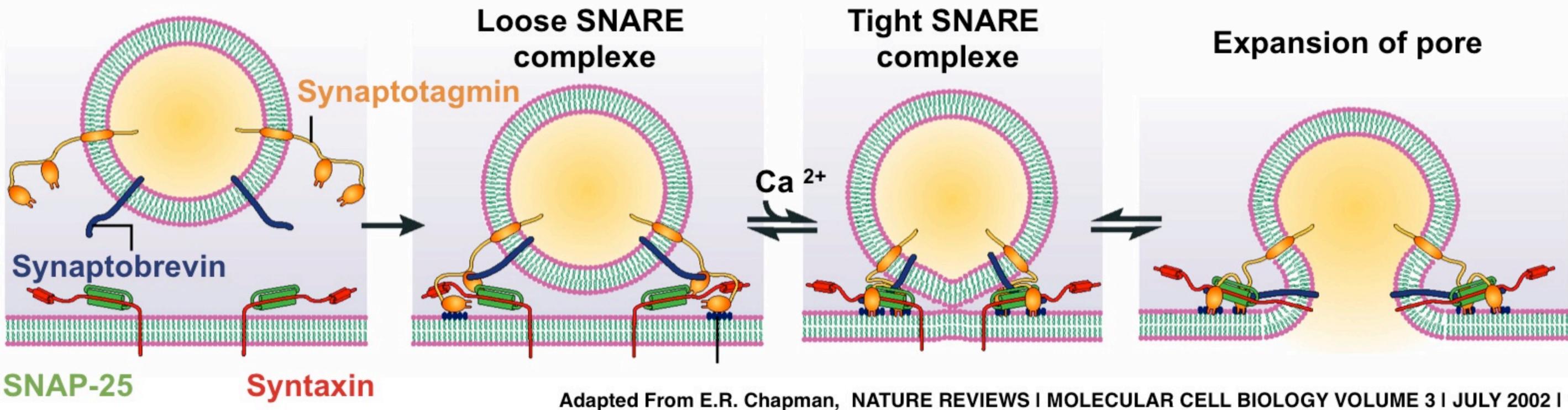
Sont des mutant nuls pour AP3 δ :
suppression du complexe AP3

Les souris MOCHA (mh)

- décoloration des yeux et des poils
- coagulation retardée (déficiency du pool des granules (plaquettes)).
- hyperactives
- rithme theta altéré dans l'electrocortigramme (hypersynchronisation).
- crises épileptiques



SNARE proteins AND Exocytosis



TI-VAMP : Tetanus Neurotoxin-Insensitive Vesicle Associated Membrane Protein

- gène lié au X
- v-SNARE de 25kDa
- ubiquitaire
- Ciblé via AP3
- Insensible aux Neurotoxines
- Presente dans les terminaisons des fibres moussues.

Synaptobrevin



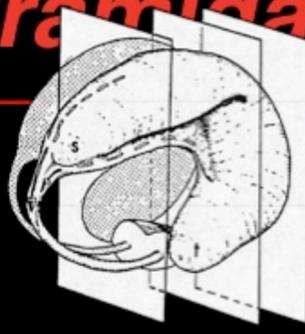
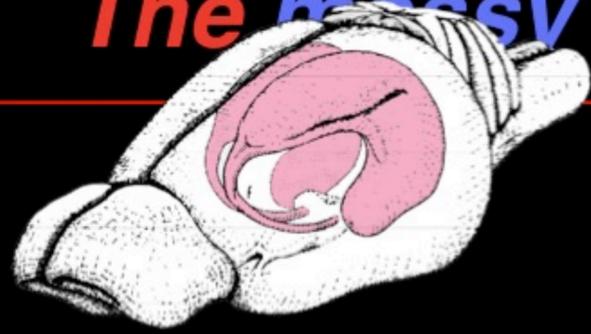
⚡ Tetanus toxin

TI-VAMP

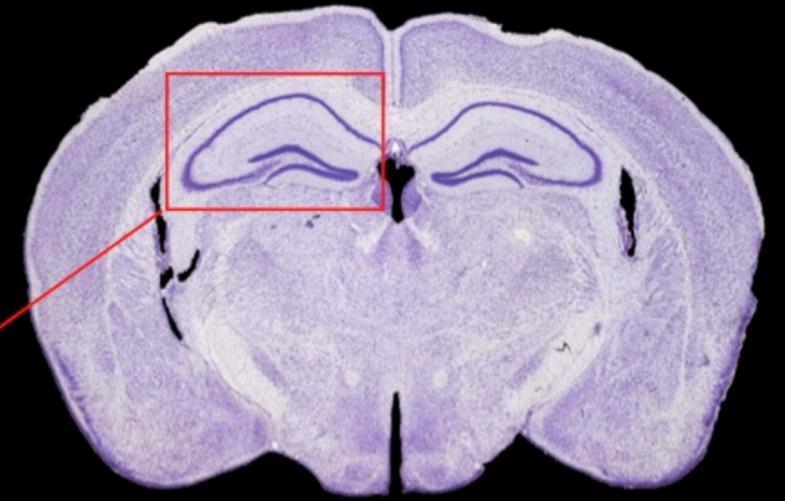


↕
δ AP-3

The messy fiber-pyramidal cell synapse



Transversal
slice



hippocampus

CA1

Pyramidal cell

CA3

s.luc

lri

Alveus

so

sr

slm

sm

Fimbria

Mossy fiber

Perforant path
(subiculum
entorhinal cortex)

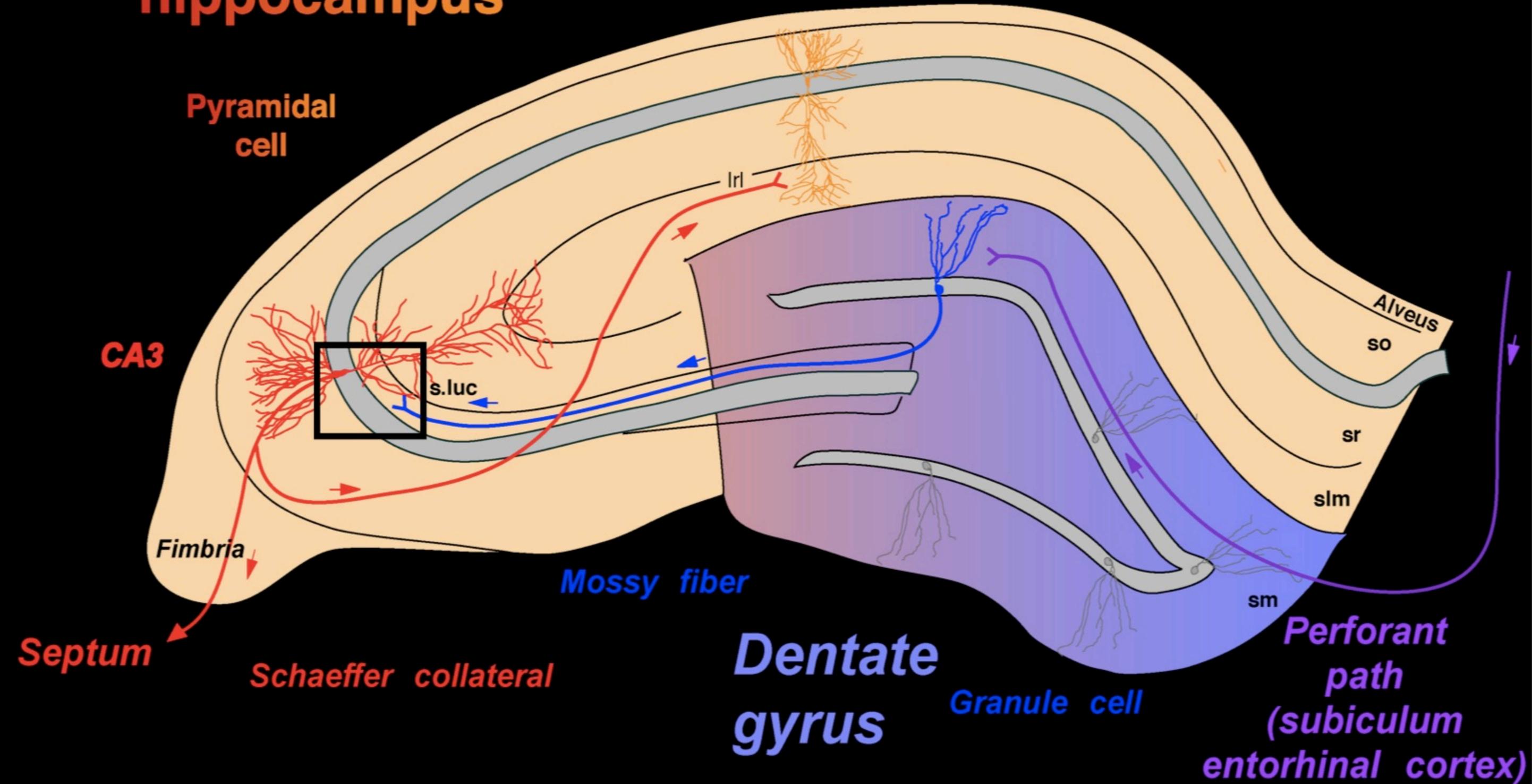
Septum

Schaeffer collateral

Dentate
gyrus

Granule cell

(subiculum
entorhinal cortex)

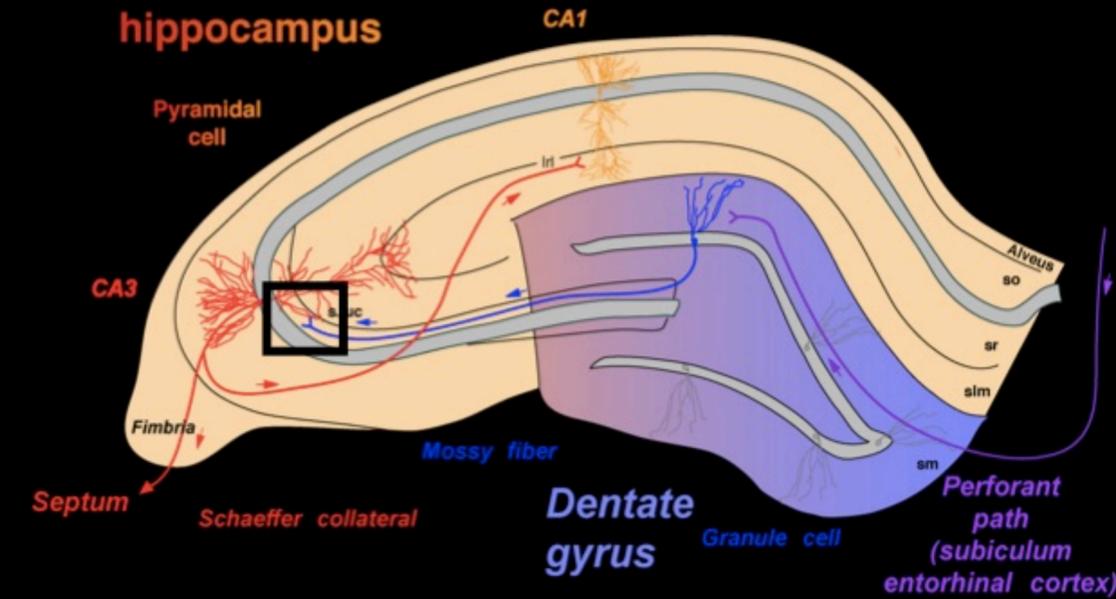
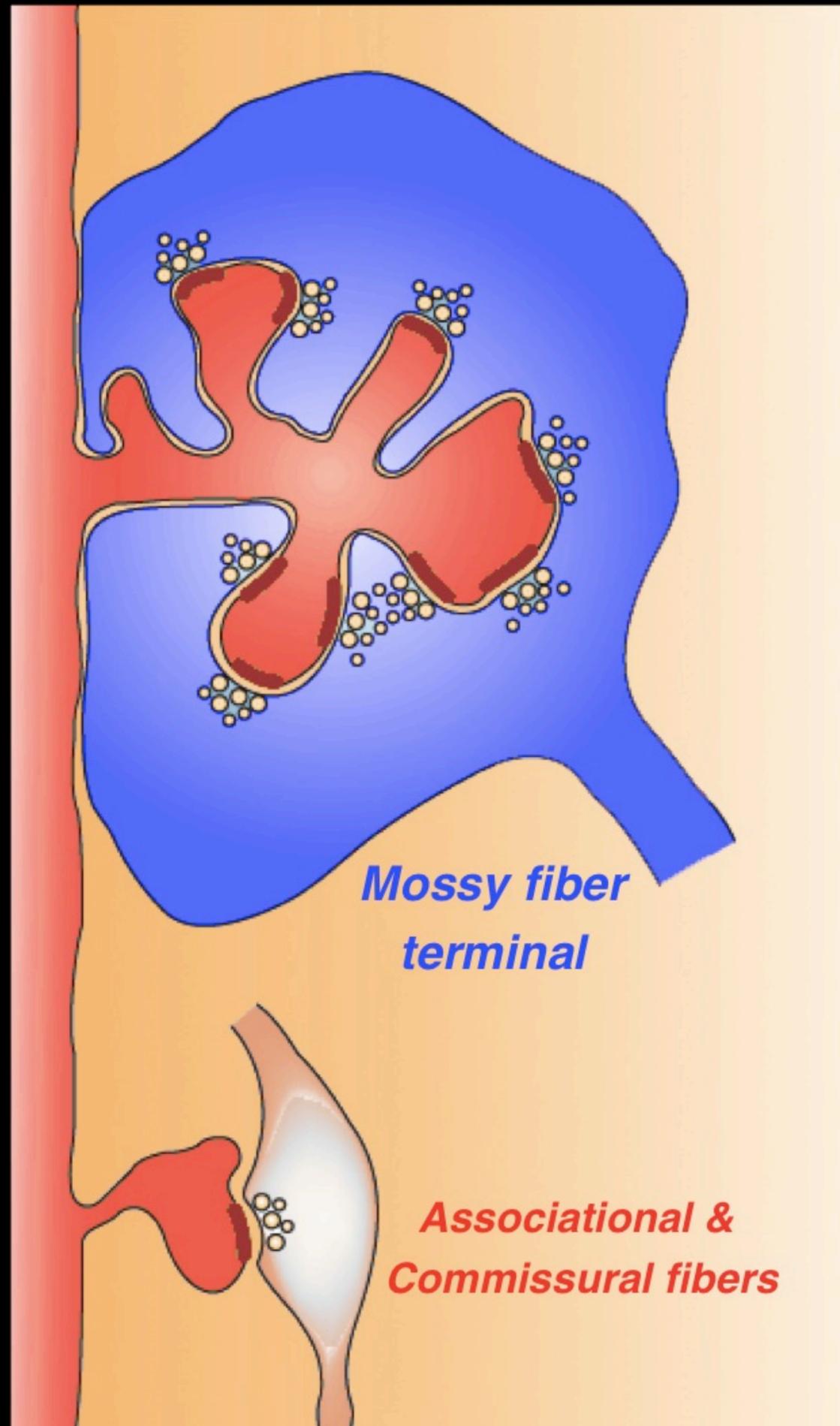


The mossy fiber-pyramidal cell synapse

Thorny excrescence

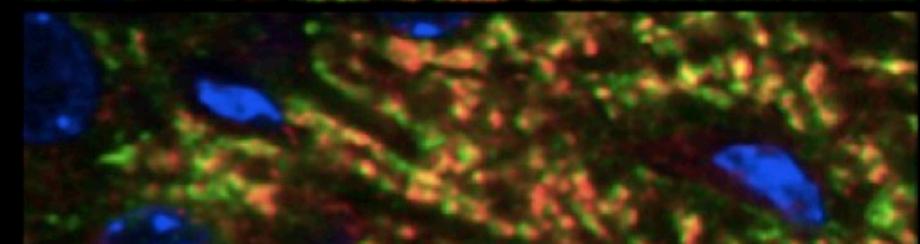
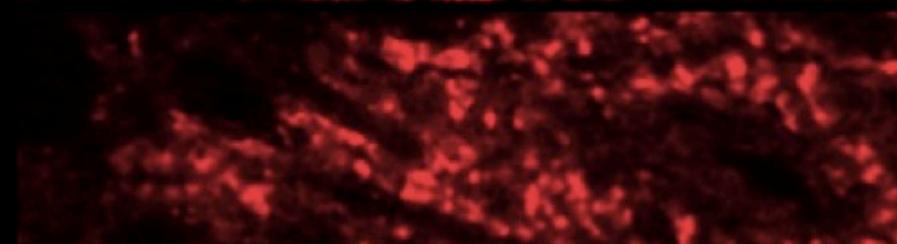
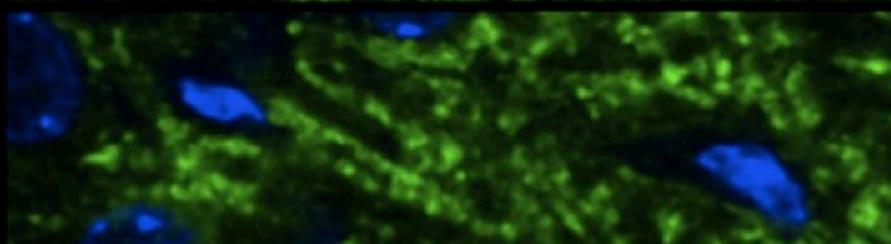
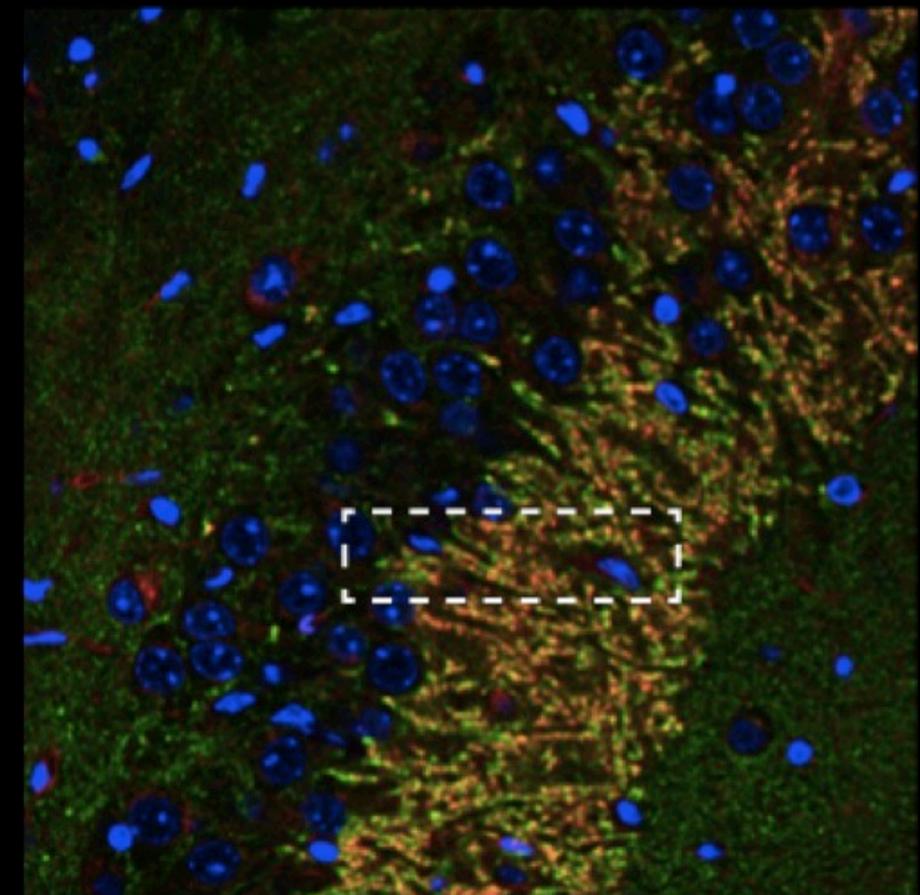
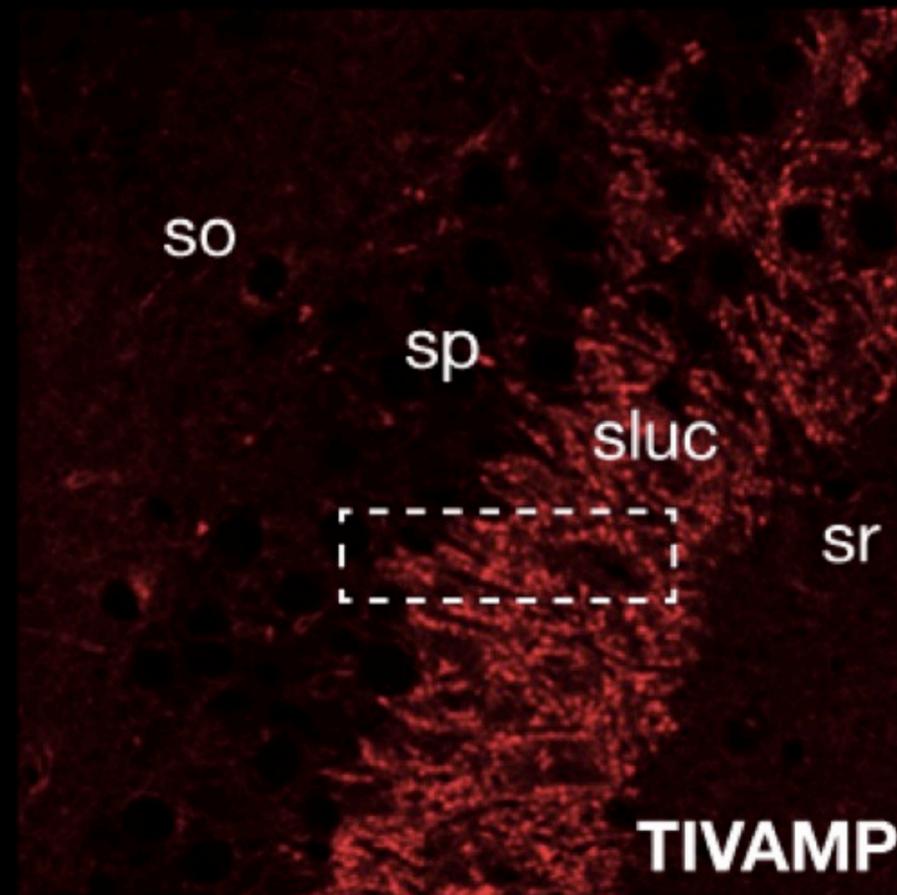
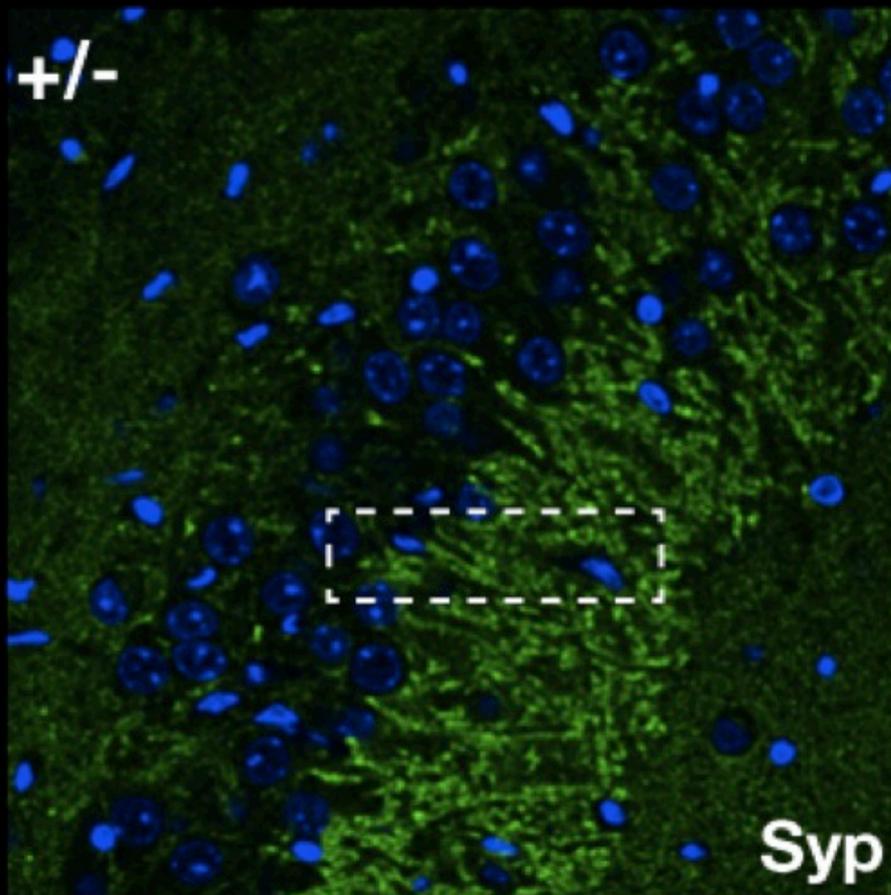
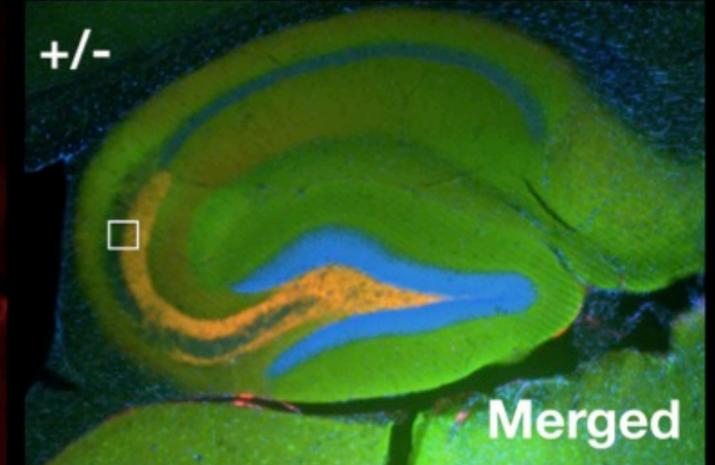
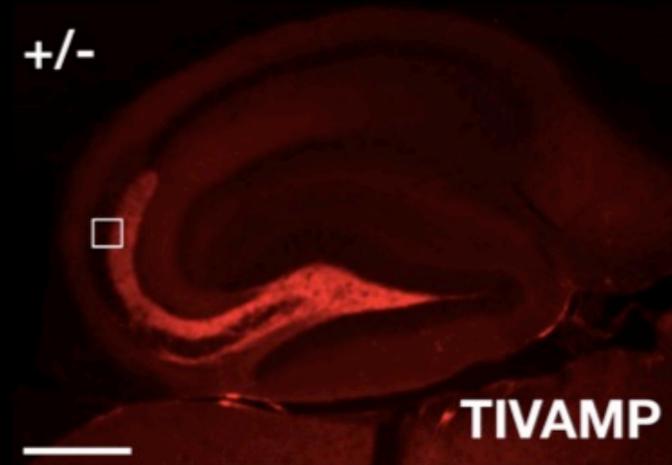
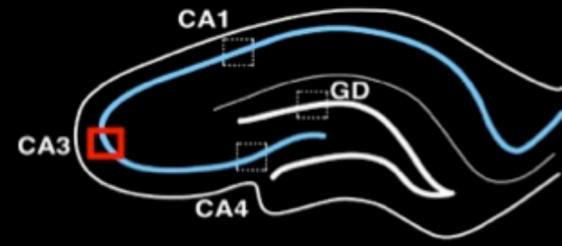
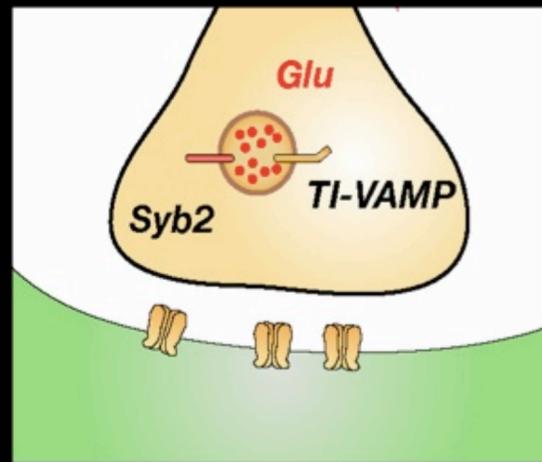
PYRAMIDAL CELL

Classical spine

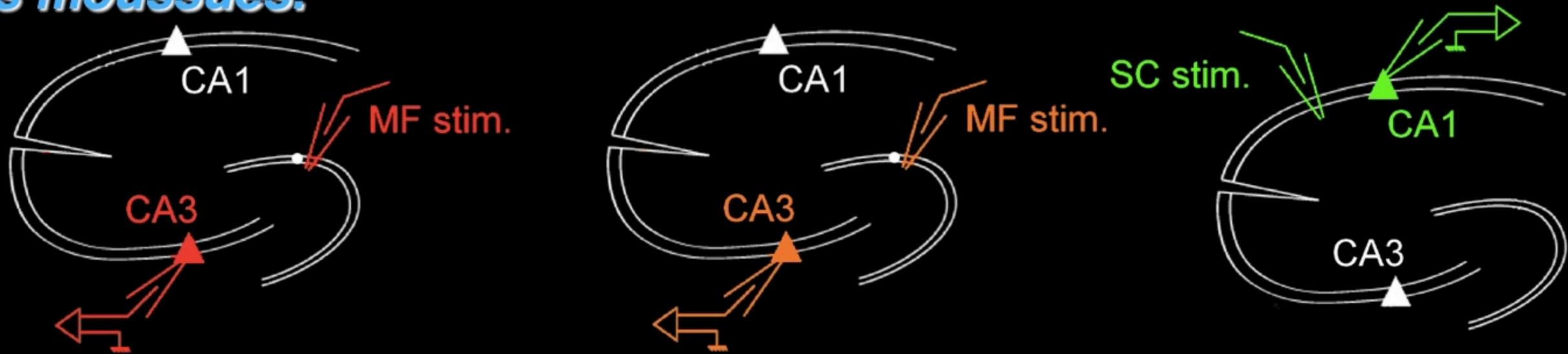


GRANULE CELL

TI-VAMP is expressed in hippocampal mossy fibers (PNAS 2006)



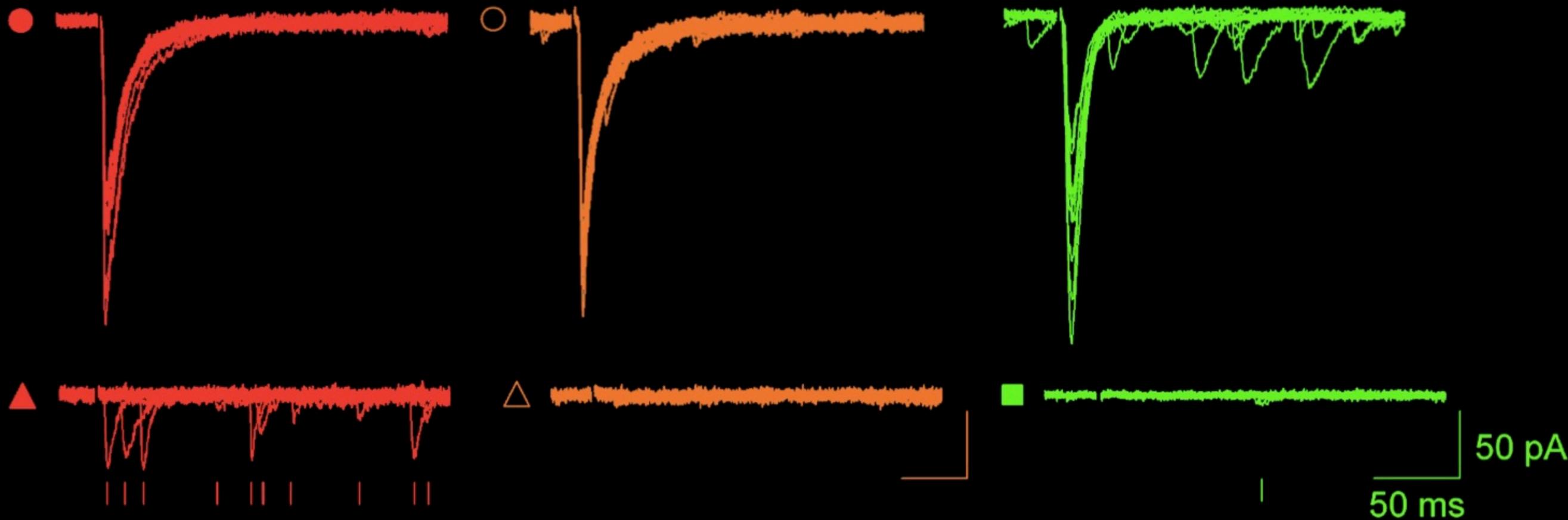
Libération Ca^{2+} -dépendente évoquée par la stimulation des fibres moussues.



Control, CA3

Mocha, CA3

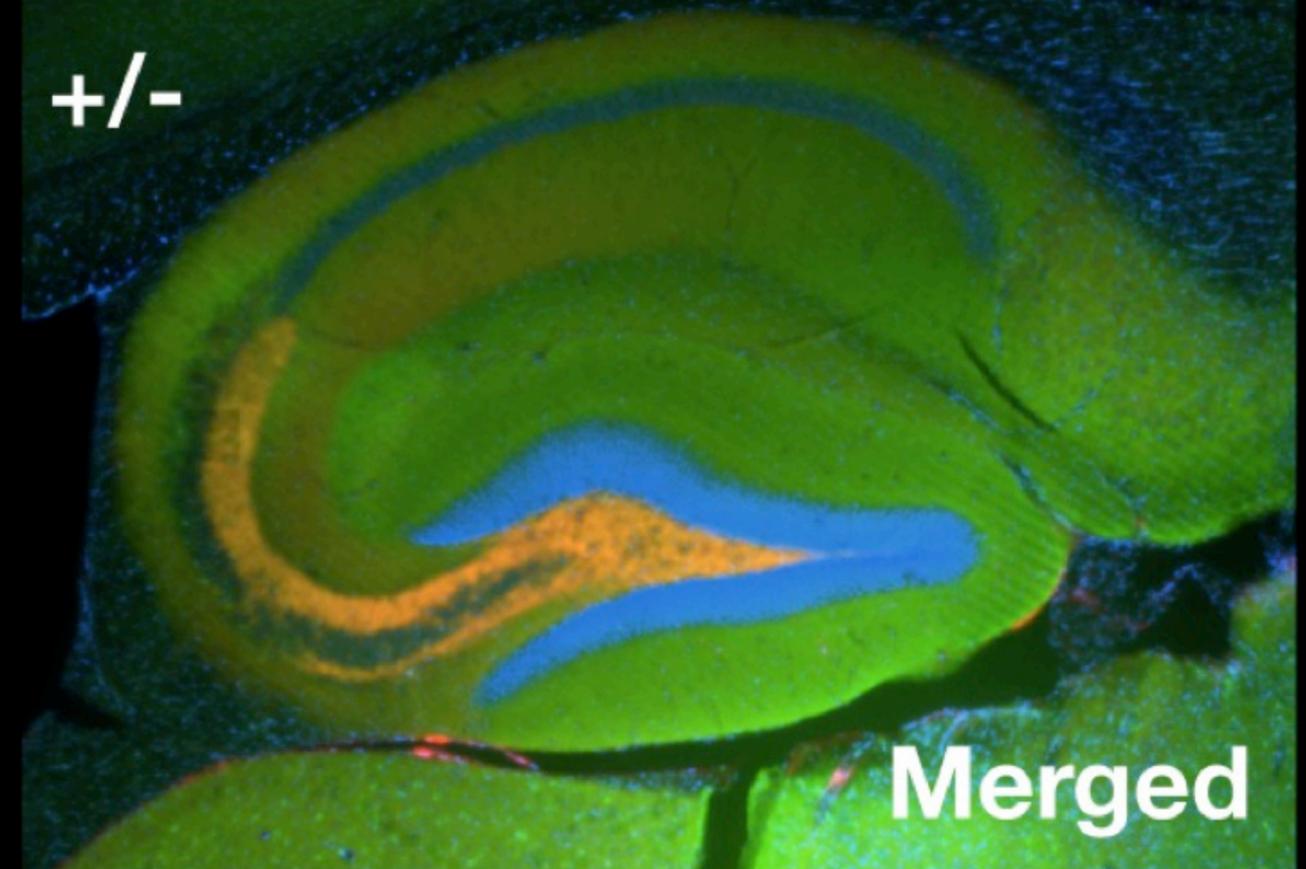
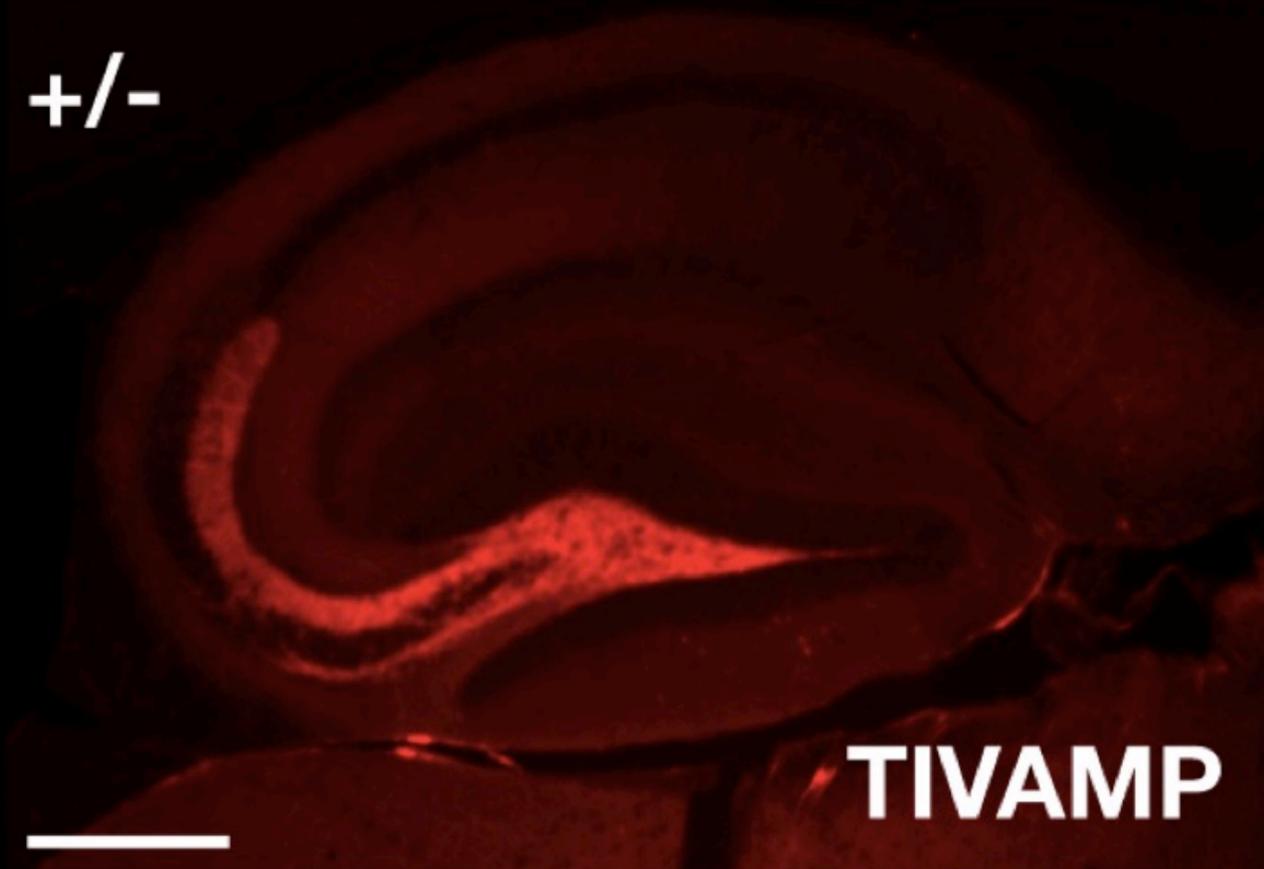
Control, CA1



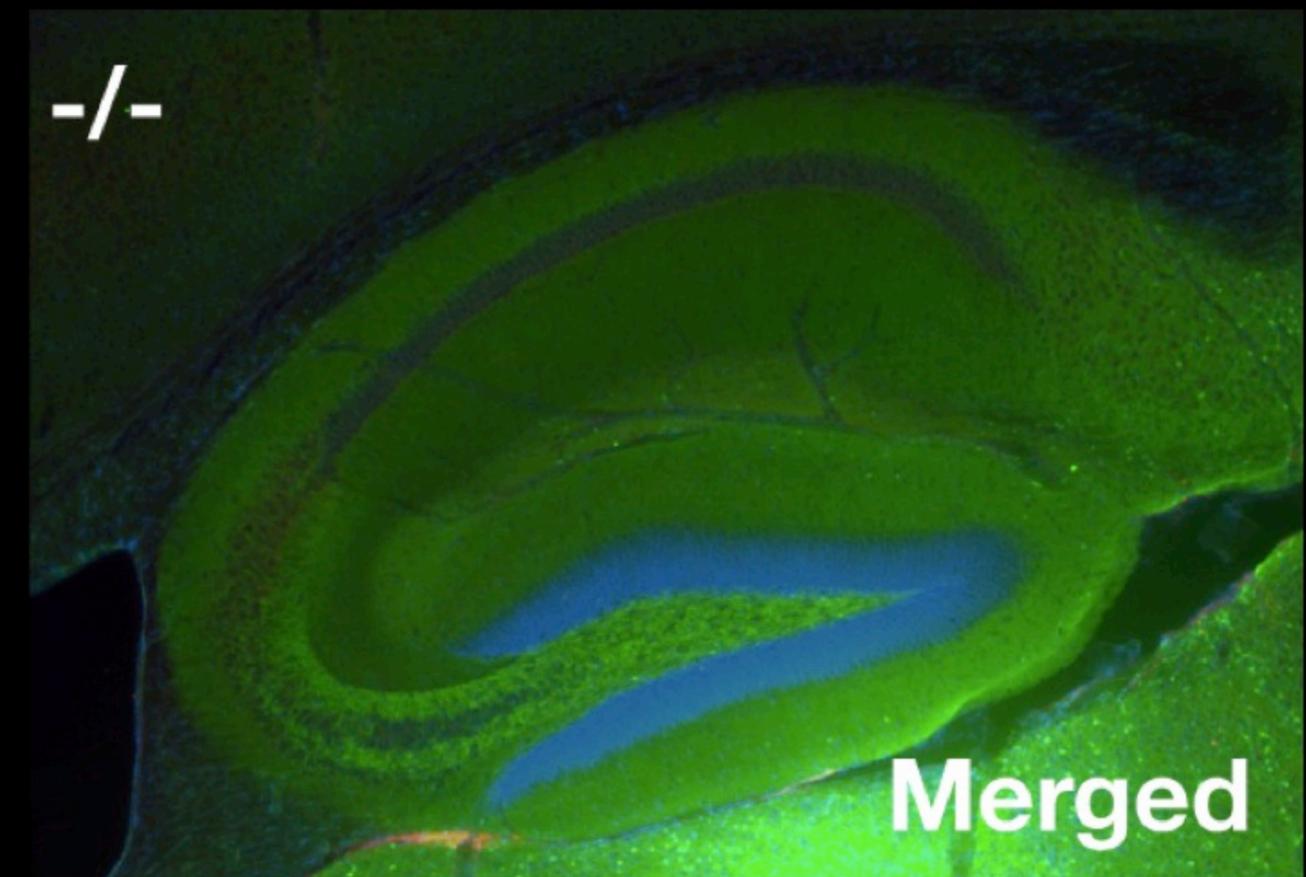
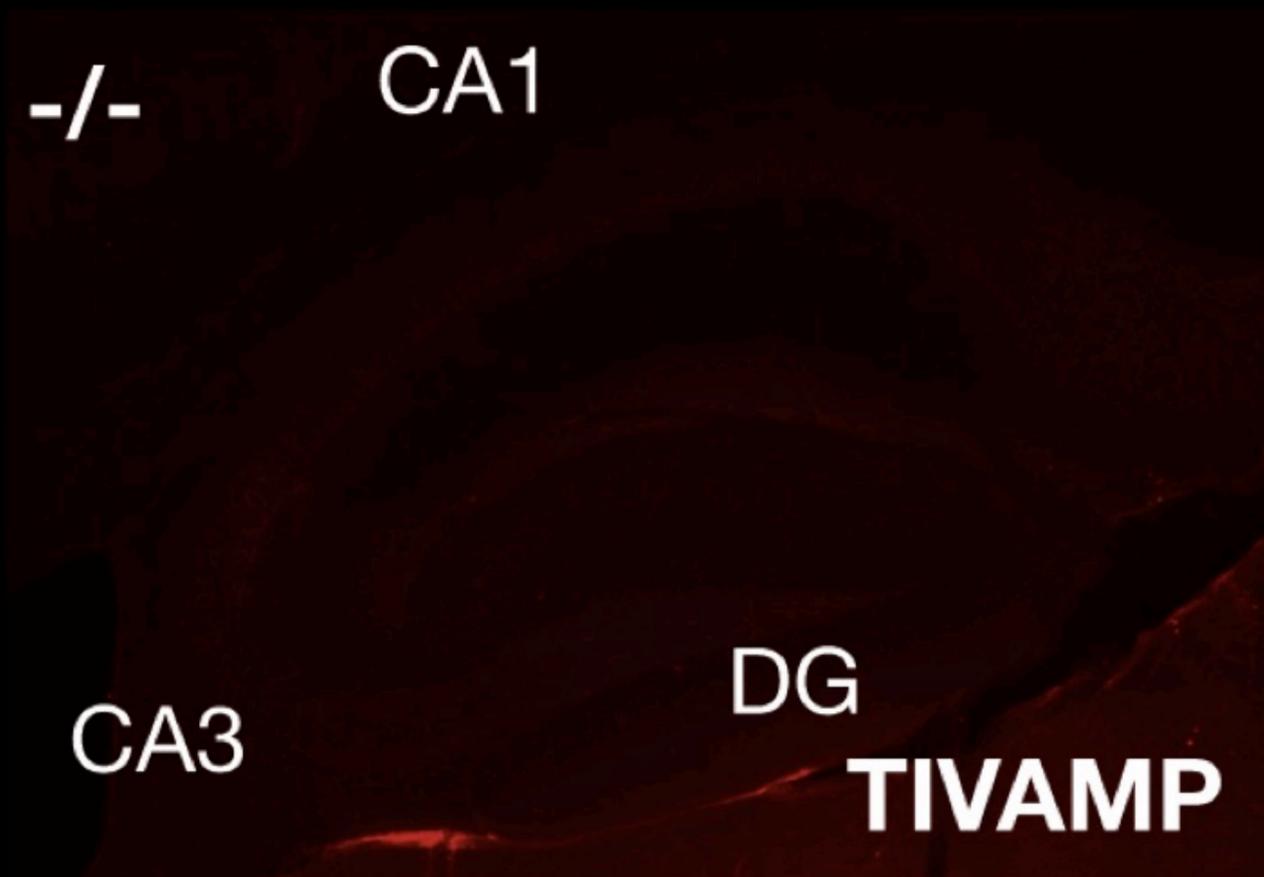
- La TeNT causes une réduction drammatique de la liberation des NT stimulée par PA.
- La libération est asynchrone, dépendante d'AP-3 et spécifique de CA3.

TI-VAMP est dans le Golgi des grains des mocha.

Control mice

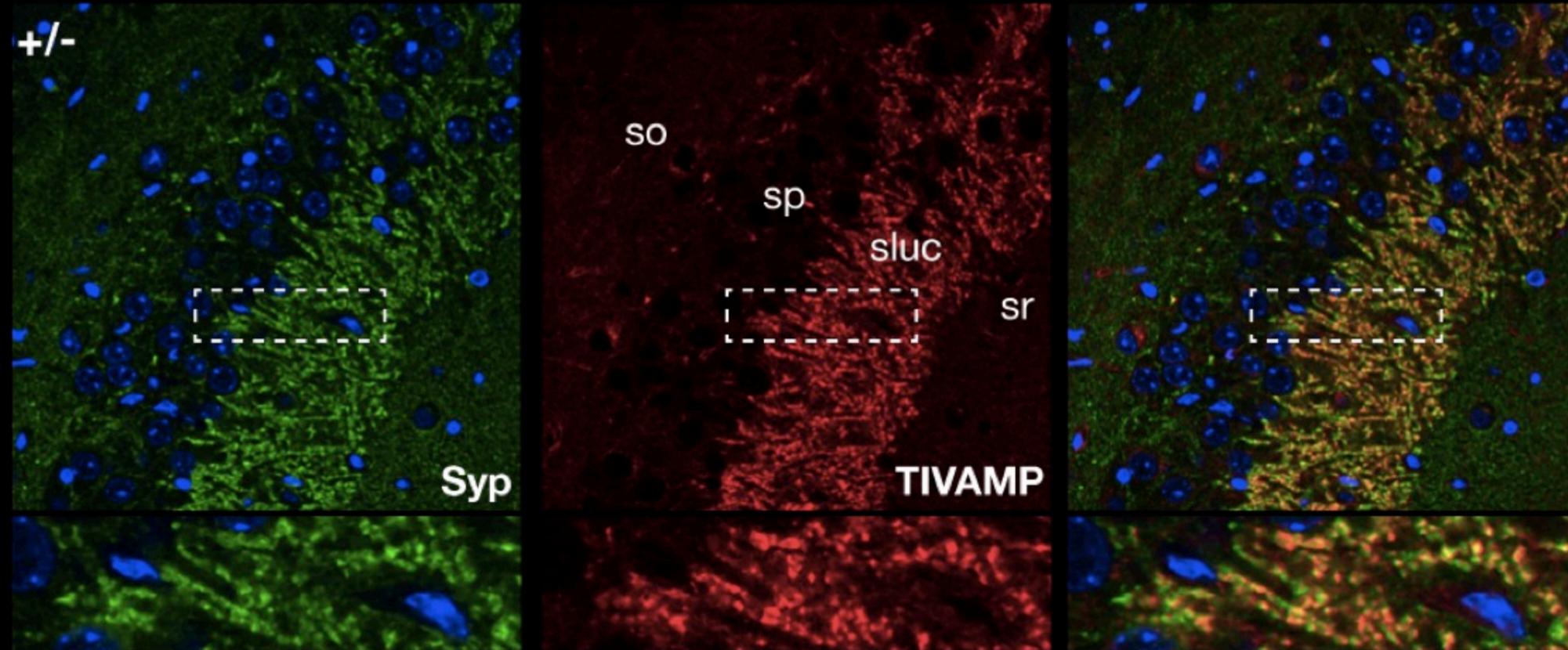
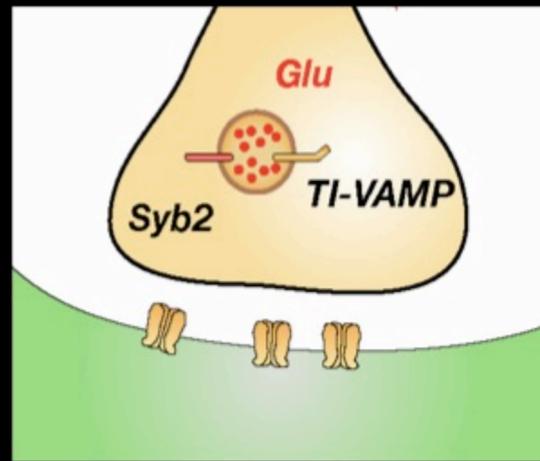
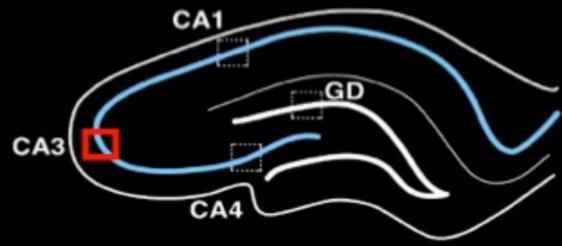


Mocha mice

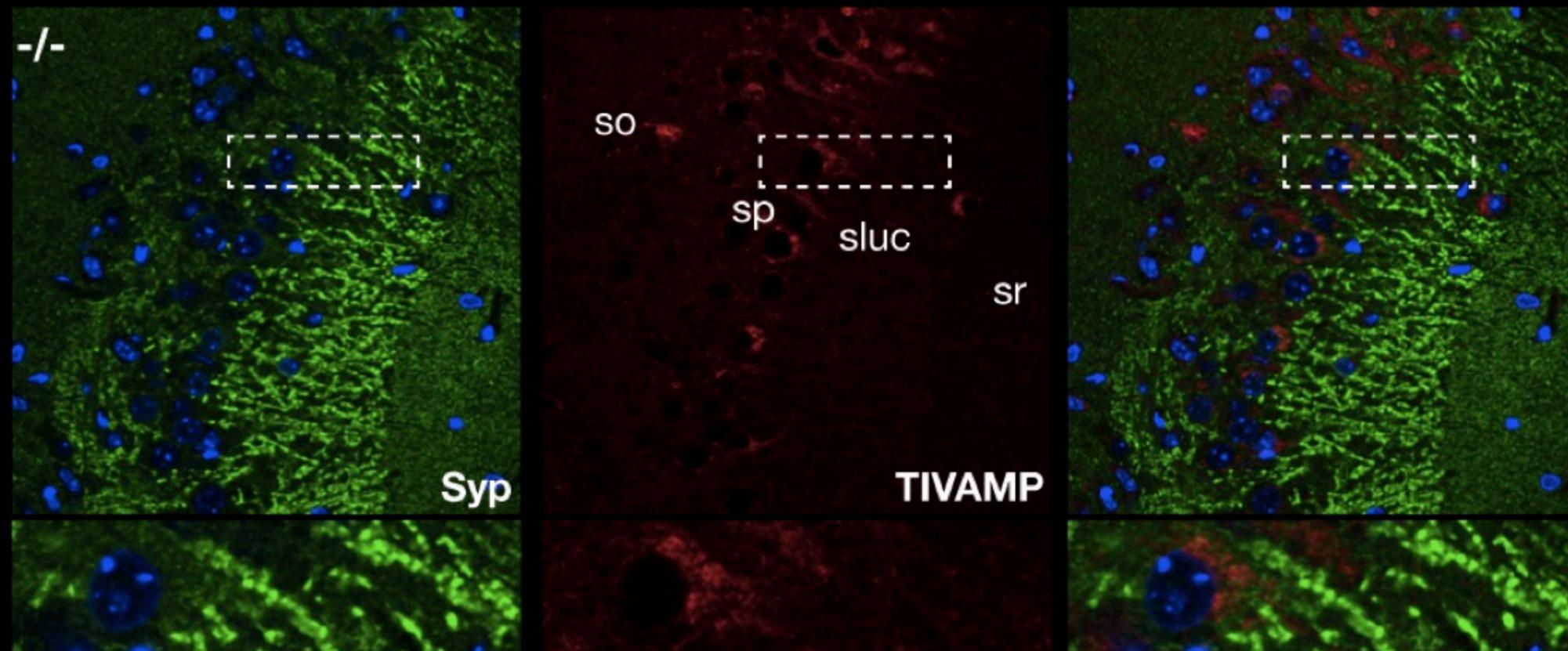
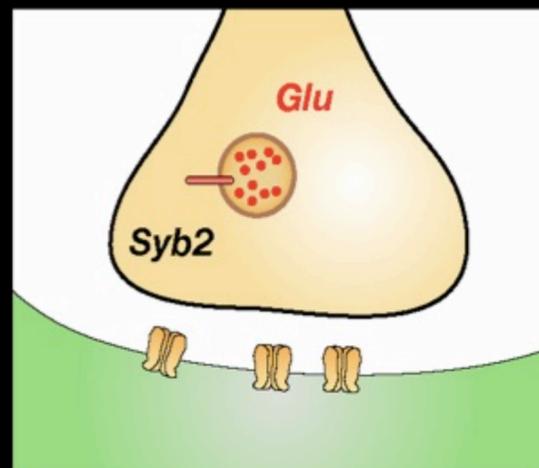


TI-VAMP est absent des terminaisons dans les mocha

Control

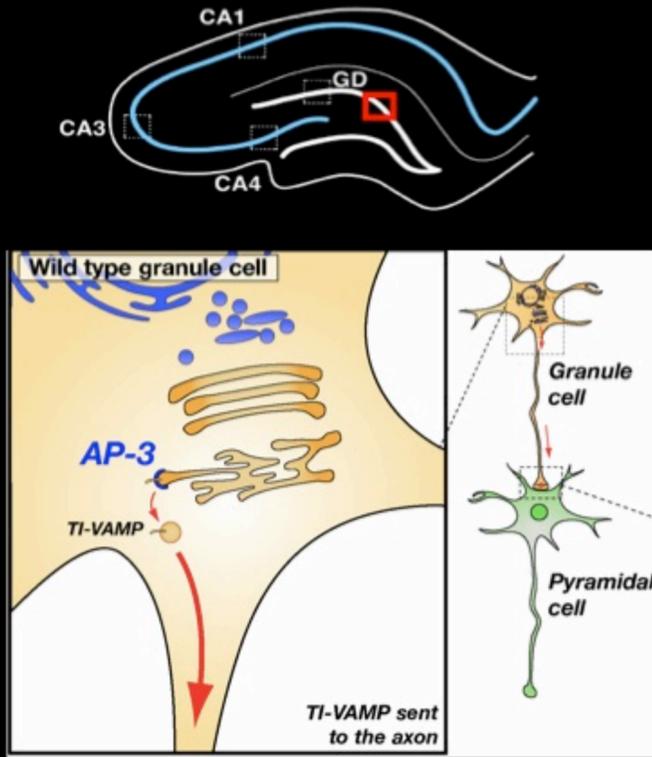


Mocha mice

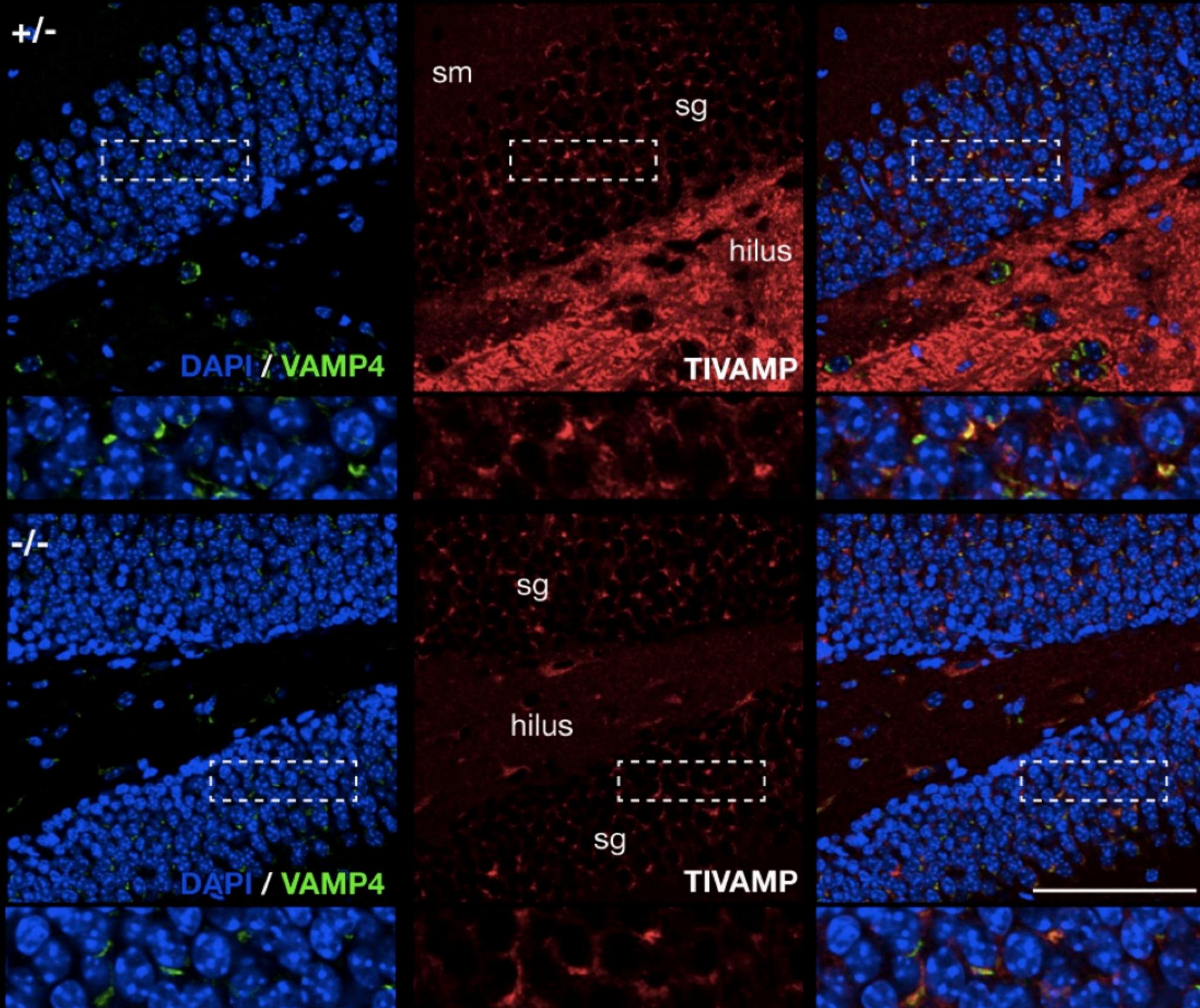
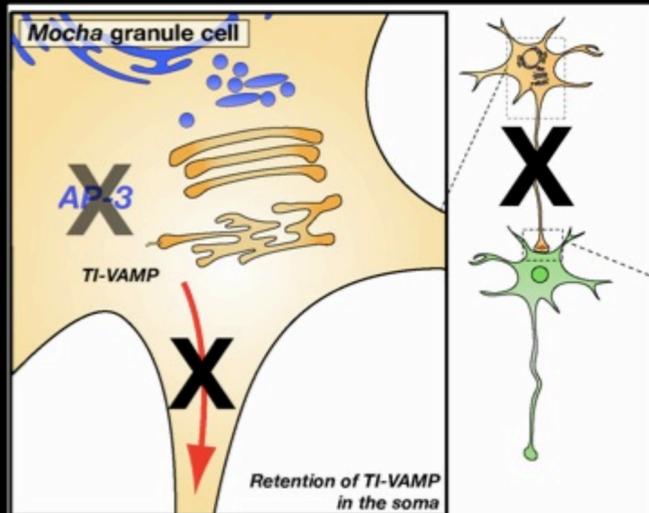


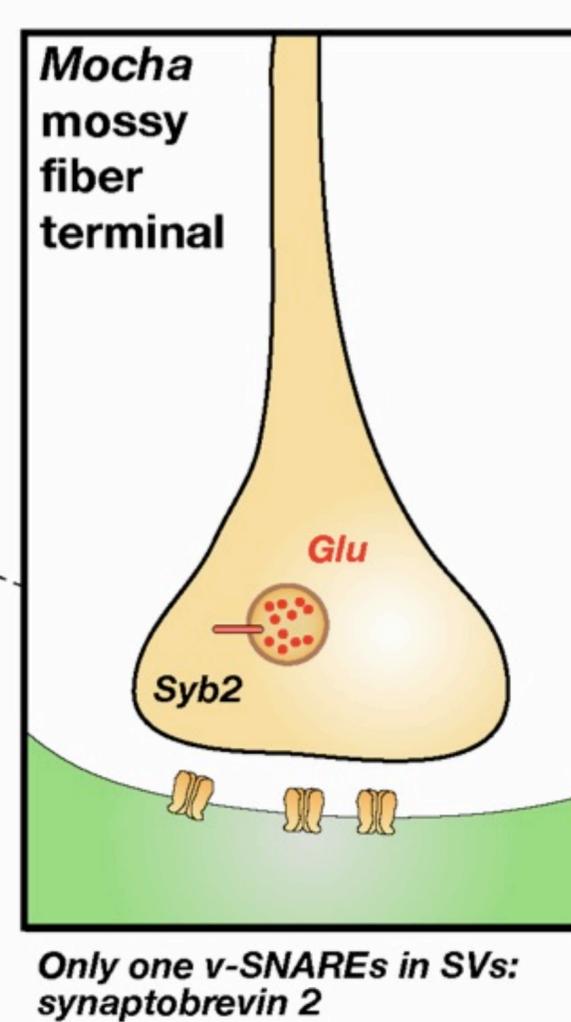
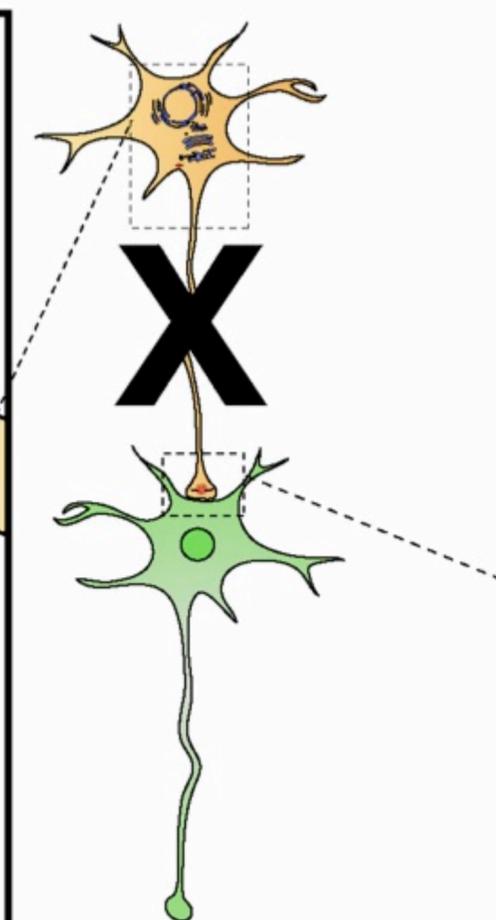
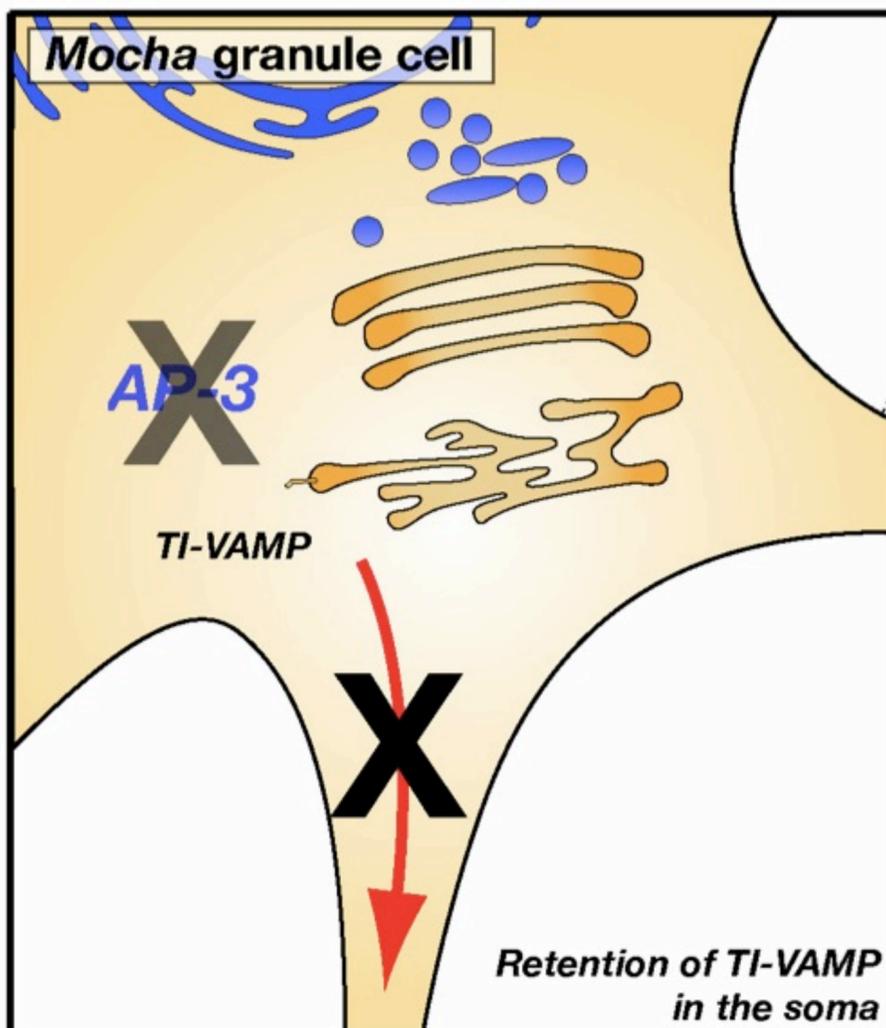
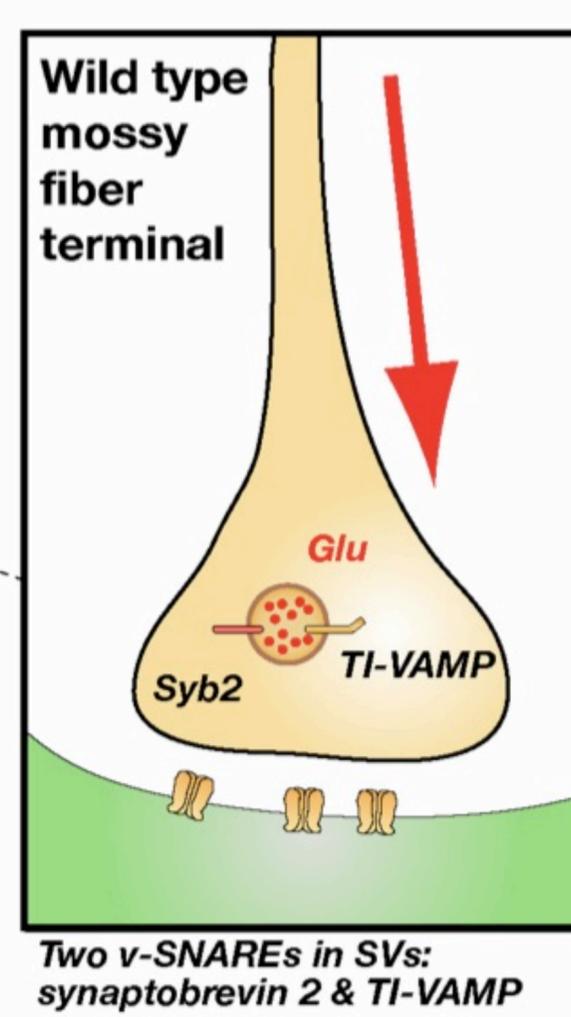
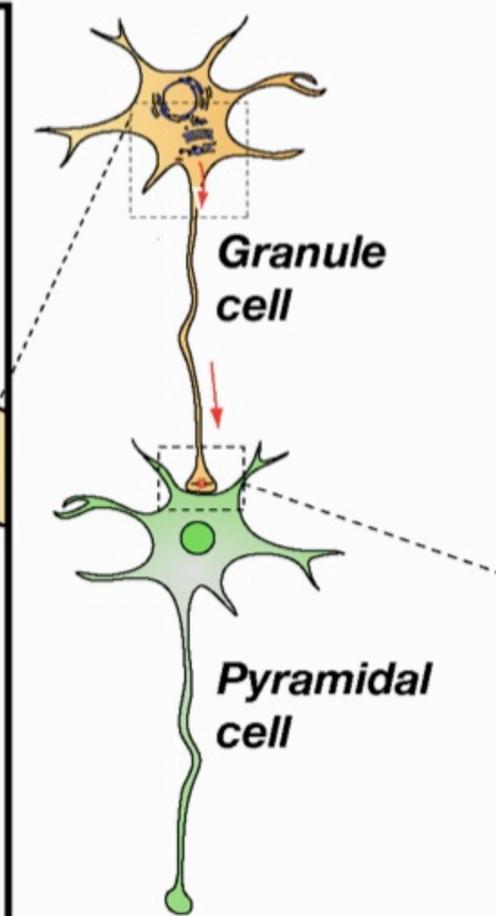
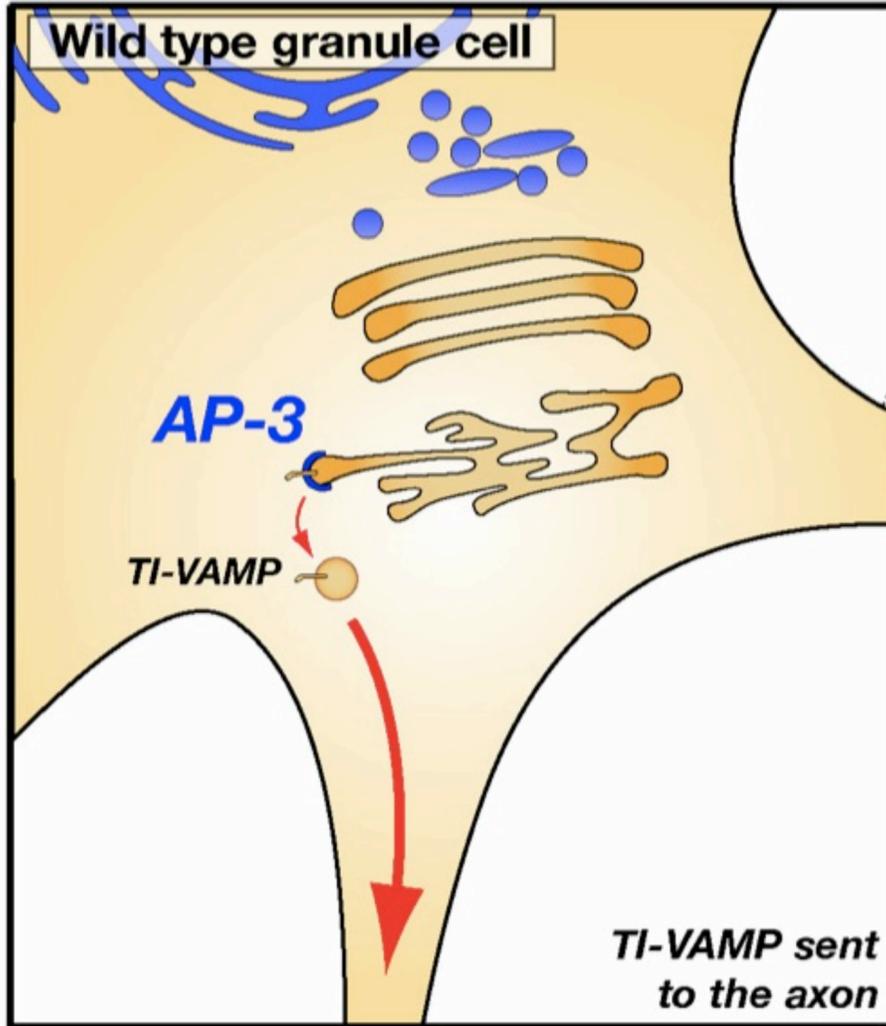
TI-VAMP est bloqué dans le soma des grains AP3 -/-

Controle



Souris mocha

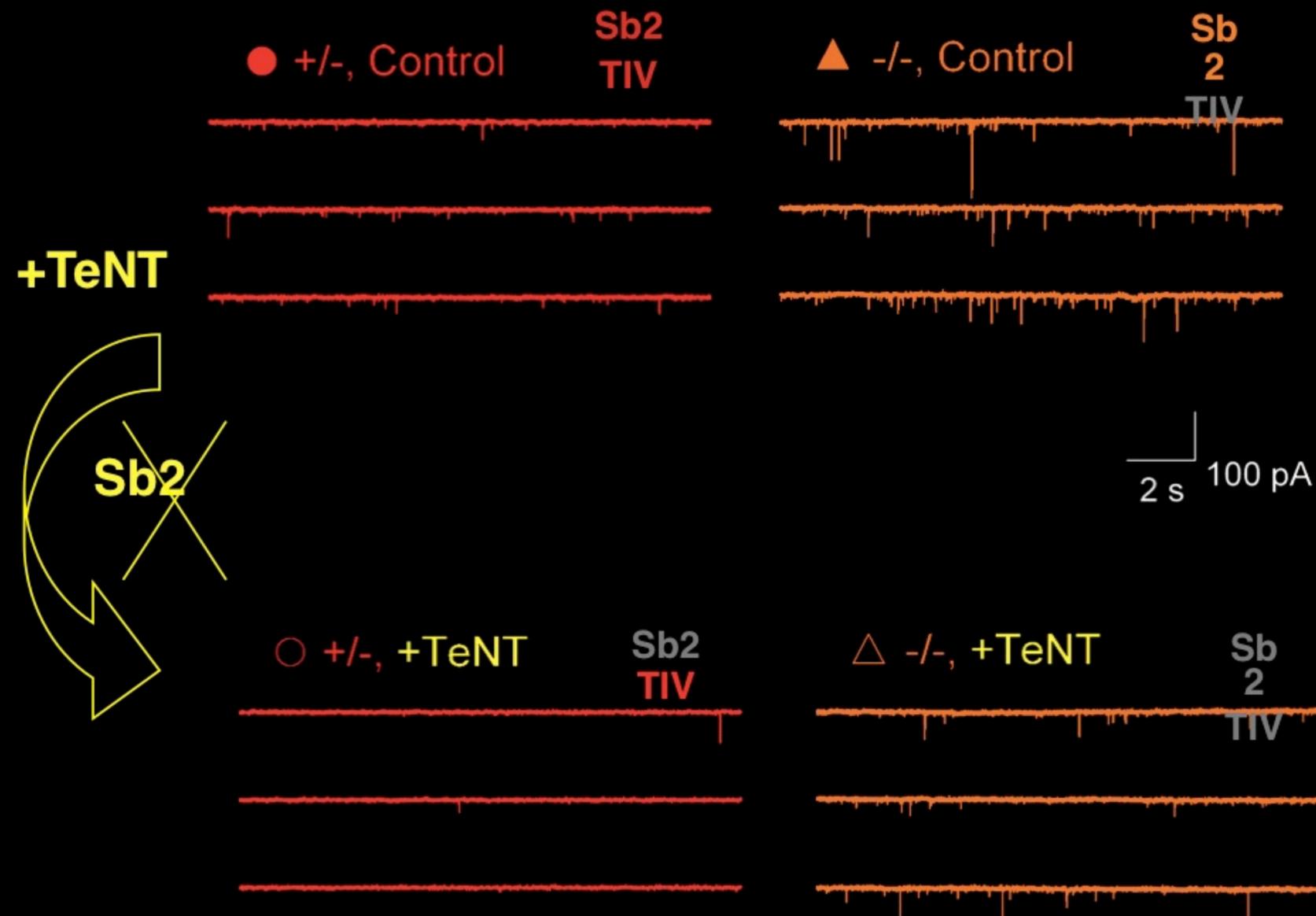
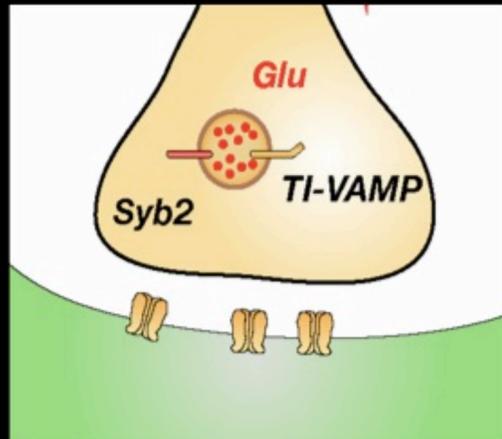




Rôle des vSNARE Syb2 et TI-VAMP dans la transmission synaptique

PNAS (2006)

Enregistrement des courants miniatures (MF)



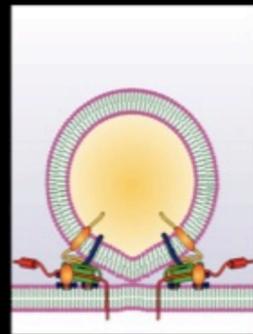
La perte de Syb2 baisse la fréquence de libération

La perte de TI-VAMP augmente la fréquence de libération

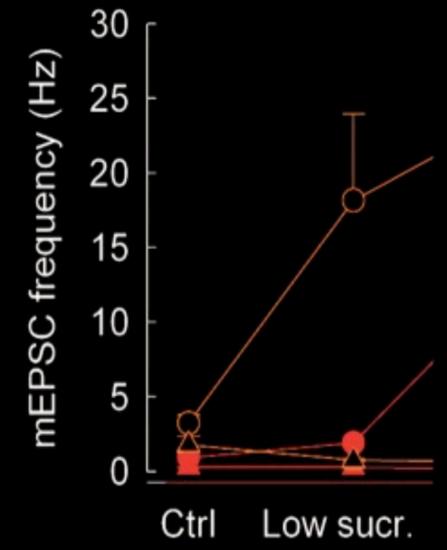
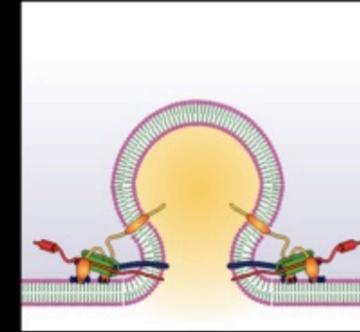
Rôle des vSNARE Syb2 et TI-VAMP dans la transmission synaptique

PNAS (2006)

Le RRP est augmenté en absence de TI-VAMP



+ sucrose



Contrôle

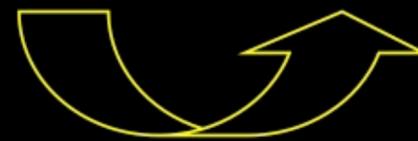


Mocha (AP3 -/-)



Perte de TI-VAMP :
Augmentation de la fréquence + grande :
le RRP est plus grand

~~TeNT~~
~~Syb2~~

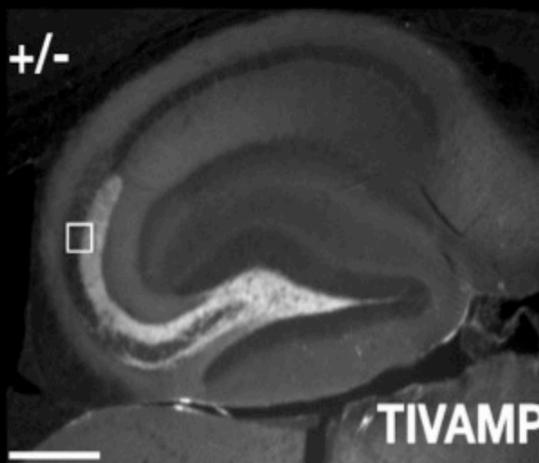
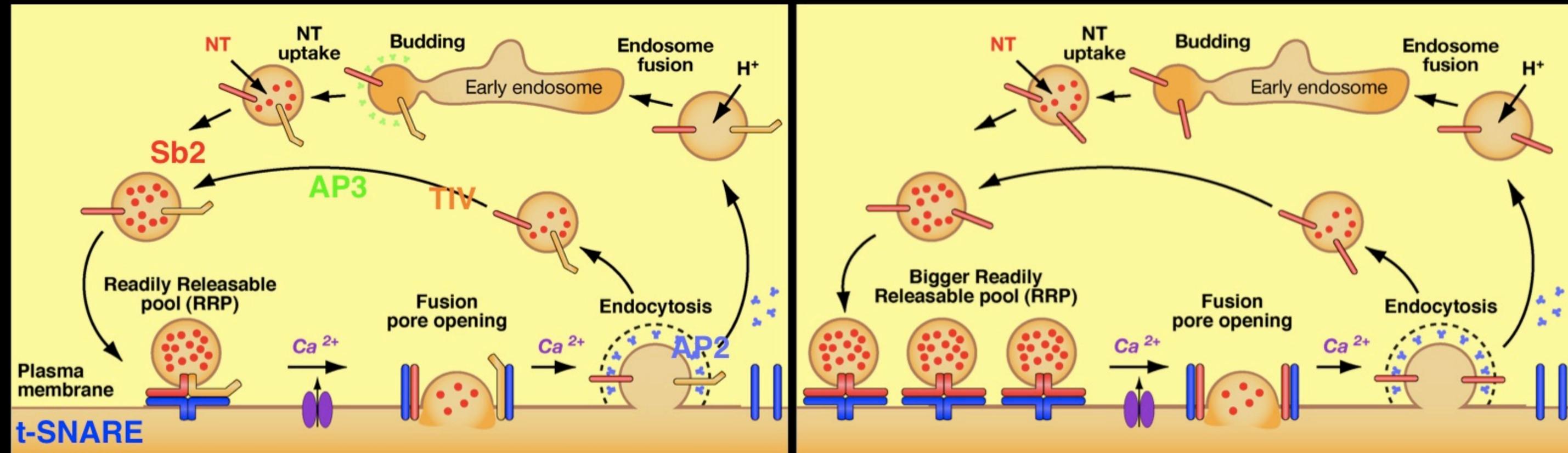


Perte de Syb2 :
Pas d'augmentation de fréquence:
Le RRP ne peut plus être recruté

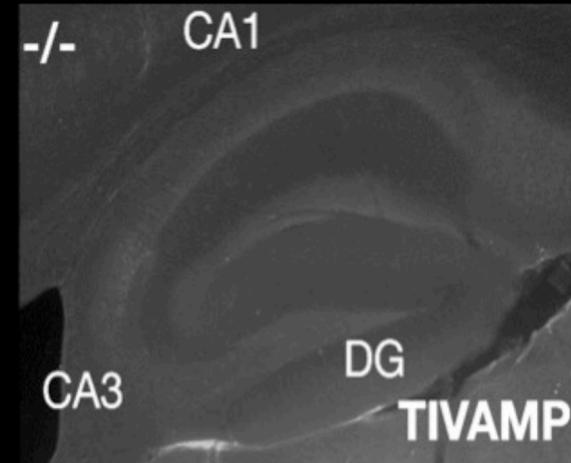
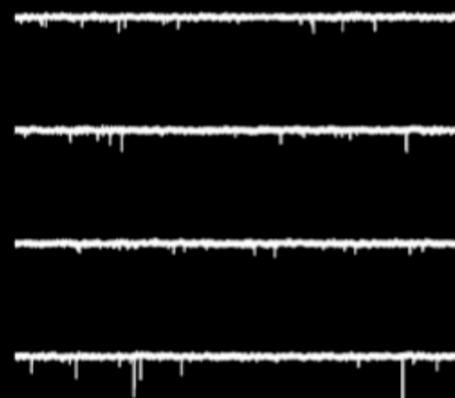
A la fois TI-VAMP & Sb2 medient la libération basale & évoquée aux terminaisons des fibres moussues

Souris control

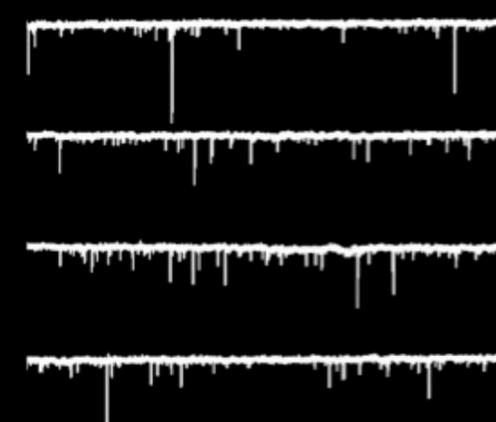
Souris mocha



Activité électrique :



Activité électrique :



En l'absence de TI-VAMP dans les terminaisons des fibres moussues (souris mocha) :

- le RRP devient plus important. La probabilité de libération basale augmente.
- La libération évoquée asynchrone, résistante à la TeNT, est perdue.

Trafic, exocytose et modèles physiopathologiques

1. Exocytose et complexe SNARE

Les voies d'exocytose régulée
 Définition du complexe SNARE
 Nomenclature v/t-SNARE et R/Q-SNARE

2. Exemple de la synapse

Découverte de NSF et SNAP
 Isolement des SNARE
 Rôle de NSF & SNAP dans la fusion

3. Le cycle des vésicules synaptiques

Voie lente: endocytose médiée par la clathrine
 Voie courte: kiss and run
 Les différents « pool » vésiculaires

4. Comment mesurer l'exocytose ?

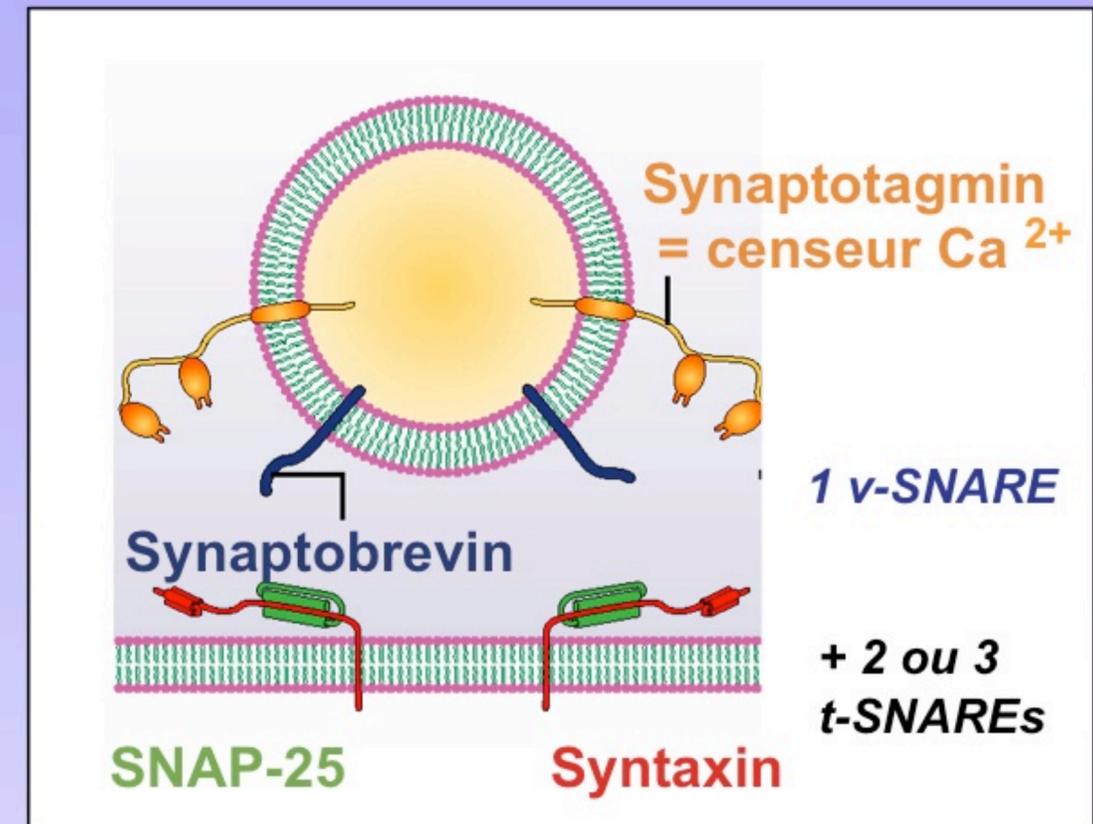
Capacitance
 Ampérométrie
 GFP pH sensible: la Phluorin
 Utilisation des SNARE inversée

5. Comment mesurer le recyclage ?

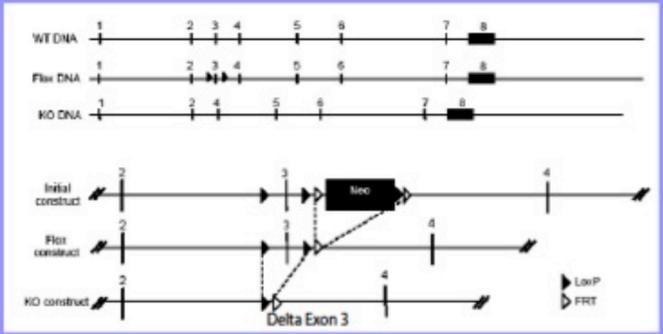
Utilisation des anti-synaptotagmine
 Sondes fluorescentes de type FM

6. Modèles physiopathologiques

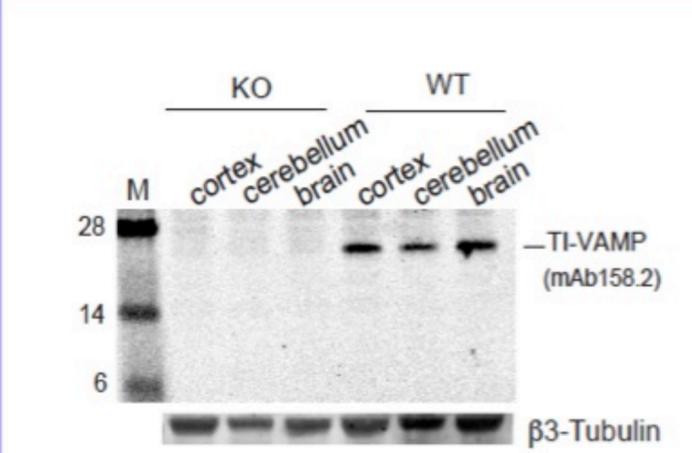
Rôle de la Cellubrevine dans la migration des cellules épithéliales
 Rôle de la synaptobrevine dans la libération des NT
 Rôles de Stx3 et SytVII dans la croissance neuritique
 Mutants de l'adaptateur AP-3: les mocha
 KO TI-VAMP
 Rôles de TI-VAMP dans l'exocytose et la diffusion des récepteurs



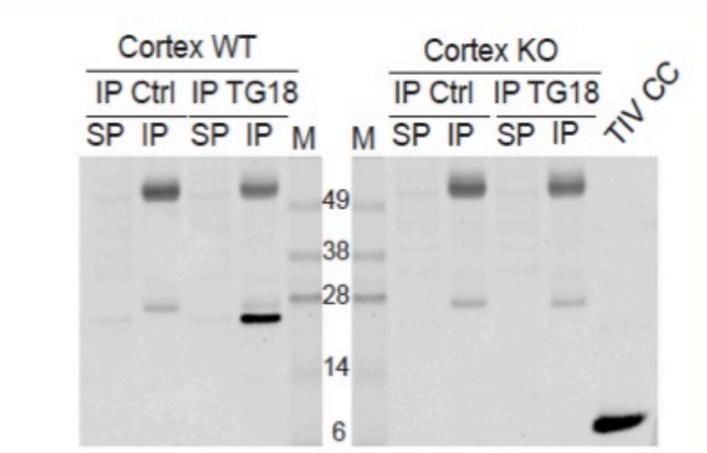
TI-VAMP/VAMP7 KO : Exon 3 deletion leads to full KO



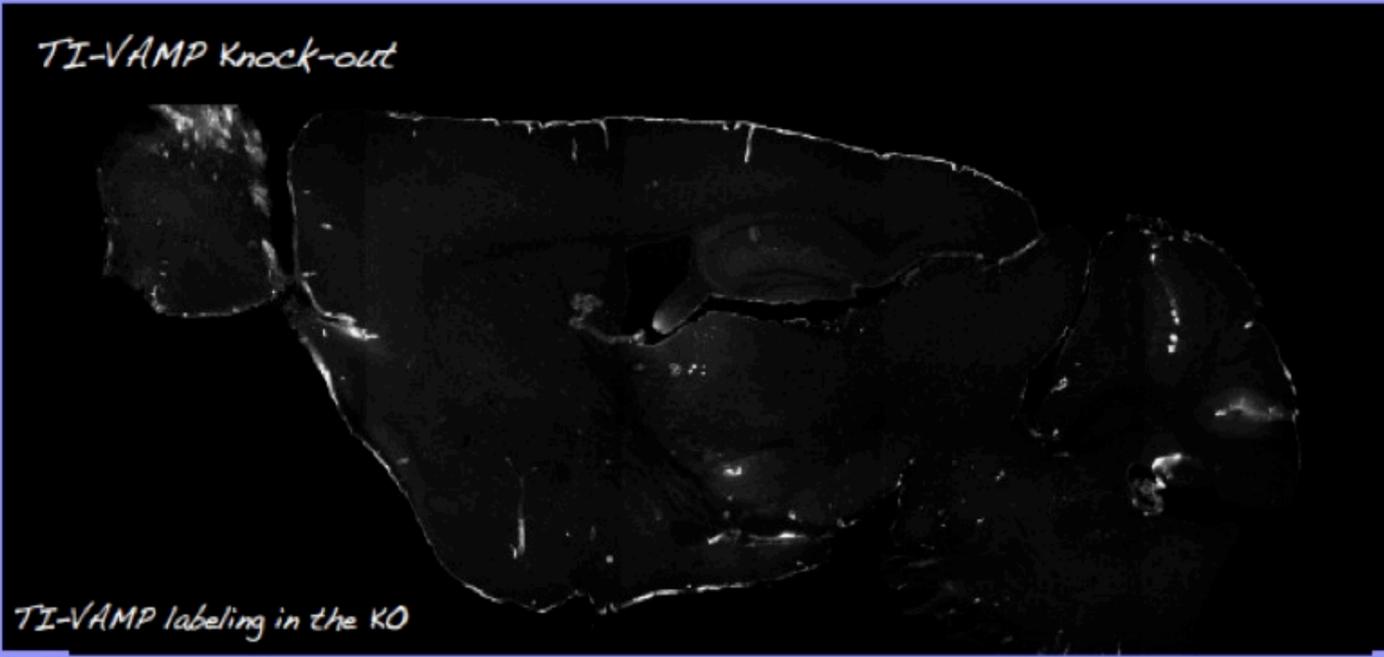
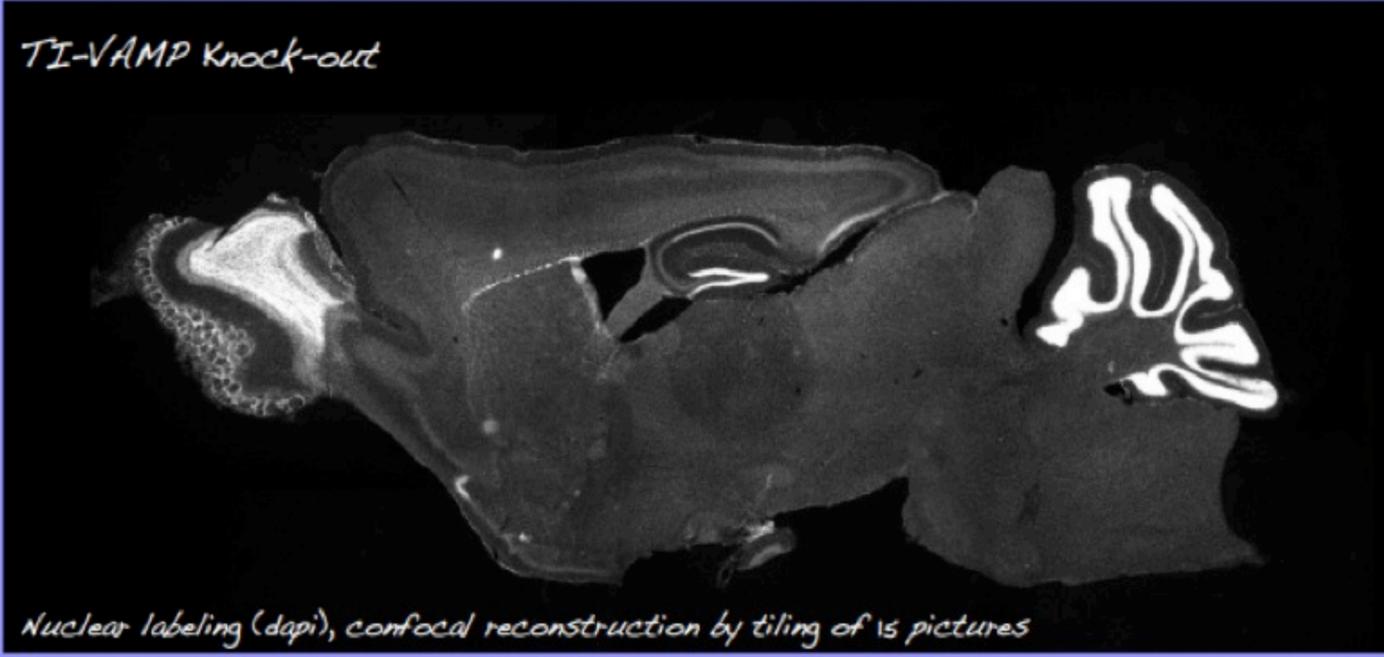
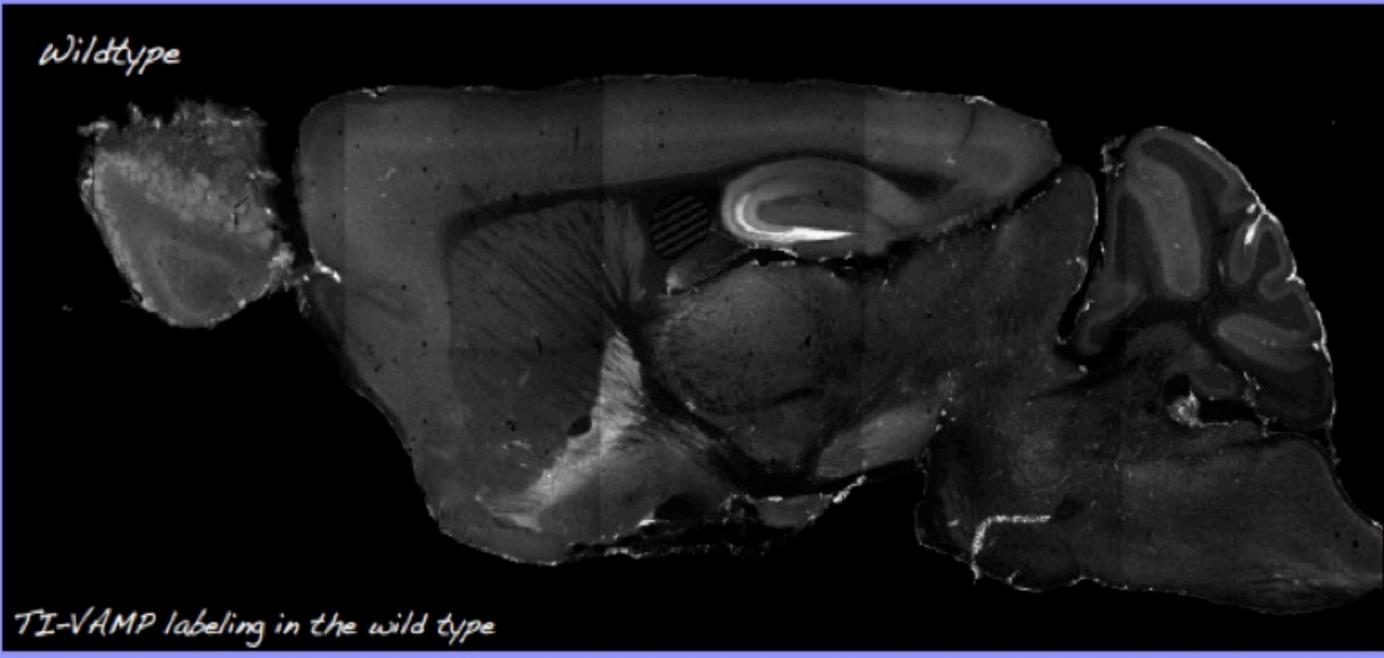
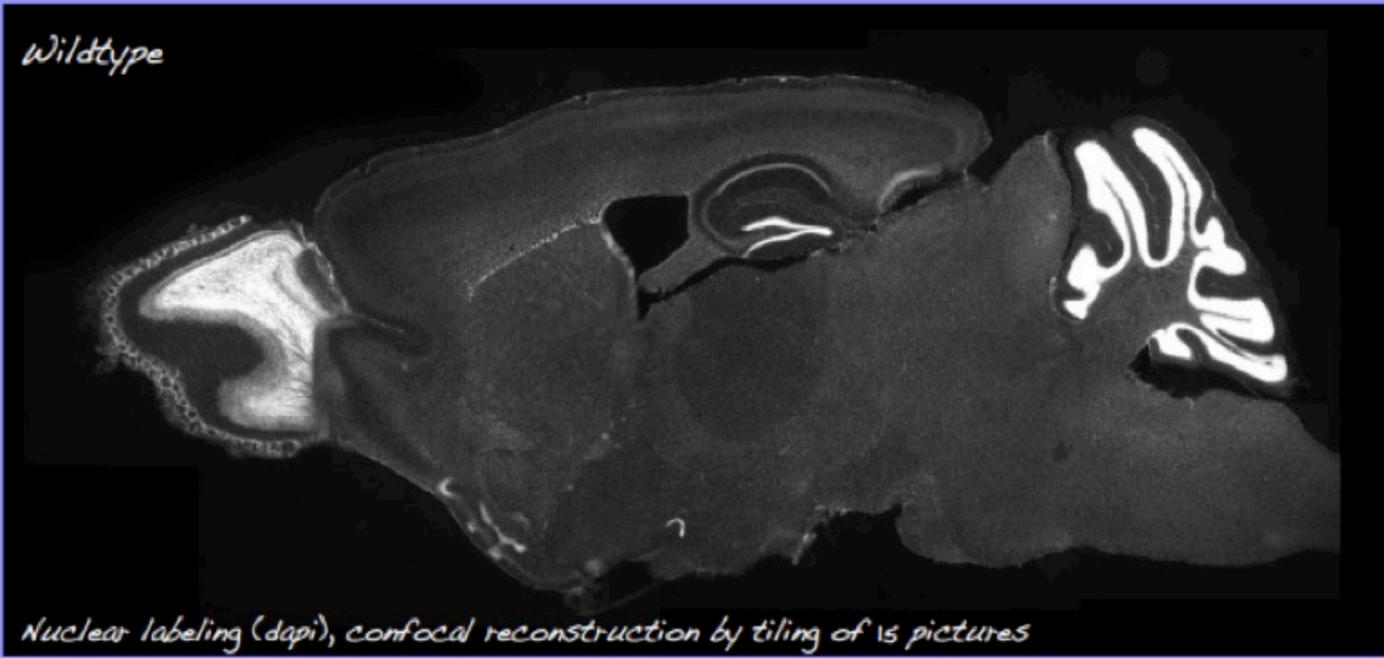
Conditional TI-VAMP KO \times CMV - Cre (ubiquitous)
 \approx Constitutive TI-VAMP KO



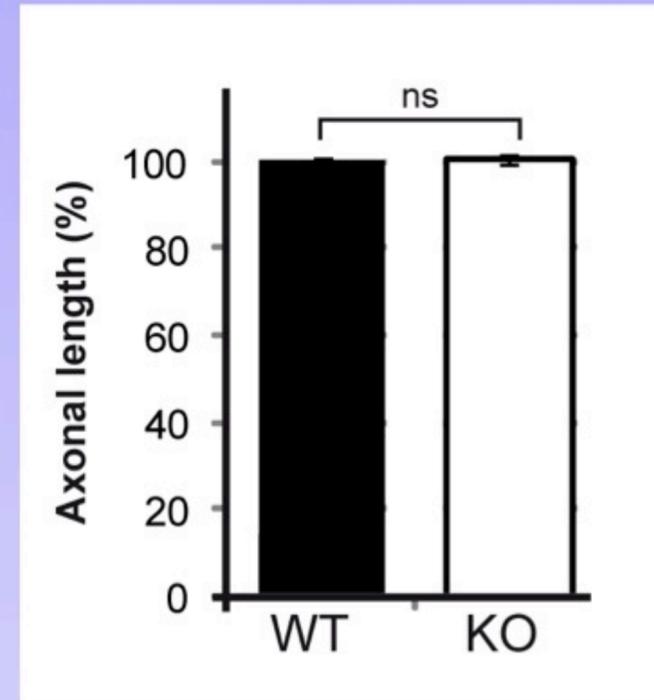
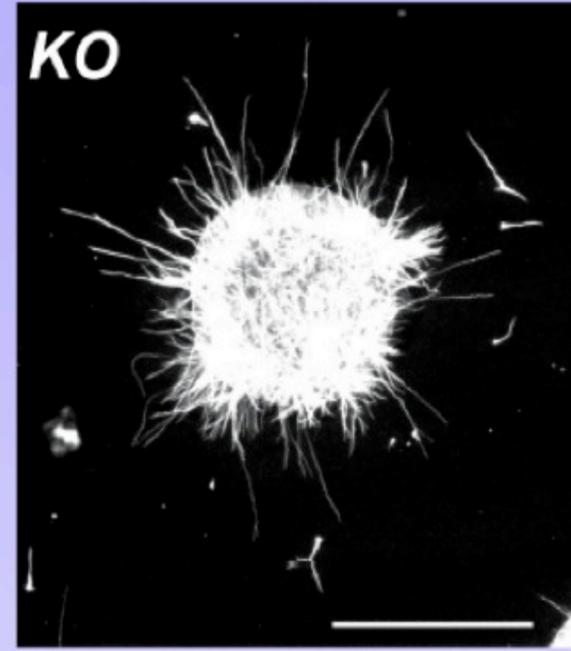
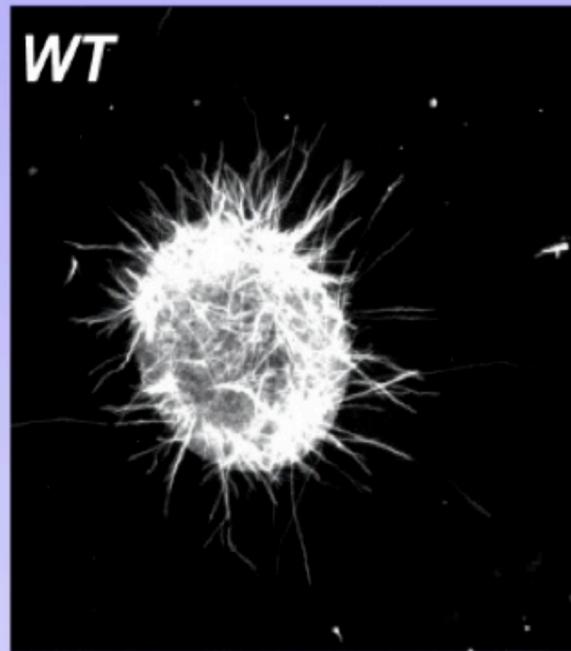
TI-VAMP protein is not detectable in the KO.



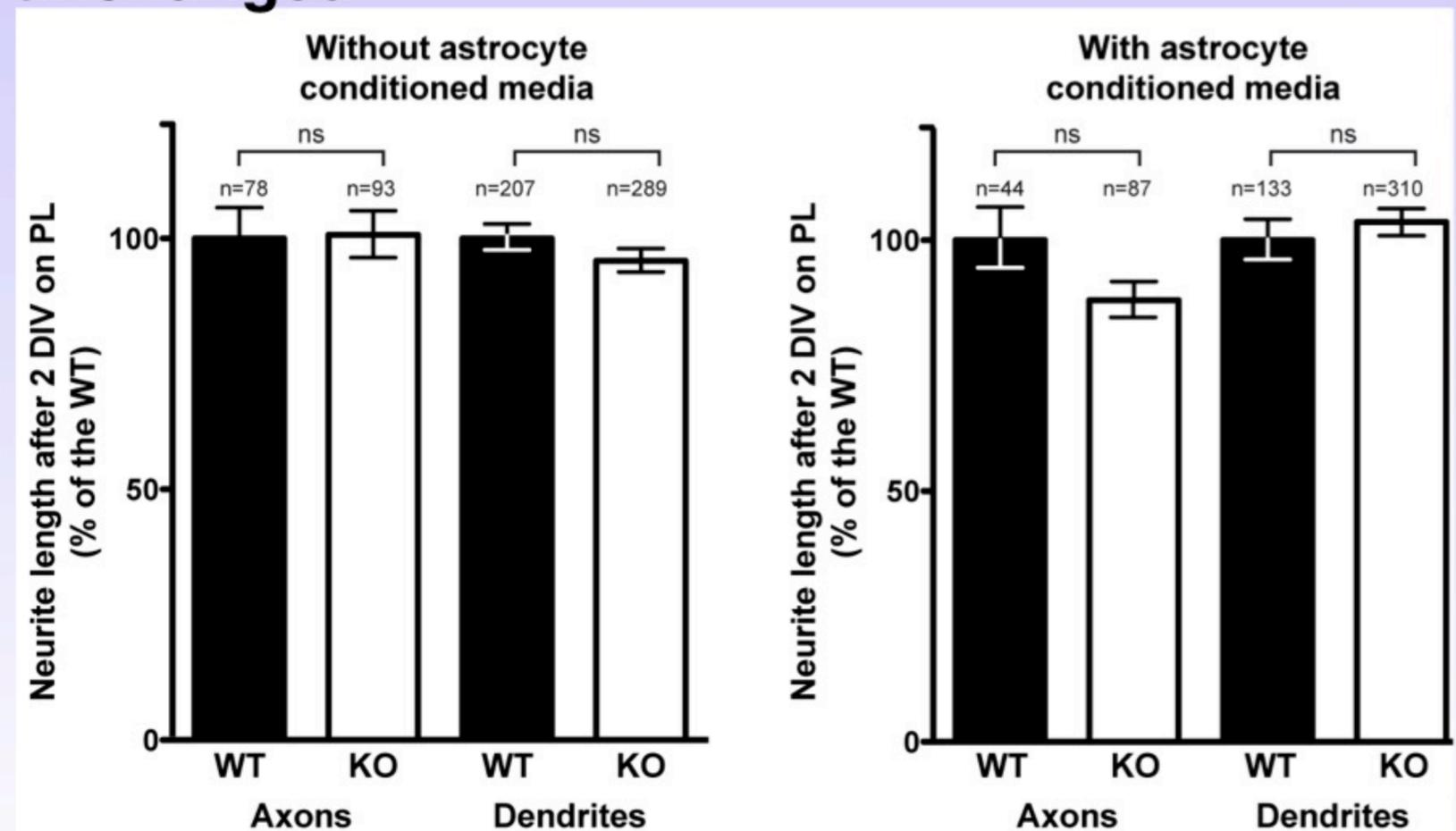
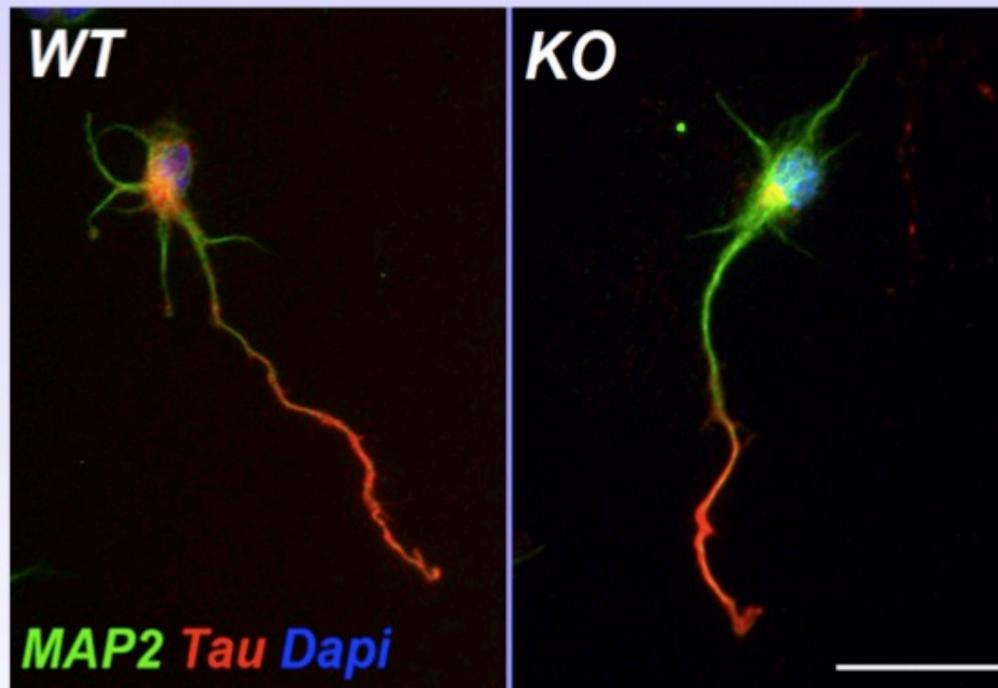
Even traces of TI-VAMP is not detectable after IP.



Cortical explant outgrowth is unchanged



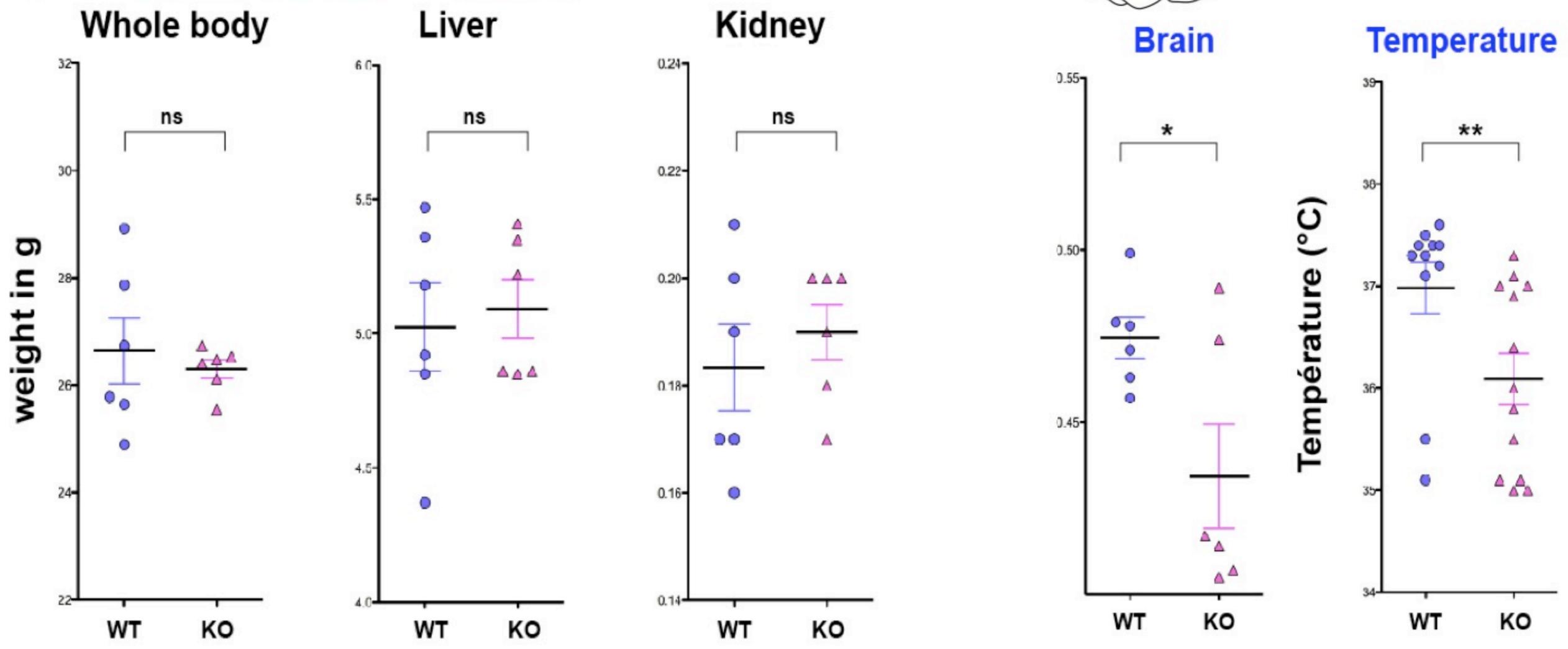
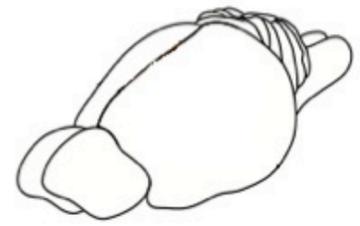
Hippocampal neurite growth is unchanged



Contrairement aux exp de siRNA, la croissance neuritique est normale chez le KO.

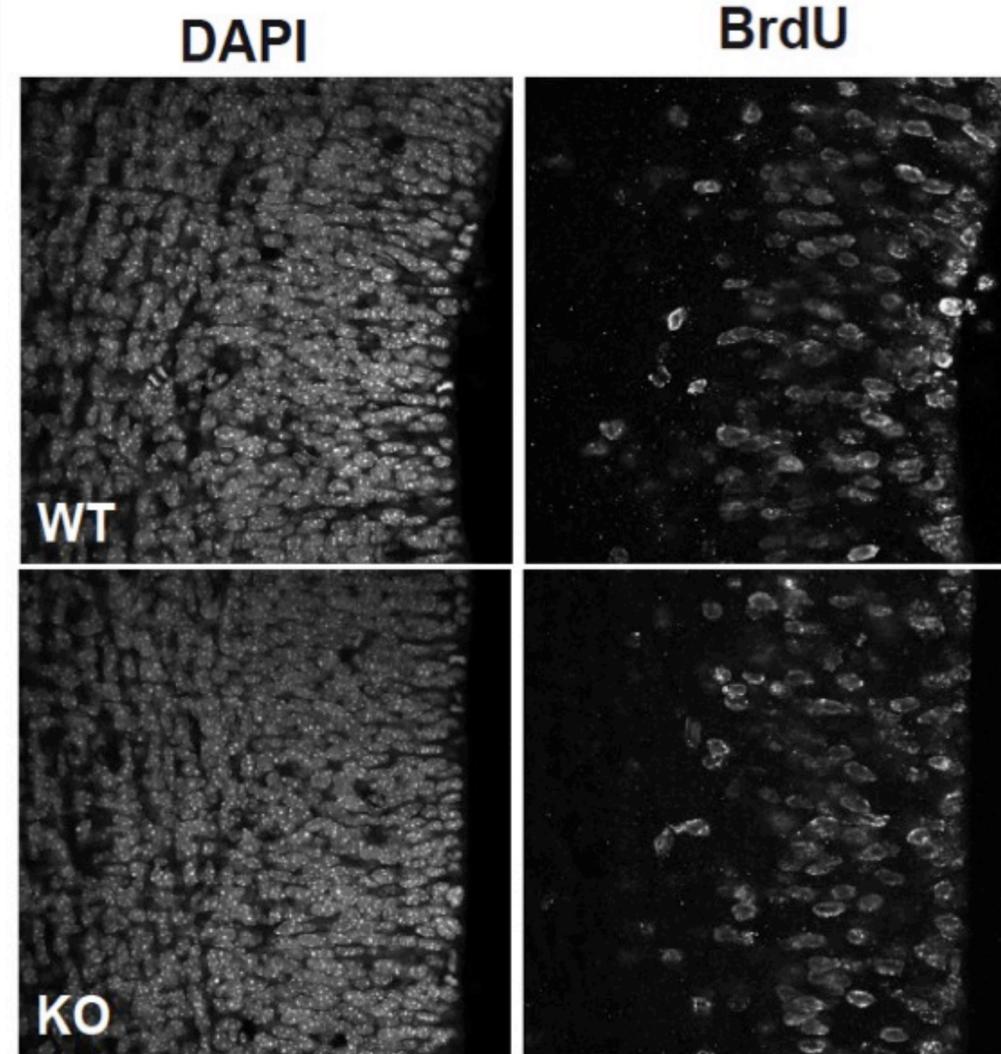
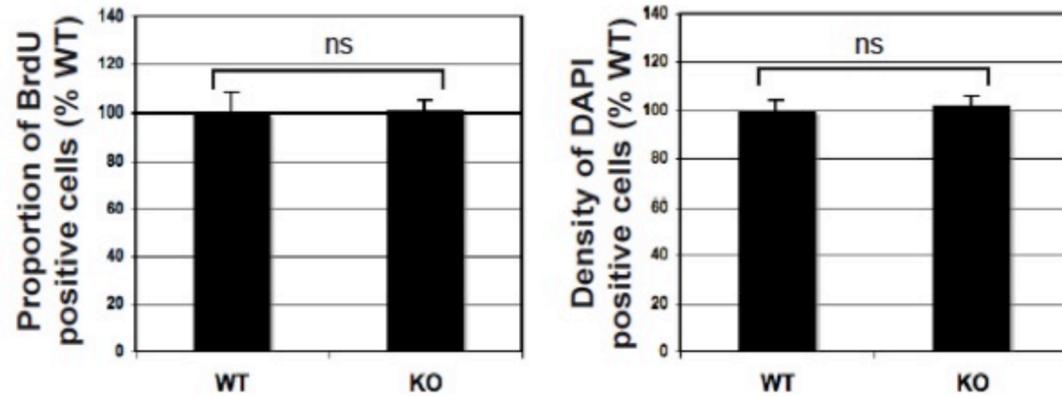
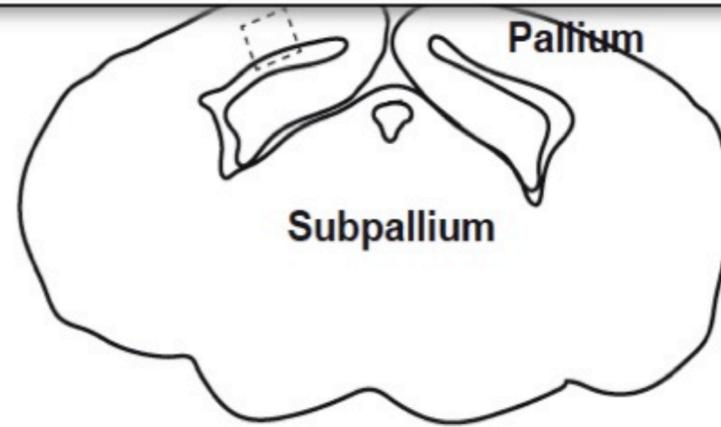


General Health	WT		KO	
	mean	±SEM	mean	±SEM
Body weight (g)	26,64	±0,62	26,30	±0,17
Brain weight (g)	0,47	±0,006	0,43	±0,015 *
Body length (cm)	9,75	±0,08	9,55	±0,11
Body temperature (°C)	36,98	±0,26	36,09	±0,25 *

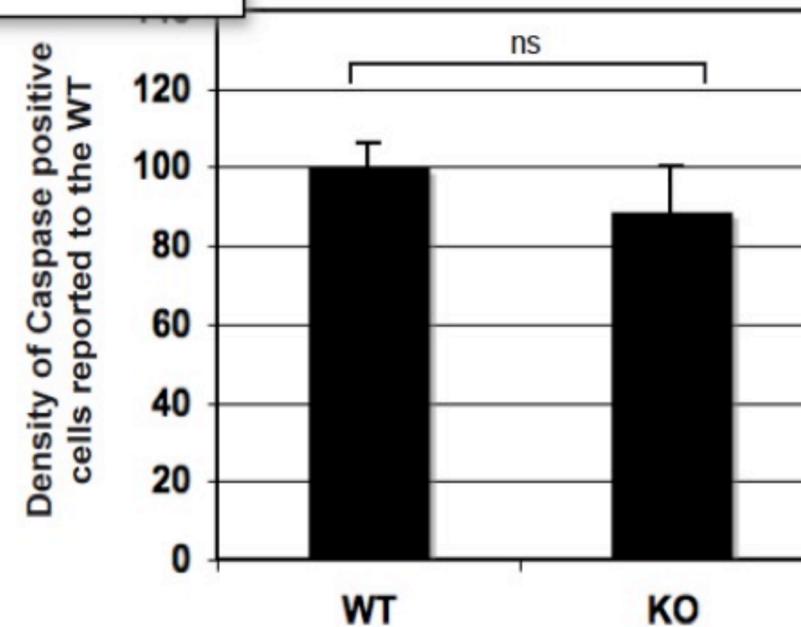
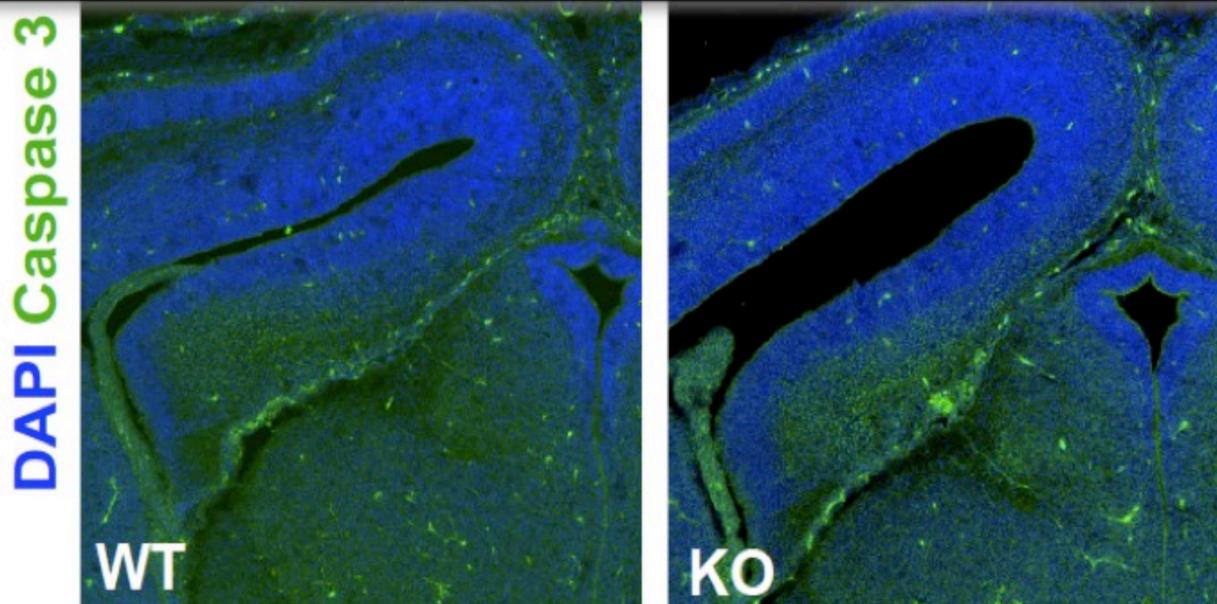


TI-VAMP KO have lighter brain and decreased temperature.

Proliferation is unchanged in cortical anlage at E15.5



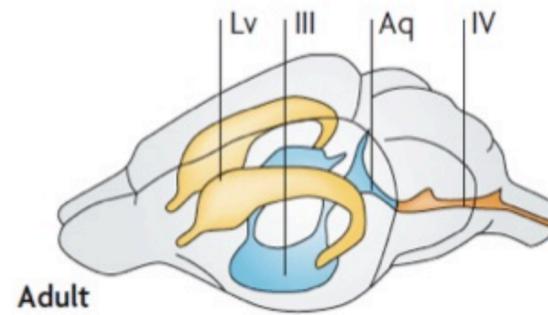
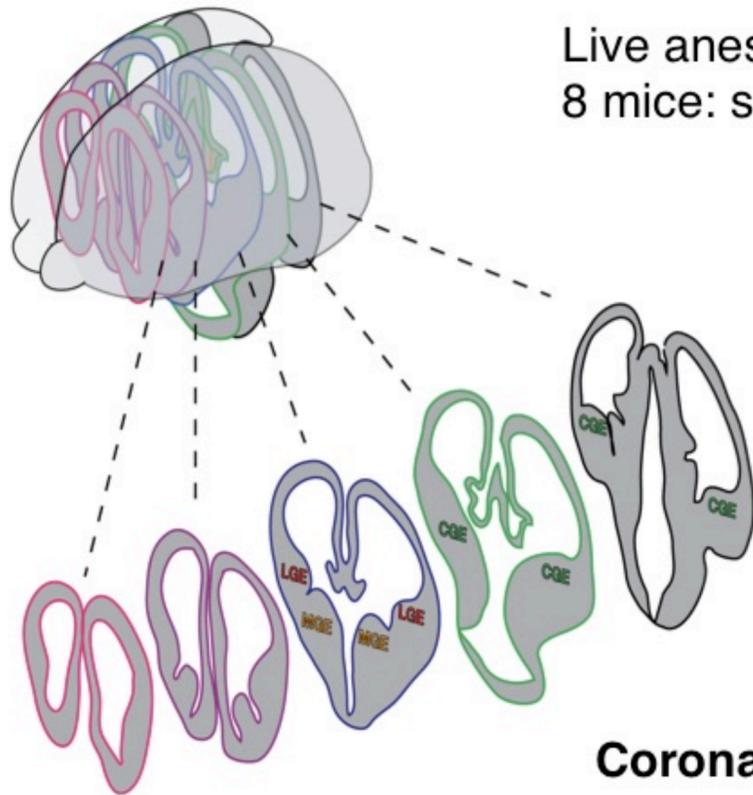
Cell death is unchanged in cortex and hippocampal anlage at E15.5



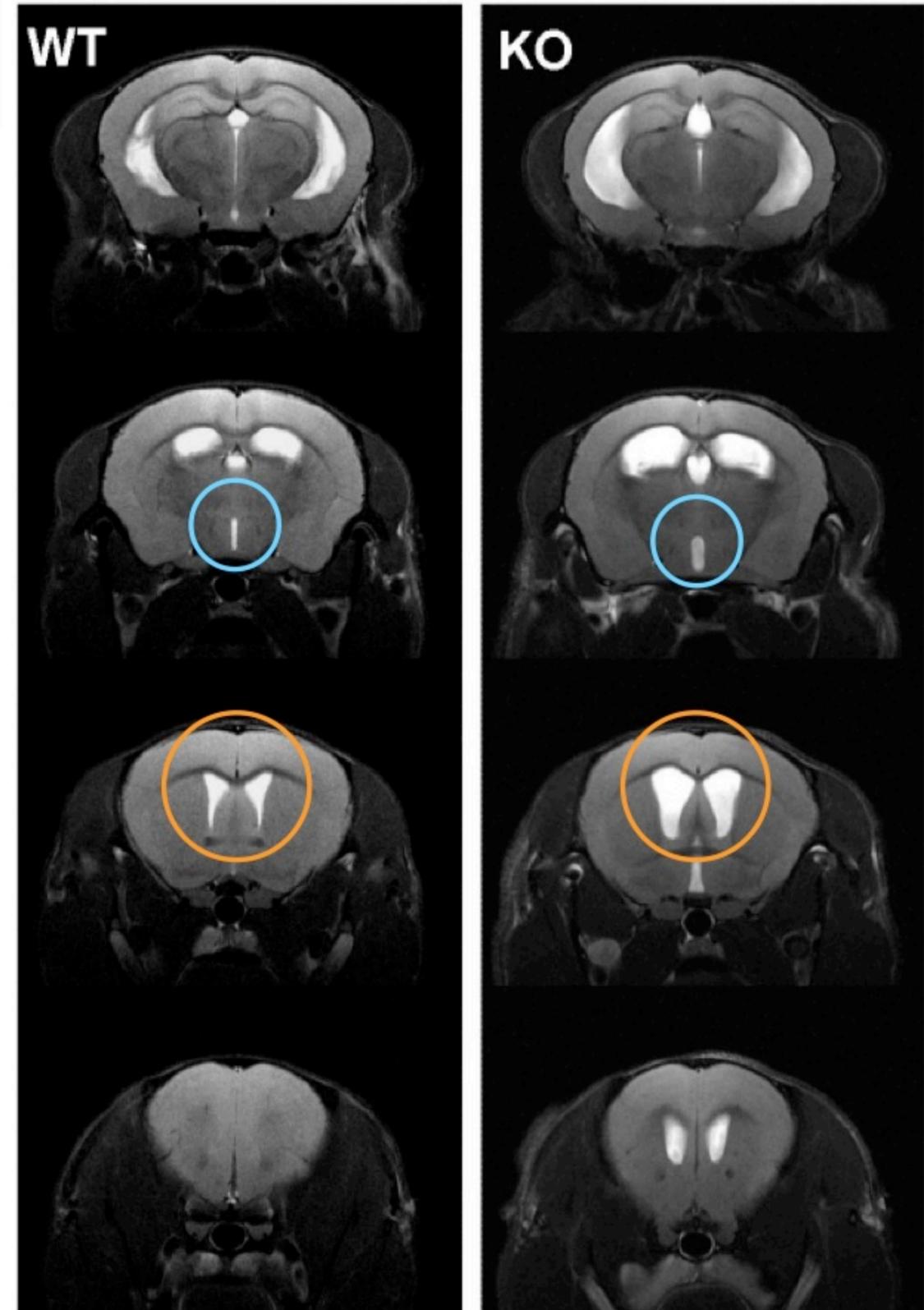
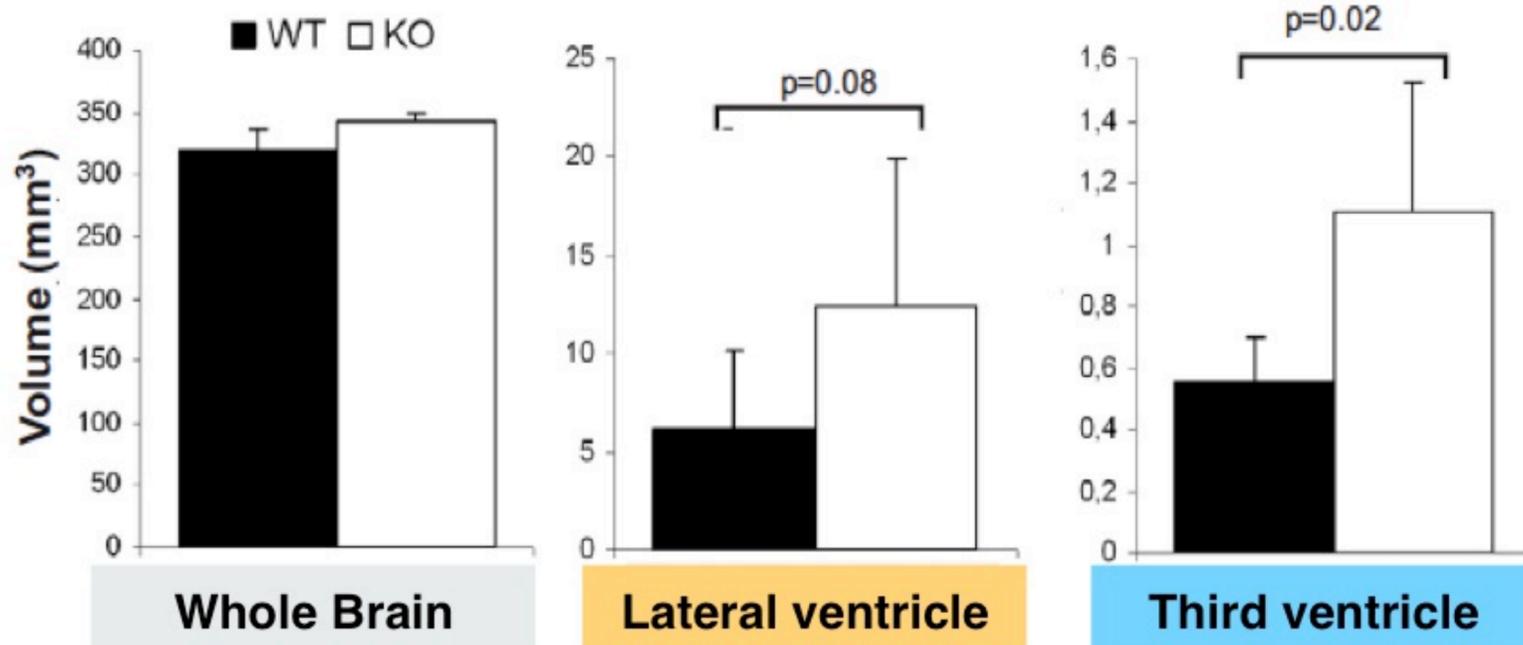


Maxime Gauberti Denis Vivien

Live anesthetized animals in 7 Teslas MRI:
8 mice: sequence T2* (fast, 1h acquisition)



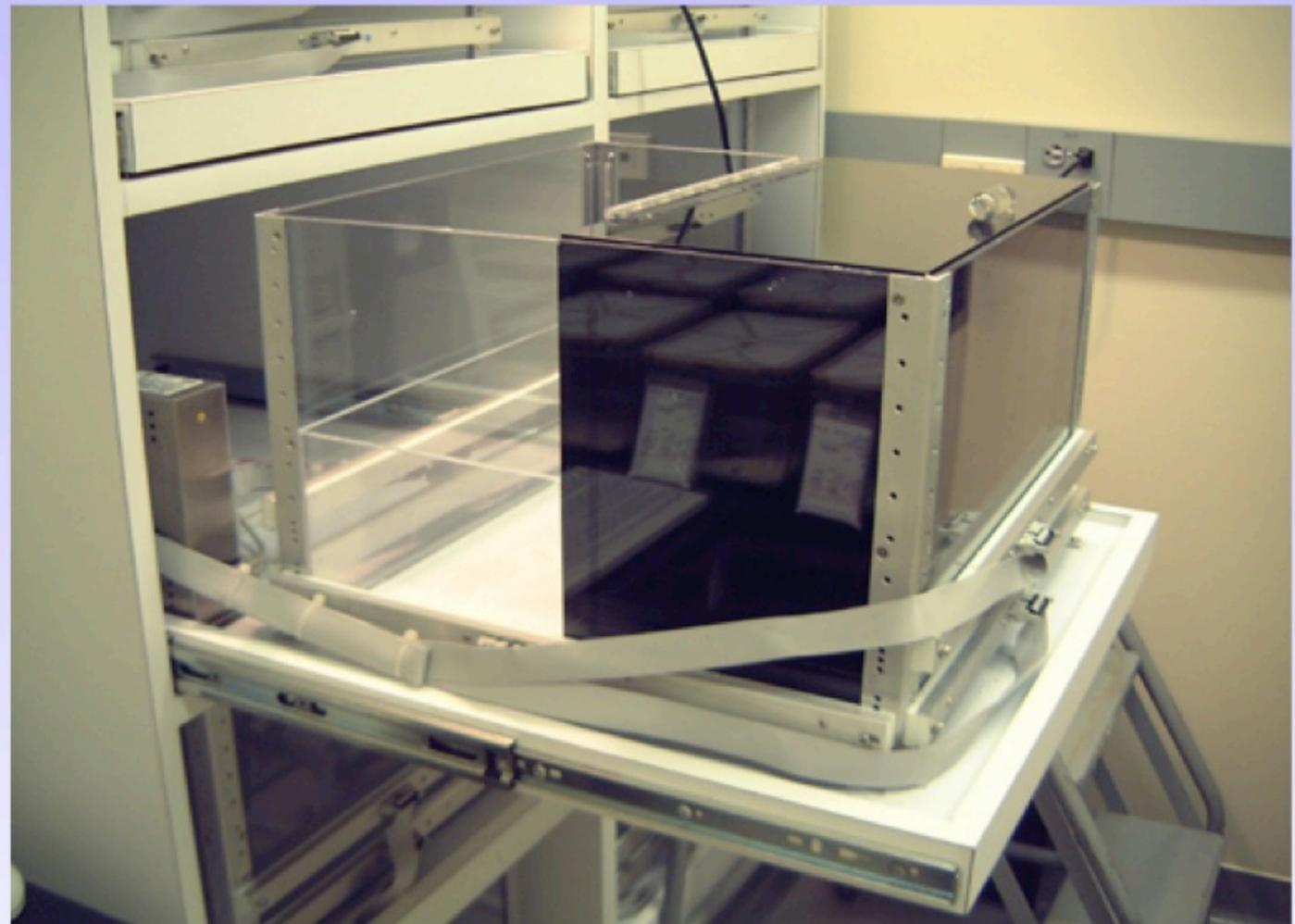
Coronal Imaging



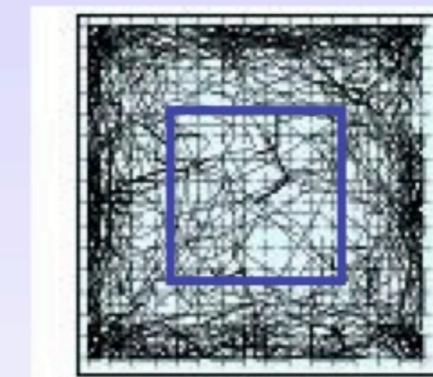
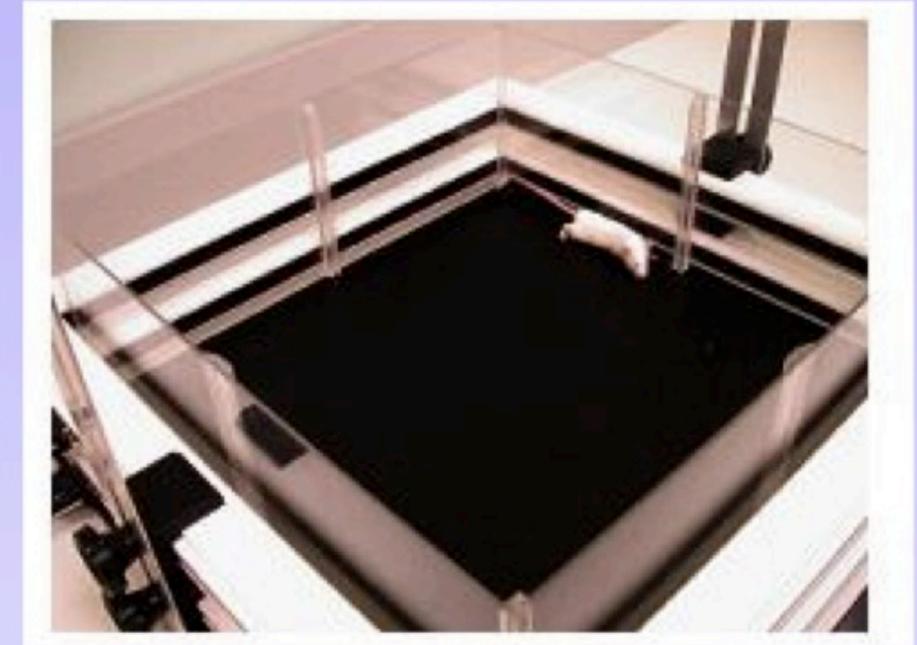
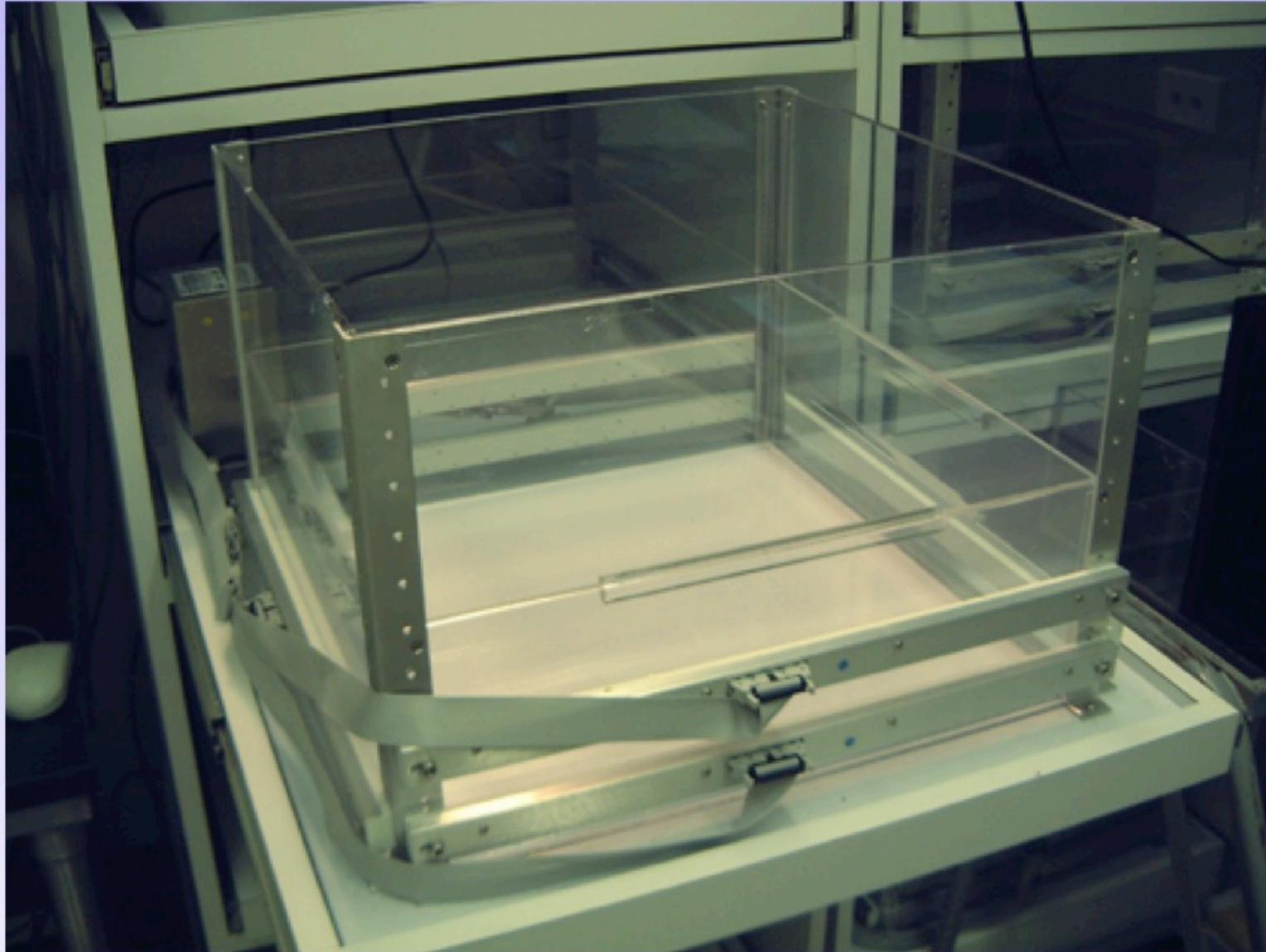
In vivo MRI (RARE).

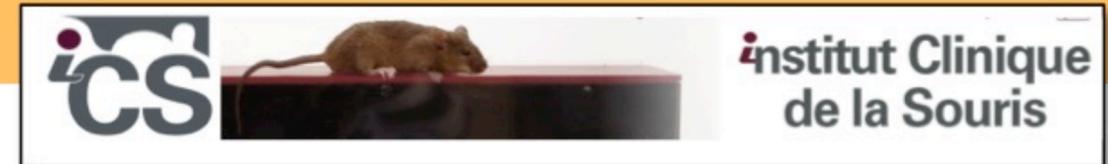
In vivo MRI reveal a conserved brain volume, but an increased ventricles size.

Light-Dark Box Test

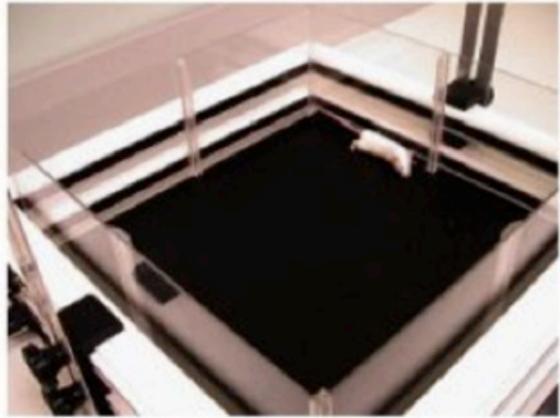


Open-Field Test

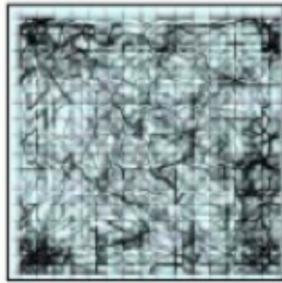




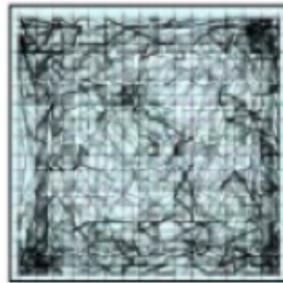
Behavioral tests with no significant difference



Open field



WT



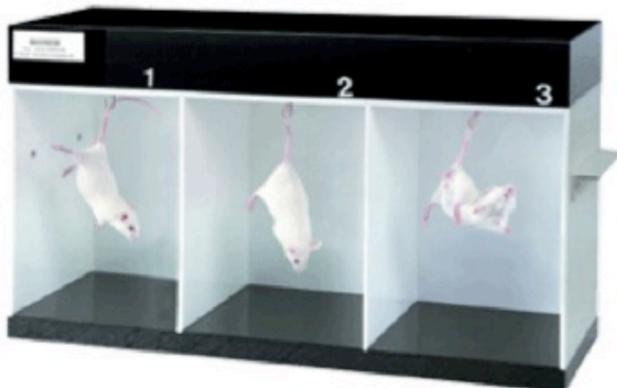
VAMP7-KO



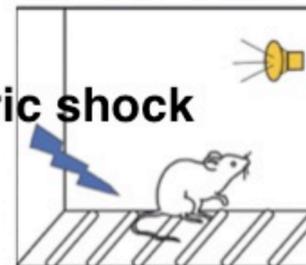
String test



**Y maze
(working memory)**



**Tail suspension test
(depression)**



Electric shock

Sound

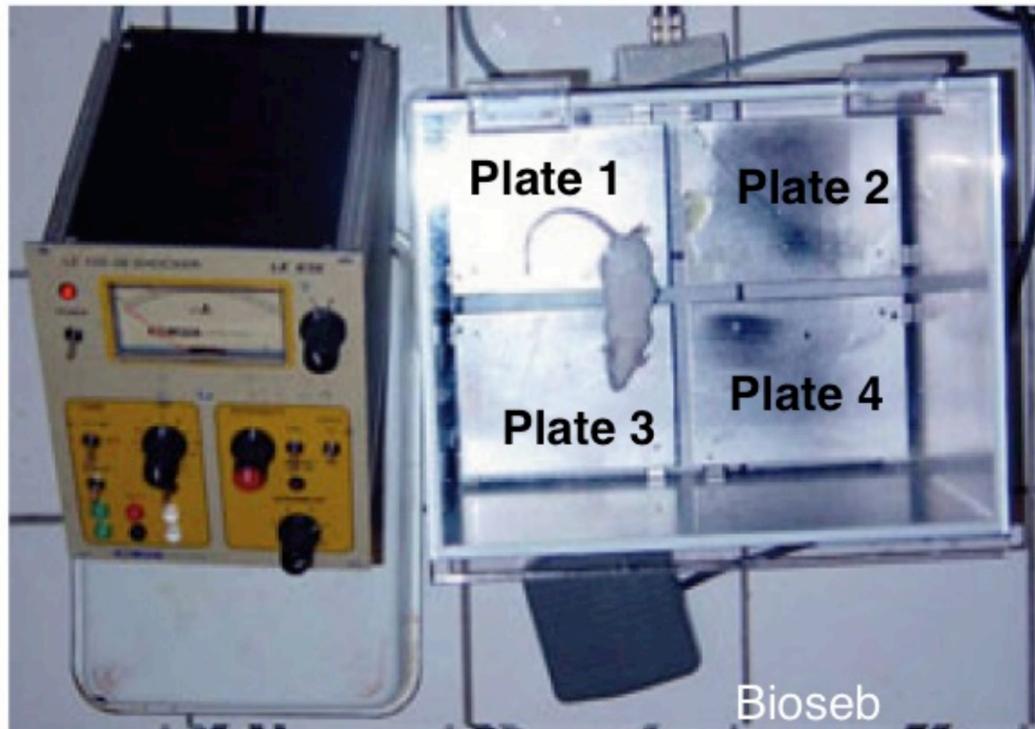
Fear conditioning

Cookie test (olfaction)

Startle reflex

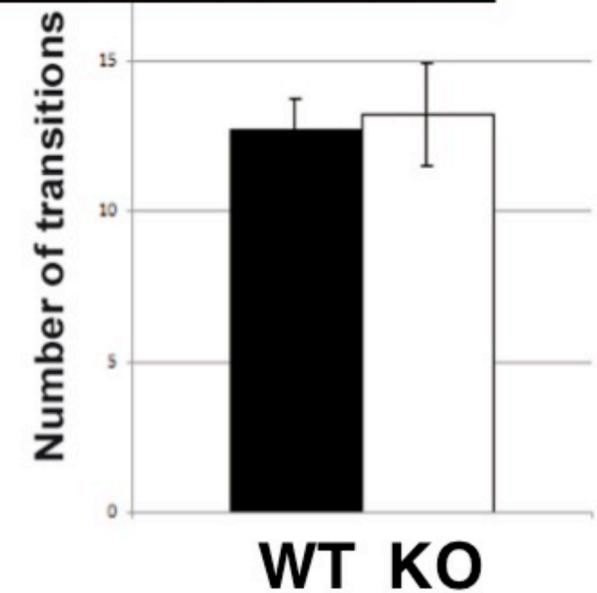
Behavioral tests	WT		KO	
	mean	±SEM	mean	±SEM
Open field				
Distance travelled (m)	106,89	±3,99	116,11	±4,10
Number of rears	261,55	±25,17	293,08	±39,43
% of time in the center	12,55	±1,35	14,66	±1,85
Number of entries	109,45	±8,91	119,23	±8,66
Rotarod- 4 to 40 rpm in 5 min (s)	132,45	±10,75	113,62	±10,54
Grip strength ratio (grip mean/weight)	7,82	±0,32	8,39	±0,20
String test (s)	2,32	±0,27	3,16	±0,36
Tail flick (s)	4,24	±0,14	4,19	±0,18
Hot plate				
First reaction (s)	9,35	±0,93	9,44	±0,72
Jump (s)	90,13	±6,63	97,64	±5,79
Shock threshold (mA)				
Flinch	0,08	±0,00	0,08	±0,00
Vocalization	0,18	±0,02	0,18	±0,02
Y-maze alternation				
% Spontaneous Alternation	60,40	±3,49	64,91	±2,76
% Alternance Arm Return	34,82	±3,75	33,52	±2,93
%Same Arm Return	2,29	±0,68	0,92	±0,49
Tail suspension test				
Duration of immobility (s)	144,80	±23,02	154,15	±20,93
Latency to first immobility (s)	70,57	±18,03	46,80	±5,89

Conflict induced anxiety test (conditioned response to stressful events): the Four plates test



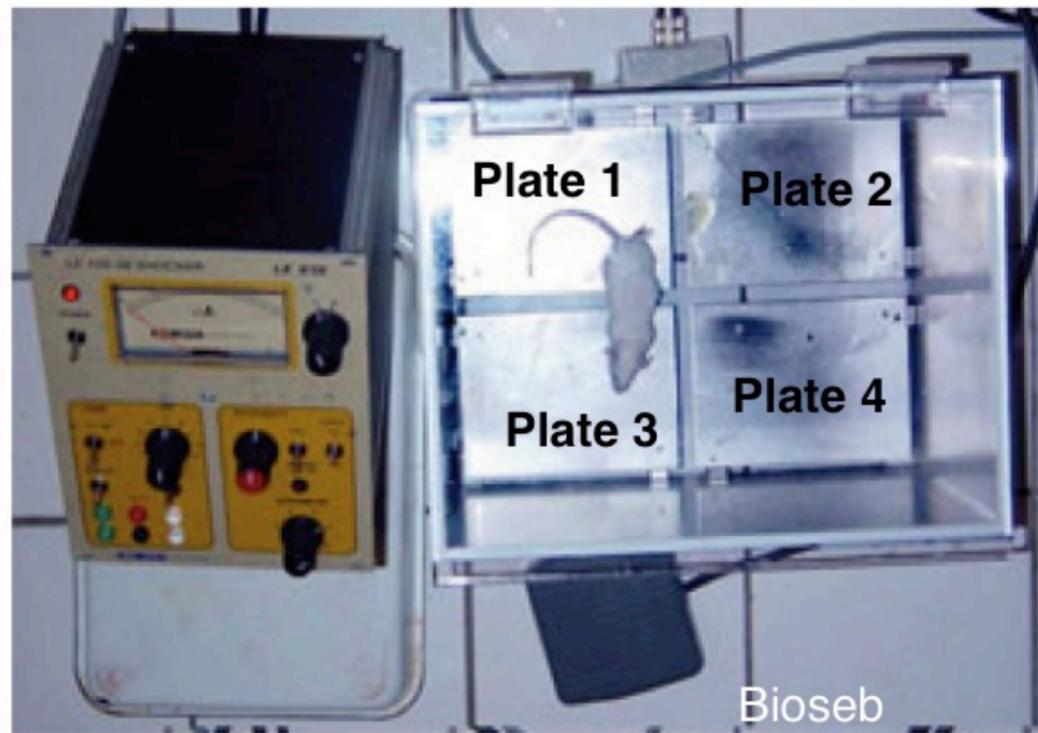
Habituation n (15s)	Shock if crossing	Number of crossing in 5 min
------------------------	----------------------	--------------------------------

Exploration of the novel surrounding is suppressed by the delivery of a mild electric foot shock (0,3mA, 0,5s).



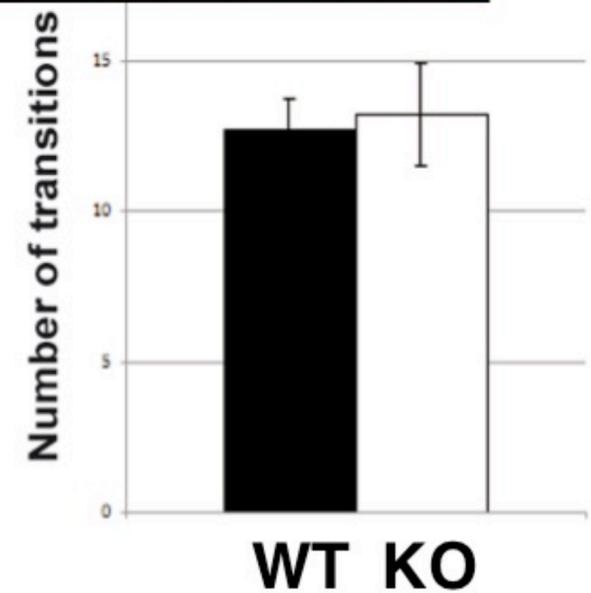
The number of crossing is not different, indicating no induced anxiety after stressful events.

Conflict induced anxiety test (conditioned response to stressful events): the Four plates test



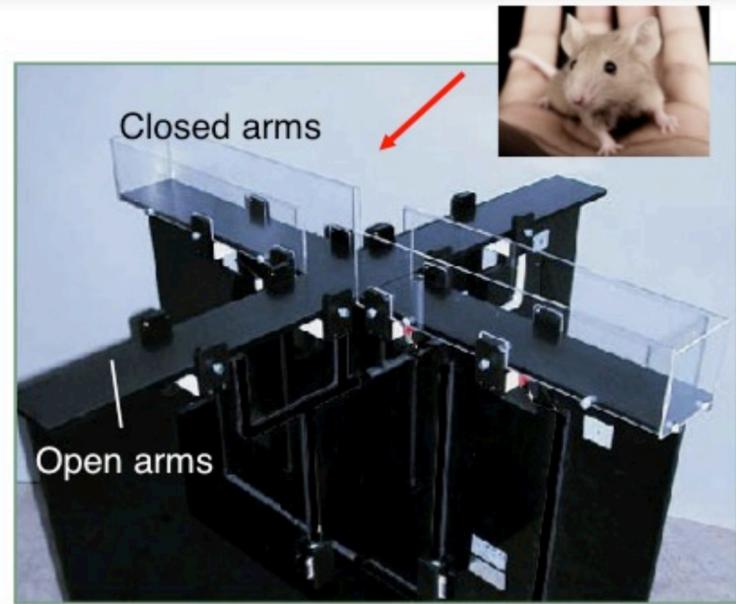
Habituation (15s)	Shock if crossing	Number of crossing in 5 min
-------------------	-------------------	-----------------------------

Exploration of the novel surrounding is suppressed by the delivery of a mild electric foot shock (0,3mA, 0,5s).

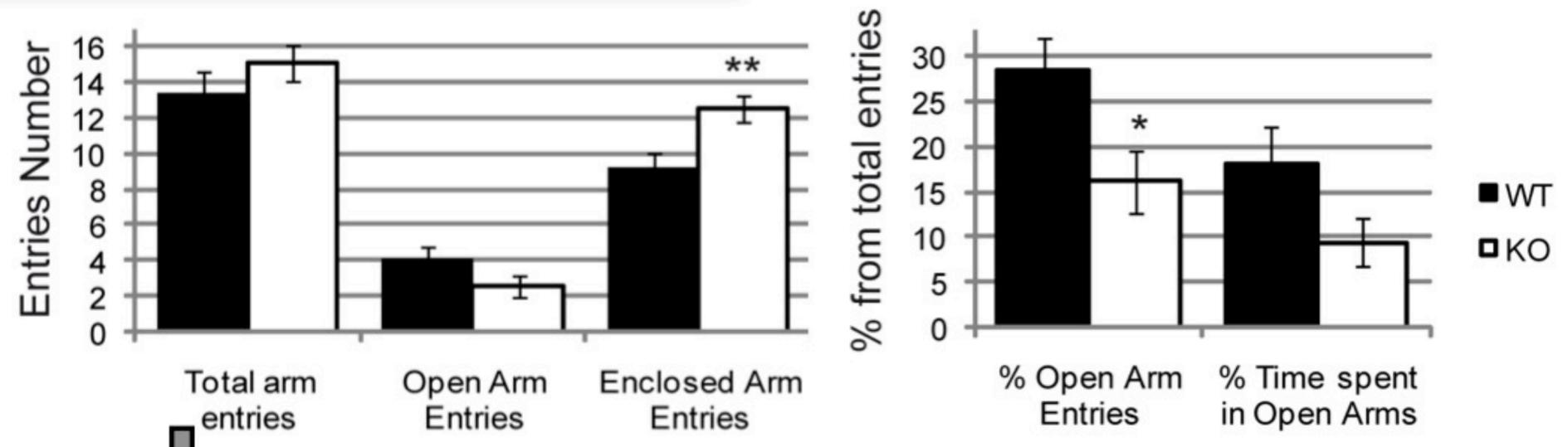


The number of crossing is not different, indicating no induced anxiety after stressful events.

Elevated plus maze (unconditioned anxiety)



Exploration/ Natural aversion for the open space.



Similar locomotion → Increased anxiety

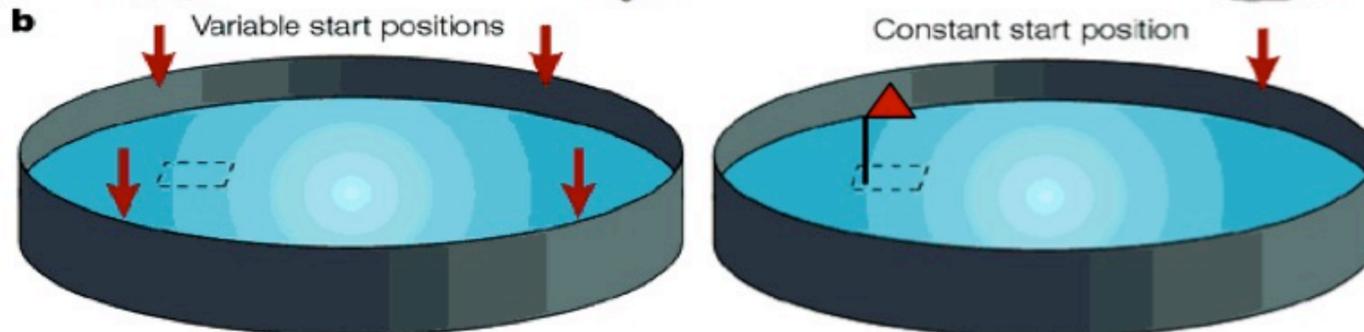
Piscine de Morris (Morris Water Maze)

ACQUISITION TRIALS

Duration of test: 90 sec

Variables:

- Inactivity (s)
- Swim speed (cm/min)
- Latency (s)
- Distance travelled (cm)
- Successful trials (proportion)



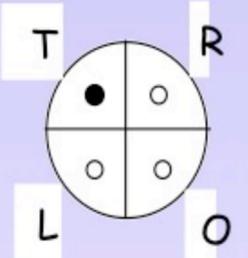
SPATIAL VERSION

Platform: fixed position

CUED VERSION

Platform: variable position

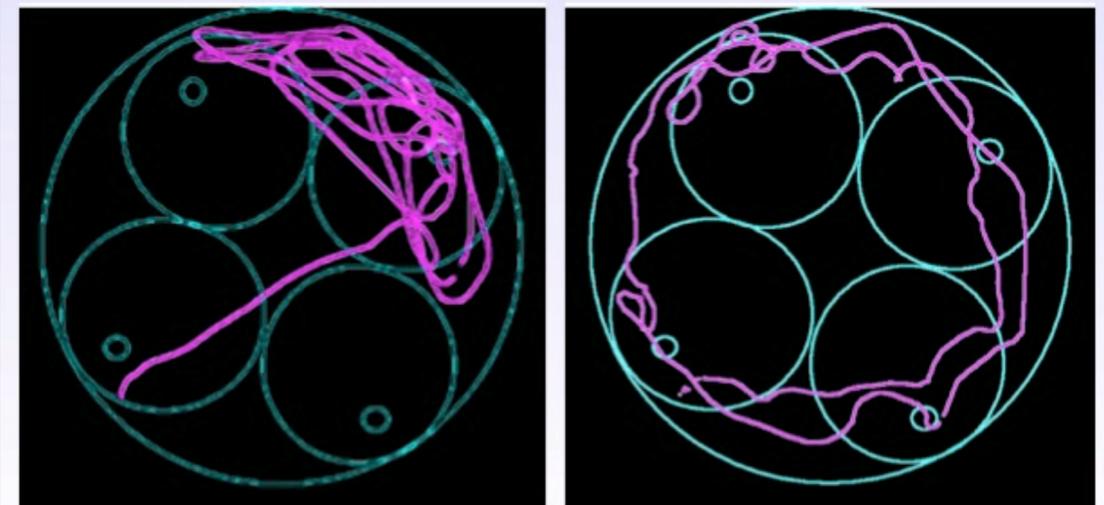
PROBE TRIAL



Duration of test: 60 sec

Variables:

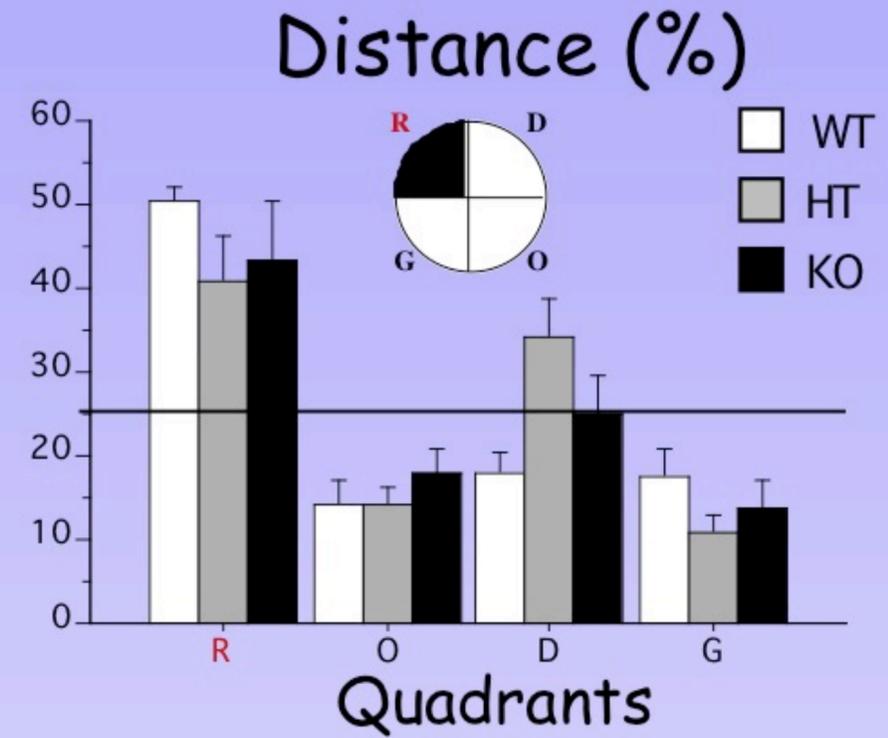
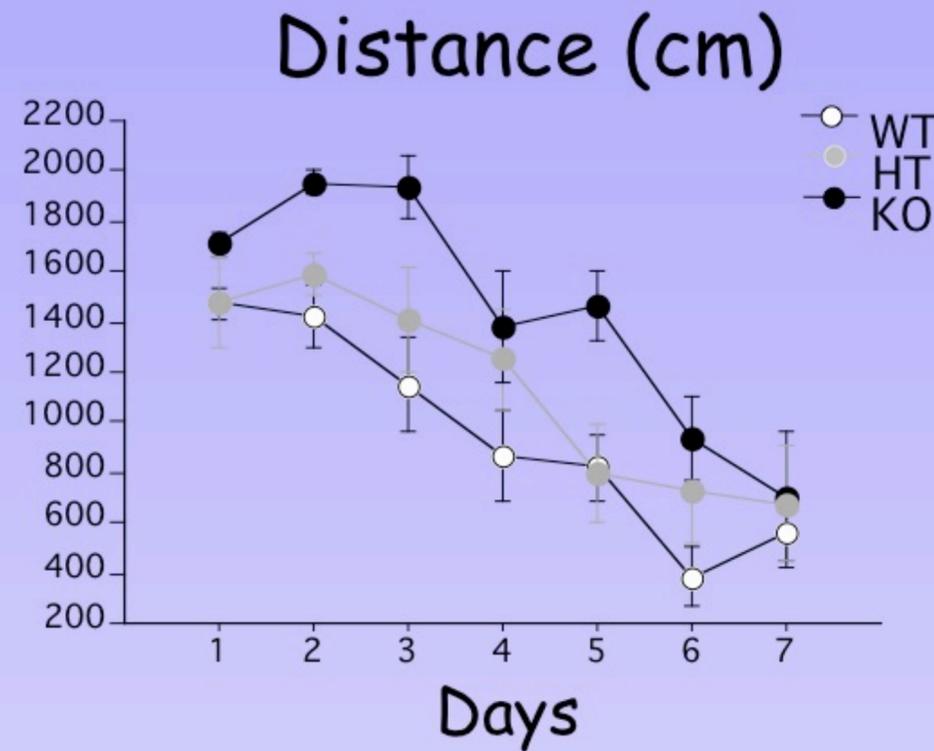
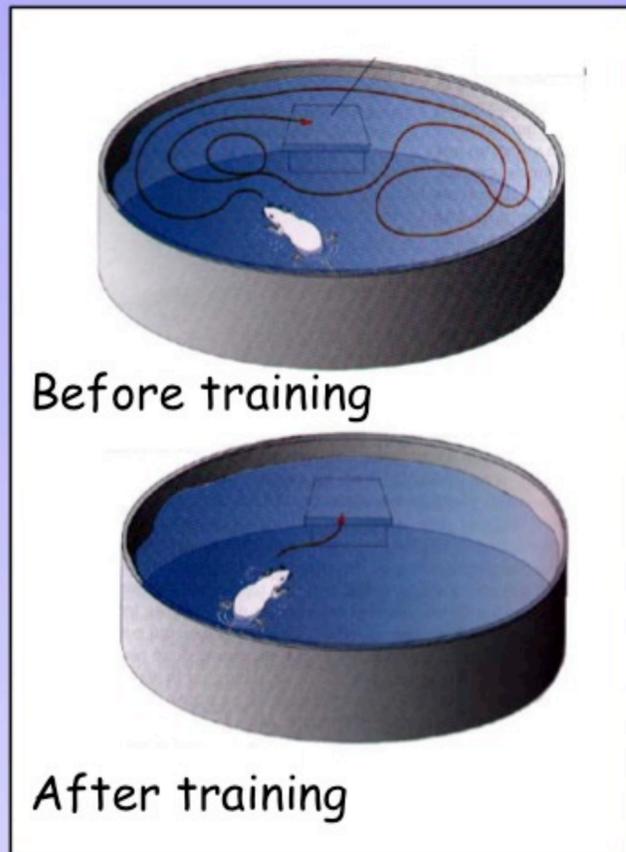
- Distance travelled (%) per quadrant
- Number of annulus crossings



Piscine de Morris (Morris Water Maze)

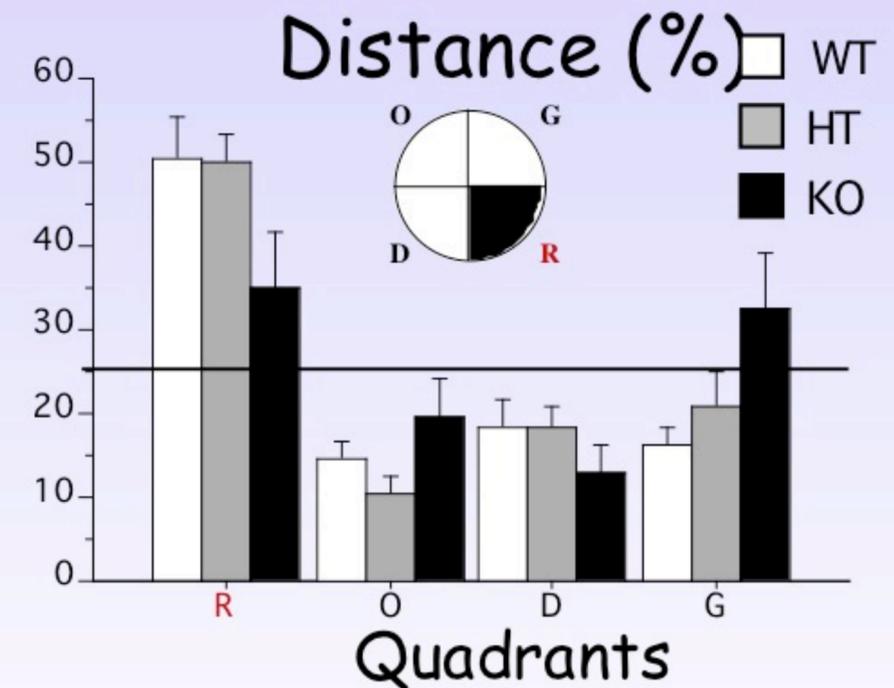
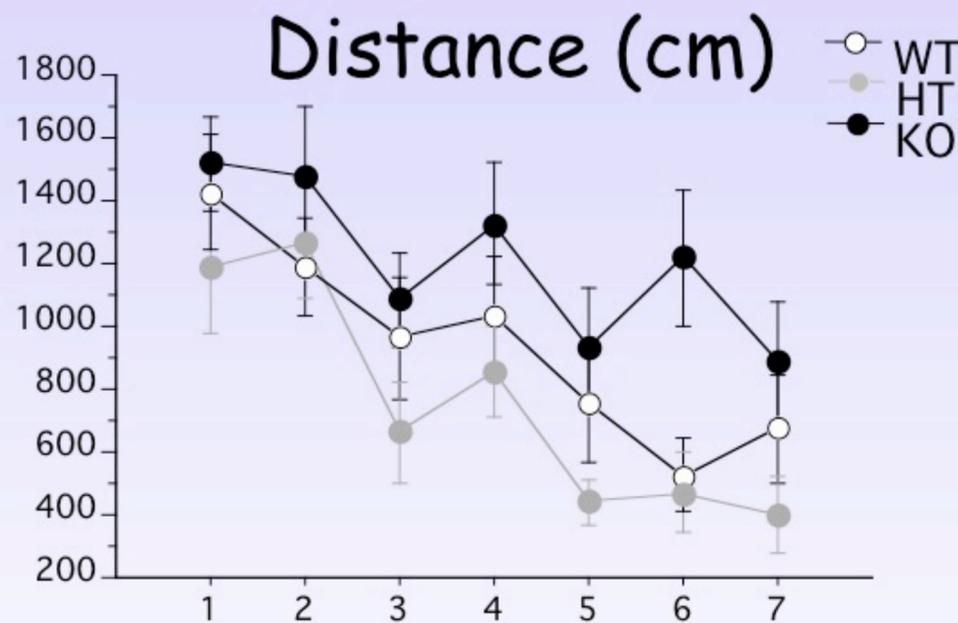
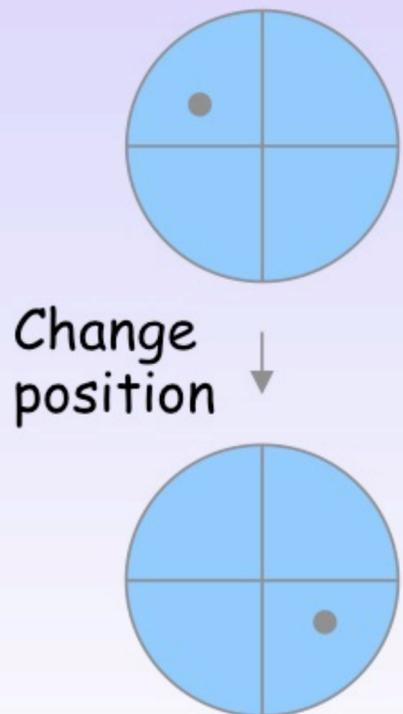


Spatial Learning and Memory



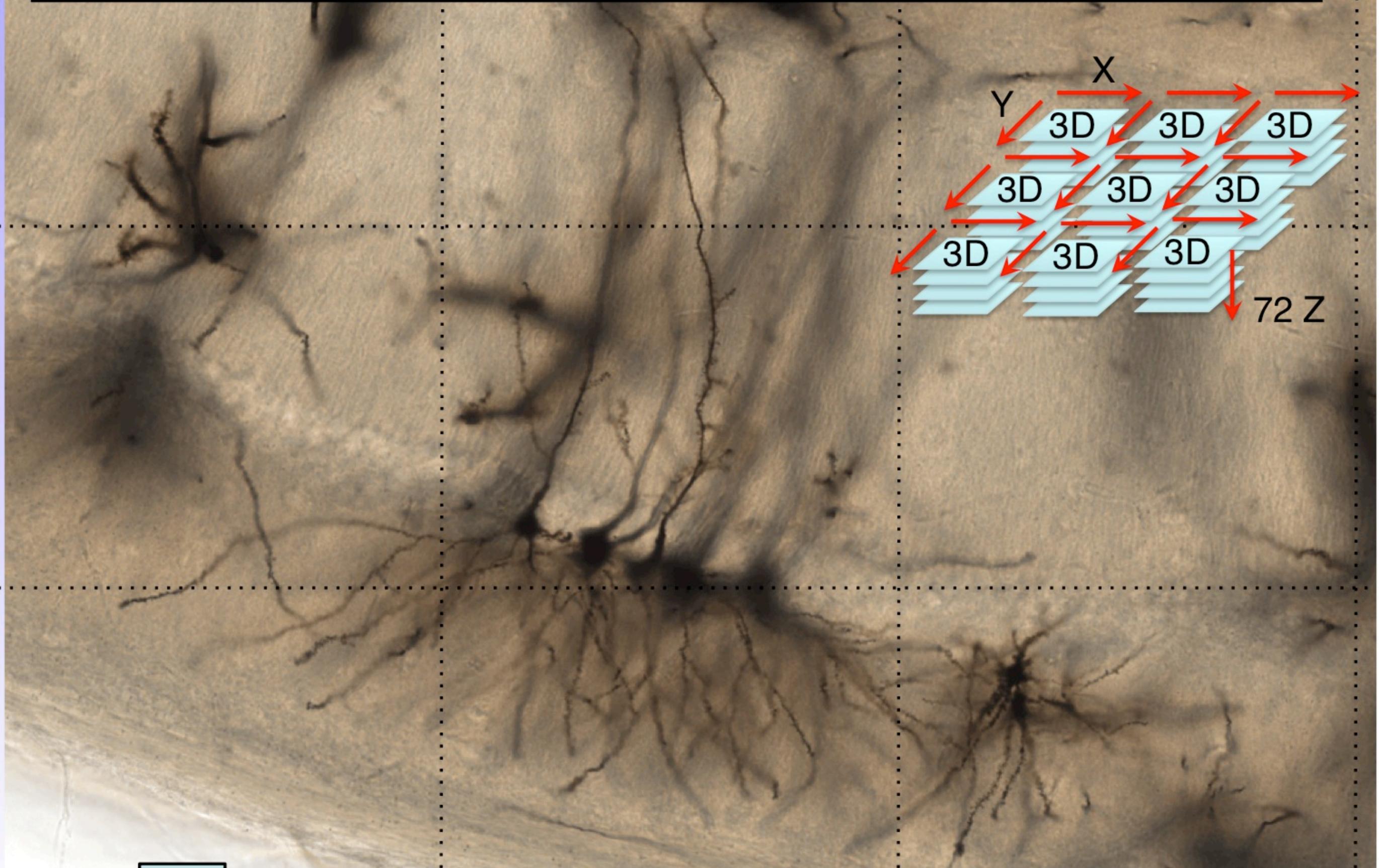
L'apprentissage est maintenu mais retardé chez le KO du transporteur de la DA.

Spatial Reversal Learning



L'apprentissage d'une nouvelle position est aléatoire: manque de flexibilité chez le KO.

What about spine morphology in TI-VAMP KO mice ?

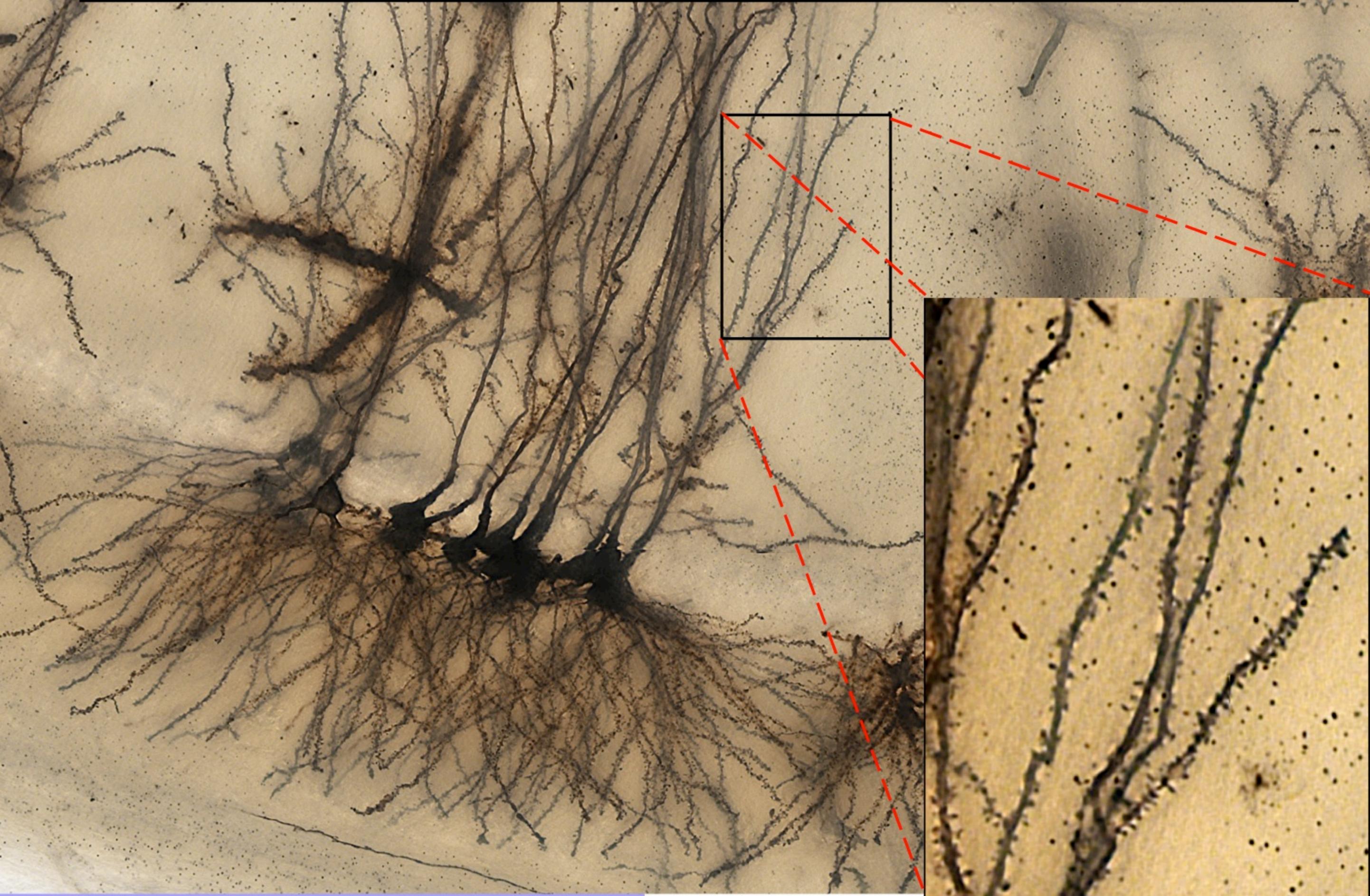


Movie

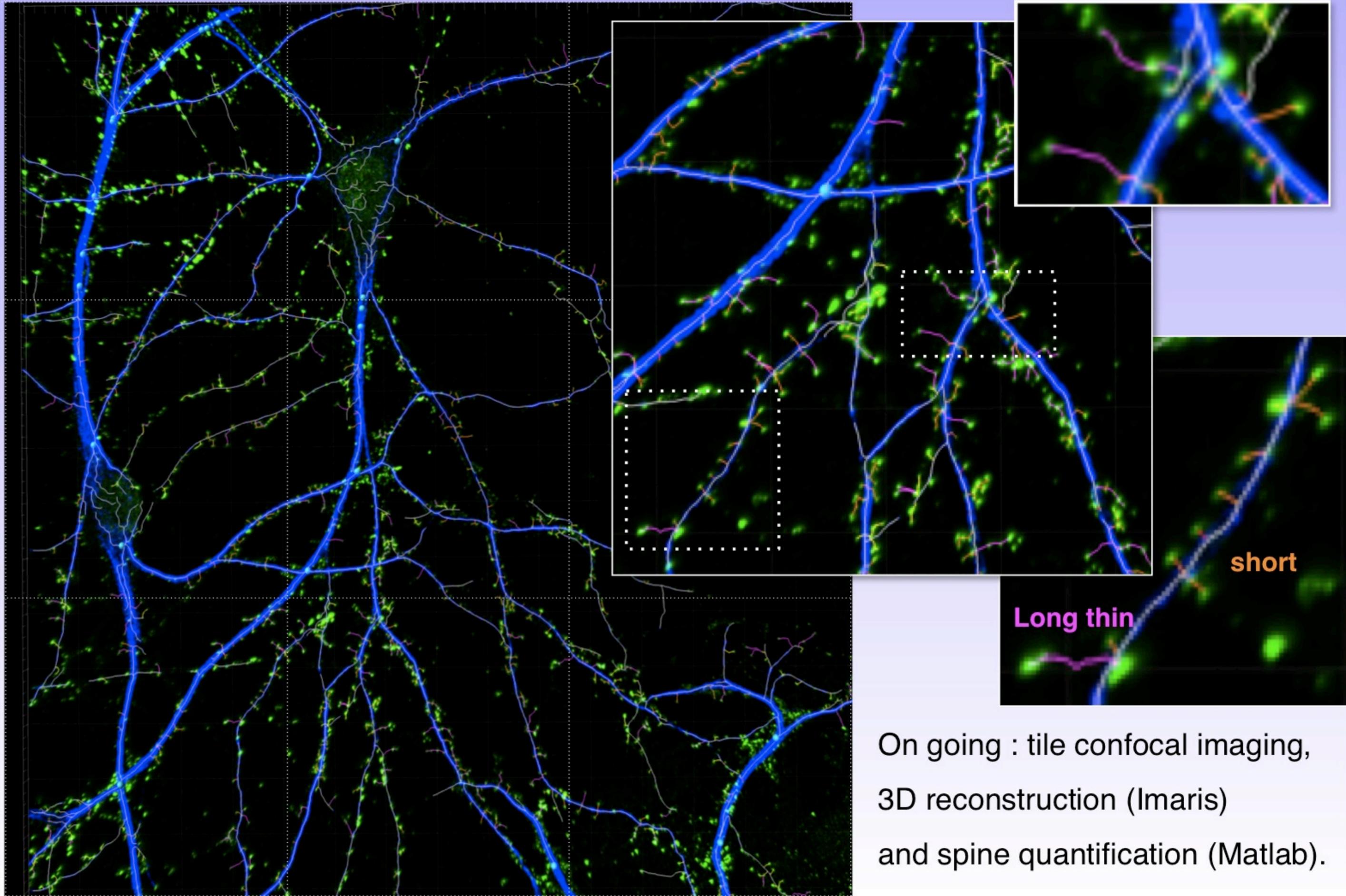


3D reconstruction of 9 Z-stacks of 72 tiled pictures taken at 40X

What about spine morphology in TI-VAMP KO mice ?

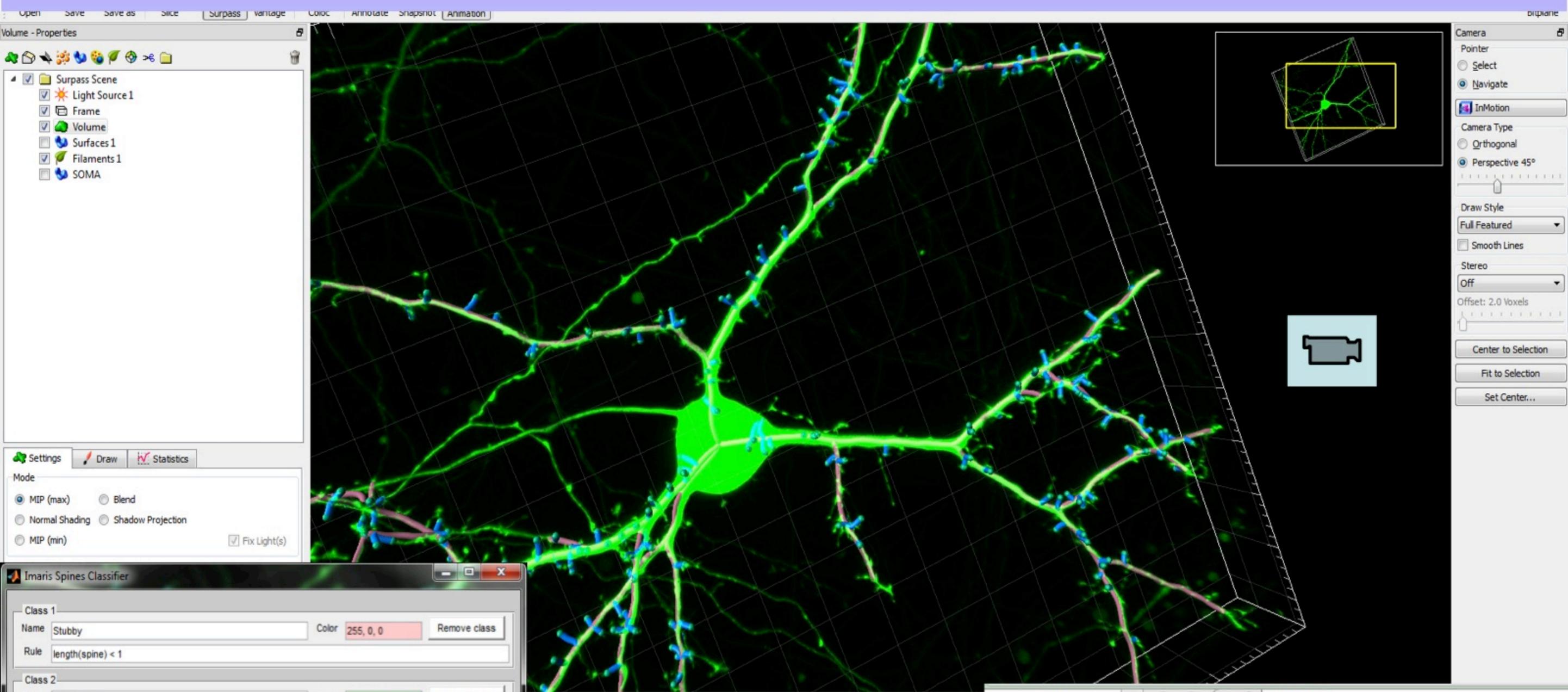


What about spine morphology in TI-VAMP KO mice ?



On going : tile confocal imaging,
3D reconstruction (Imaris)
and spine quantification (Matlab).

What about spine morphology (Imaris & Matlab spine classifier)



Imaris Spines Classifier

Class 1
 Name: Stubby
 Color: 255, 0, 0
 Rule: length(spine) < 1

Class 2
 Name: Mushroom
 Color: 0, 255, 0
 Rule: length(spine) < 3 and max_width(head) > mean_width(neck) * 2

Class 3
 Name: Long Thin
 Color: 0, 0, 255
 Rule: mean_width(head) >= mean_width(neck)

Class 4
 Name: Filopodia / Dendrite
 Color: 255, 0, 255
 Rule: true

Variables:
 spine, head, neck, ground, volume, length, mean_width, max_width, min_width

Create one Filament:
 ... for each class
 ... with custom stats
 Plot class statistics

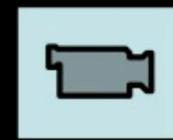
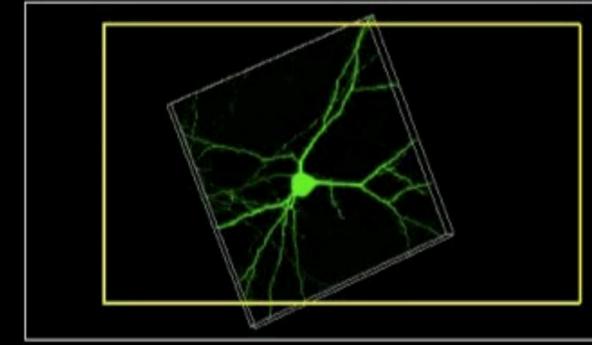
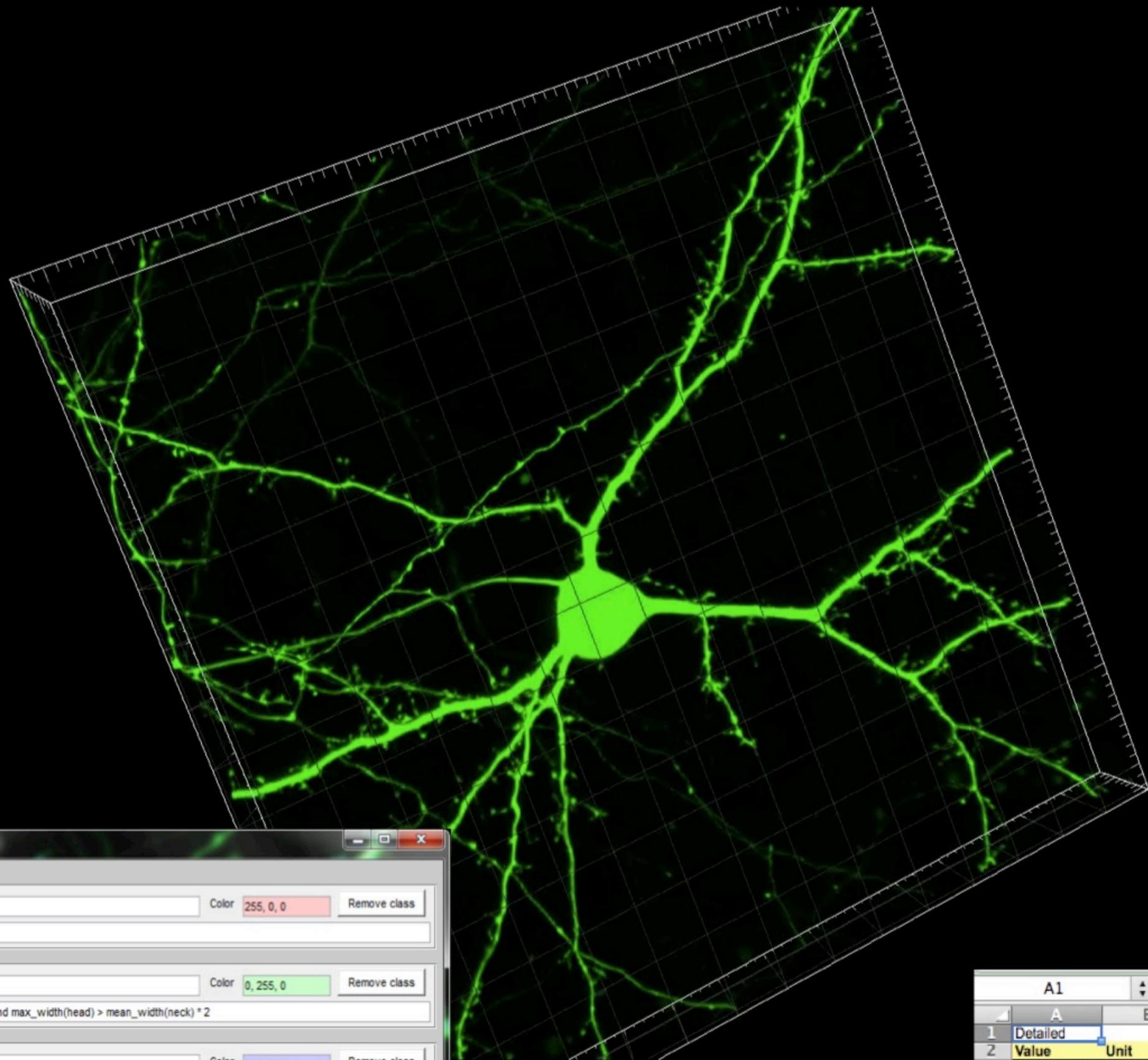
Head length (%) 25
 Ground length (%) 25

Add new class
 Classify spines



Excel file

	A1						
	A	B	C	D	E	F	G
1	Detailed						
2	Value	Unit	Category	Depth	Level	Surpass Obj	Time
3	0,92	um^2	Spine	21	3	Stubby	1
4	1,41	um^2	Spine	21	3	Long Thin	1
5	0,49	um^2	Spine	21	3	Stubby	1
6	3,21	um^2	Spine	21	3	Long Thin	1
7	0,22	um^2	Spine	20	2	Stubby	1
8	3,24	um^2	Spine	21	3	Long Thin	1
9	0,94	um^2	Spine	20	3	Stubby	1
10	1,92	um^2	Spine	21	3	Long Thin	1
11	0,35	um^2	Spine	20	3	Stubby	1
12	1,16	um^2	Spine	21	3	Long Thin	1
13	1,02	um^2	Spine	20	3	Stubby	1
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Imaris Spines Classifier

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11	0,35	um^2	Spine	20	3	Stubby	1
12	1,16	um^2	Spine	21	3	Long Thin	1
13	1,02	um^2	Spine	20	3	Stubby	1
14	2,30	um^2	Spine	20	2	Long Thin	1
15	0,77	um^2	Spine	21	3	Stubby	1

Trafic, exocytose et modèles physiopathologiques

1. Exocytose et complexe SNARE

Les voies d'exocytose régulée
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Découverte de NSF et SNAP
 Isolement des SNARE
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3. Le cycle des vésicules synaptiques

Voie lente: endocytose médiée par la clathrine
 Voie courte: kiss and run
 Les différents « pool » vésiculaires

4. Comment mesurer l'exocytose ?

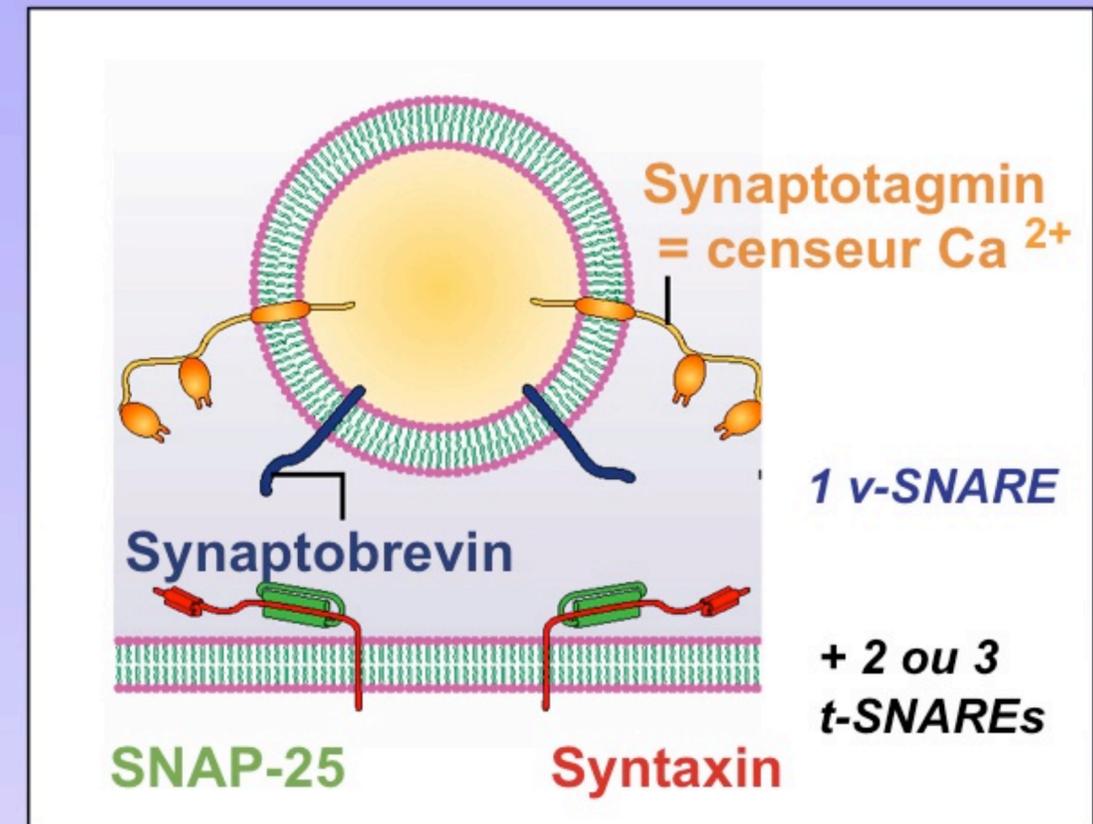
Capacitance
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 GFP pH sensible: la Phluorin
 Utilisation des SNARE inversée

5. Comment mesurer le recyclage ?

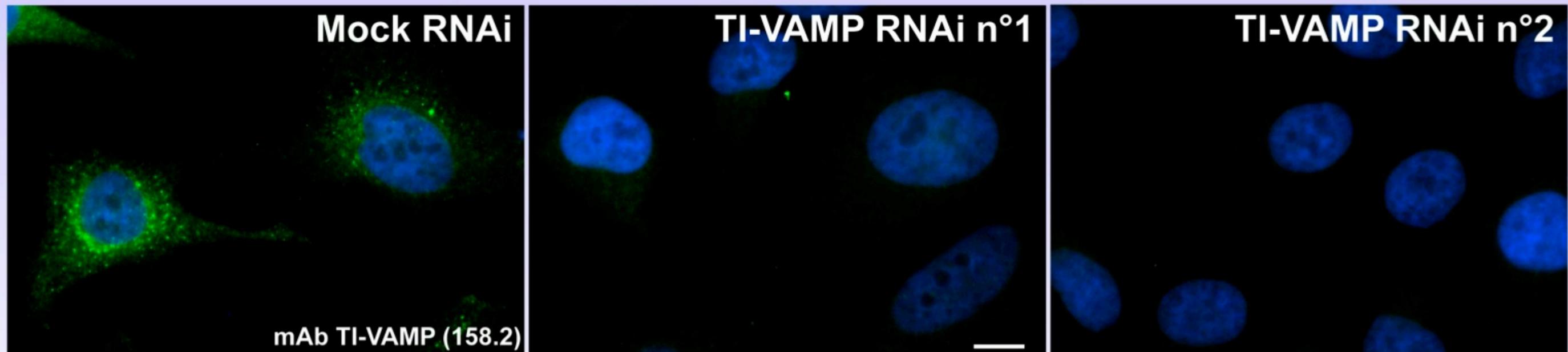
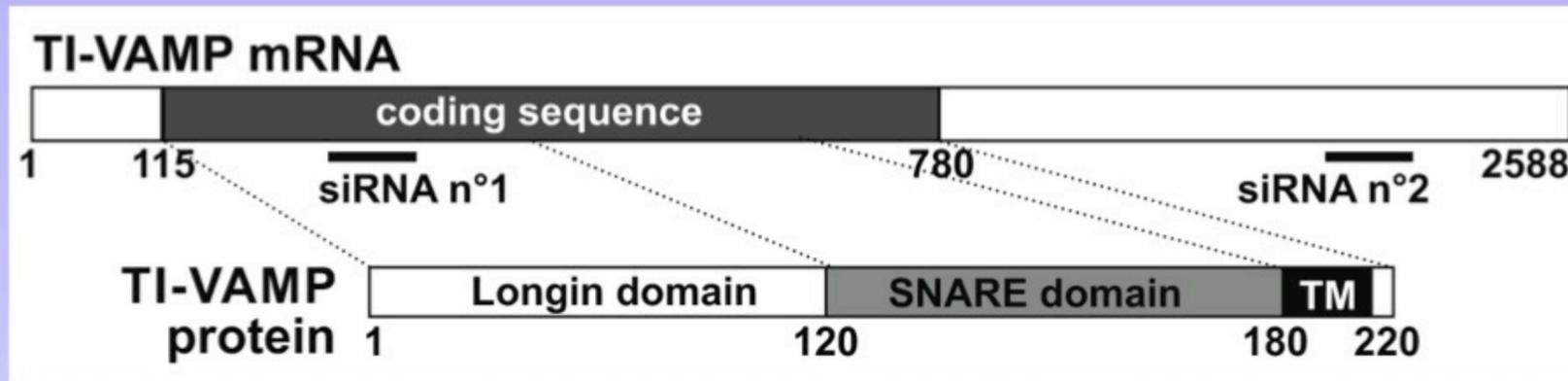
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 Rôles de Stx3 et SytVII dans la croissance neuritique
 Mutants de l'adaptateur AP-3: les mocha
 KO TI-VAMP
 Rôles de TI-VAMP dans l'exocytose et la diffusion des récepteurs

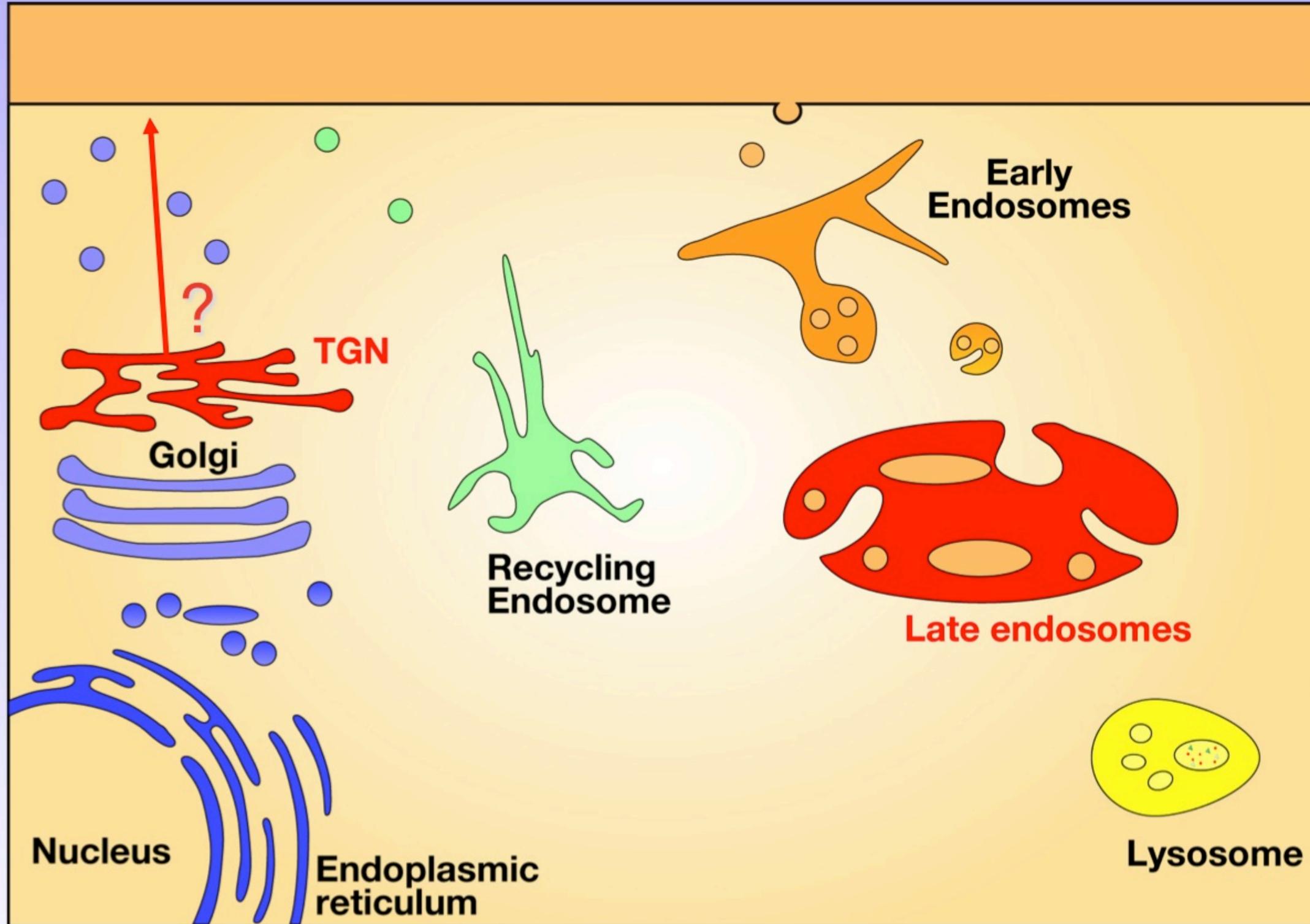


Extinction of vSNARE by RNAi

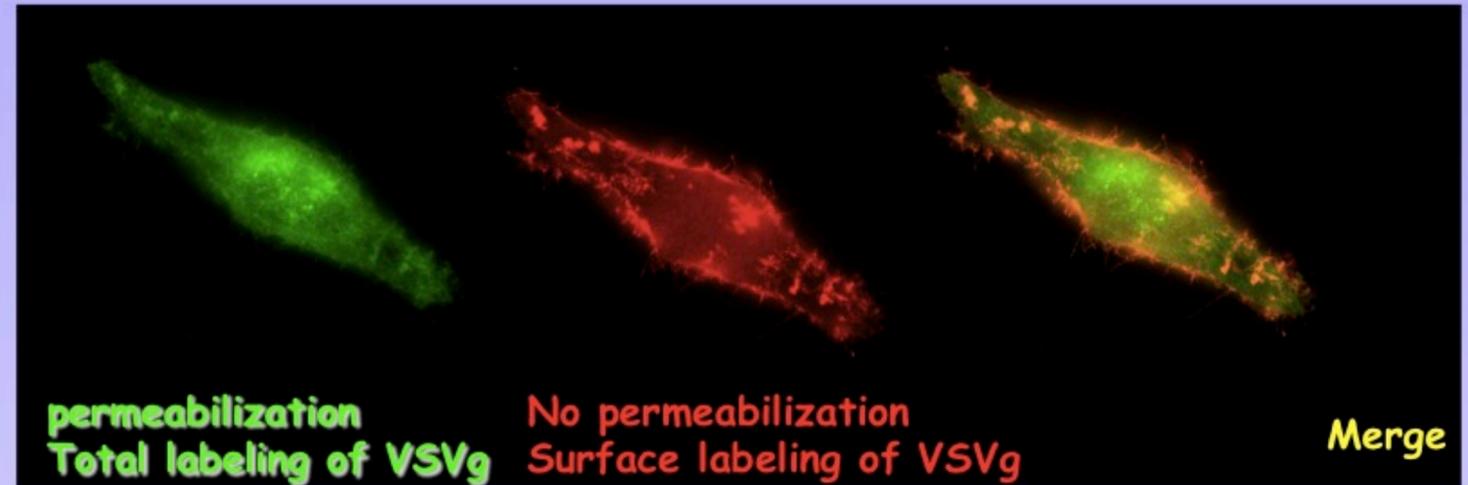
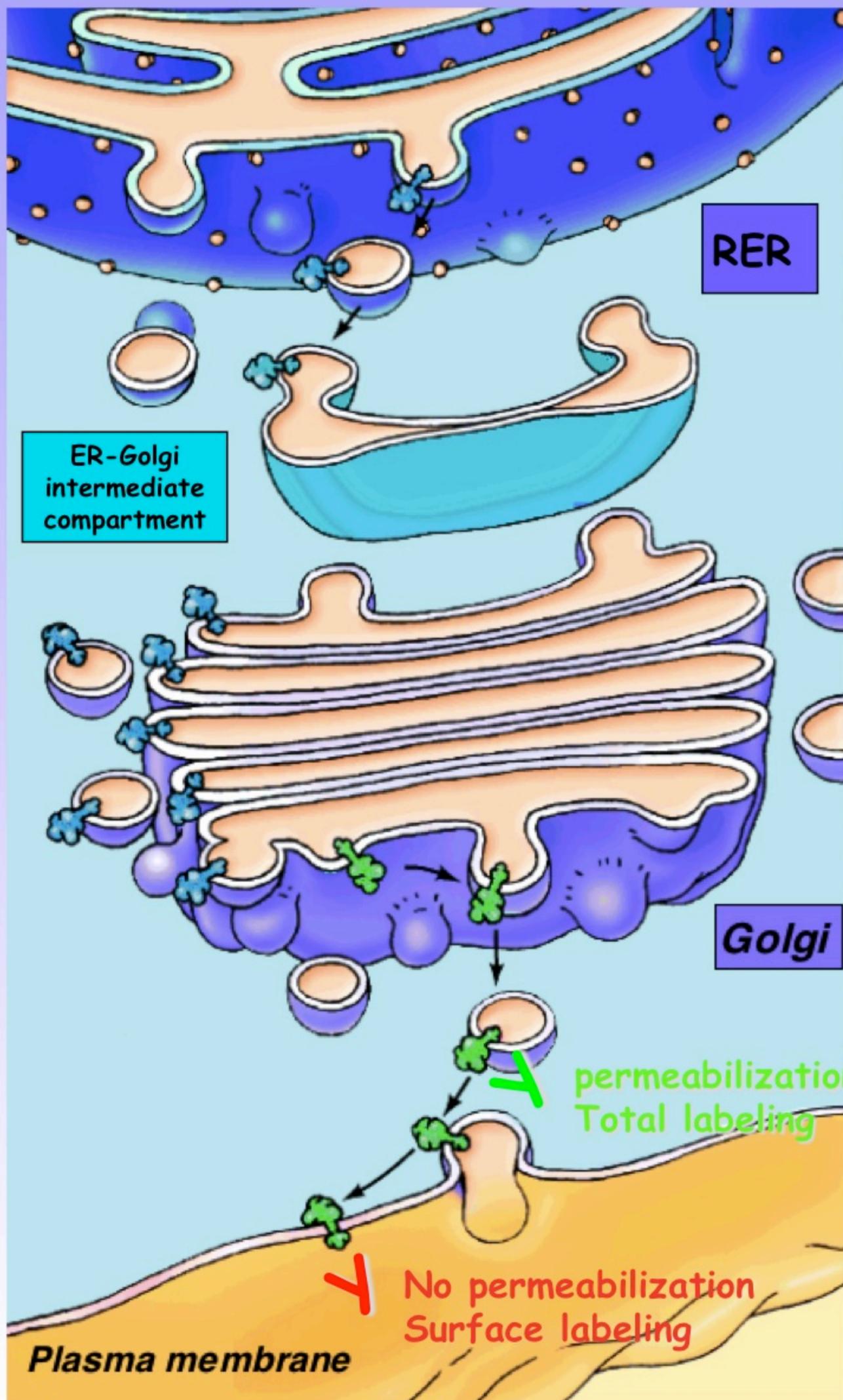


→ The extinction is very efficient (between 97 & 99 % with 2 oligos).

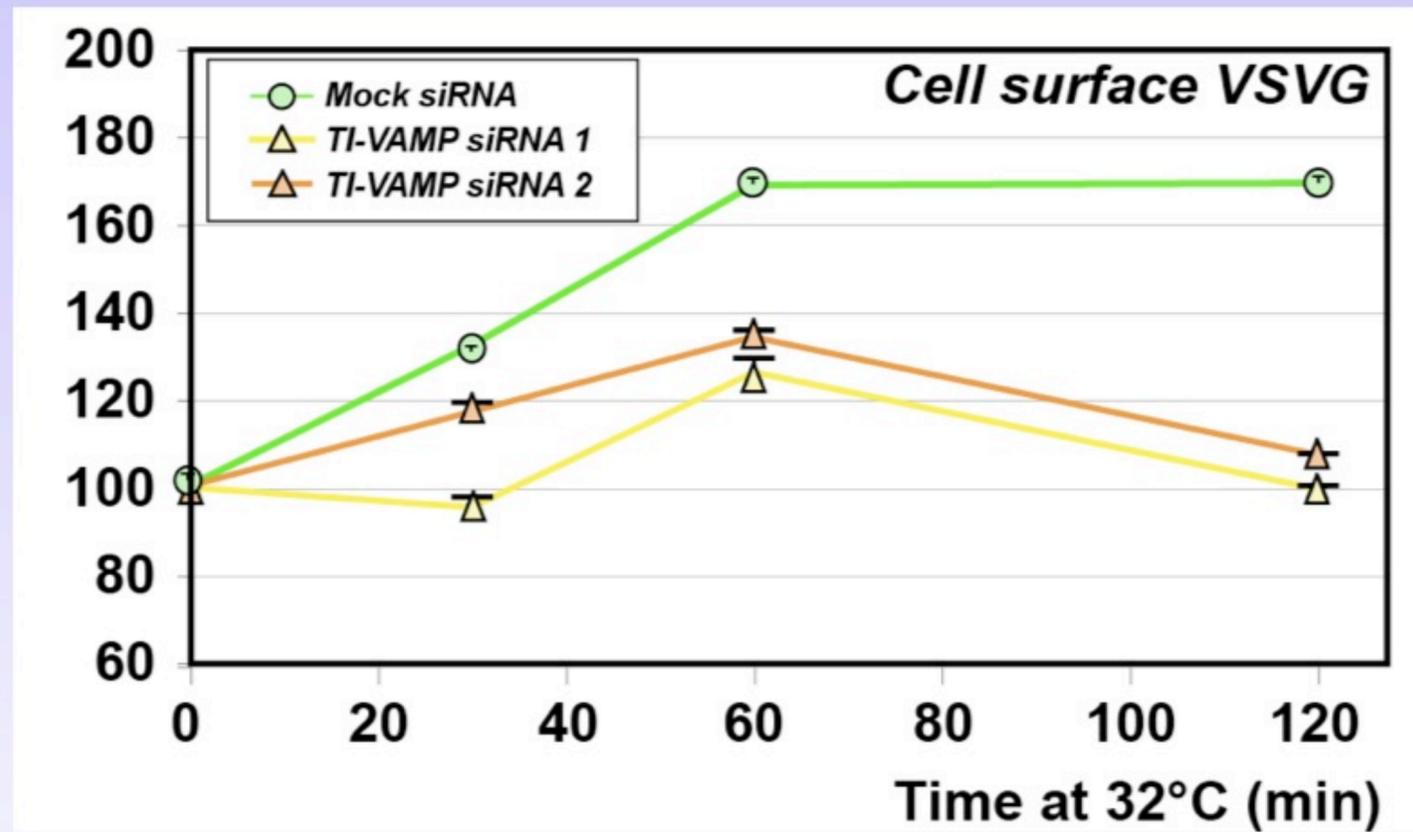
Role de TI-VAMP dans la voie exocytique ?



Role on exocytosis



Selection of the transfected cells by facs (green)
Quantification of intensity of VSVG (red) at the cell surface

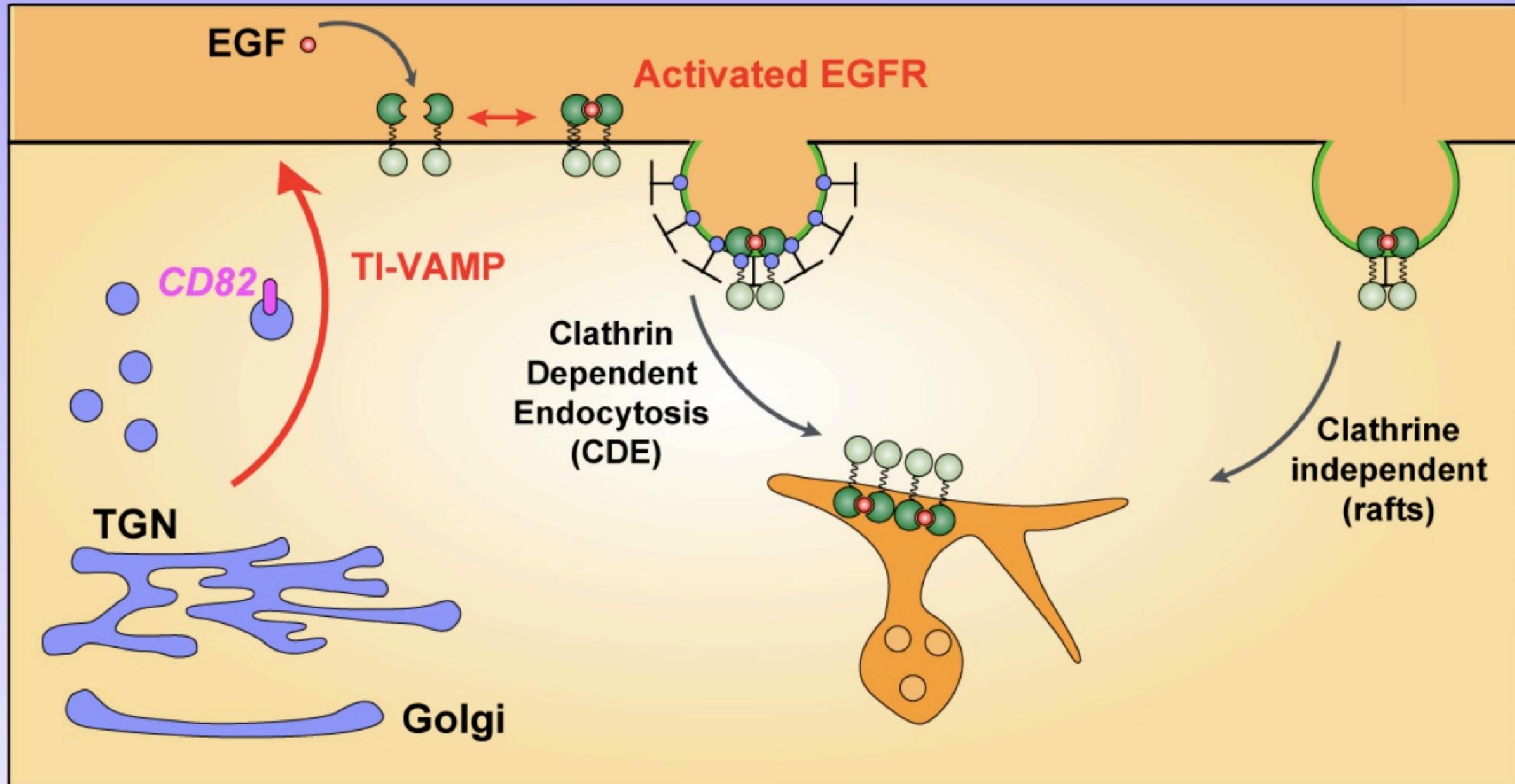


→ **TI-VAMP assure l'exocytose entre l'appareil de Golgi et la membrane.**

Role de TI-VAMP sur la dynamique de l'EGFR

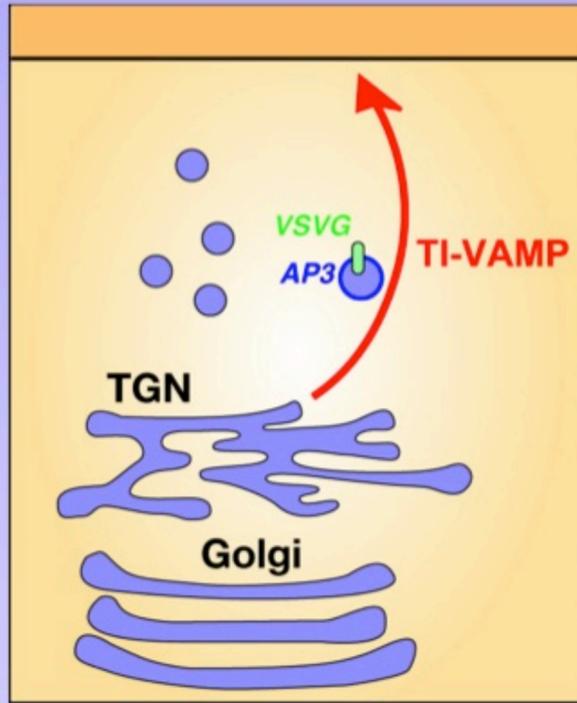
Danglot et al., (2010)

Insight on <http://www.cnrs.fr/insb/>

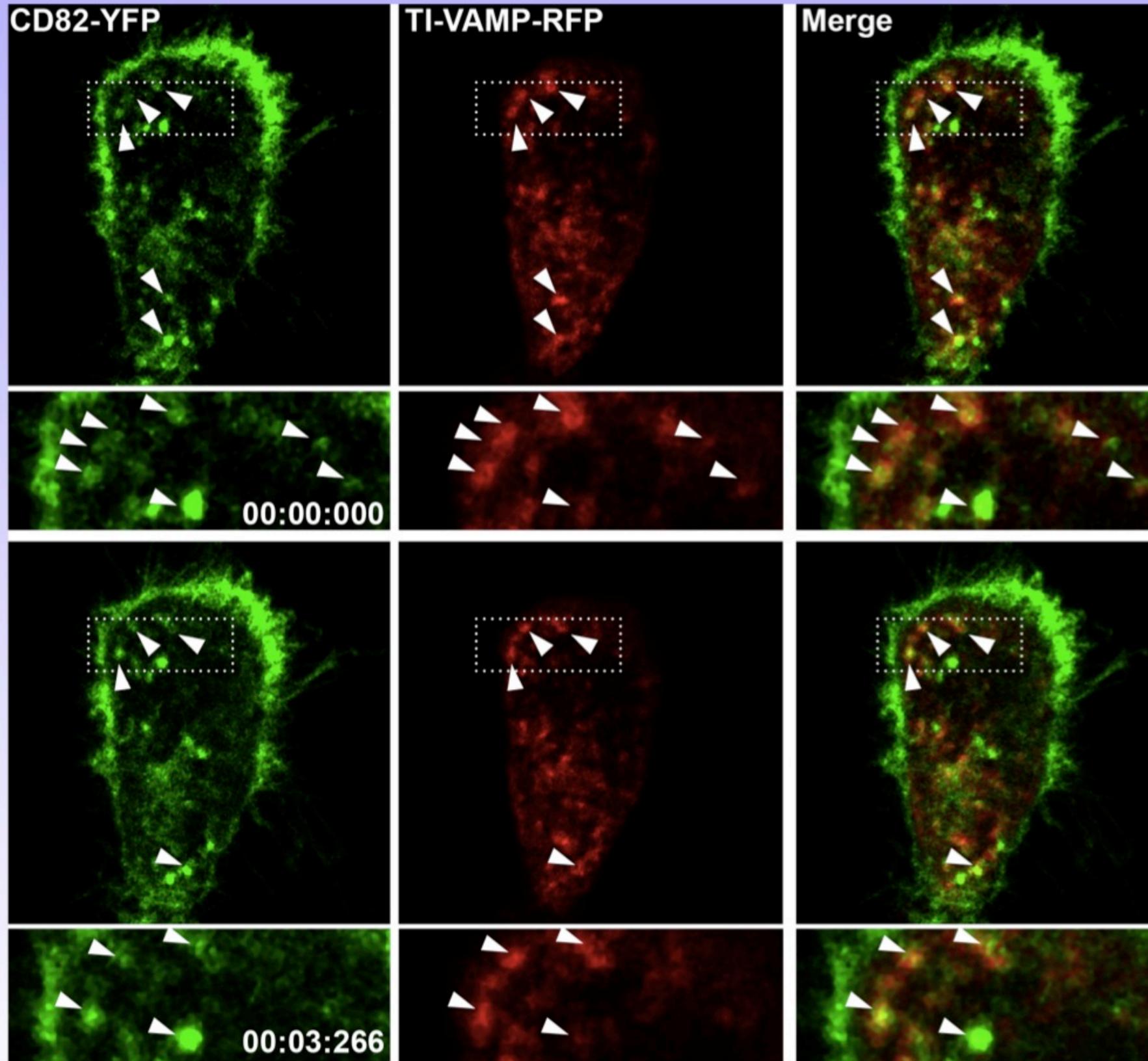


- Role of TI-VAMP in Golgi > plasma membrane transport

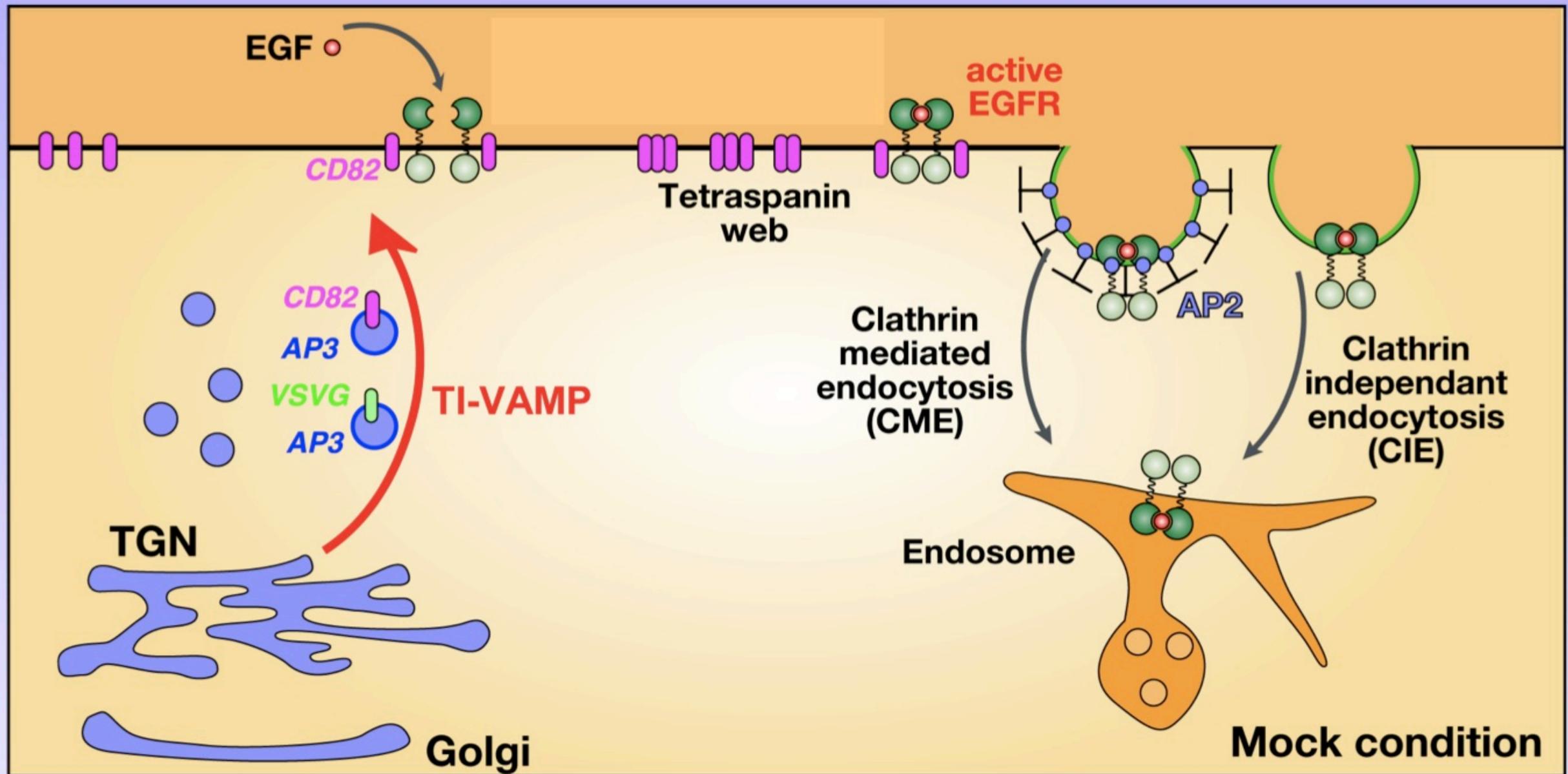
Transport de tetraspanine a la membrane plasmique ?



Ultra fast sequential confocal video-microscopy on SP5

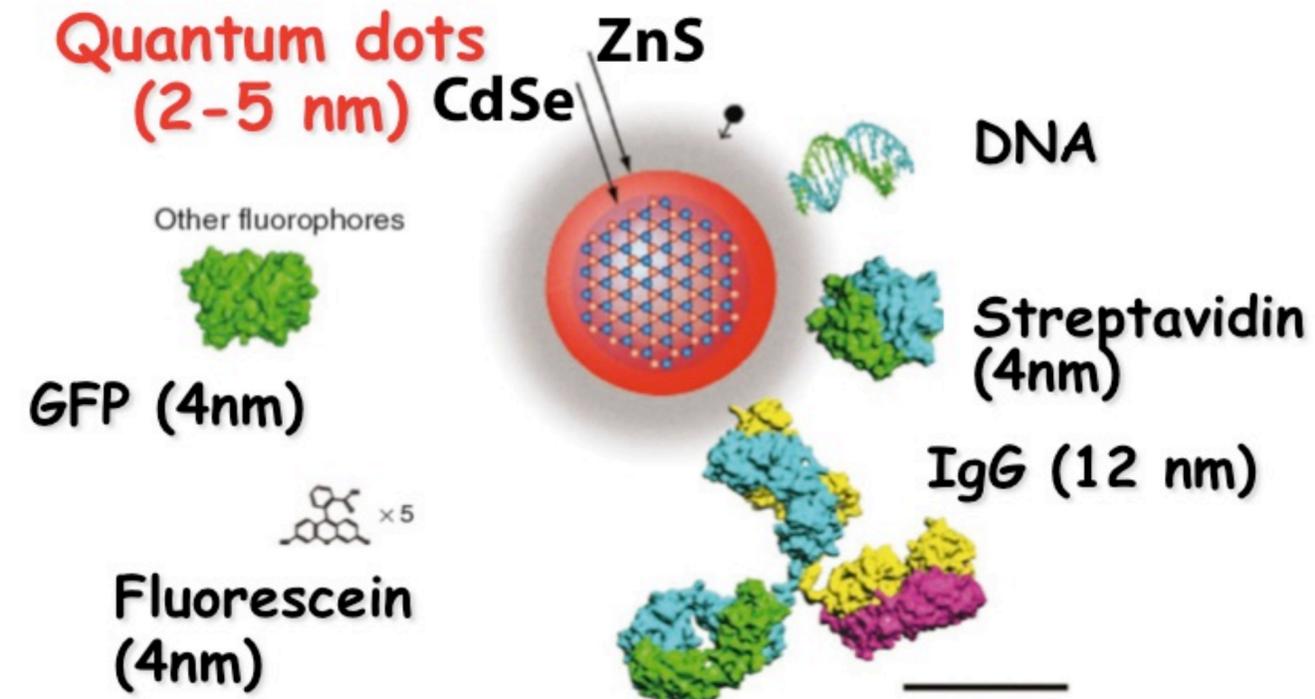
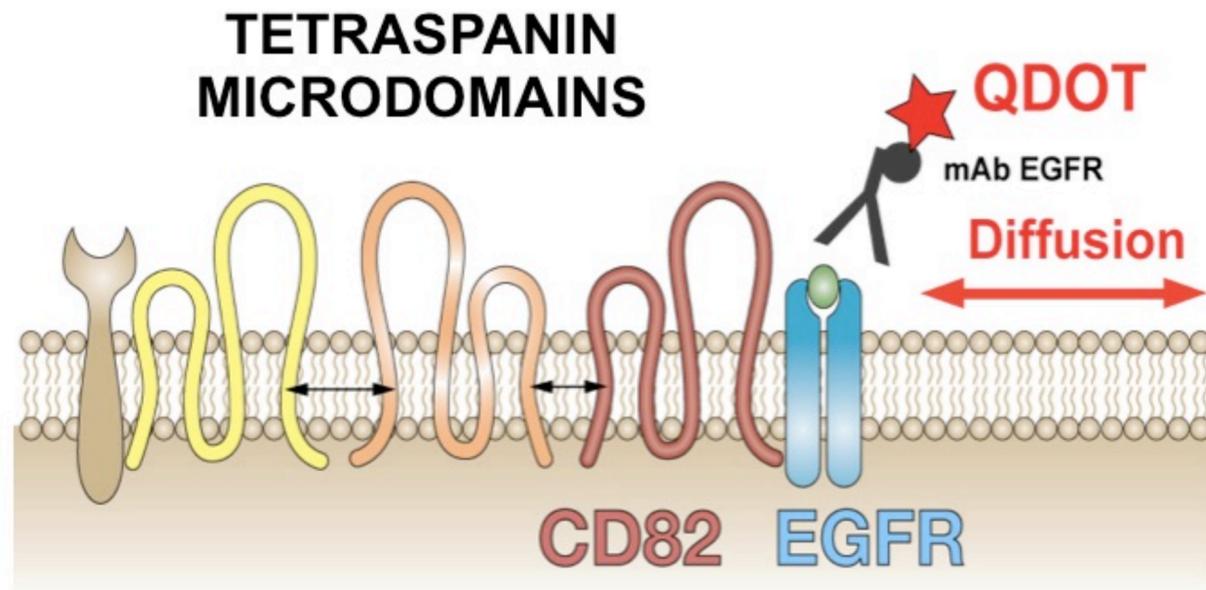


→ **CD82 est transporté dans les vésicules TI-VAMP**



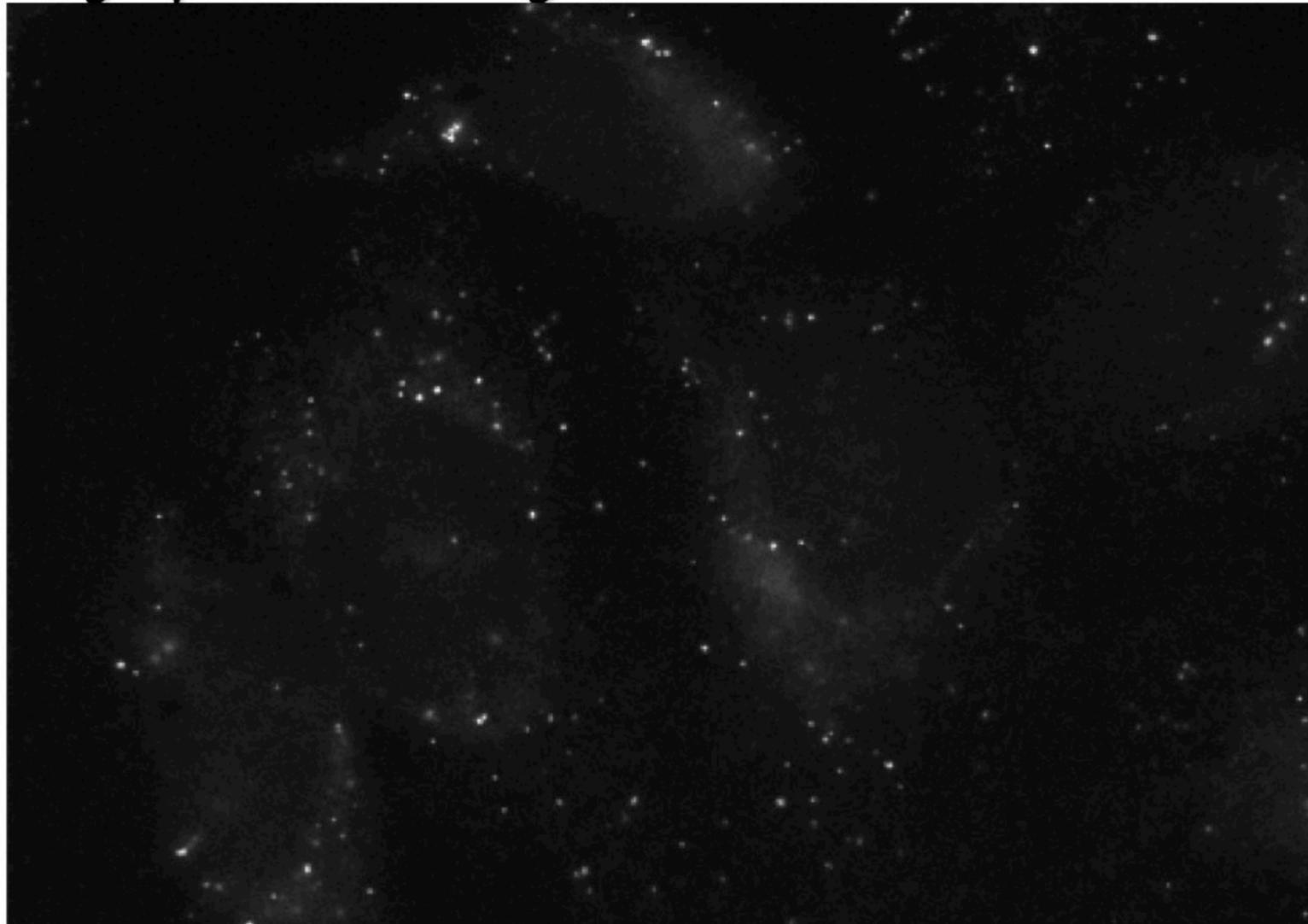
Est ce que la diffusion de l'EGFR est altéré par le défaut de CD82 ?

Single particle tracking of EGFR by quantum dots



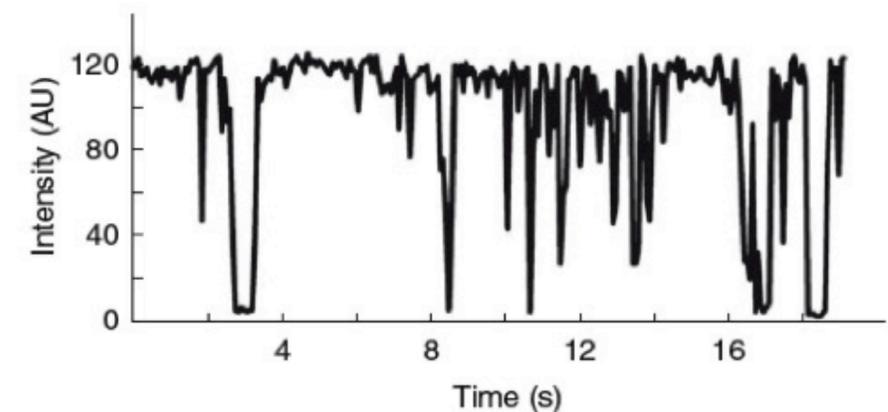
Adapted from Nat. Meth. 2010, 7:275.

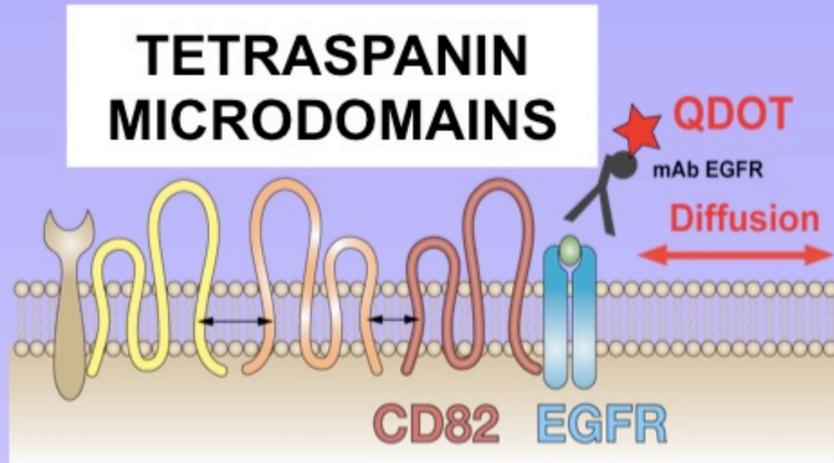
Single particle tracking of EGFR on fixed cells



Fluorophores	Photostability
CY3	5 s
GFP	100 ms
QDOT	> 20 min

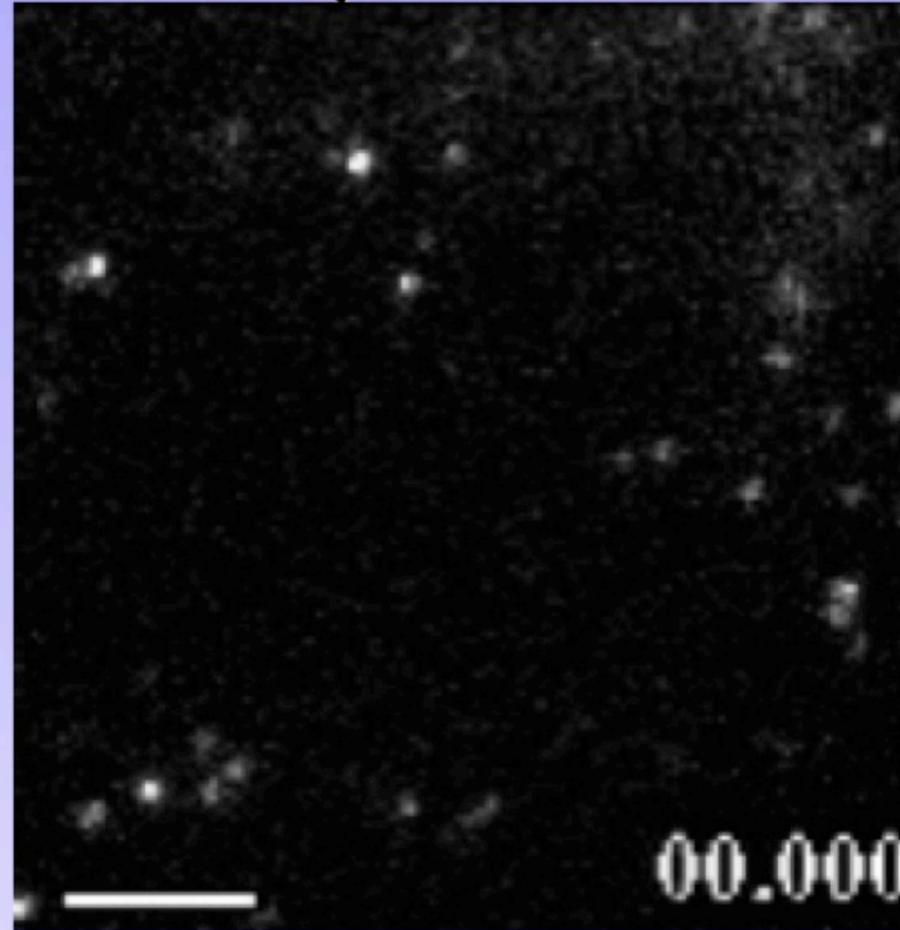
Blinking effect of quantum dots



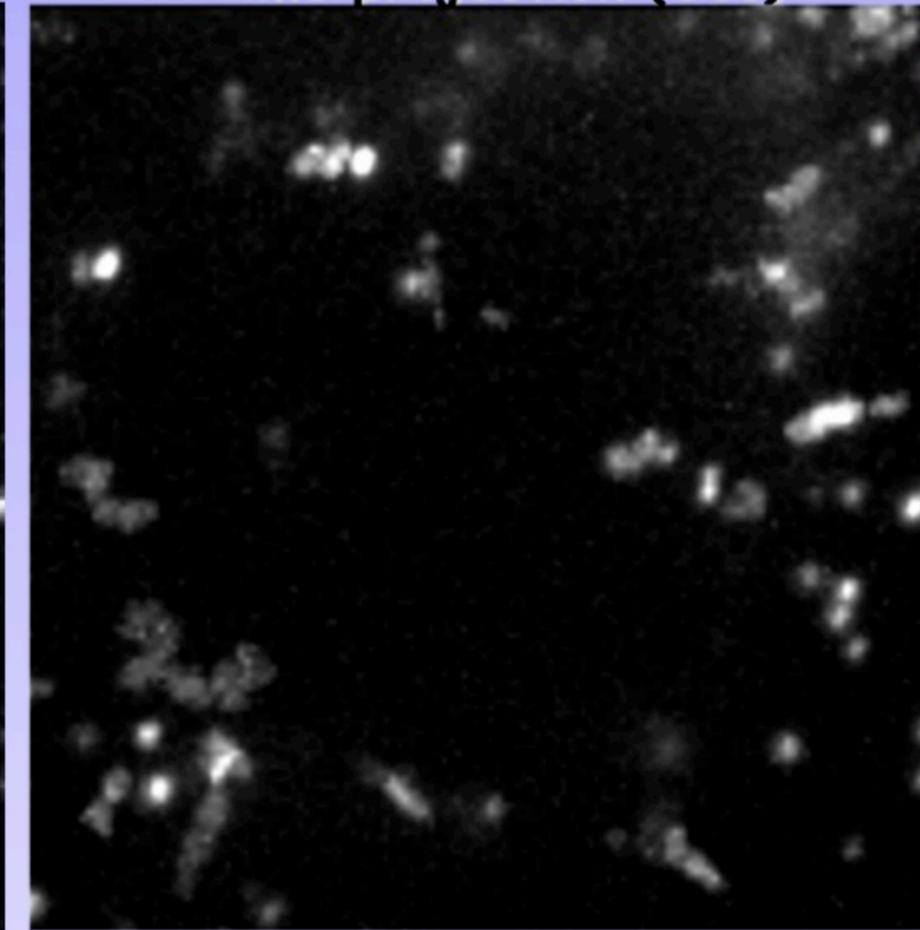


Single particle tracking

EGFR-QDOT 605 movie



Time projection (30s)

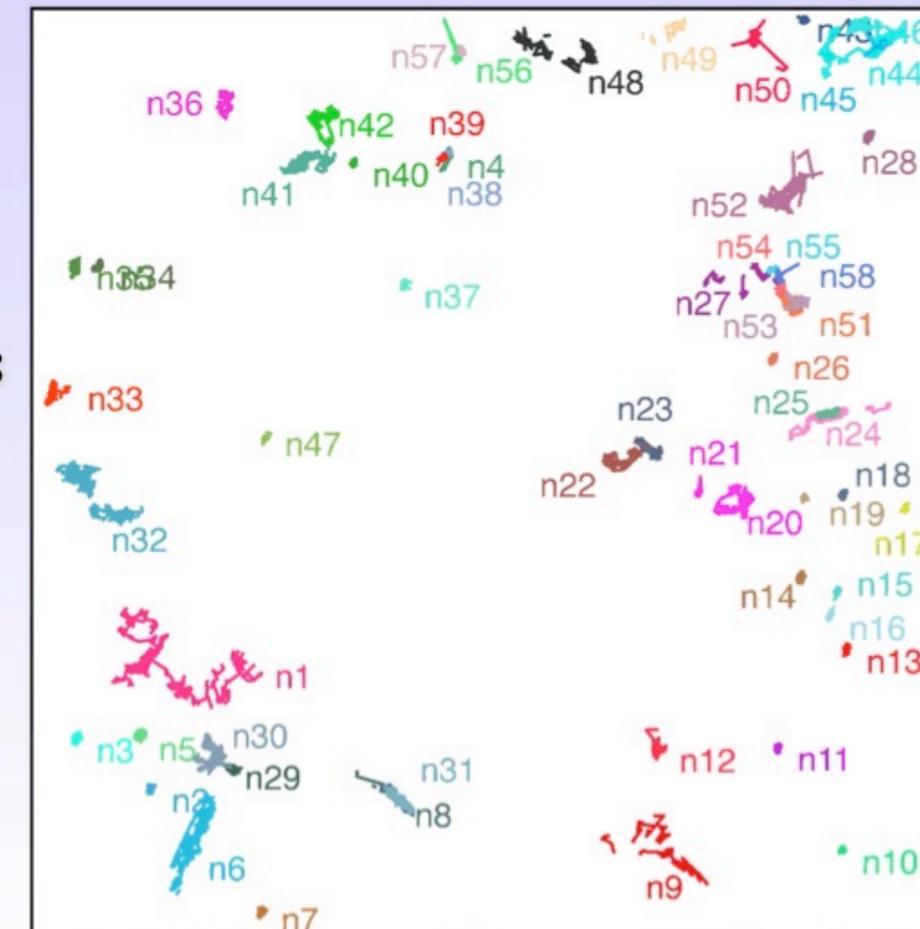


MATLAB

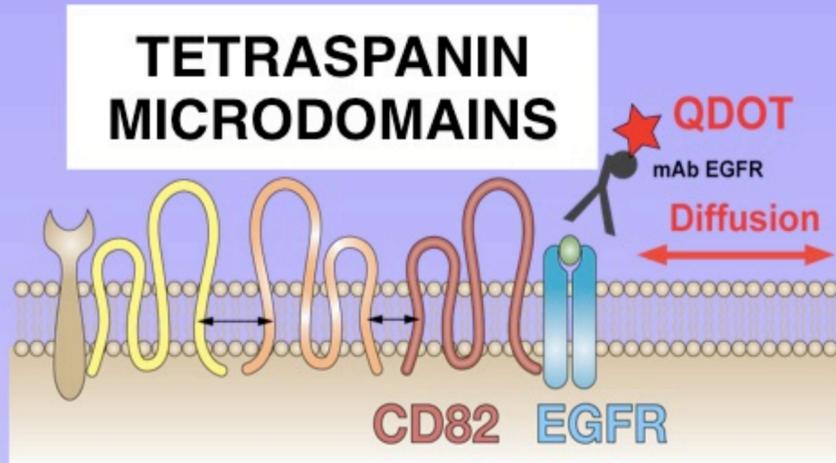
Mean Square
Displacement

Trajectories

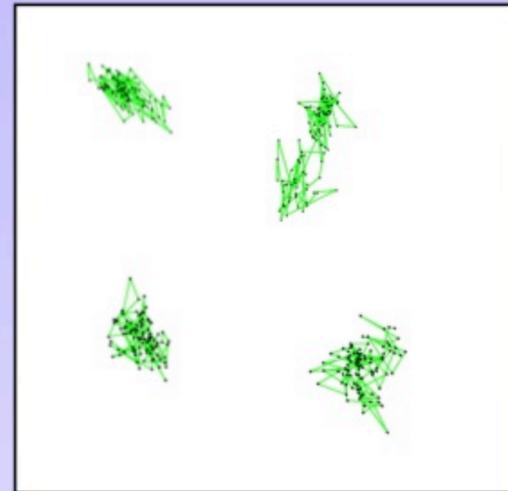
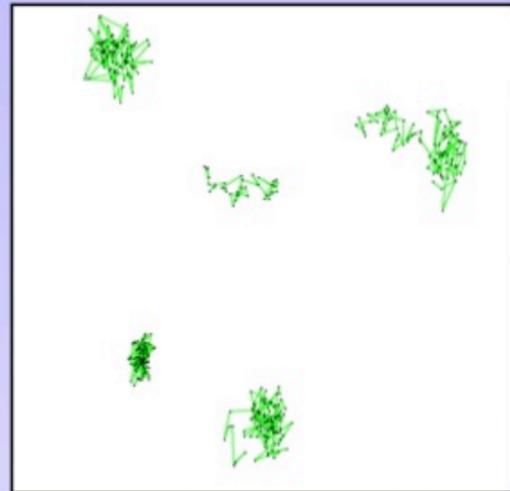
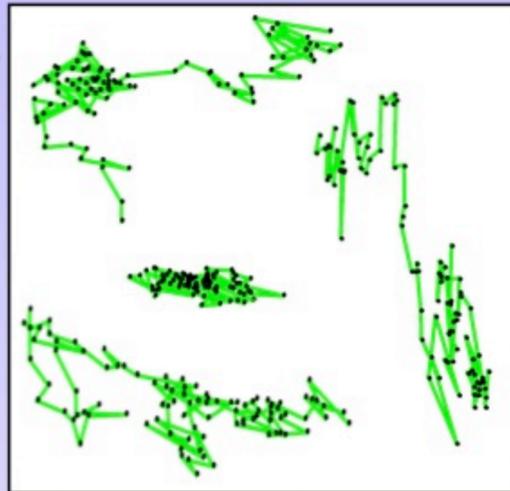
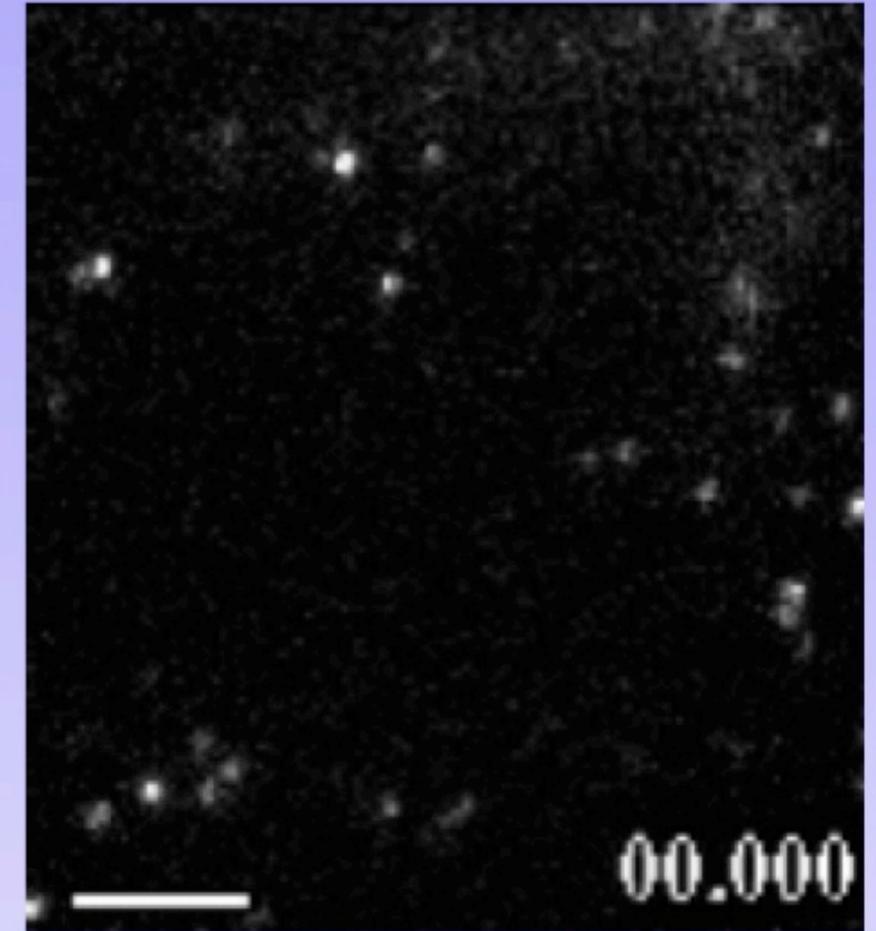
Diffusion coefficient
($\mu\text{m}^2/\text{s}$)



La diffusion de l'EGFR est elle altérée ?



Single particle tracking by video-microscopy (QDOT)

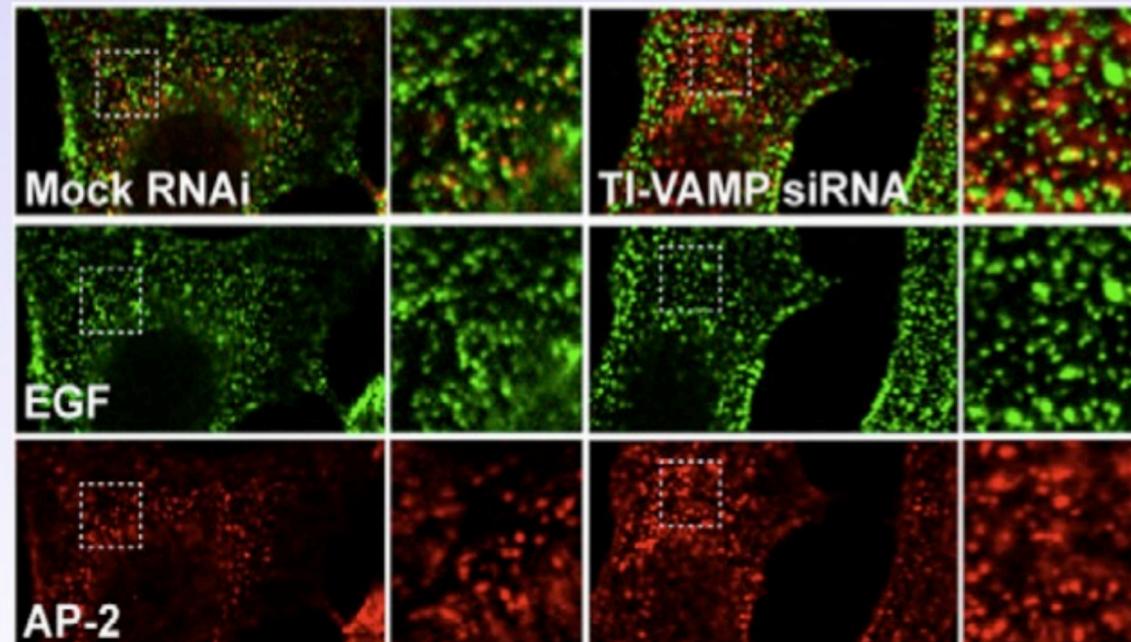
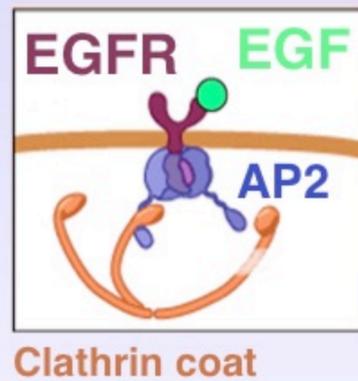
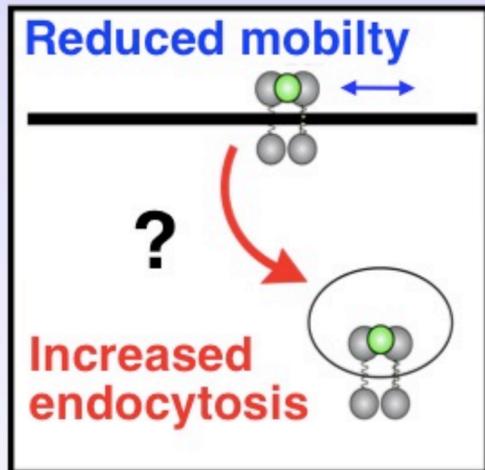


Mock siRNA

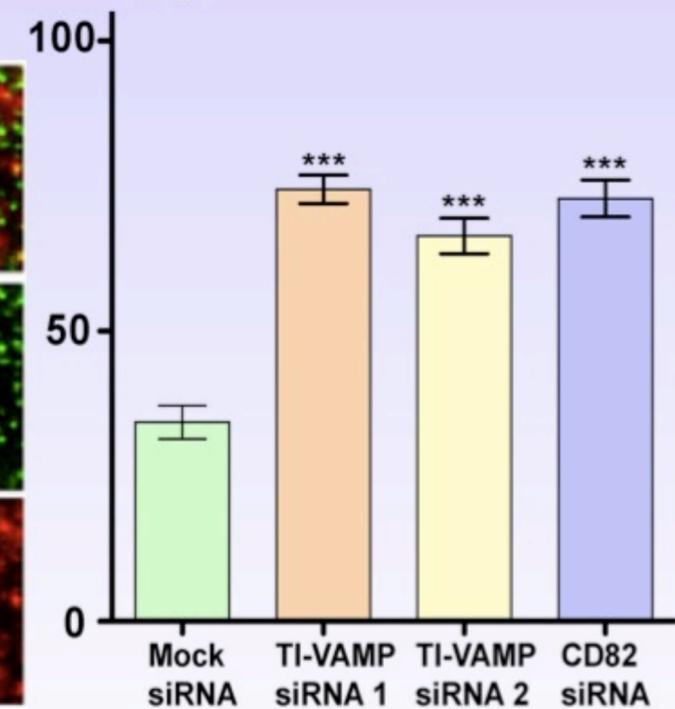
TI-VAMP siRNA

CD82 siRNA

→ Le KD de TI-VAMP ou CD82 diminue la diffusion de l'EGFR....



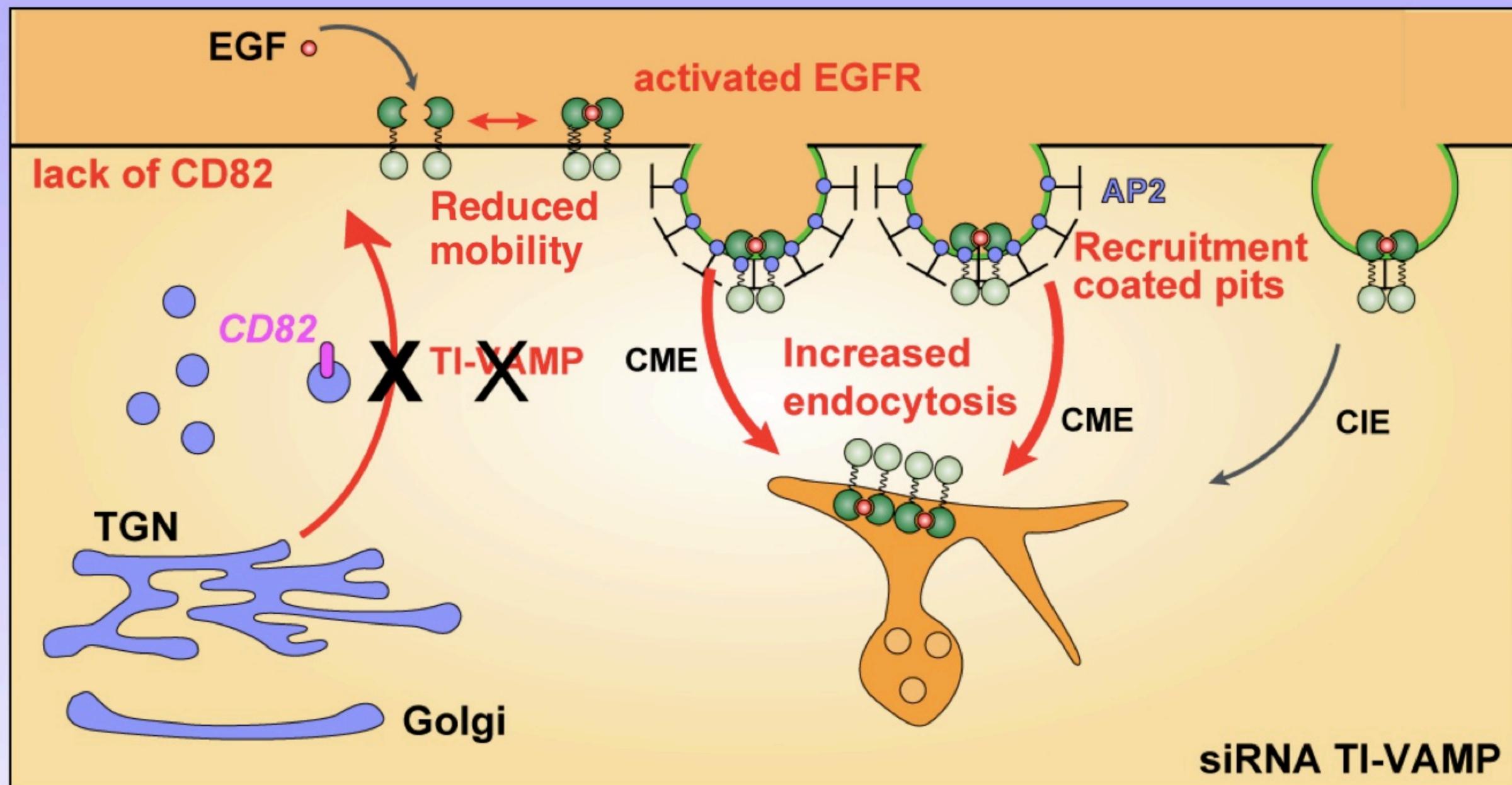
Apposition AP2-EGFR



... et favorise son endocytose par la machinerie AP2

Role de TI-VAMP sur la dynamique de l'EGFR

Danglot et al., (2010)

 Insight on <http://www.cnrs.fr/insb/>


- Role de TI-VAMP sur le transport Golgi > plasma membrane transport
- En absence de TI-VAMP :
 - le transport Golgi > membrane diminue
 - Diminution de CD82 en surface
 - Recrutement accru d'AP2
 - Endocytose de l'EGFR accrue

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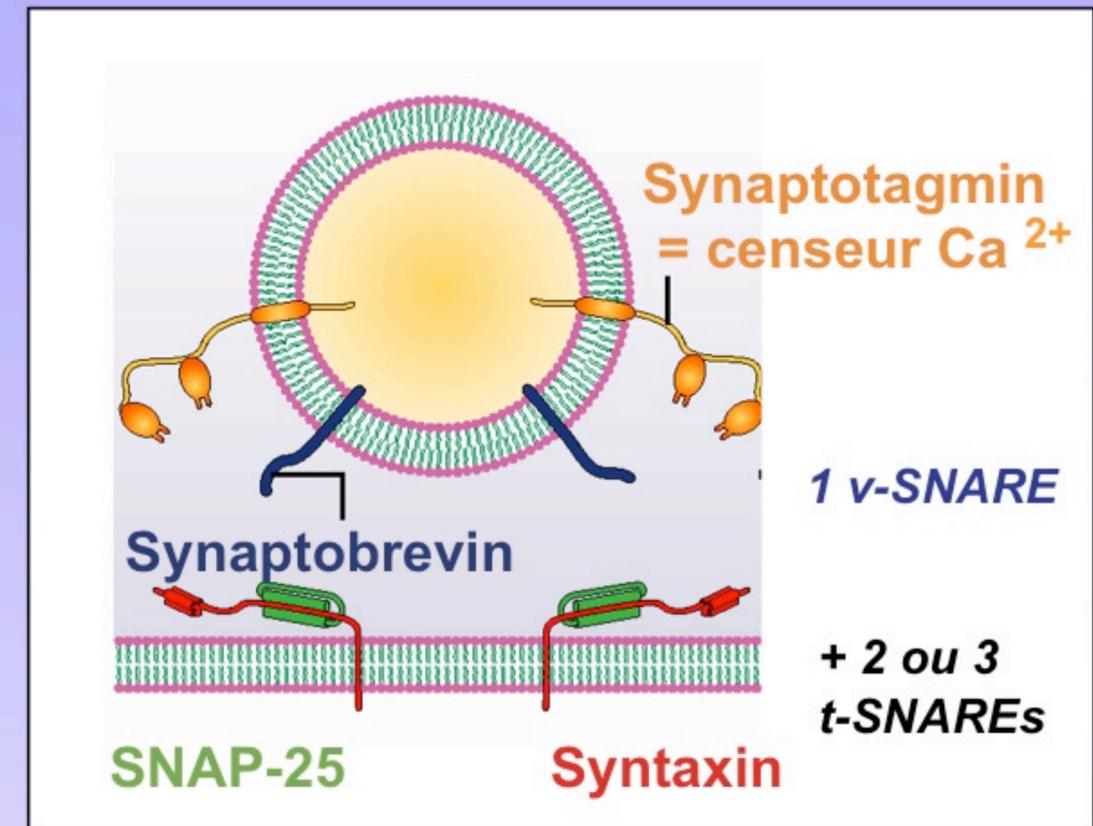
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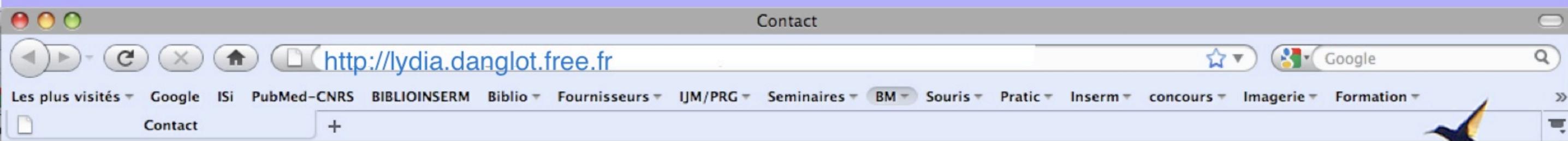
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Si vous avez des questions ...



Lydia Danglot web page

Life Science & Imaging

Octobre 31, 2010

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[English](#)

Contact

Lydia DANGOLOT, PhD

[Institut Jacques Monod](#), CNRS UMR7592

Inserm U950 [Thierry Gallj](#):

"Membrane traffic and epithelial and neuronal morphogenesis"

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