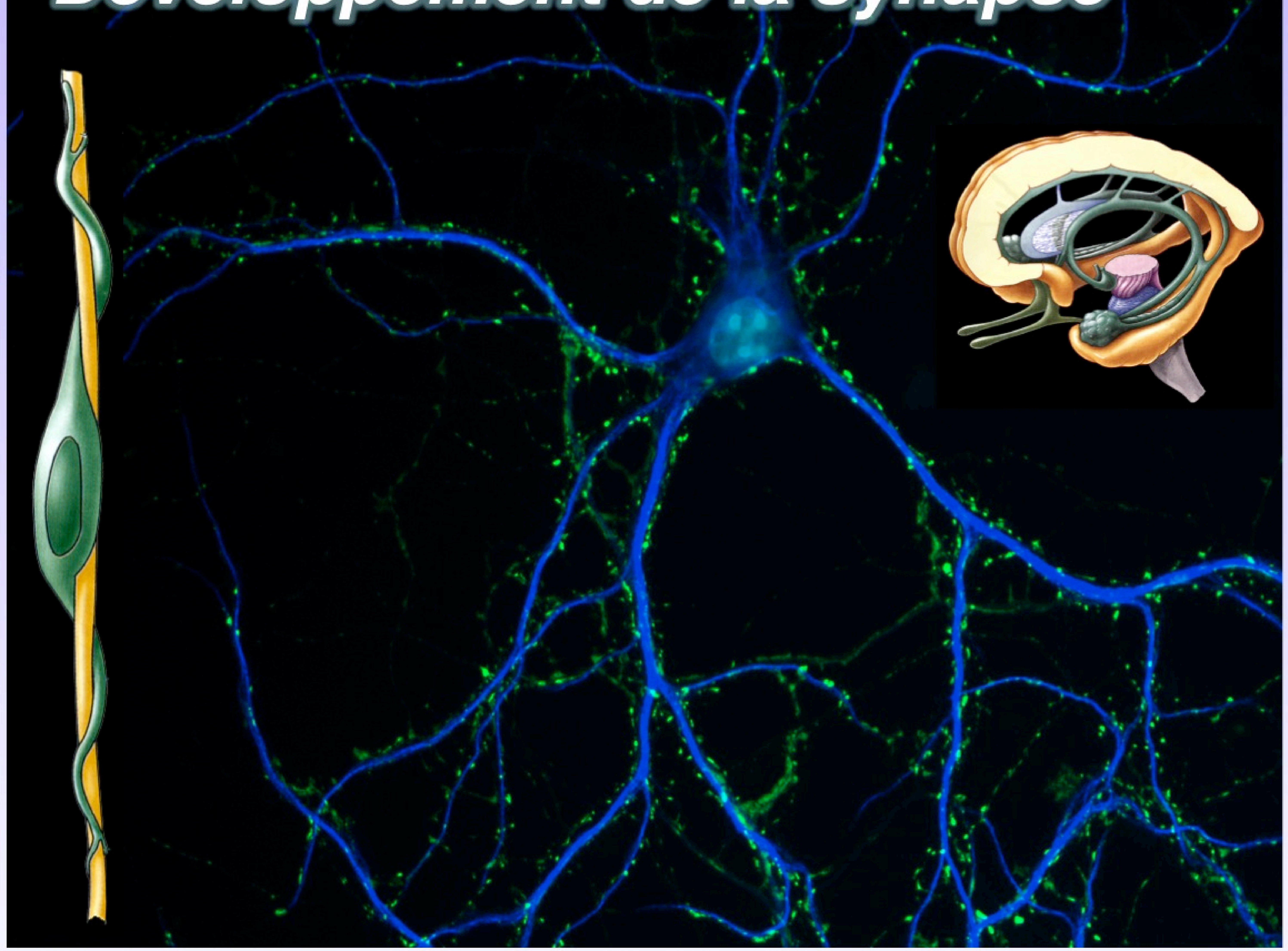
INSTITUT JACQUES MONOD

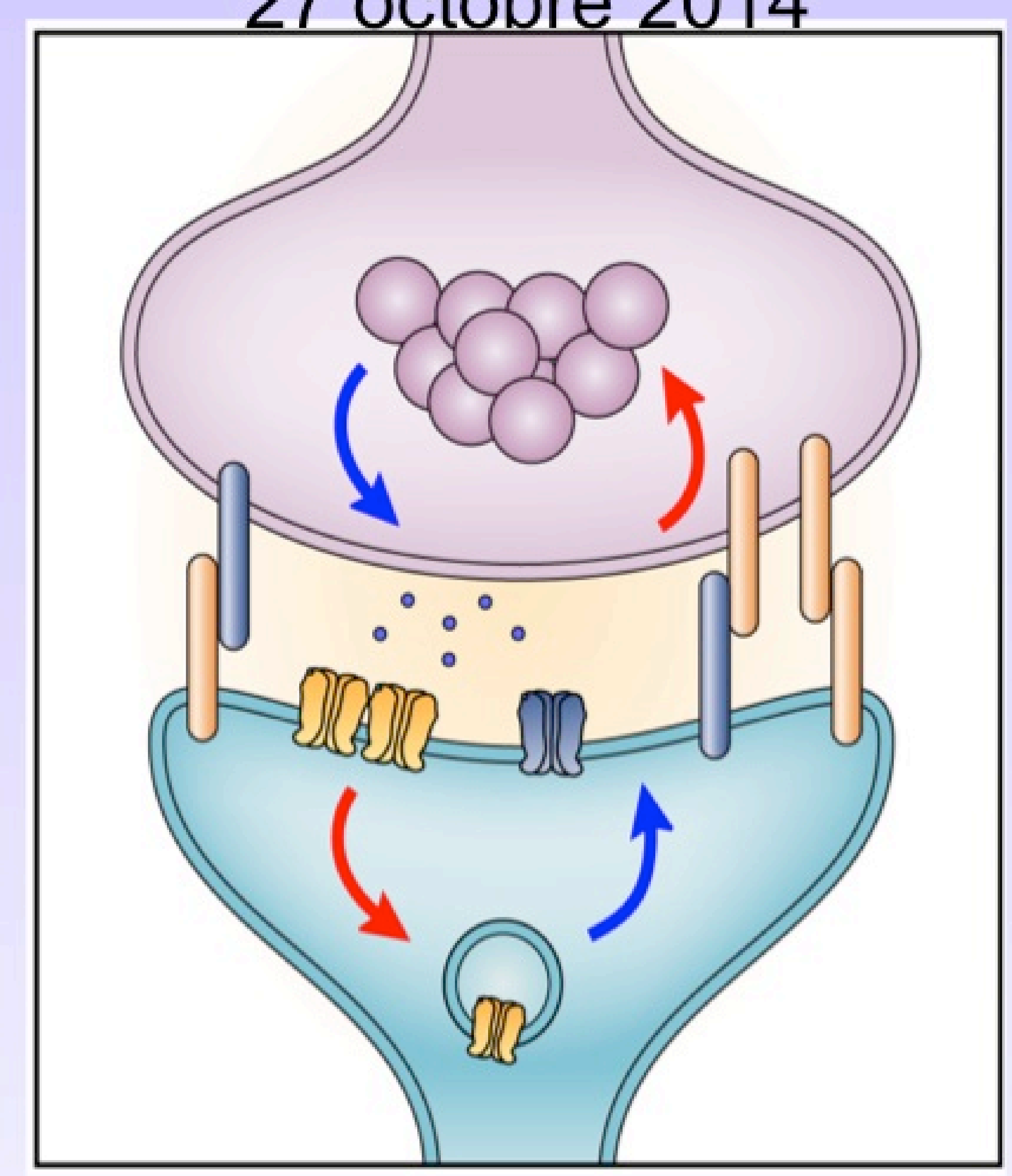
Développement de la synapse



MODULE DÉVELOPPEMENT NORMAL ET PATHOLOGIQUE DU SYSTEME NERVEUX

Master BCPP

27 octobre 2014



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Life Science & Imaging

Novembre 2, 2009

- Thème de recherche
- Publications
- Enseignement
- Liens favoris
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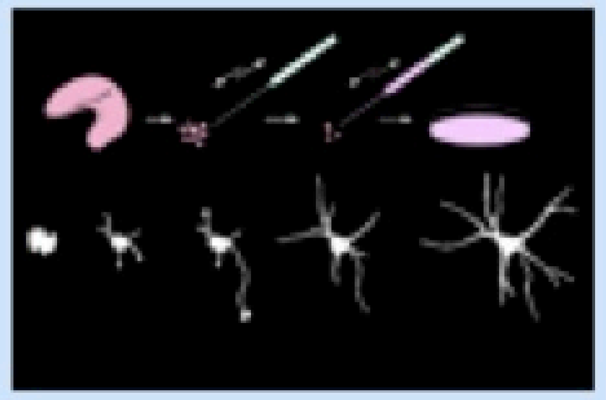
French English

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.***

MANUEL de cours



Master2- Paris 6 Neuritogenèse et polarité neuronale.
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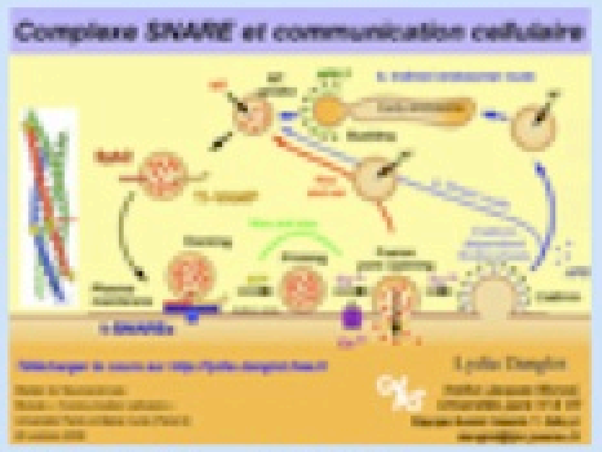

Master2- Paris 6 Complexe SNARE et communication cellulaire.
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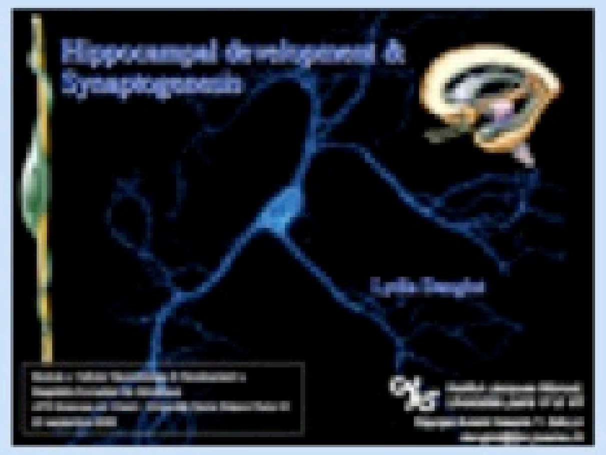

Master2- Paris 7 Développement de l'hippocampe et synaptogenèse
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Formation et développement de la synapse

1. **Le neurone: unité fonctionnelle polarisée**
2. **La croissance neuritique**
3. **Transport sélectif ou rétention**
4. **Le recyclage des vésicules synaptiques**
5. **Le cycle des vésicules synaptiques**
   - Voie lente: endocytose médiée par la clathrine
   - Voie courte: kiss and run
   - Les différents « pool » vésiculaires
6. **Comment mesurer l'exocytose ?**
   - Capacitance
   - Ampérométrie
   - GFP pH sensible: la Phluorin
   - Utilisation des SNARE inversée
7. **Comment mesurer le recyclage ?**
   - Utilisation des anti-synaptotagmine
   - Sondes fluorescentes de type FM
8. **Formation de la Synapse dans les neurones en culture**
9. **Structure des épines dendritiques**
10. **Formation de la synapse plasticité synaptique in vivo**

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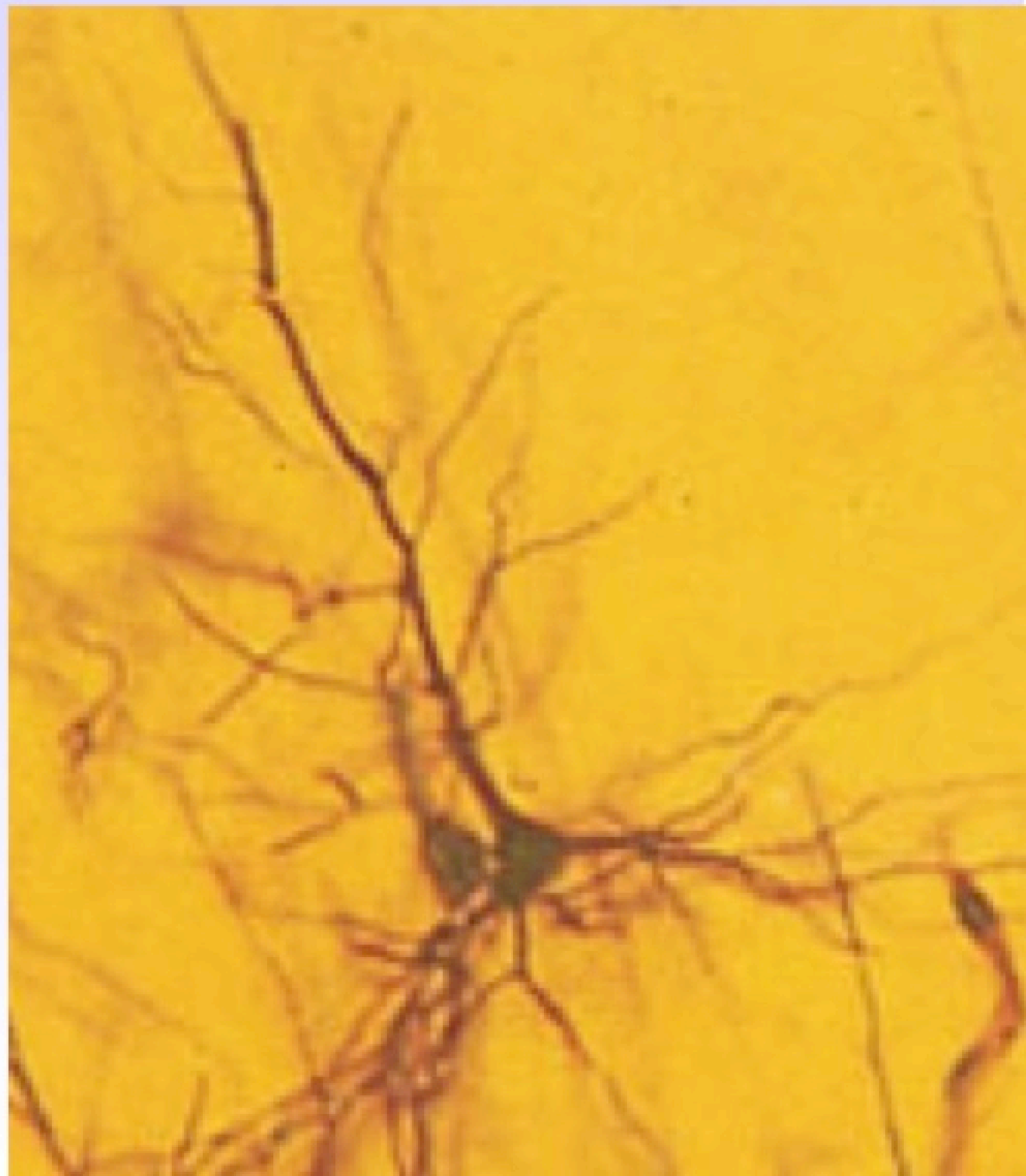
1. Le Neuron: unité fonctionnelle polarisée

- 1800's: les techniques de microscopies permettent l'examen de tissus nerveux
  Refinement of the microscope allows scientists to examine brain tissue

- Les tissus sont composés d'unités nommées: les cellules
  All tissues are composed of microscopic units called cells

- Les cellules du cerveau sont nommés neurones
  Cells in the brain are termed "neurons"



Un peu d'histoire...

- Théorie réticulaire (C. Golgi): Il existe une continuité cytoplasmique entre les neurones (tunnel).

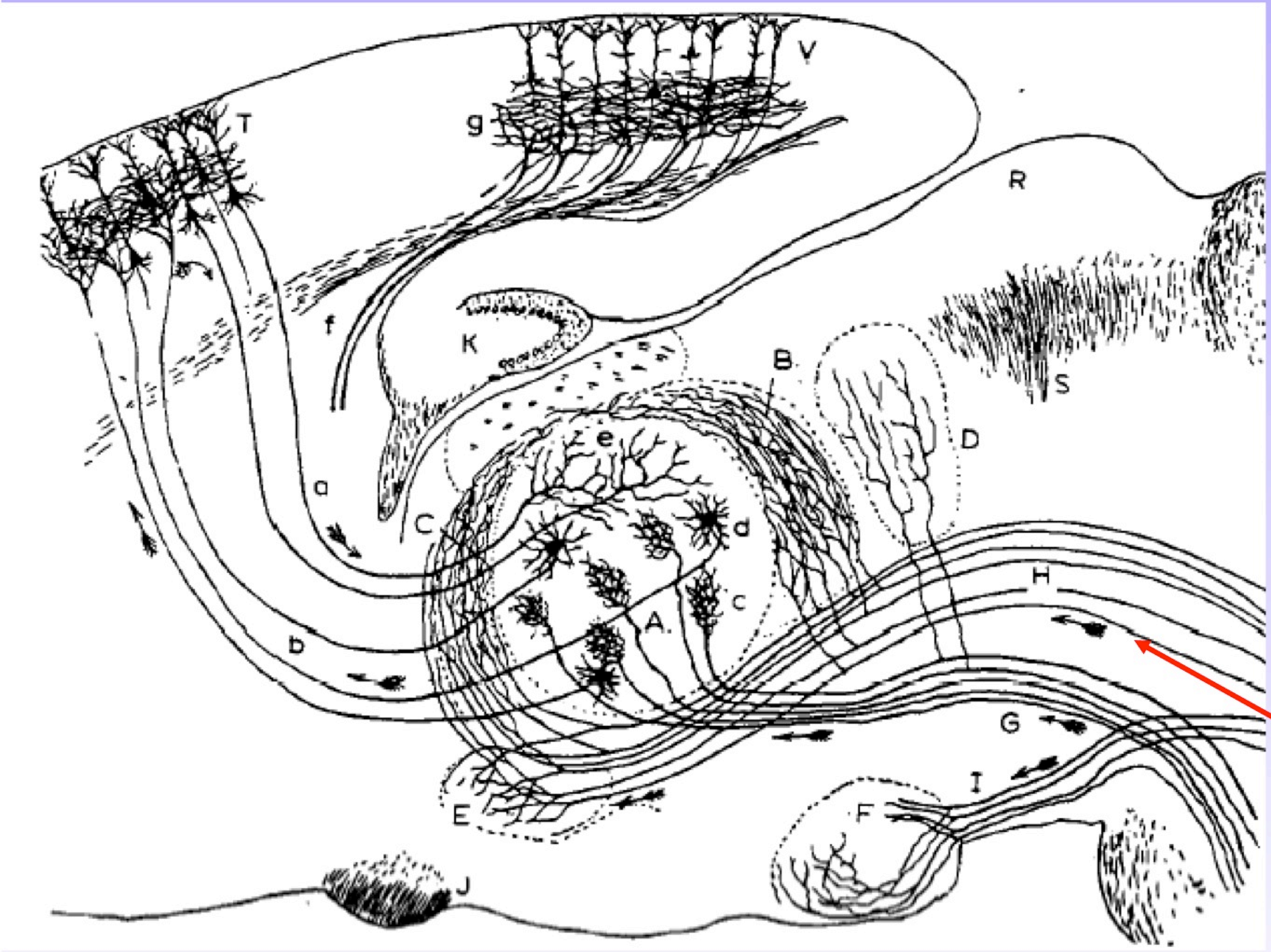
- Théorie du neurone (Cajal): Les neurones sont bel et bien séparé, sans continuité, leur membrane sont complètes.

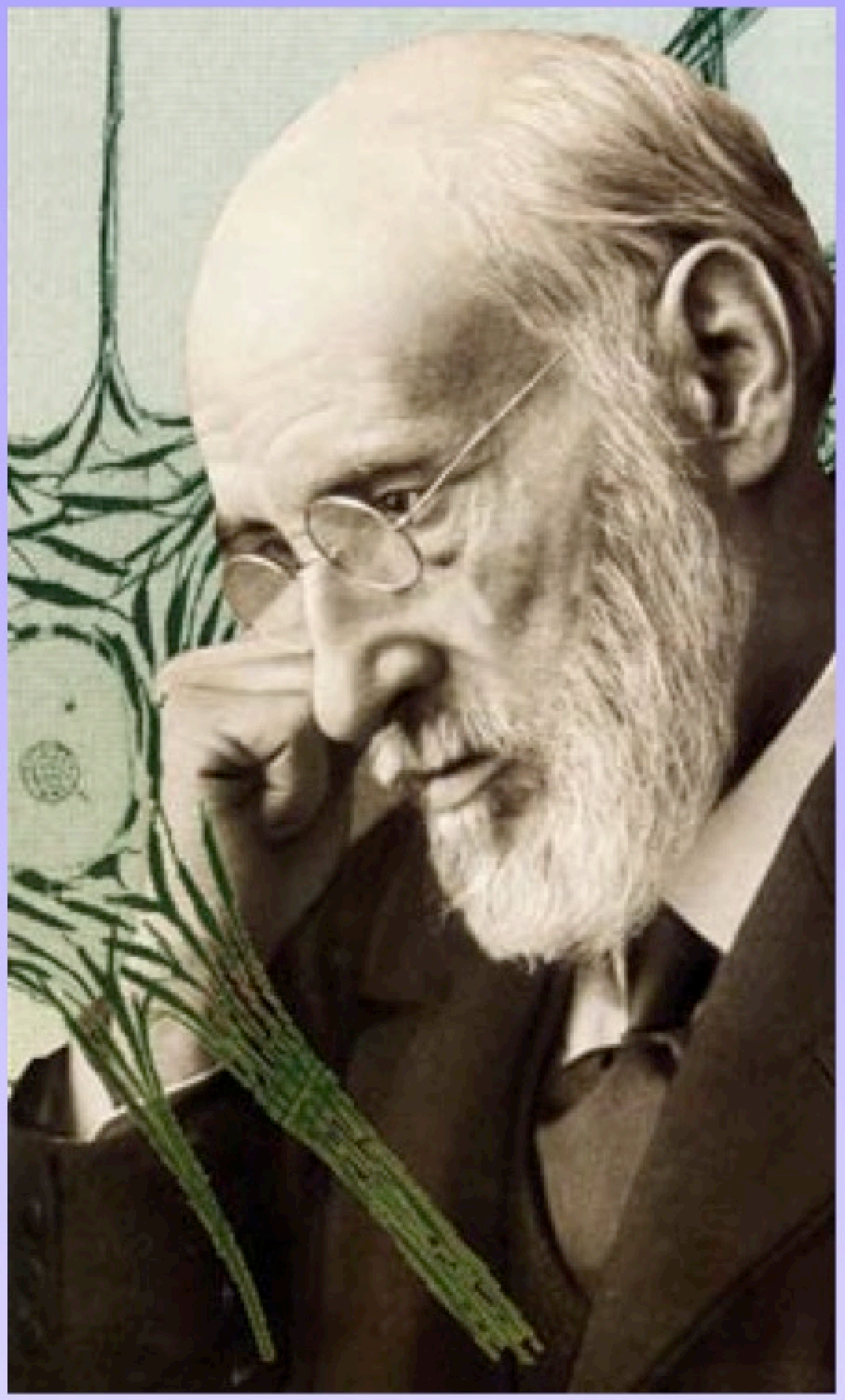
➡ Aujourd'hui, les scientifiques donnent raison à Cajal

Ironie: Cajal a réussi à convaincre la communauté scientifique grâce à une technique histologique inventée par Golgi !

(Les deux ont reçu le prix Nobel en 1906)

Les neurones sont des cellules polarisées





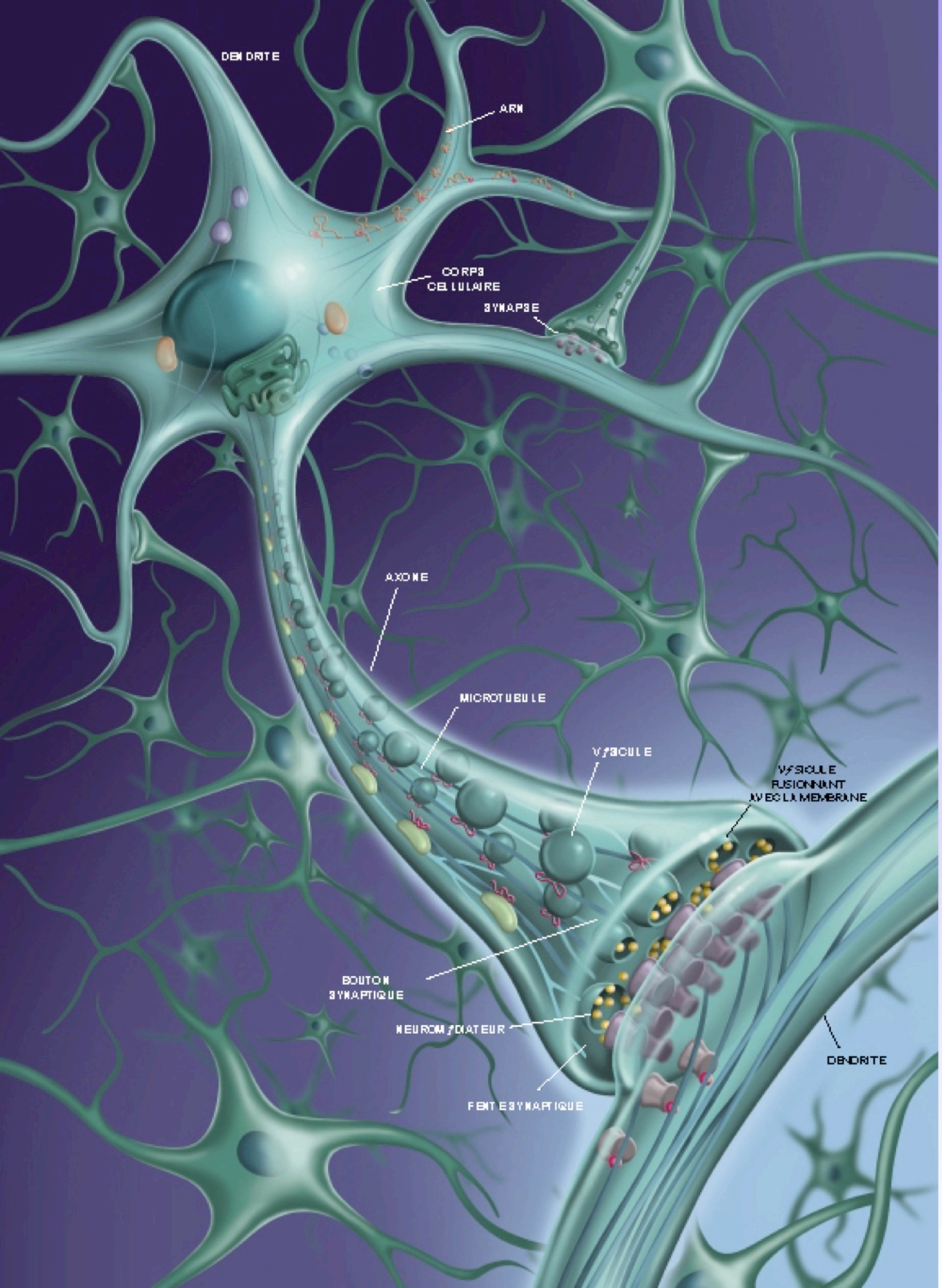
Santiago Ramón y Cajal

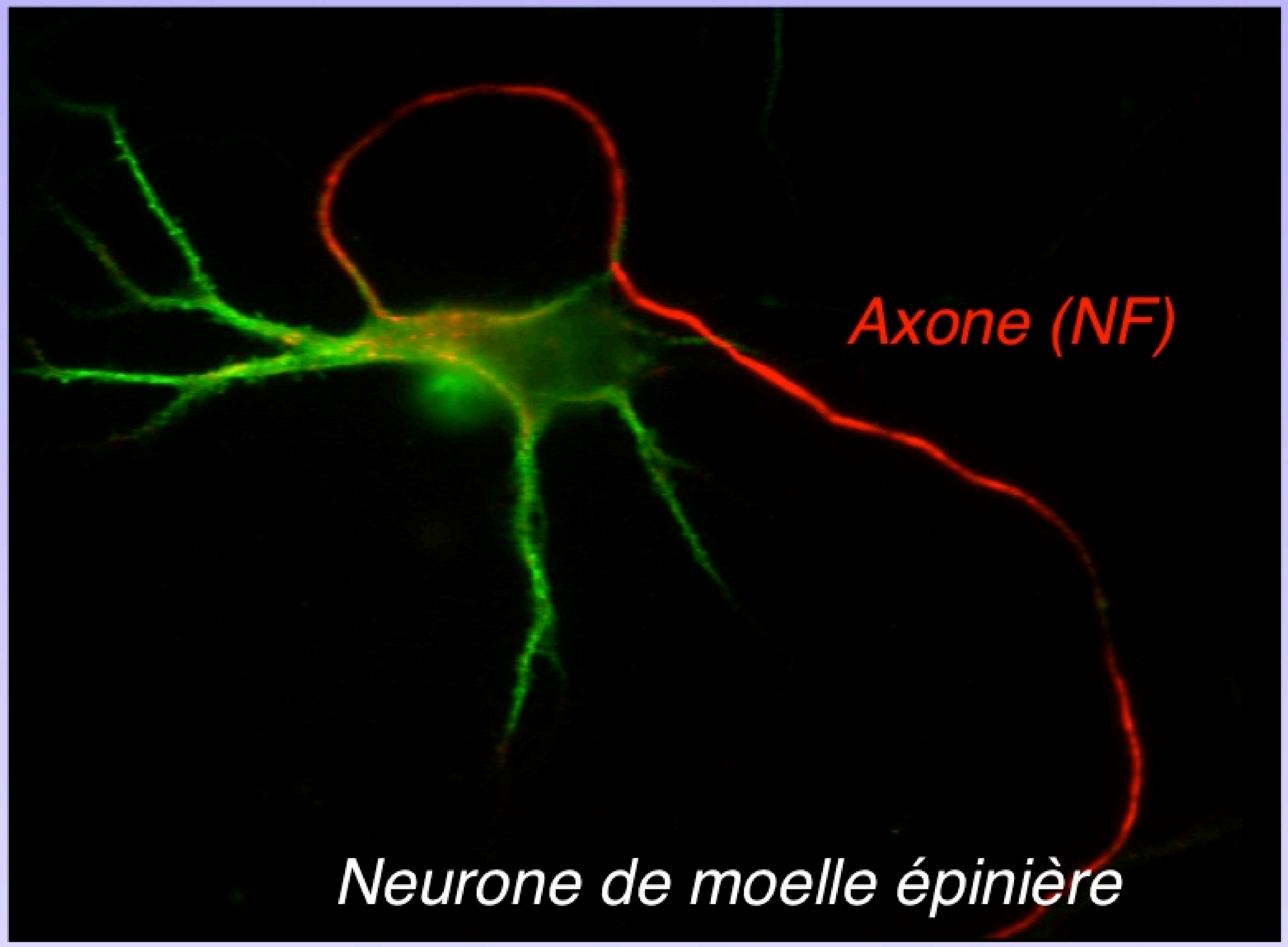
(1852-1934)

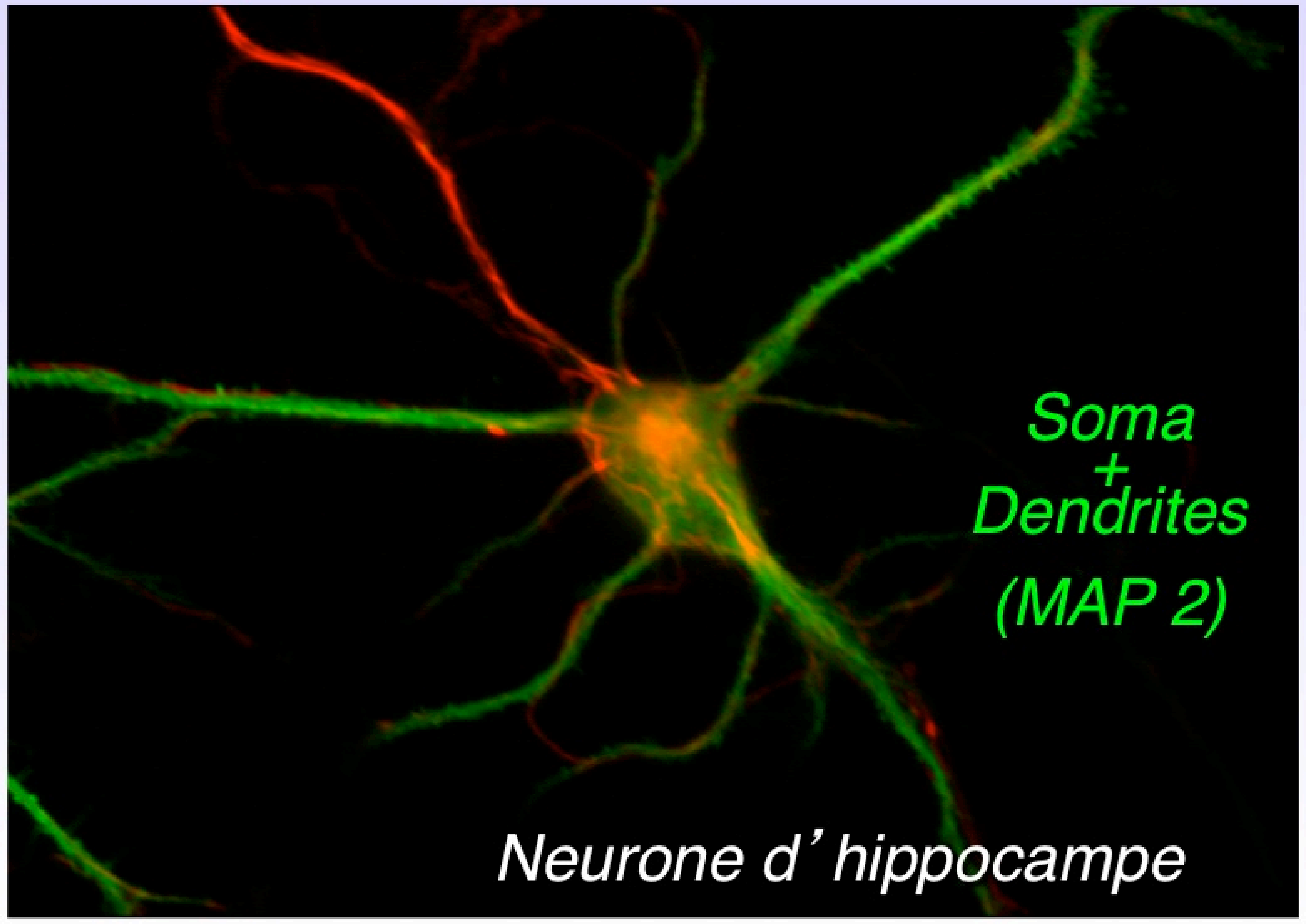
Direction de l'influx nerveux

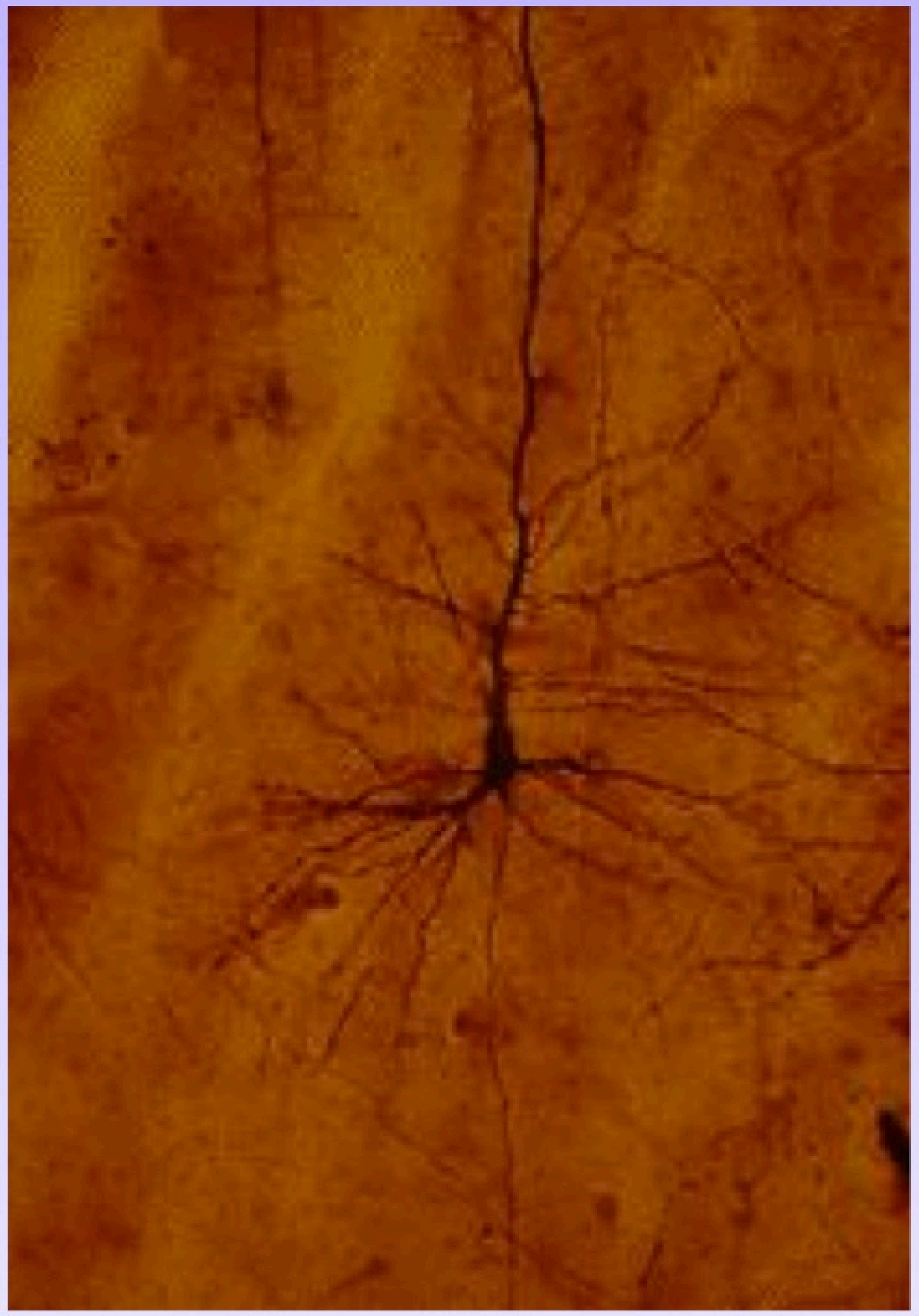
2 compartiments:

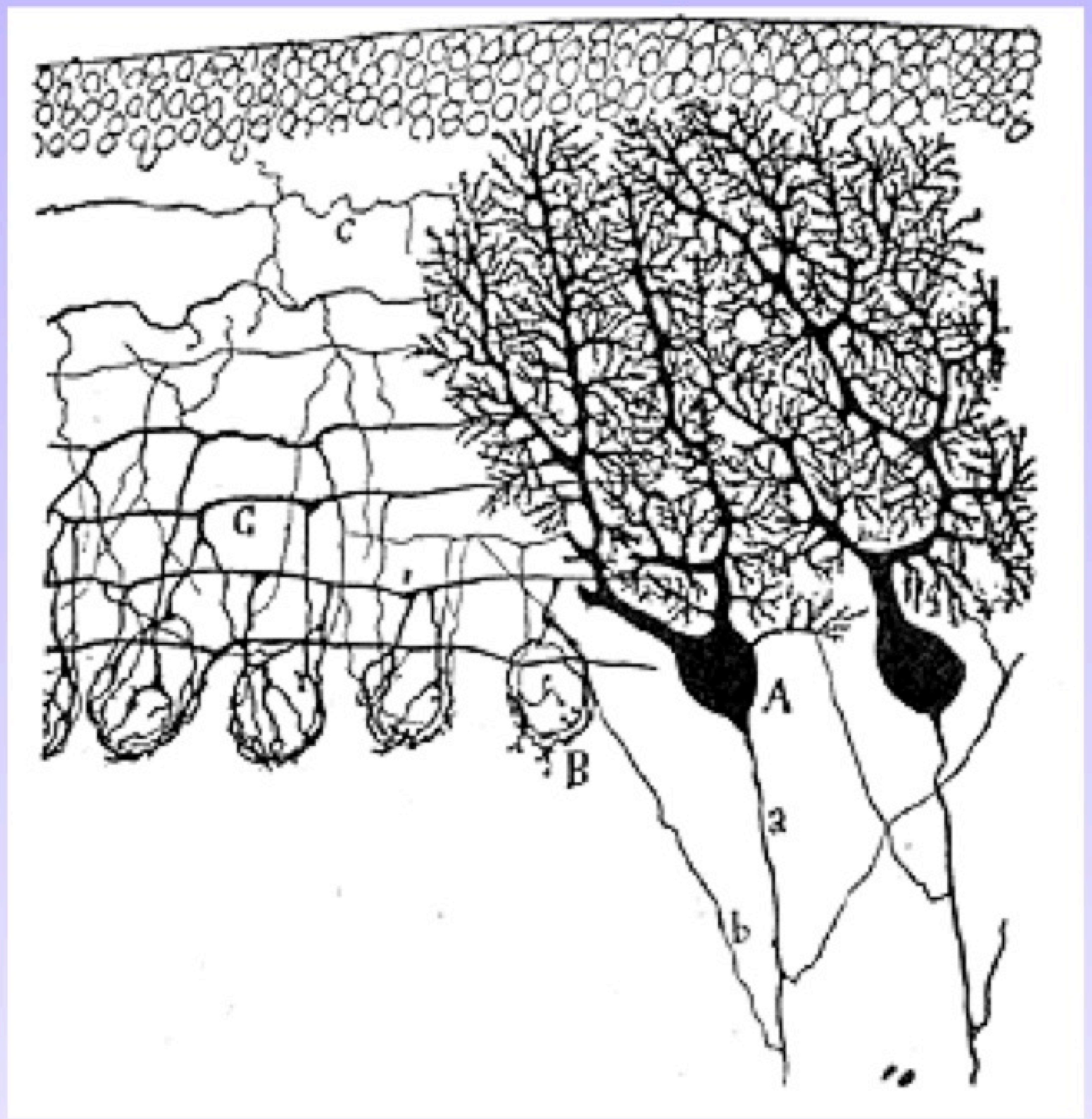
- *Axonal*
- *Somatodendritique*







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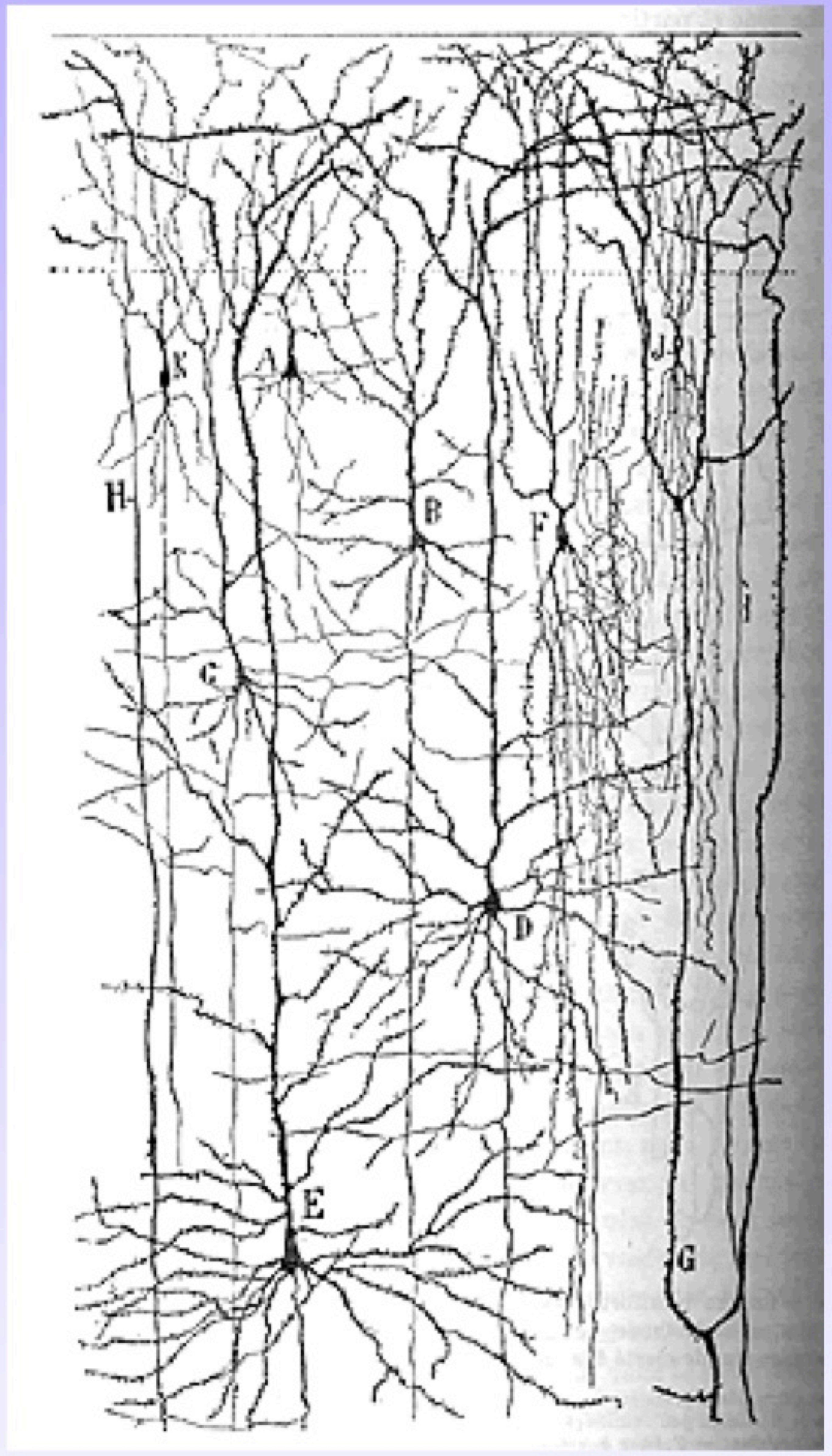
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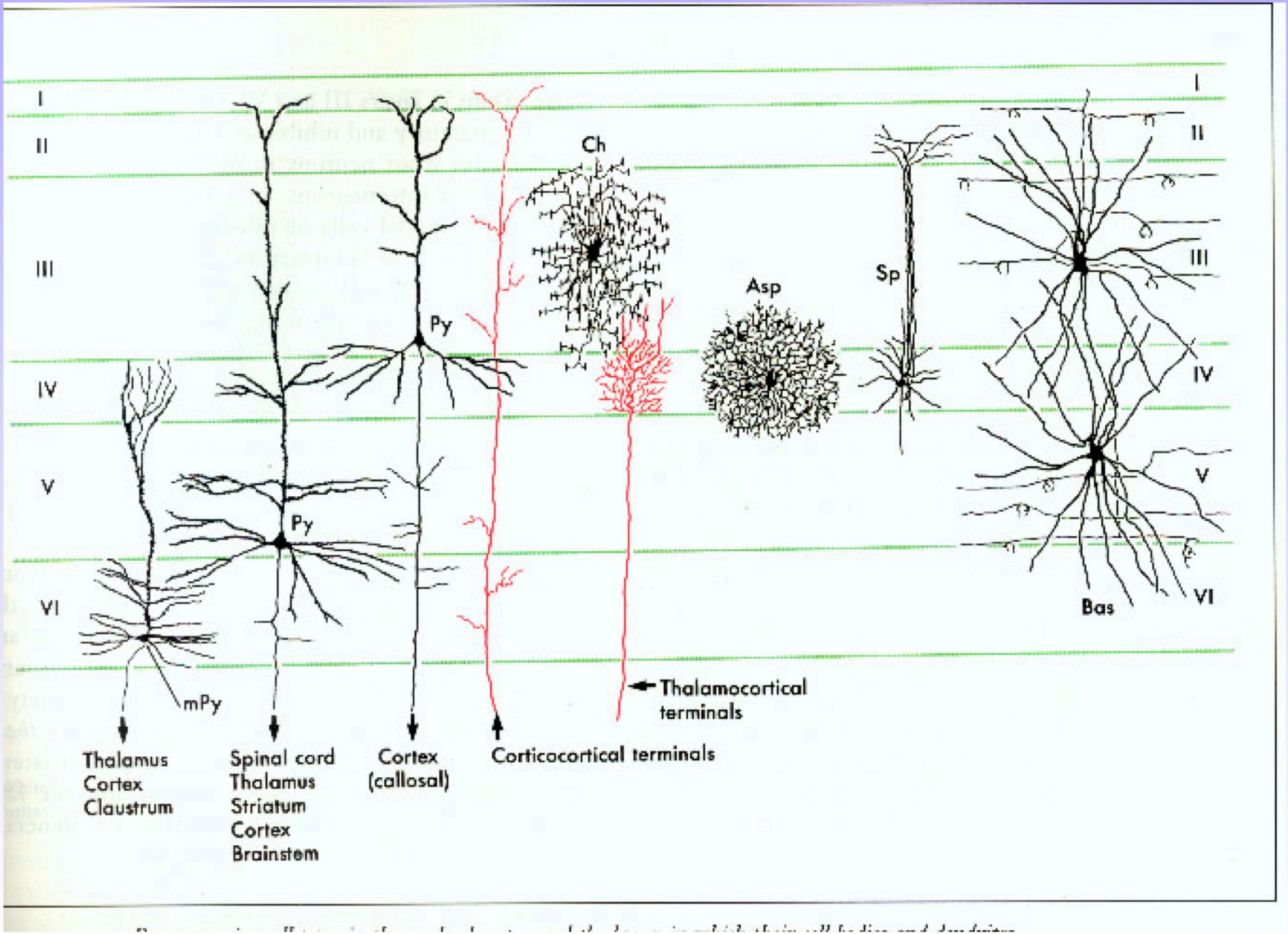
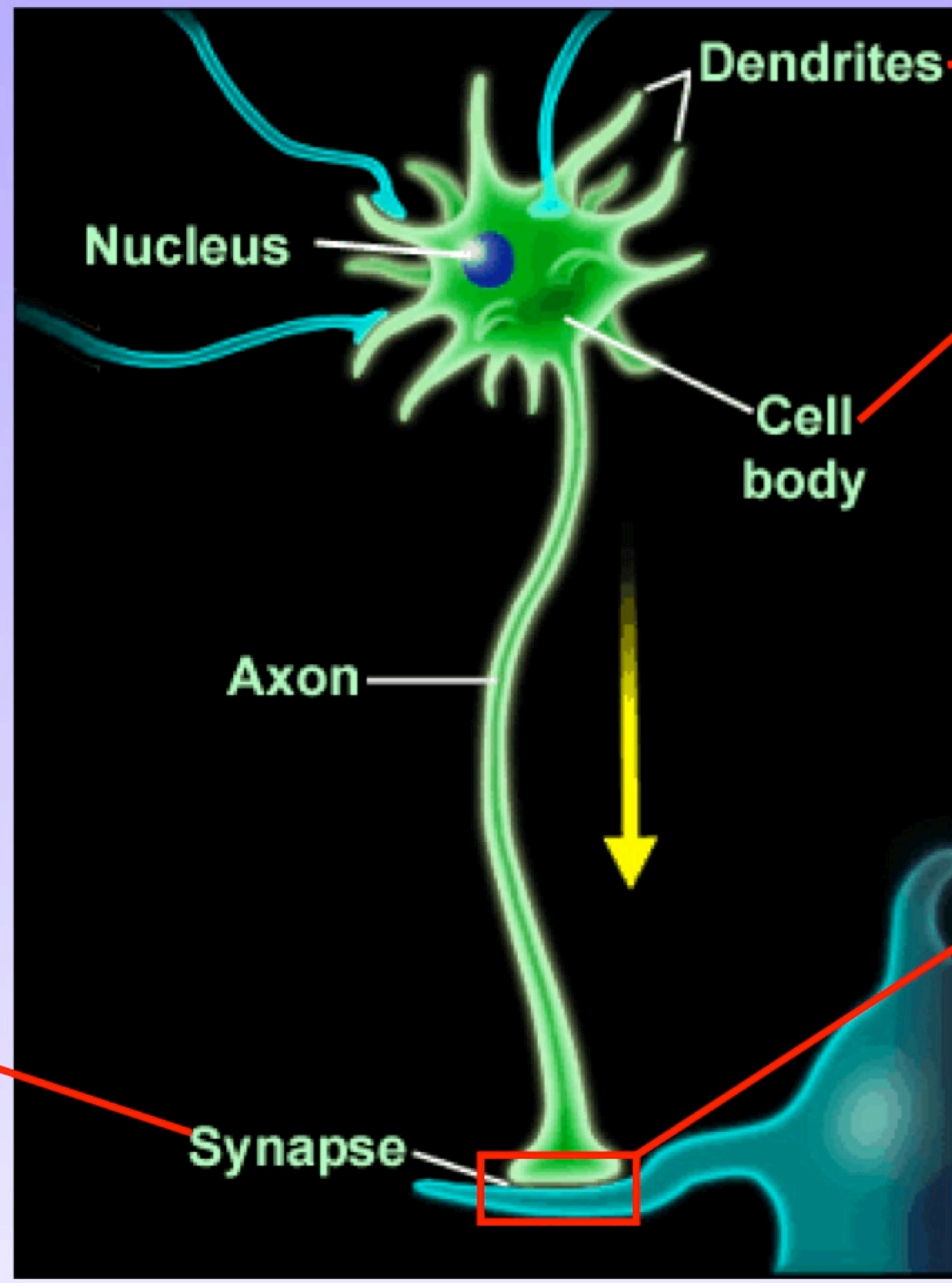
Cerebral Cortex



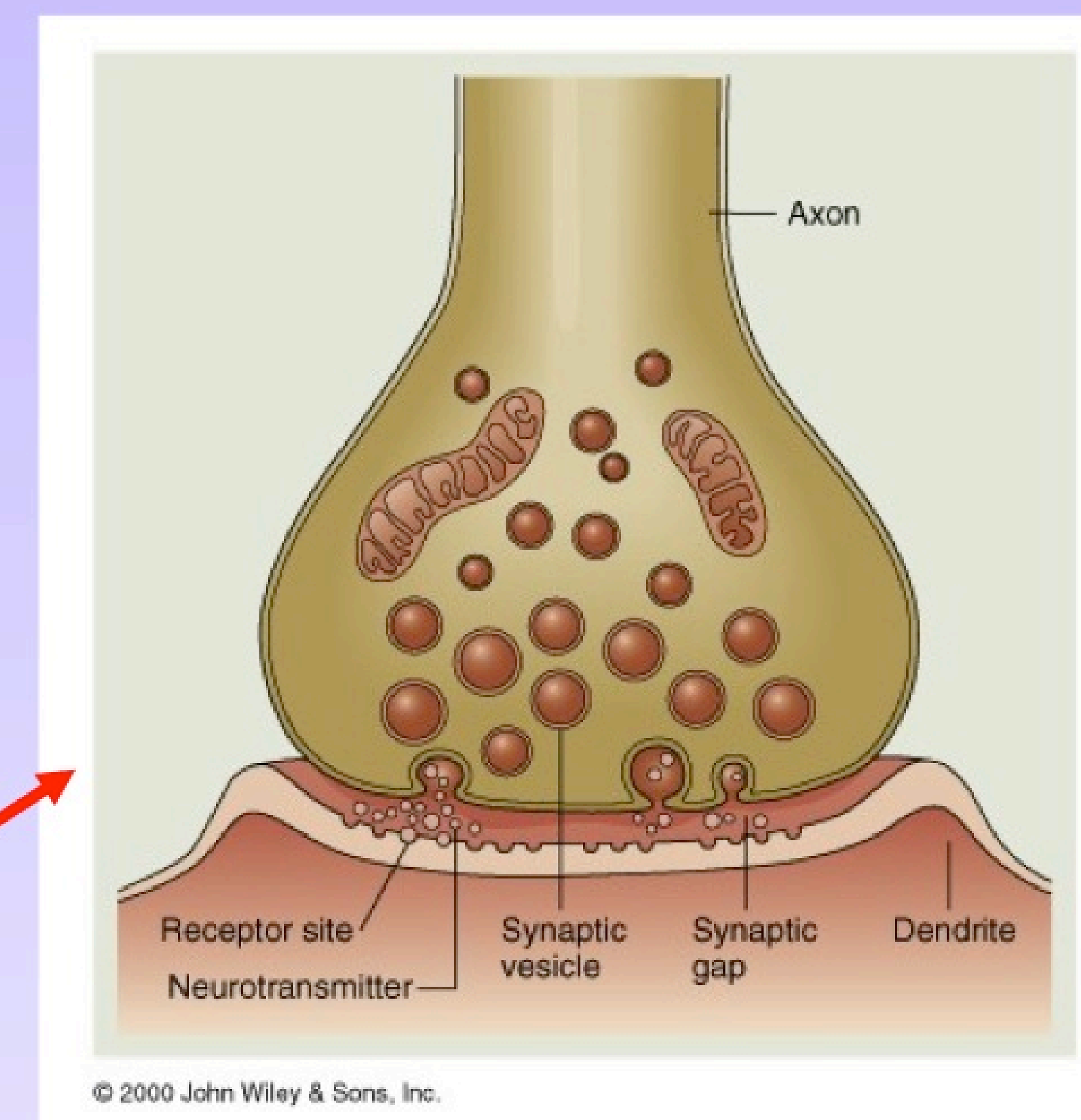
Domaines du neurones

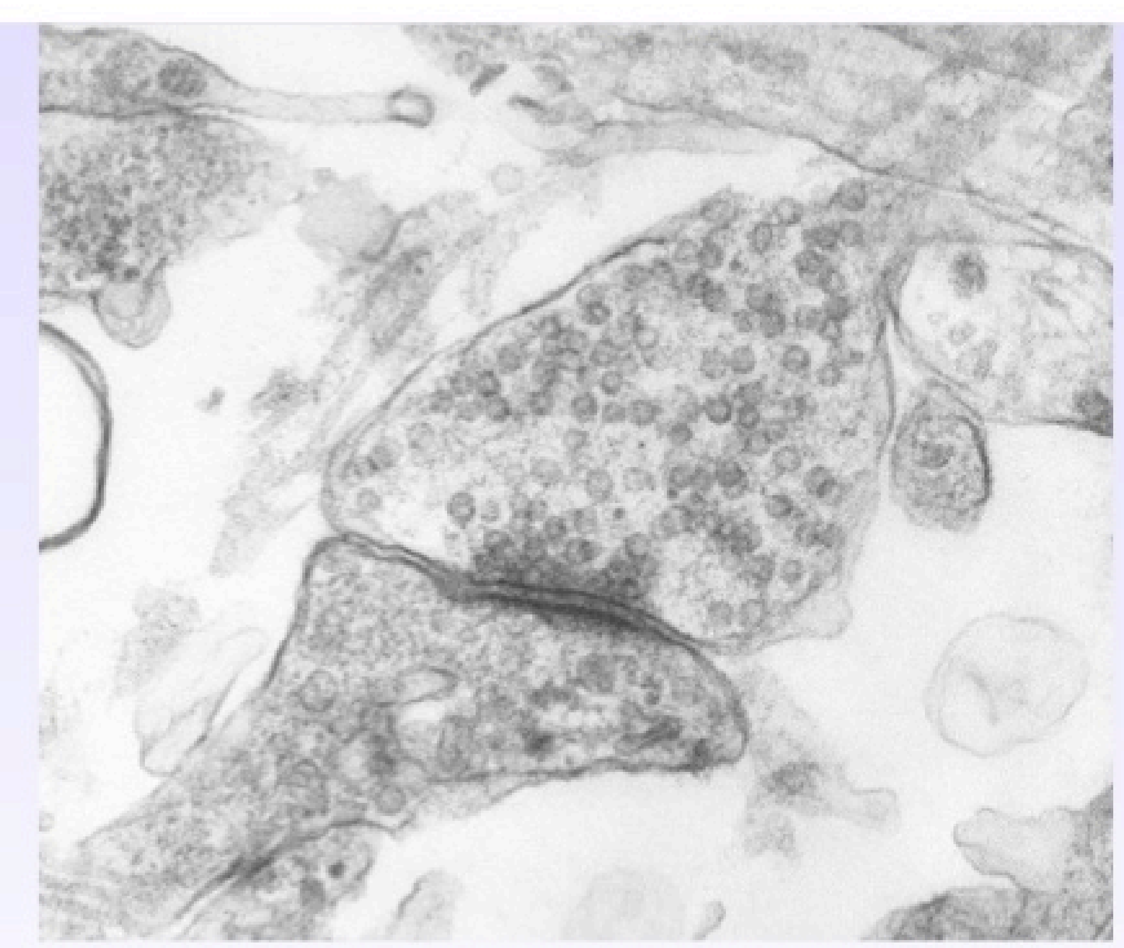
1 mm³ de substance grise du cortex peut contenir 5 milliards de synapses.

Pôle récepteur



Pôle émetteur





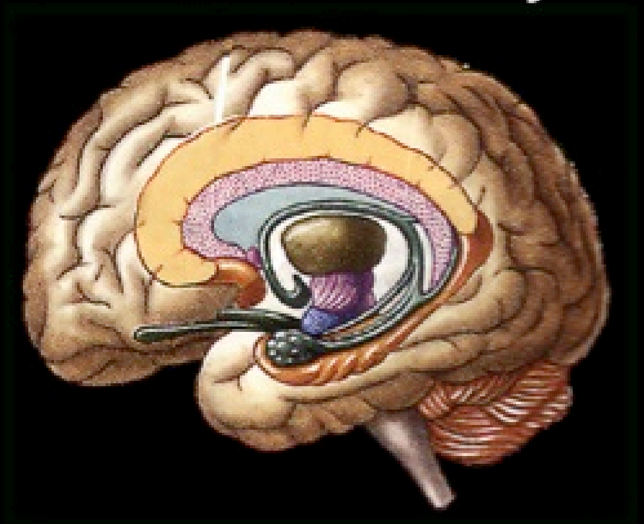
La question centrale :

Polarisation neuronale: développement de l'axone et des dendrites.

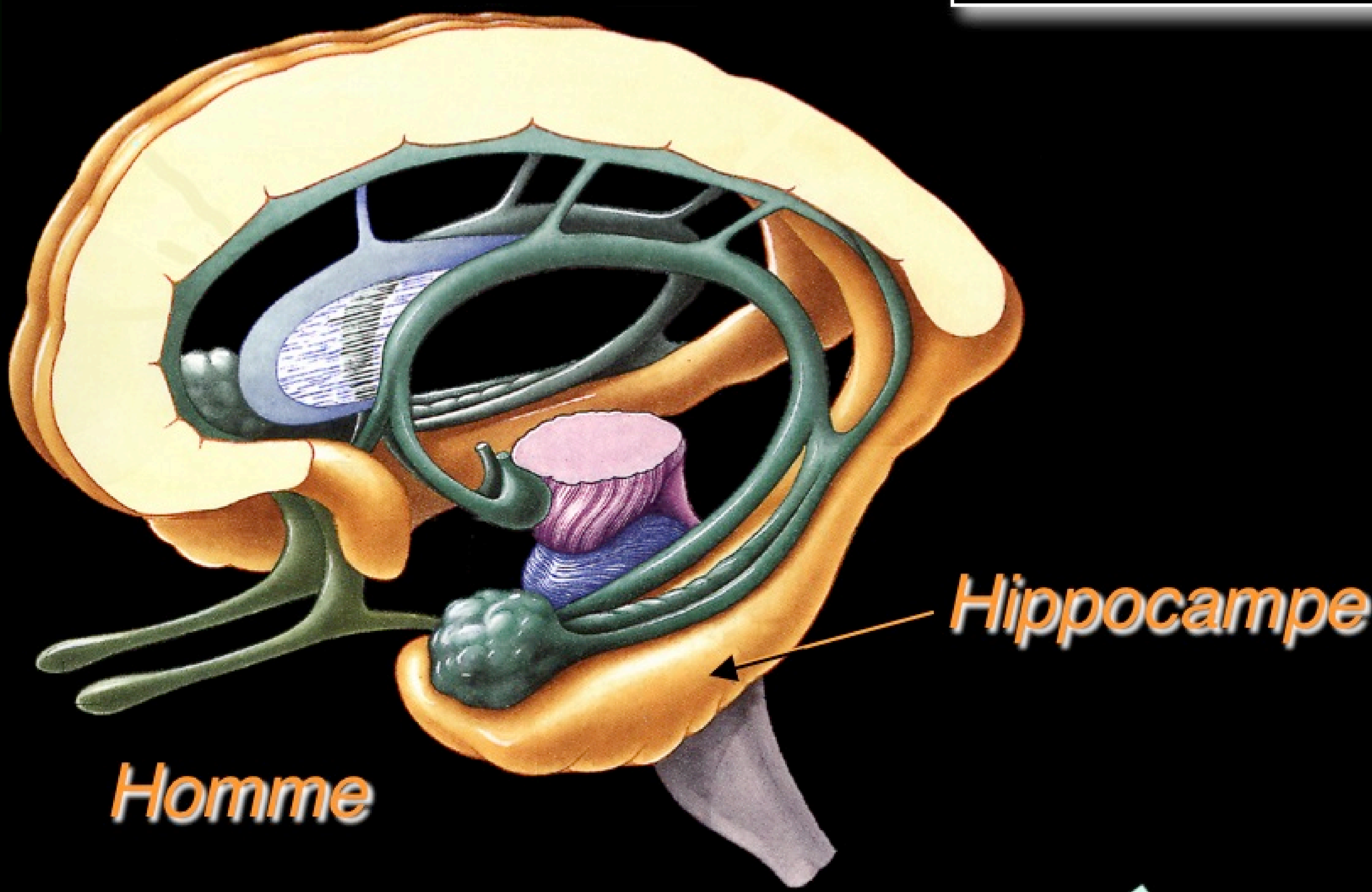
Les marqueurs classiques des domaines polarisés

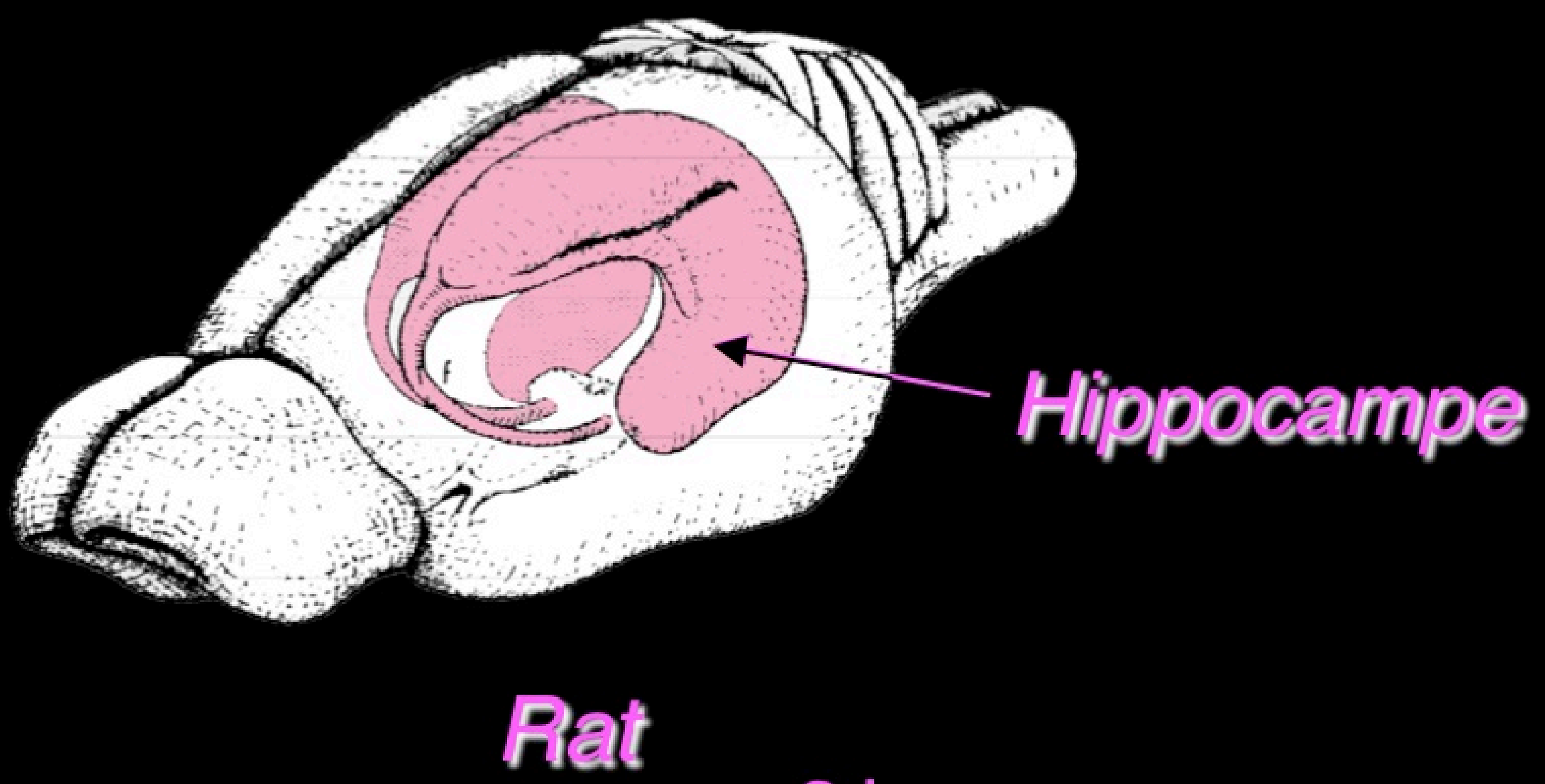
Axone: Tau1, GAP43, synapsine, synaptotagmine…

Dendrite: MAP2, Glycine receptor, GABAa receptor …

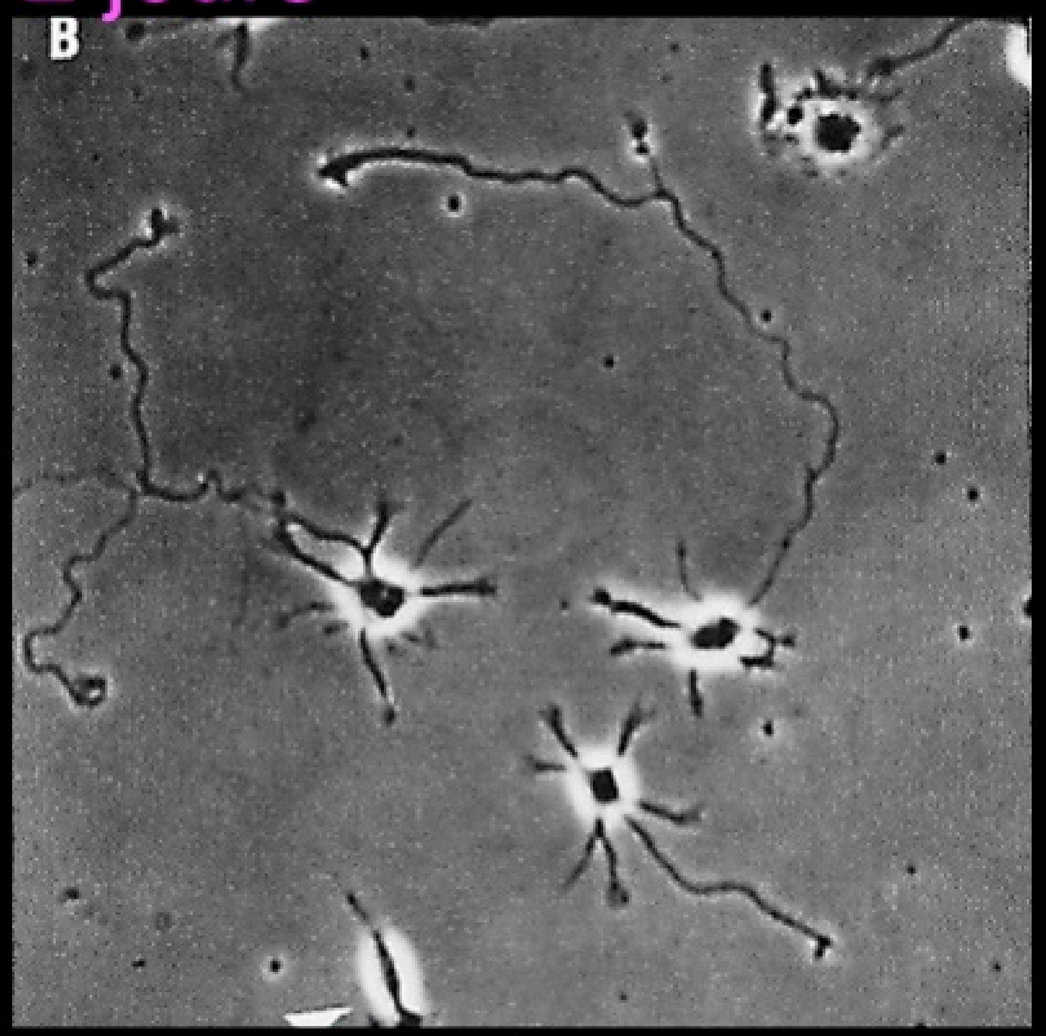


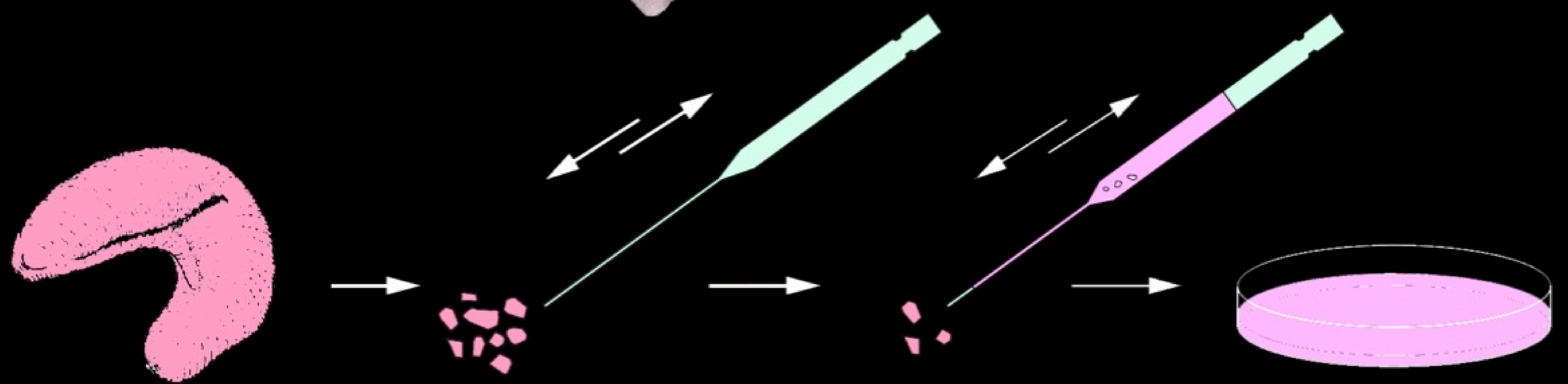
Neurones d' hippocampe en culture



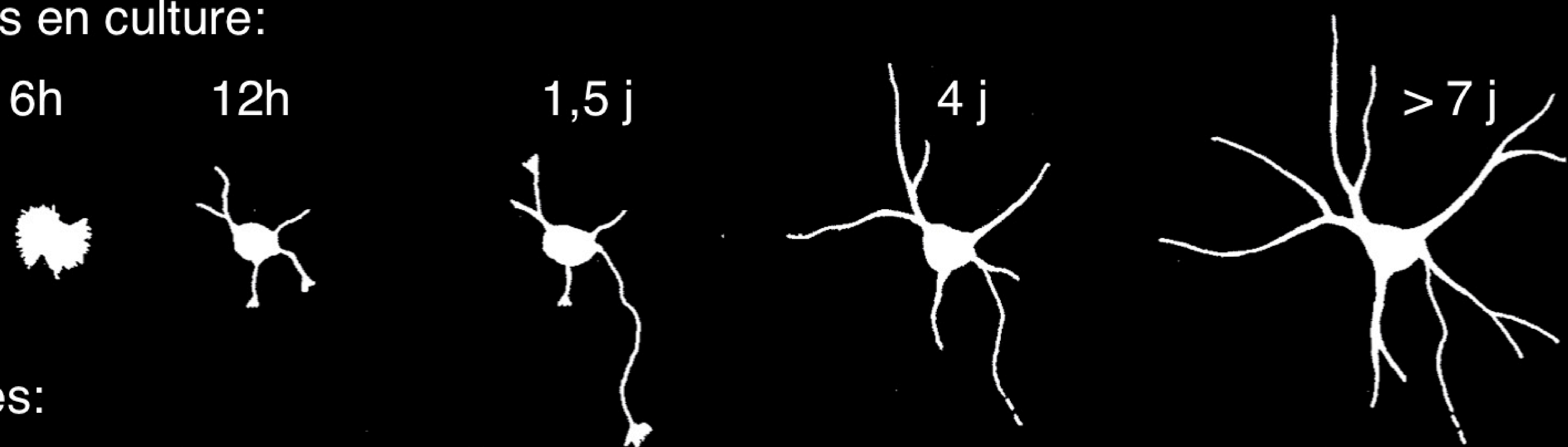


2 jours

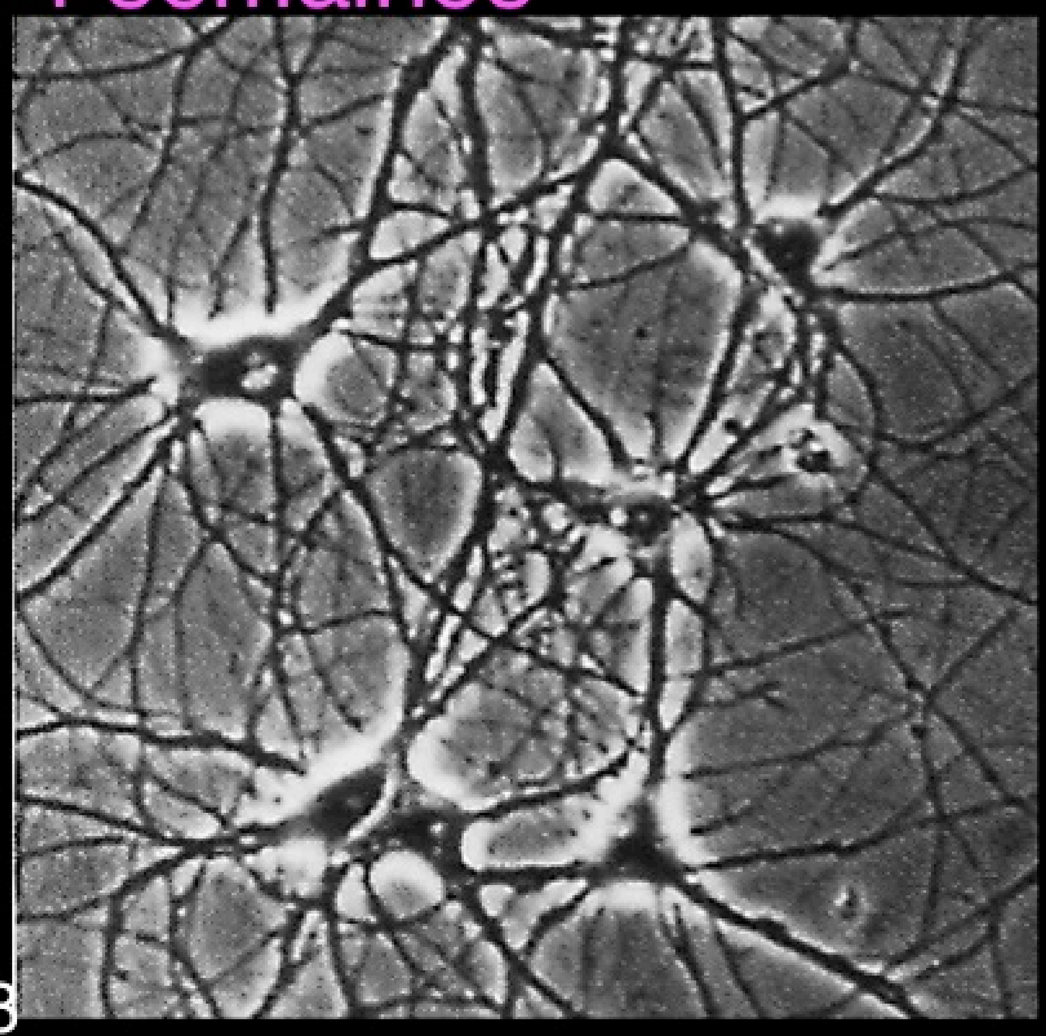




Temps en culture:



4 semaines



Stades:

1 Lamellipodes

2 Neurites mineurs

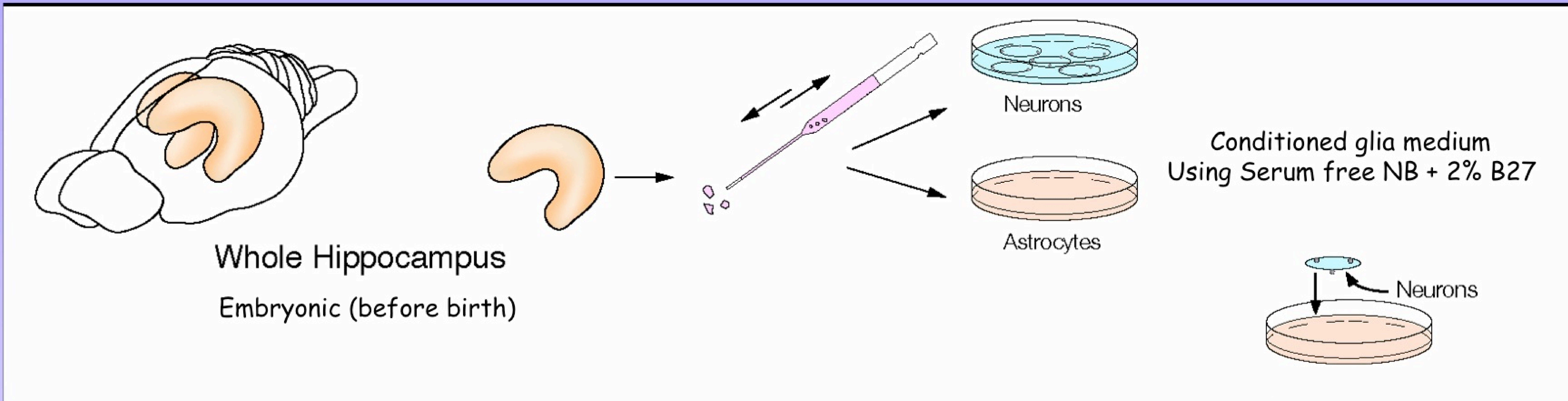
3 Croissance axonale

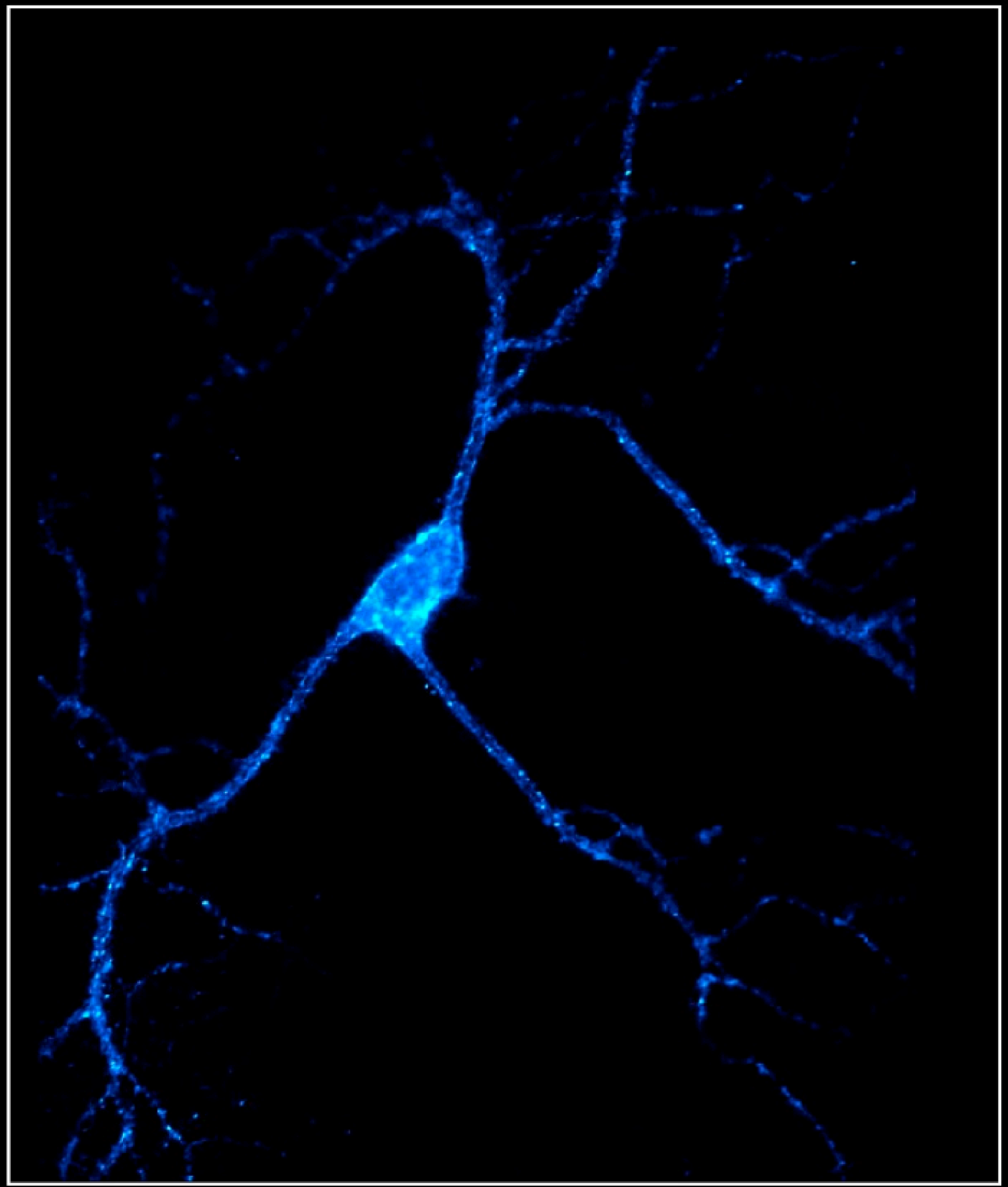
4 Croissance dendritique

5 Maturation

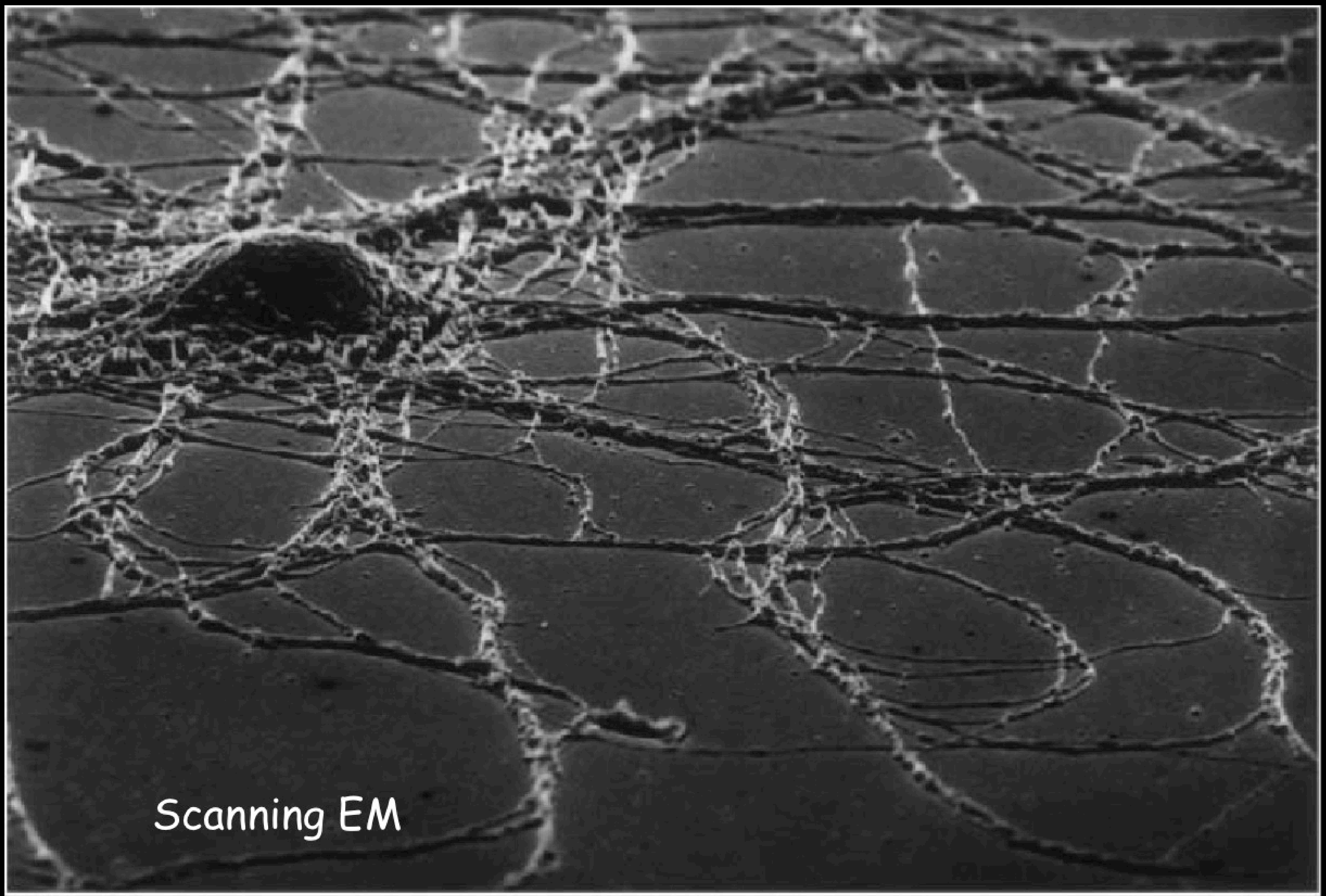
D' après Dotti et al. 1988

Culture « à la Banker » dite « en sandwich » ou « glial feeder layer »



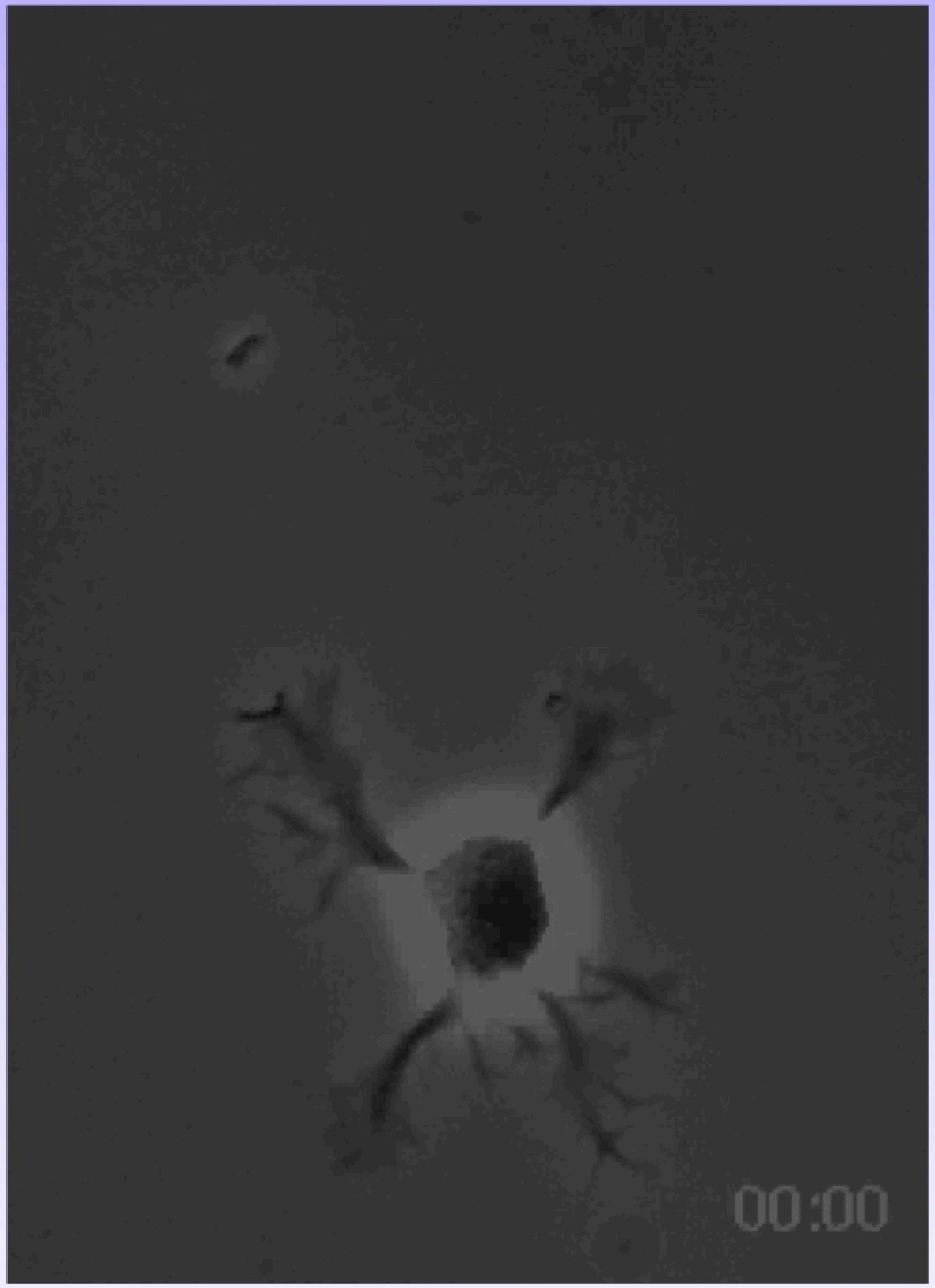


Danglot et al (2003), Mol Cell Neurosci. 23:264-278.



C. Verderio et al., Cell. Mol. Life Sci. 55 (1999) 1448–1462.

2. La croissance neuritique

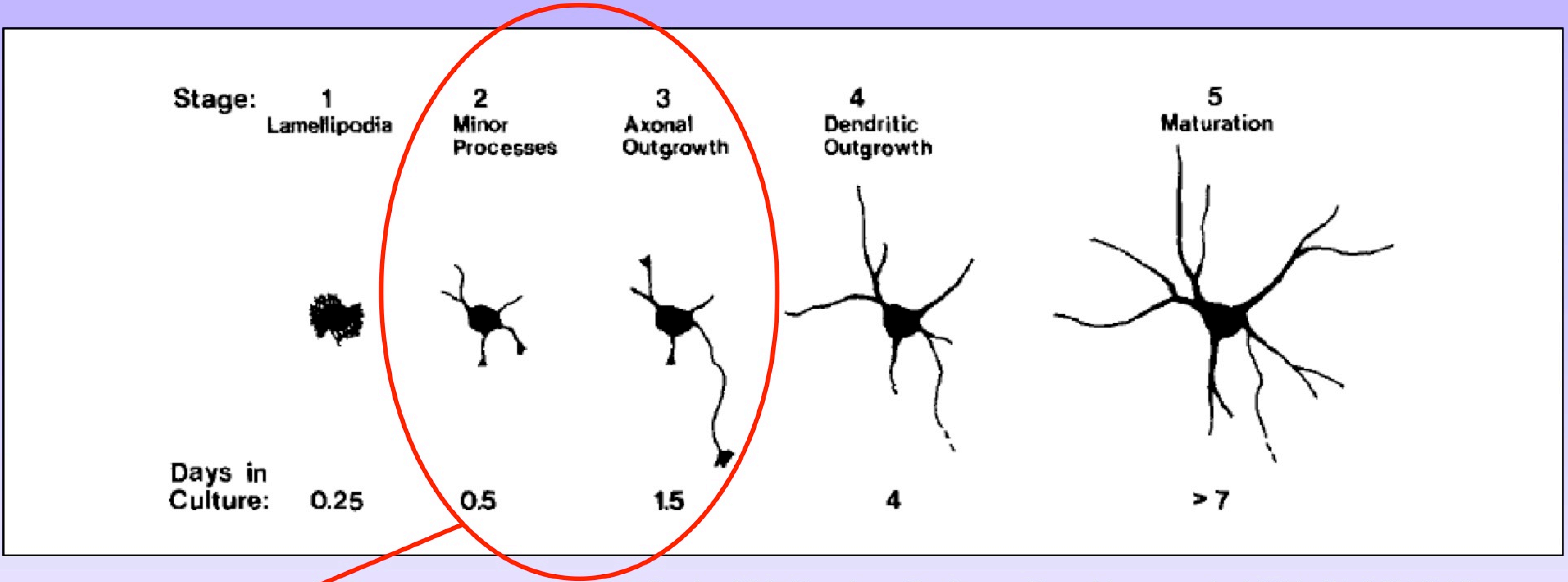


Movie from http://www.silvermanlab.orgl

Phase contrast time-lapse recording of a neuron forming an axon. The video shows a rapid playback of a 16 hour recording with one image taken every 10 minutes.

http://www.silvermanlab.org/gal_mov.html

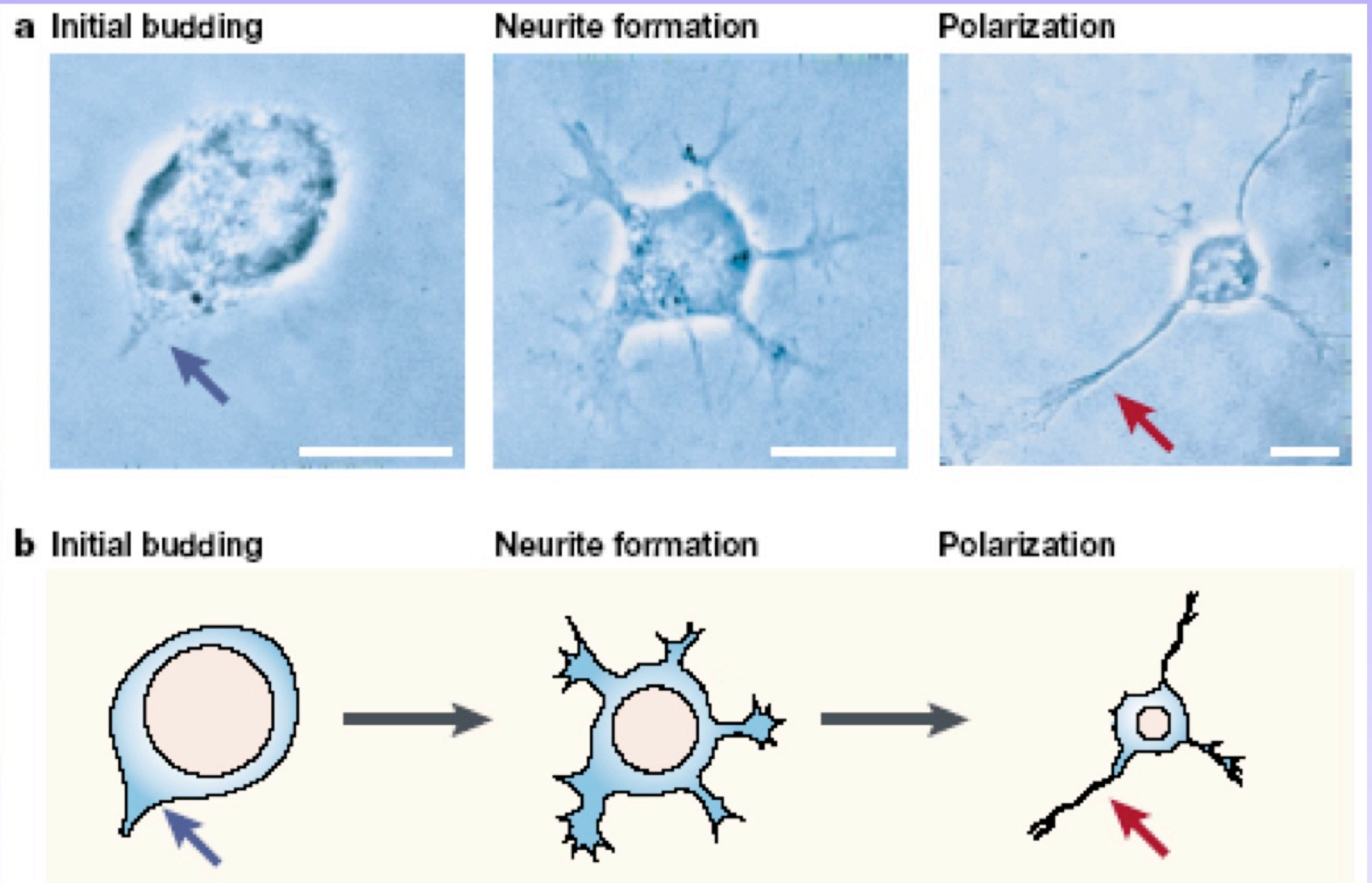
Etudes pionnières *in vitro* par Gary Banker dans les années 1980's-1990's

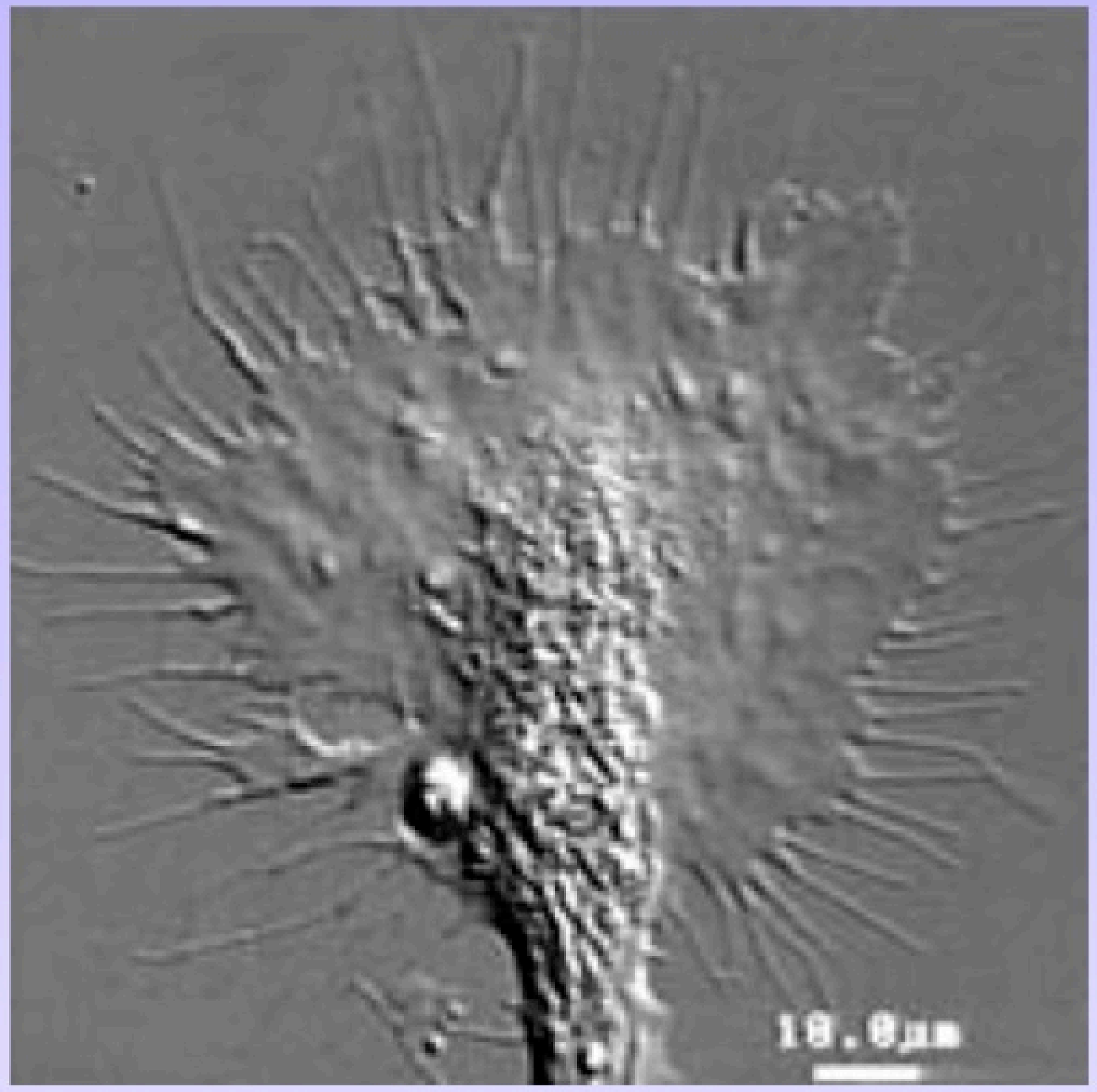


Craig AM, Banker G. Annu Rev Neurosci. 1994;17:267-310.

L'acquisition de la polarité réside en la sélection d'un neurite, qui après une croissance accélérée, donnera l'axone pendant la transition du stade 2 au stade 3.

Acquisition de la polarité entre les stades 2 & 3 dans les neurones d'hippocampe





Cône de croissance

da Silva JS, Dotti CG. Nat Rev Neurosci. 2002 Sep;3(9): 694-704.

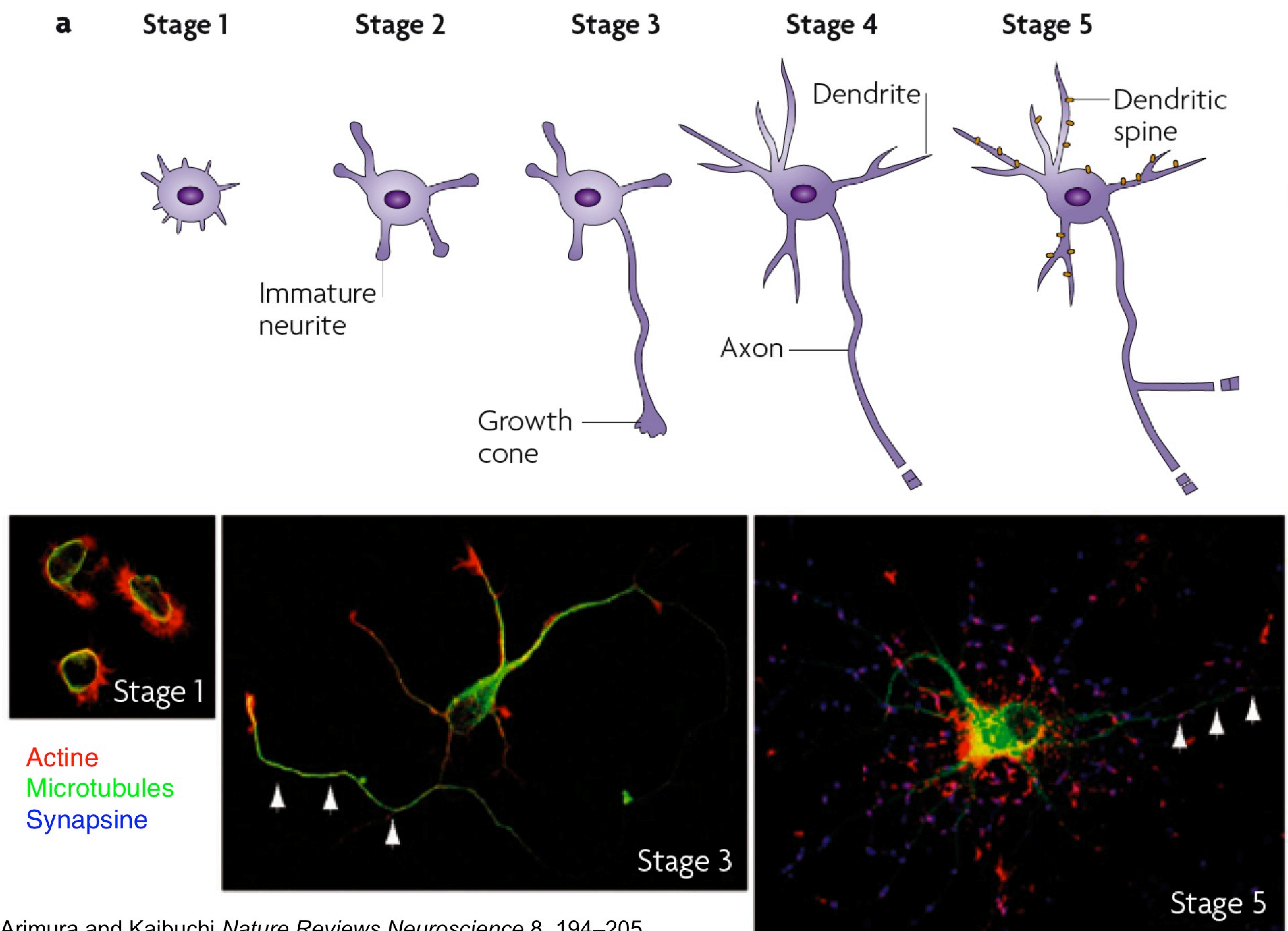
Des études extensives ont montrées que la croissance du neurite était étroitement controlée par la structure de son extrémité : le cone de croissance. La question de la polarité pourrait se simplifier à la sélection du cône de croissance.

Croissance neuritique:

Elle est menée par 4 grandes étapes:

- Une augmentation de la membrane plasmique (recrutement de vésicules et fusion)
- Augmentation de la dynamique des filaments d'actine
- Augmentation de la formation des microtubules
- Augmentation locale de molécules de signalisation (PI3K, Rho GTPase,…)

Arimura and Kaibuchi *Nature Reviews Neuroscience* 8, 194–205 (March 2007)

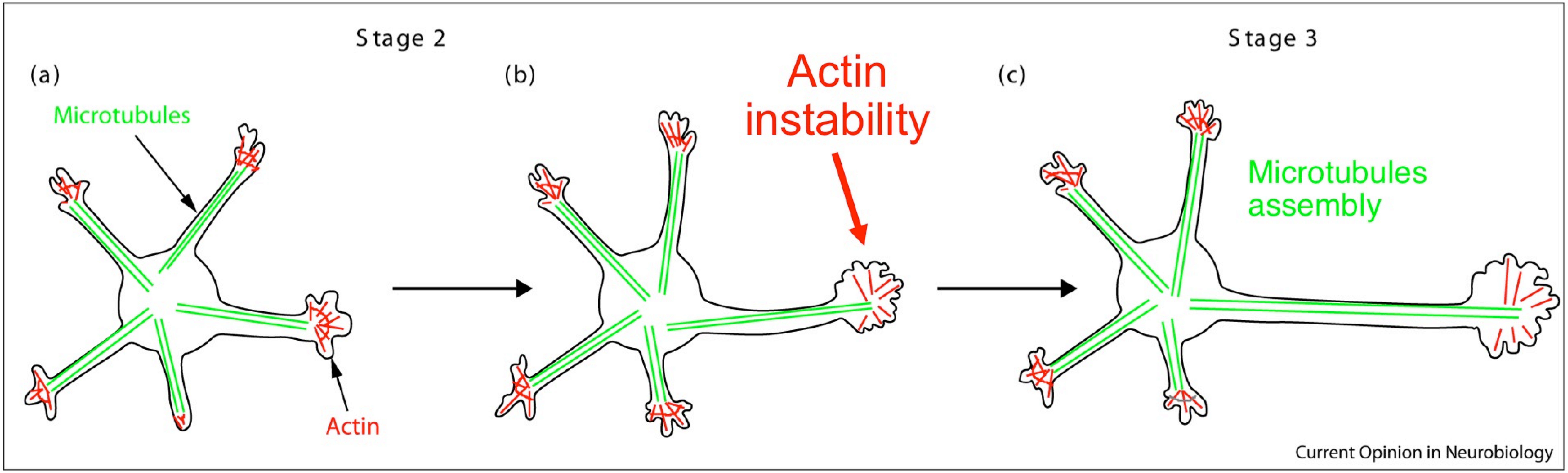


Arimura and Kaibuchi *Nature Reviews Neuroscience* 8, 194–205

-The Role of Local Actin Instability in Axon Formation. Frank Bradke and Carlos G. Dotti. (1999) *Science* 283: 1931-1934

-Establishment of neuronal polarity: lessons from cultured hippocampal neurons, Frank Bradke and Carlos G Dotti (2000). *Curr Opin. Neurobiol. 10: 574-581*

Figure 3



Actin instability causes axon formation. (a) The growth cones of the different neurites of unpolarized stage 2 cells show similar activities over time early in development. The actin filaments comprise the peripheral area of the growth cones whereas the microtubules enter only at the central domain of the growth cone. (b) One of the growth cones of the neurites of morphologically unpolarized stage 2 cells becomes highly dynamic and displays a less-stable actin cytoskeleton. The actin filaments appear to restrict the protrusion of the microtubules to a lesser extent. Thus, the microtubule may protrude and polymerize more distally. (c) Eventually, this neurite elongates by further polymerization/protrusion of microtubules enabled by a growth cone that has a high actin turnover. Thus, neuronal polarization takes place.

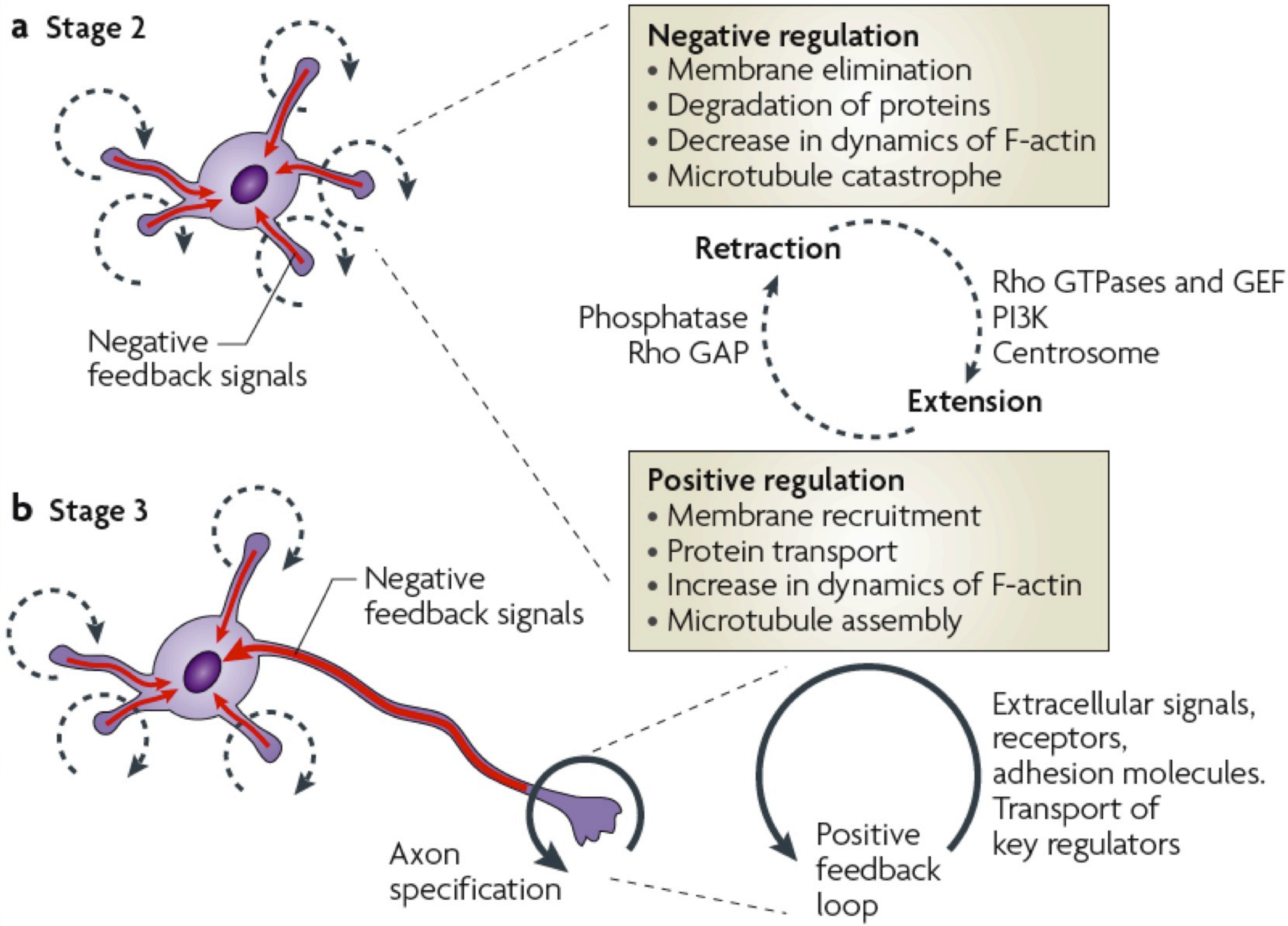


Figure 2 | **A tentative model for axon specification in neuronal polarization.**

Arimura and Kaibuchi *Nature Reviews Neuroscience* 8, 194–205 (March 2007)

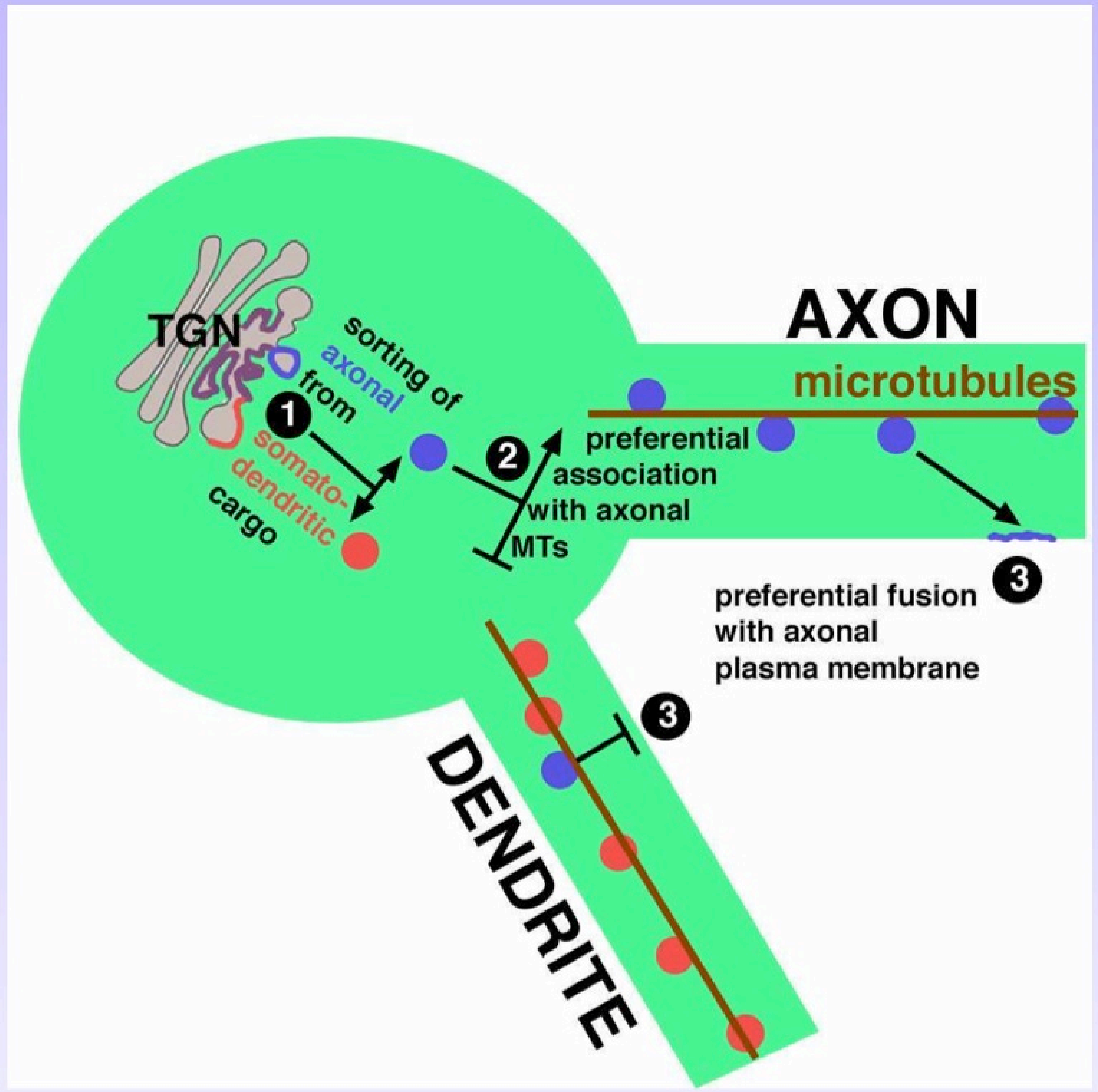
Molécules régulant la polarité

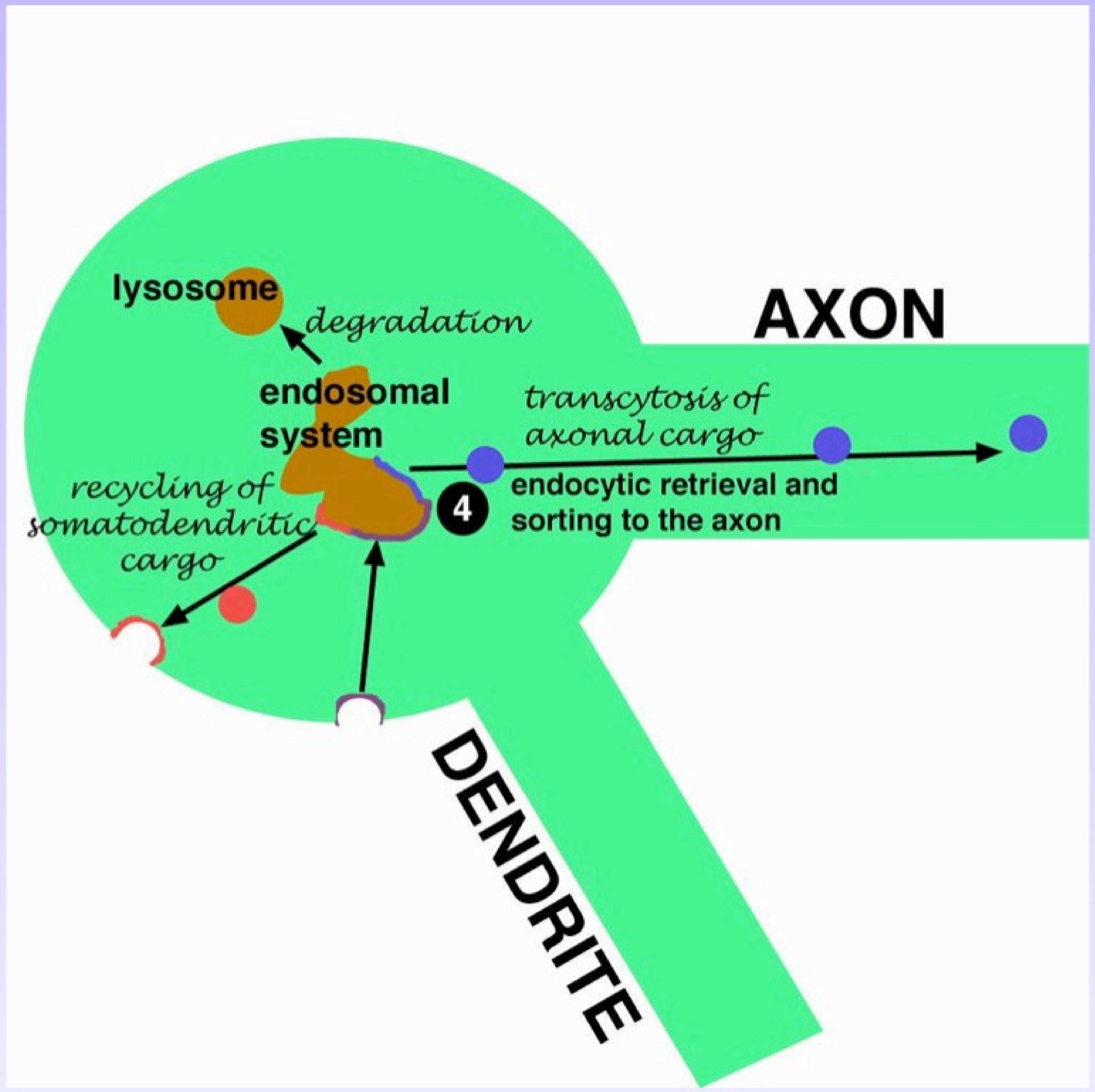
	Protein (function)	Subcellular localization	*Effect of upregulated activity	*Effect of down-regulated activity	Criteria used to score effects	Association with other molecules	Refs
+	PI3K (kinase; produces PtdIns(3,4,5)P_3)	Activited at the tip of future axon (st.2,3)	Myr-p110: multiple axons	Inhibitor (LY294002 etc.): no axon, or delay of polarization	Morphological and molecular (tau-1)	PtdIns(3,4,5)P_3, Akt	9,25, 38,40
-	PTEN (PtdIns(3,4,5)P_3 degradating enzyme)	Uniform (EGFP–PTEN)	WT: unpolarized	siRNA: increased number of axons	Morphological and molecular (PAR3, tau-1, MAP2, pAkt, synapsin 1, GAP43)	PI3K, Akt, GSK3β	25,38
+	R-Ras (small GTPase)	Single neurite (st.2); axon (st.3)	WT, DA (Q87L): multiple axons	siRNA: unpolarization; R-RasGAP-Myr: prevention of axon formation	Morphological and molecular (tau-1, synapsin 1, GAP43, FM4-64 uptake, MAP2, pAkt, pGSK3β)	GSK3β, Akt	51
+	H-Ras (small GTPase)	ND	WT, DA (V12): multiple axons	DN(N17): no axon	Morphological and molecular (tau-1)	PI3K, MEK, GSK3β, CRMP2	40
+	RAP1B (small GTPase)	Tip of single minor process (st.2); tip of axon (st.3)	DA (V12): super-numerary axons, or reduced number of minor processes	siRNA: loss of polarity, no axon	Morphological and molecular (tau-1, MAP2, PAR3, p-aPKC, pAkt)	PI3K, Cdc42, PAR3, PAR6, aPKC, Akt	26
+	Cdc42 (small GTPase)	Tip of single minor process (st.2); tip of axon (st.3)	DA (L28): supernumerary axons; V12: unpolarized	siRNA: no axon; N17: no effect	Morphological and molecular (tau-1, MAP2, PAR3, p-aPKC, pAkt, synapsin 1)	PI3K, PAR3, PAR6, aPKC, Akt	26,63
+	Rac1 (small GTPase)	ND	DA (V12): increased the length of minor neurite, or unpolarized	DN (N17): reduced the length of minor process, or unpolarized	Morphological and molecular (tau-1, MAP2, synapsin 1)	STEF	26,63
+	Akt (mediates the signals of growth factors)	Tips of minor processes (st.2); tip of axon (st.3)	WT, Myr-Akt: multiple axons, or increased number of neurites	shRNA: neuronal death	Morphological and molecular (tau-1, MAP2, pAkt, synapsin 1, GAP43)	PI3K, Ras, GSK3β, tau, MAP1B	38,40, 42
-	GSK3β (glycogen synthesis)	Tips of all neurites (st.2); tip of axon (st.3); pGSK3β: tip of axon (st.3); cell body	WT, S9A: no axon or unpolarized	shRNA, inhibitor GID5–6 peptide: multiple axon GSK3α/β knock-in mice: no effect	Morphological and molecular (tau-1, MAP2, pAkt, synapsin 1, GAP43, synaptophysin)	PAR3, PI3K, Akt, CRMP2, tau, BDNF, NT3, MAP1B, APC	38,39, 41,42, 83
+	PAR3 (involved in asymmetric cell division and cell polarization)	Tips of minor processes (st.2); tip of axon (st.3)	WT, 4N-1: unpolarized, or multiple axon-like neurites	delC, delN: no axon, or increased length of minor processes	Morphological and molecular (tau-1, MAP2, synapsin 1)	PAR6, aPKC, STEF	25,26, 63
+	PAR6 (involved in asymmetric cell division and cell polarization)	Tip of axon (st.3)	ND	WT, delCREB: unpolarized	Morphological and molecular (tau-1, MAP2, synapsin 1)	PAR3, aPKC, Cdc42, STEF	25,26, 63
+	aPKC (involved in asymmetric cell division and cell polarization)	ND	ND	Inhibitor (Bis): unpolarized	Morphological and molecular (tau-1, synapsin 1, MAP2)	PAR3, PAR6	25,26, 63
+	SAD (presynaptic differentiation)	Diffuse (st.3)	ND	KO: immature axon formation, enhancement of dendritic formation	Morphological and molecular (tau-1, MAP2)	Tau	94
-	MARK2 (microtubule affinity-regulating kinase)	All neurites, or tip of longest neurite (st.2); tip of axon (st.3)	WT or kinase dead mutant: unpolarized	siRNA: multiple axon-like neurites	Morphological and molecular (tau-1, synapsin 1)	Tau, aPKC	89
+	CRMP2 (mediator in semaphorin 3A signalling; cargo receptor)	Diffuse (st.2); distal part of axon (st.3)	WT: multiple axons, or increased length of axon	siRNA: unpolarized, or decreased length of axon	Morphological and molecular (tau-1, synapsin 1, synaptophysin, MAP2)	Tubulin, kinesin 1, numb, GSK3β, Akt, PI3K, BDNF, NT3	39,98, 99,104, 132

Arimura and Kaibuchi *Nature Reviews Neuroscience* 8, 194–205 (March 2007)

nature REVIEWS NEUROSCIENCE

3.Transport sélectif ou rétention ?

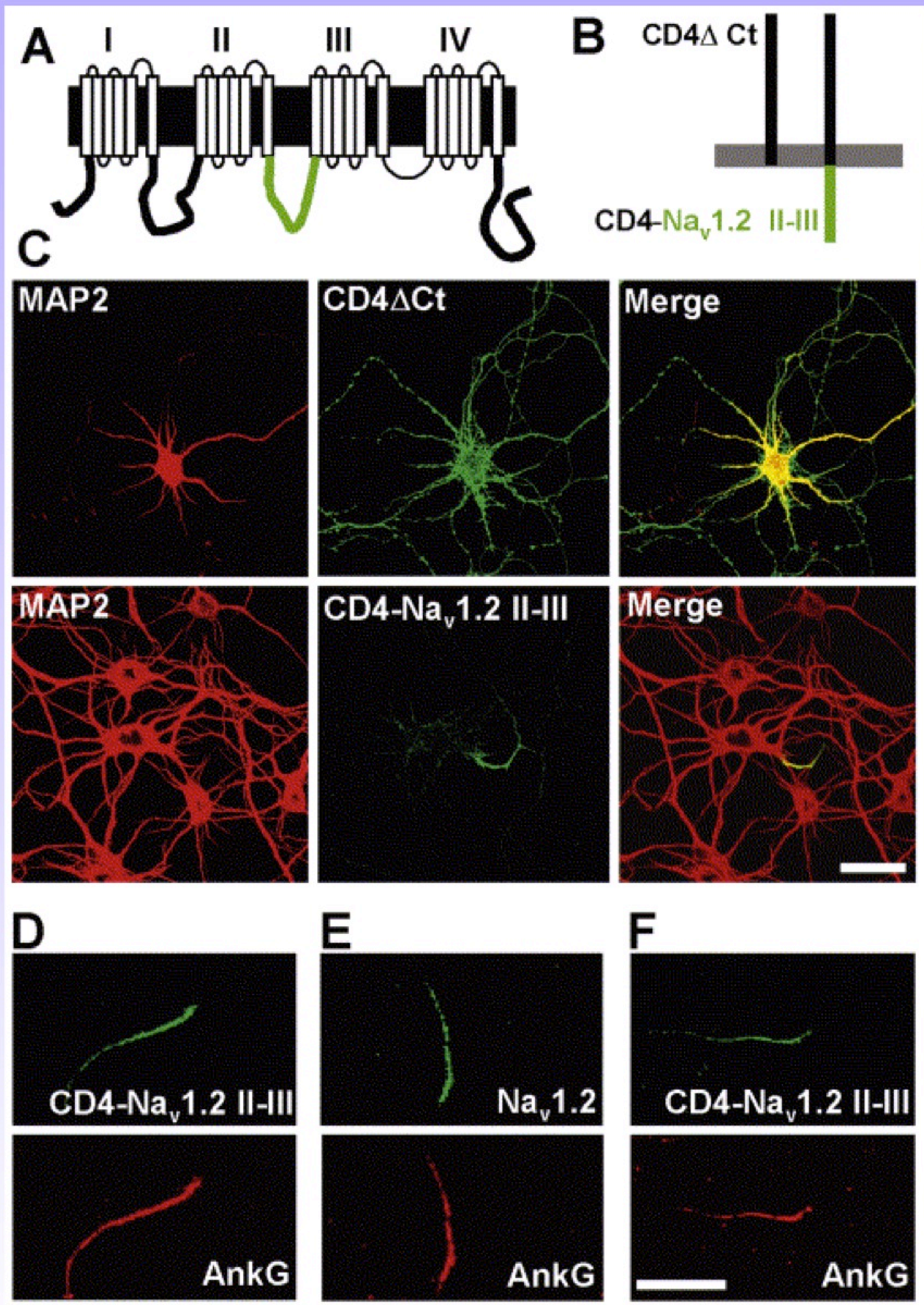


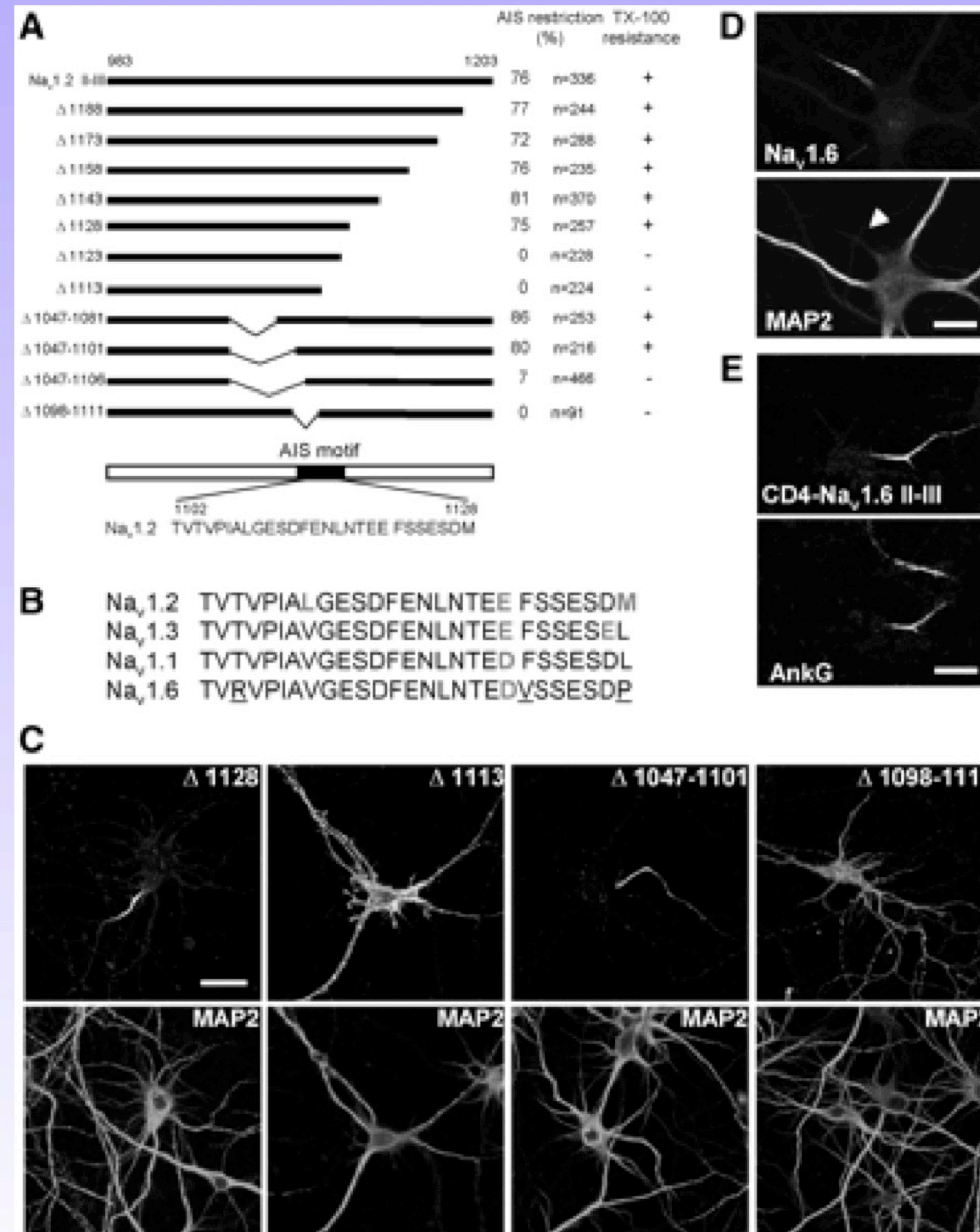


RTf

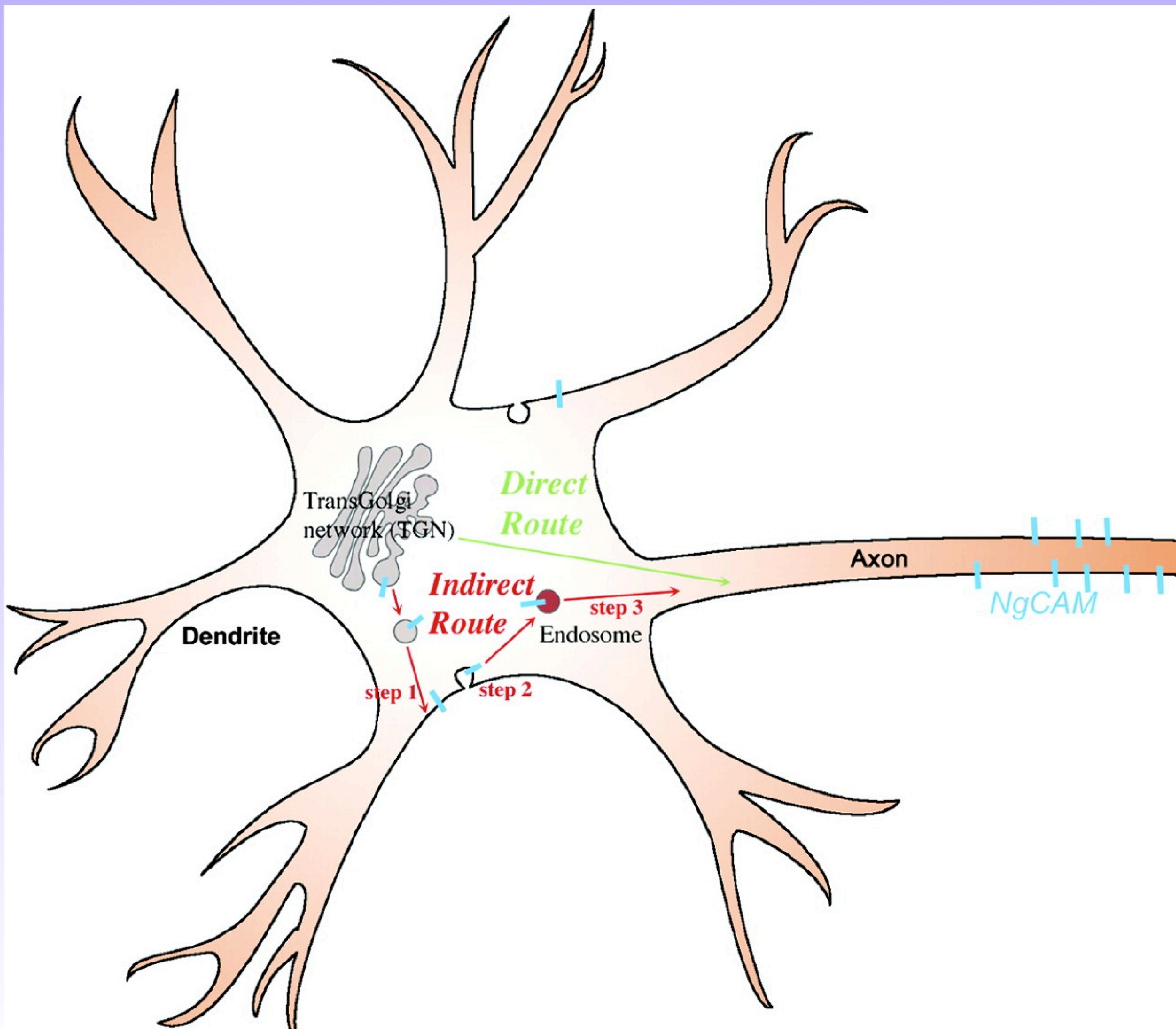
NgCAM dendritique
puis NgCAM axonal

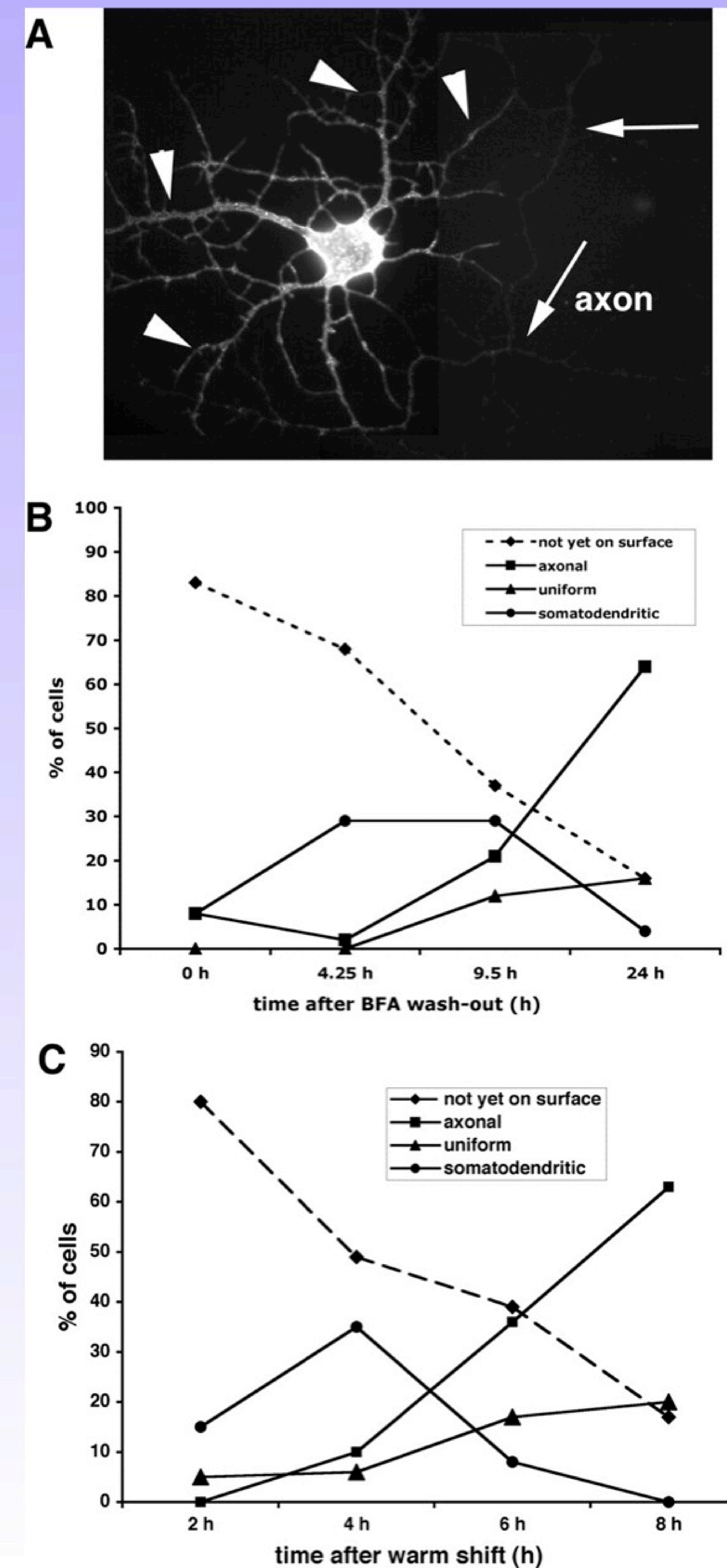
Example de signal: pour rejoindre le segment initial de l'axone (AIS) par l'équipe de B. Dargent & coll.



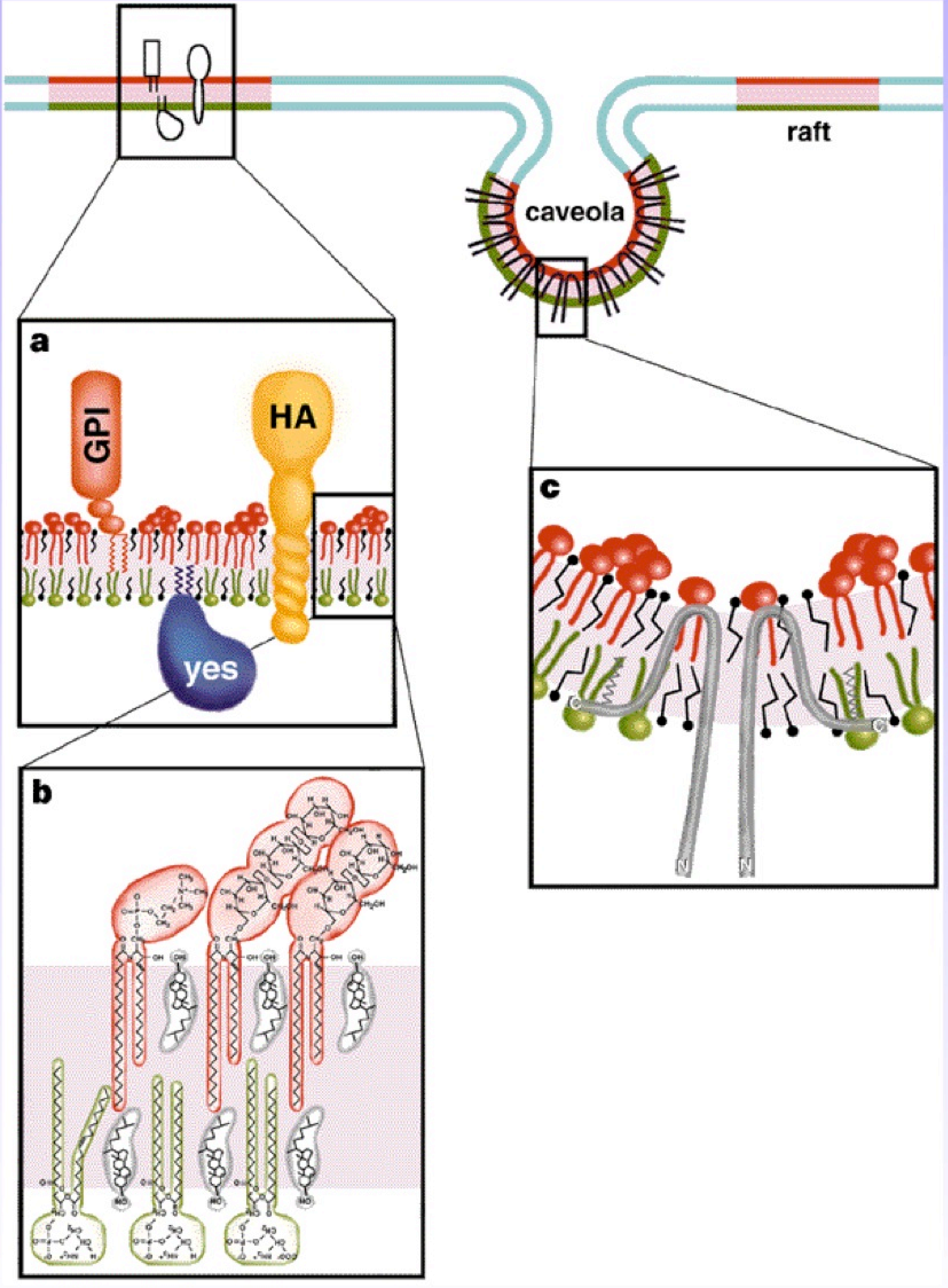


Transcytosis of NgCAM in neurons



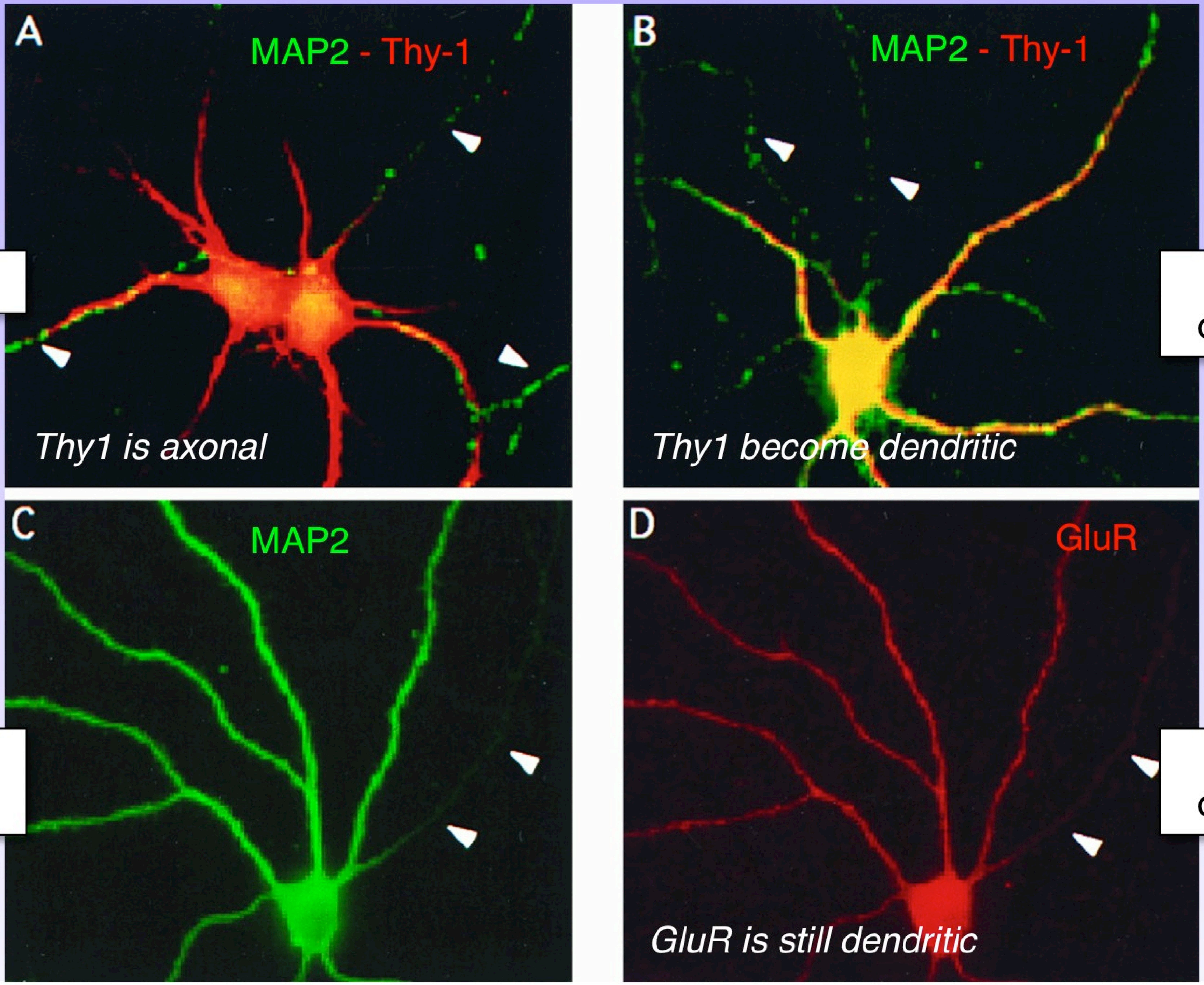


Raft: axonal signals?



Missorting of the axonal Thy-1 but not of a dendritic membrane protein occurred in sphingolipid-deprived cells. These results indicate that neurons sort a subset of axolemmal proteins by a mechanism that requires the formation of protein-lipid rafts. The involvement of rafts in axonal membrane sorting may explain the neurological deficits observed in patients with certain types of Niemann-Pick disease.

Control cells



sphingolipid-deprived cells

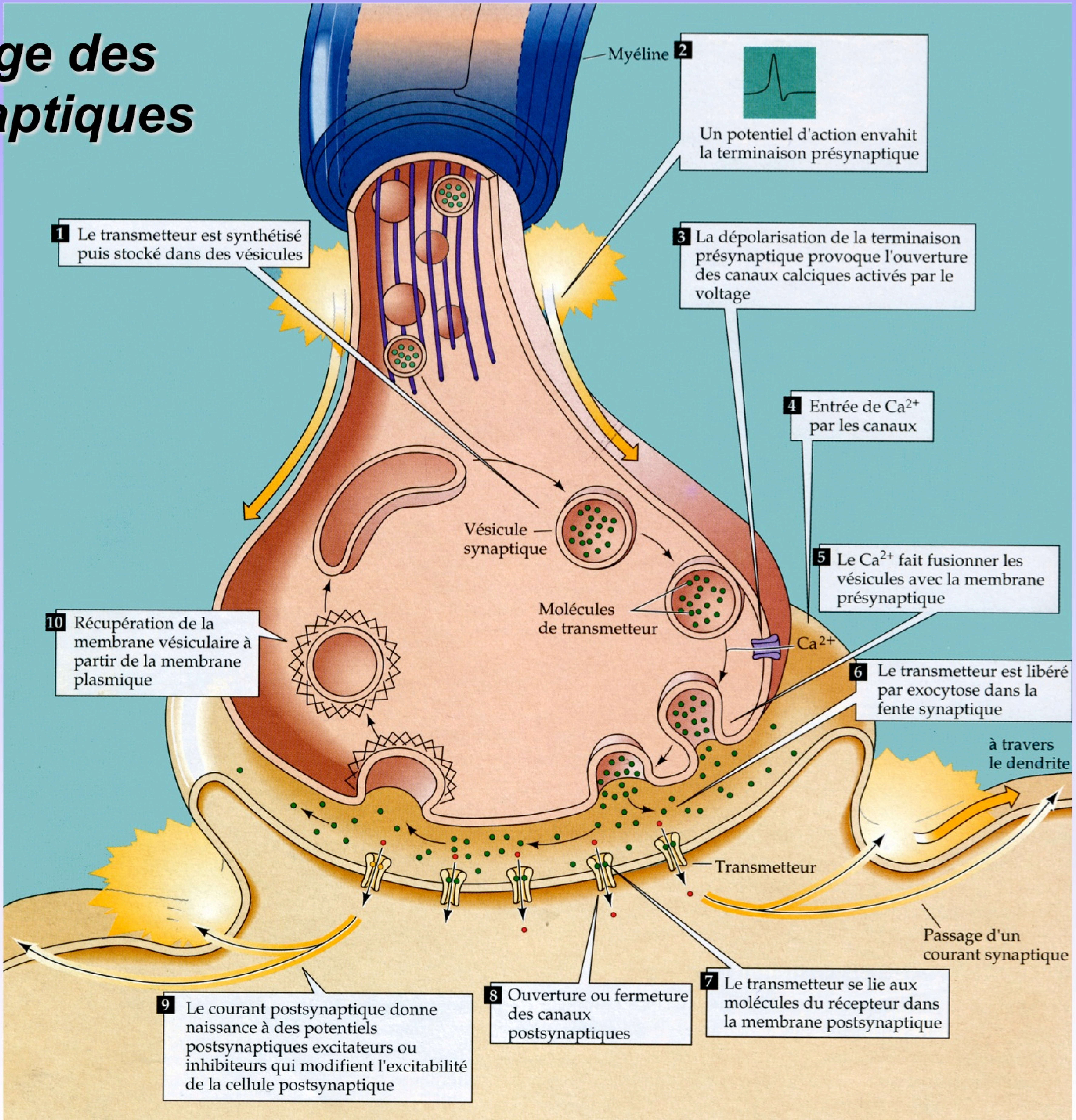
sphingolipid-deprived cells

sphingolipid-deprived cells

Ledesma, Maria Dolores et al. (1998) Proc. Natl. Acad. Sci. USA 95, 3966-3971

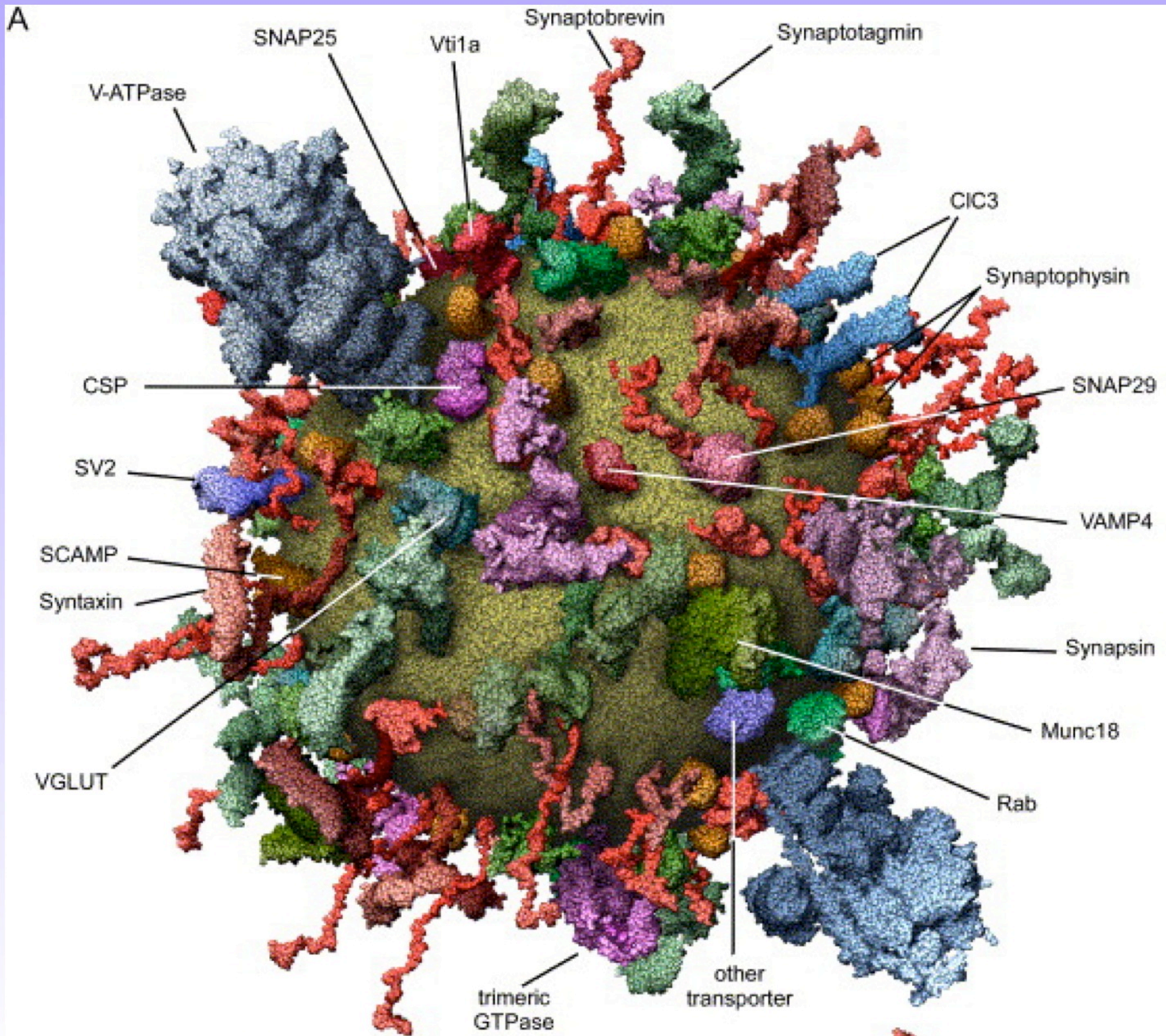
PNAS

4. Le recyclage des vésicules synaptiques



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

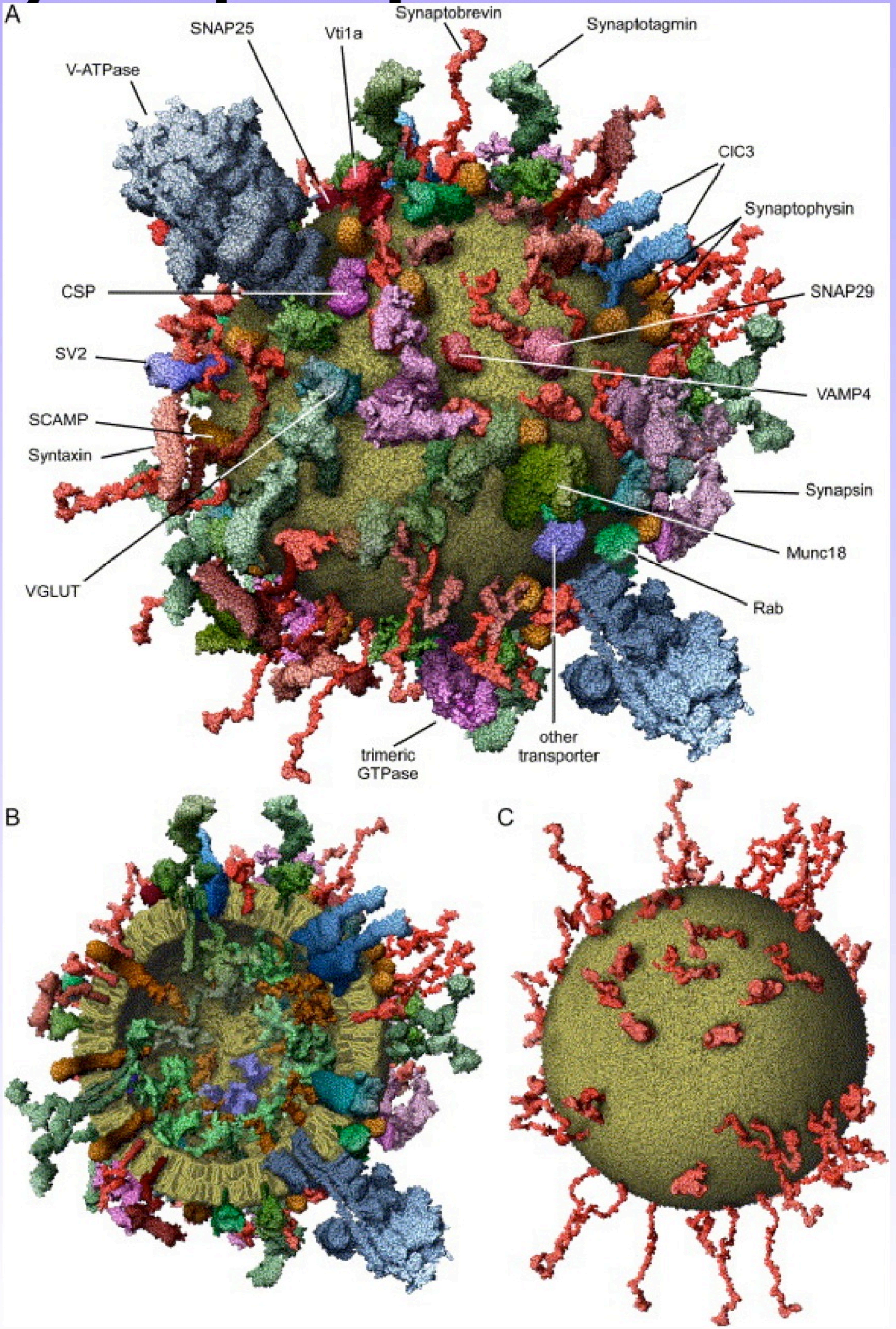
Vésicules Synaptiques



Takamori et al. Cell (2006)127:831.

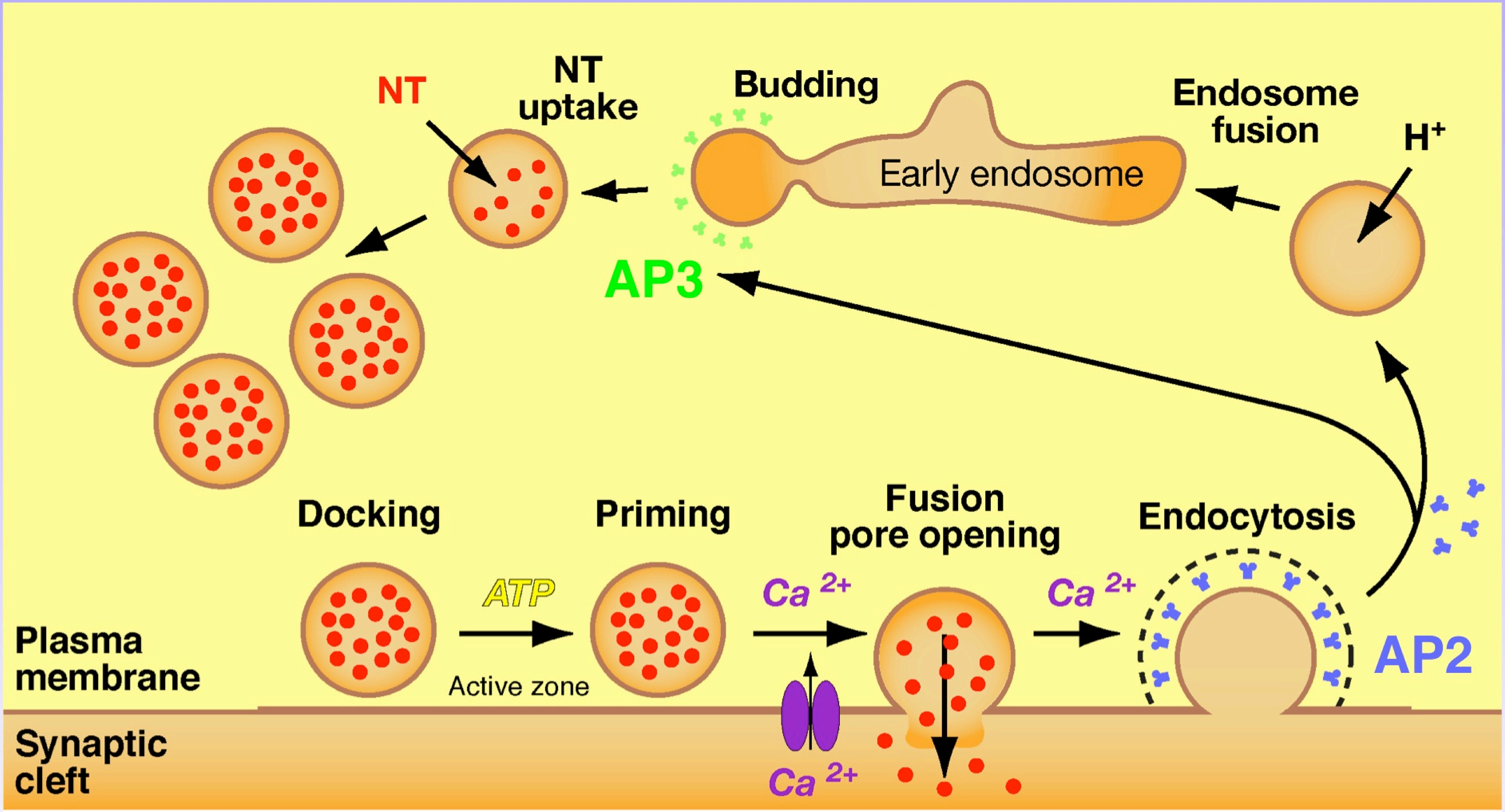
Vésicules Synaptiques

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]



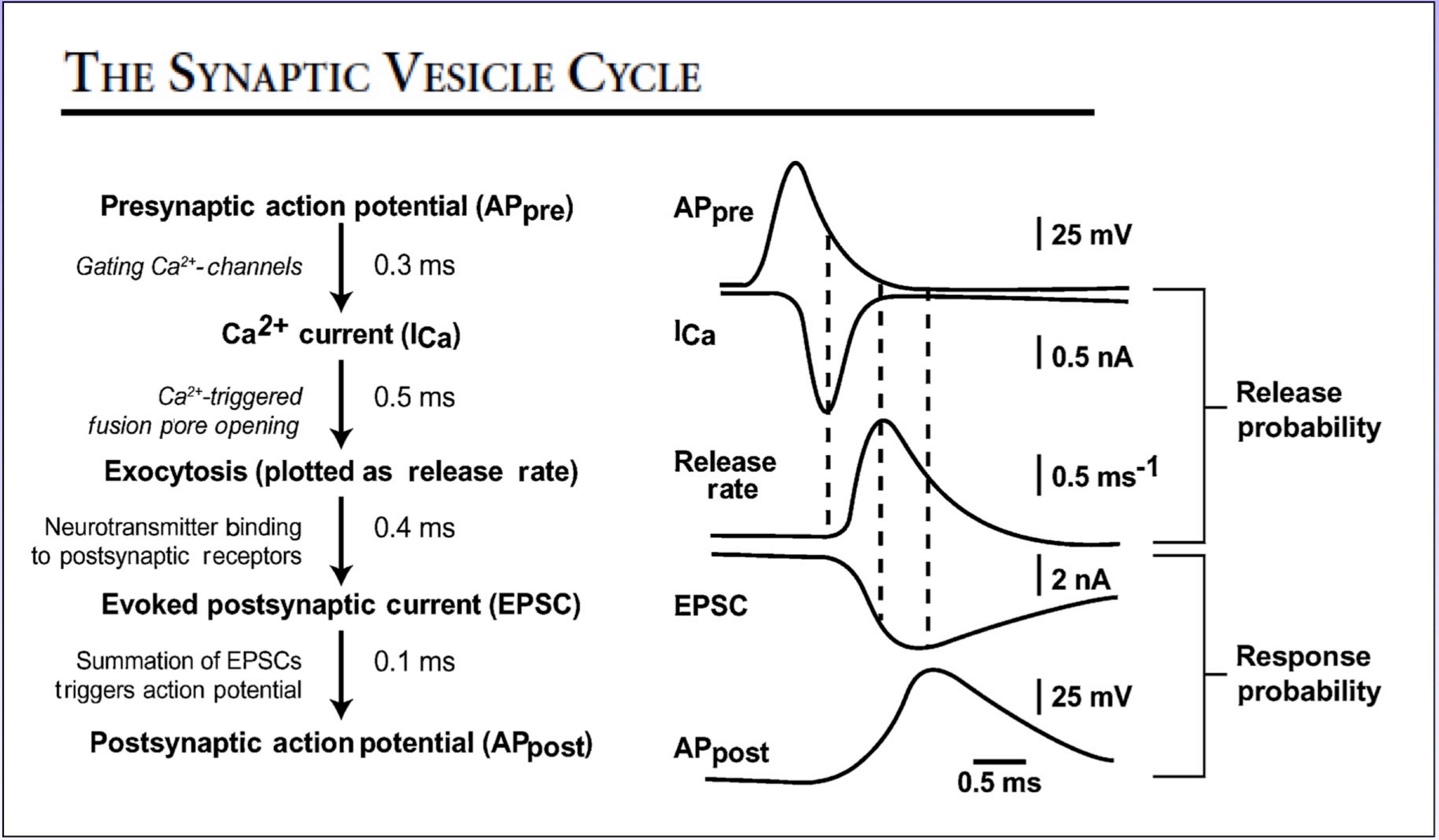
Takamori et al. Cell (2006)127:831.

Recyclage des vésicules synaptiques



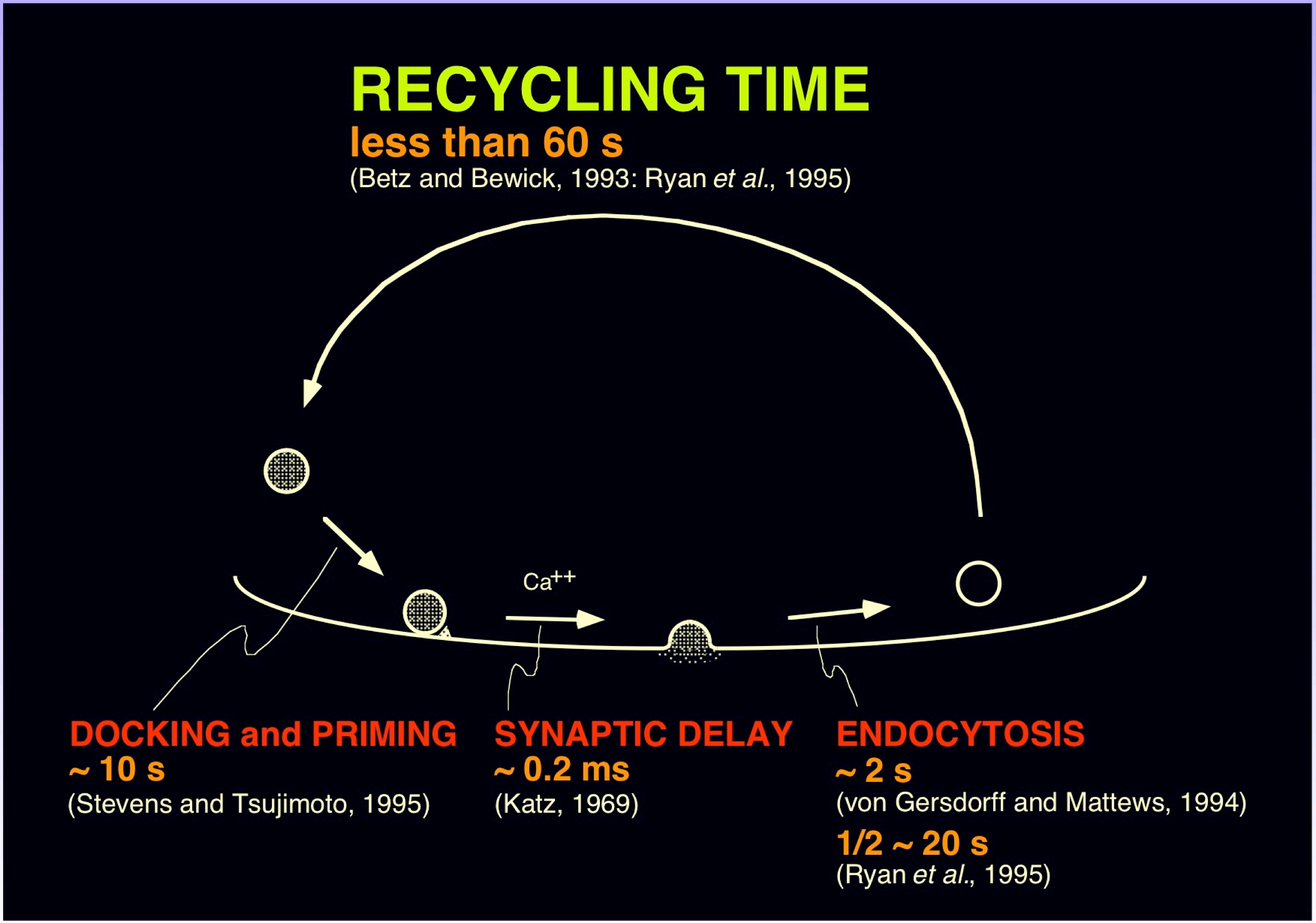
Danglot & Galli, Biology of the cell, 2003.

Chronologie: rapidité! Et rôle du calcium

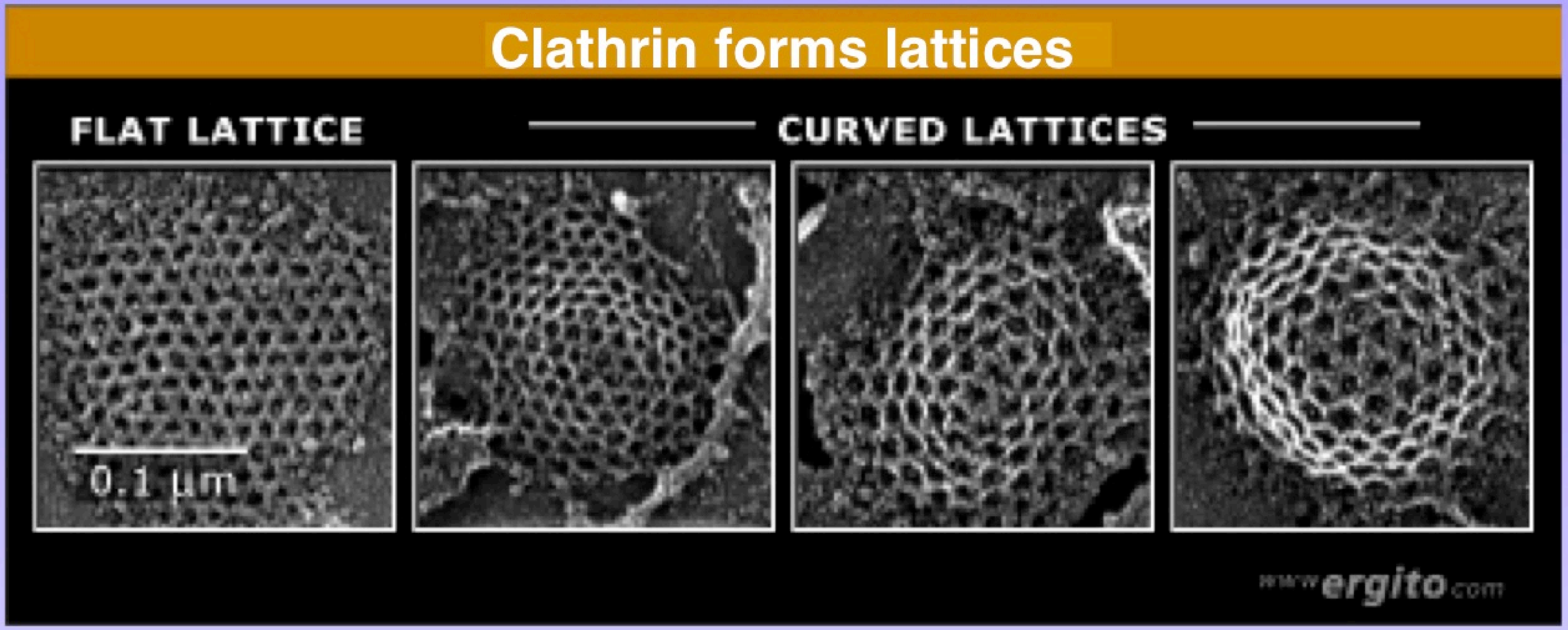


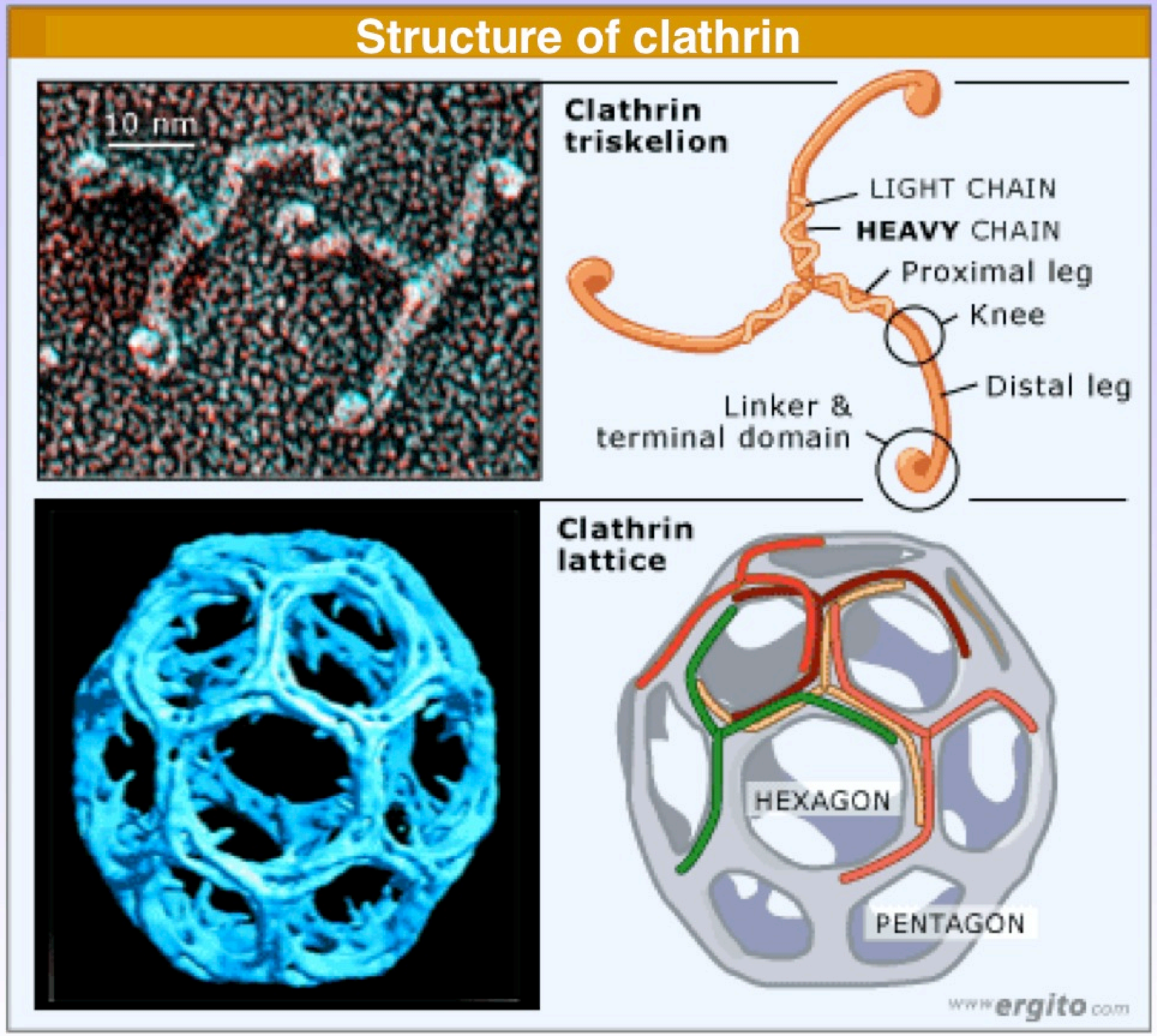
Südhof, Annu. Rev. Neurosci. 2004.27:509-547.

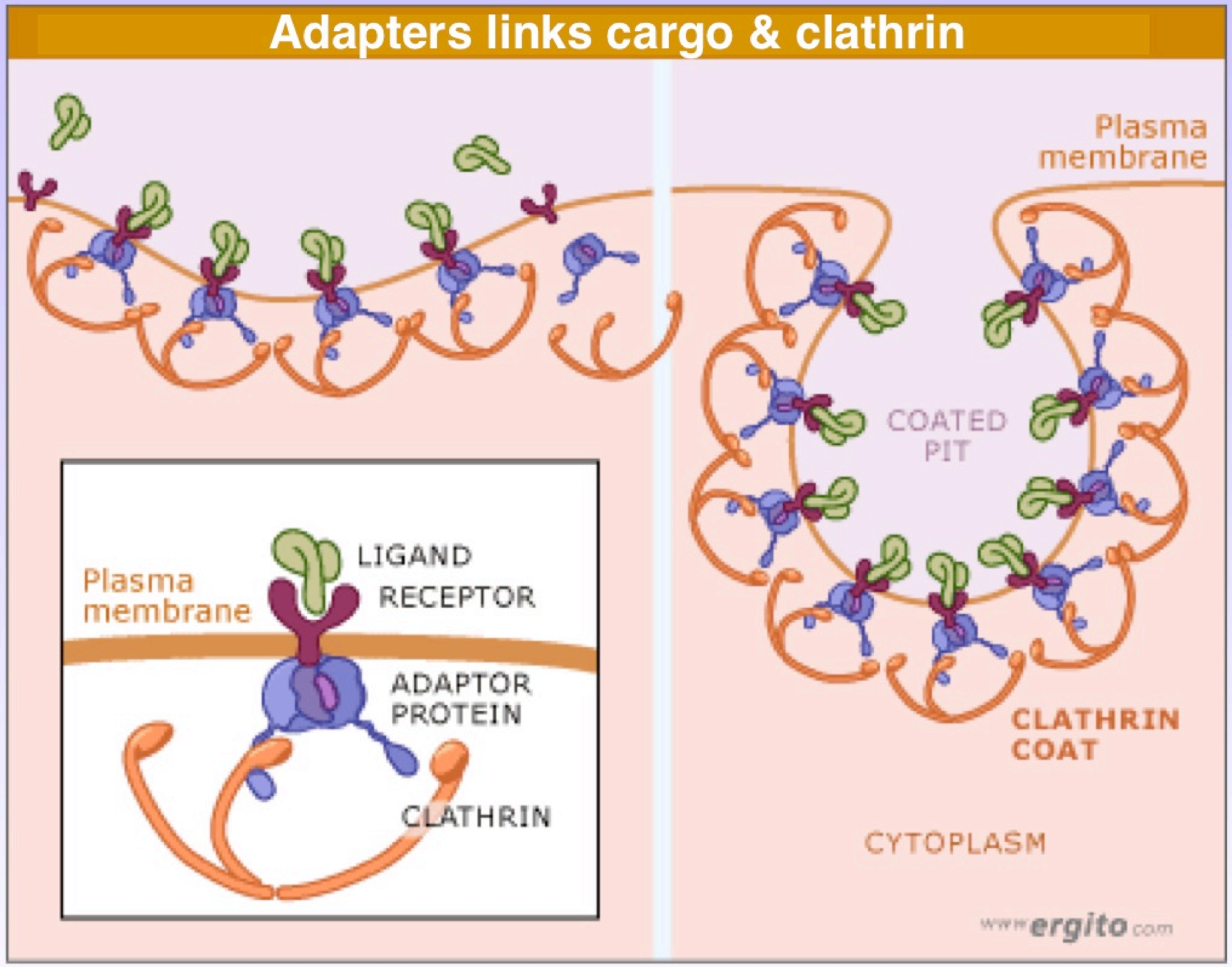
Recyclage des vésicules synaptiques



Clathrine



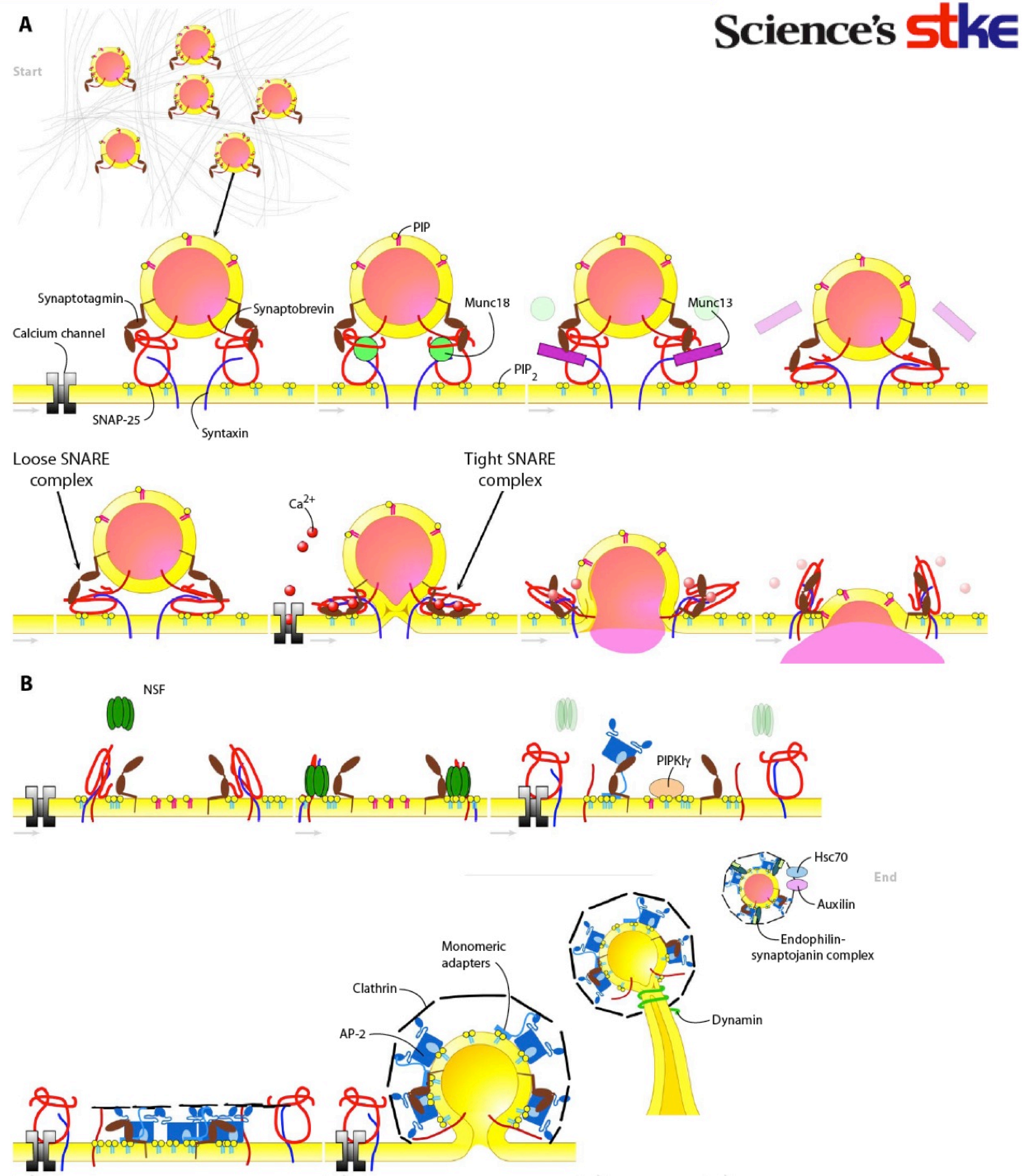




www.ergito.com/index.jsp

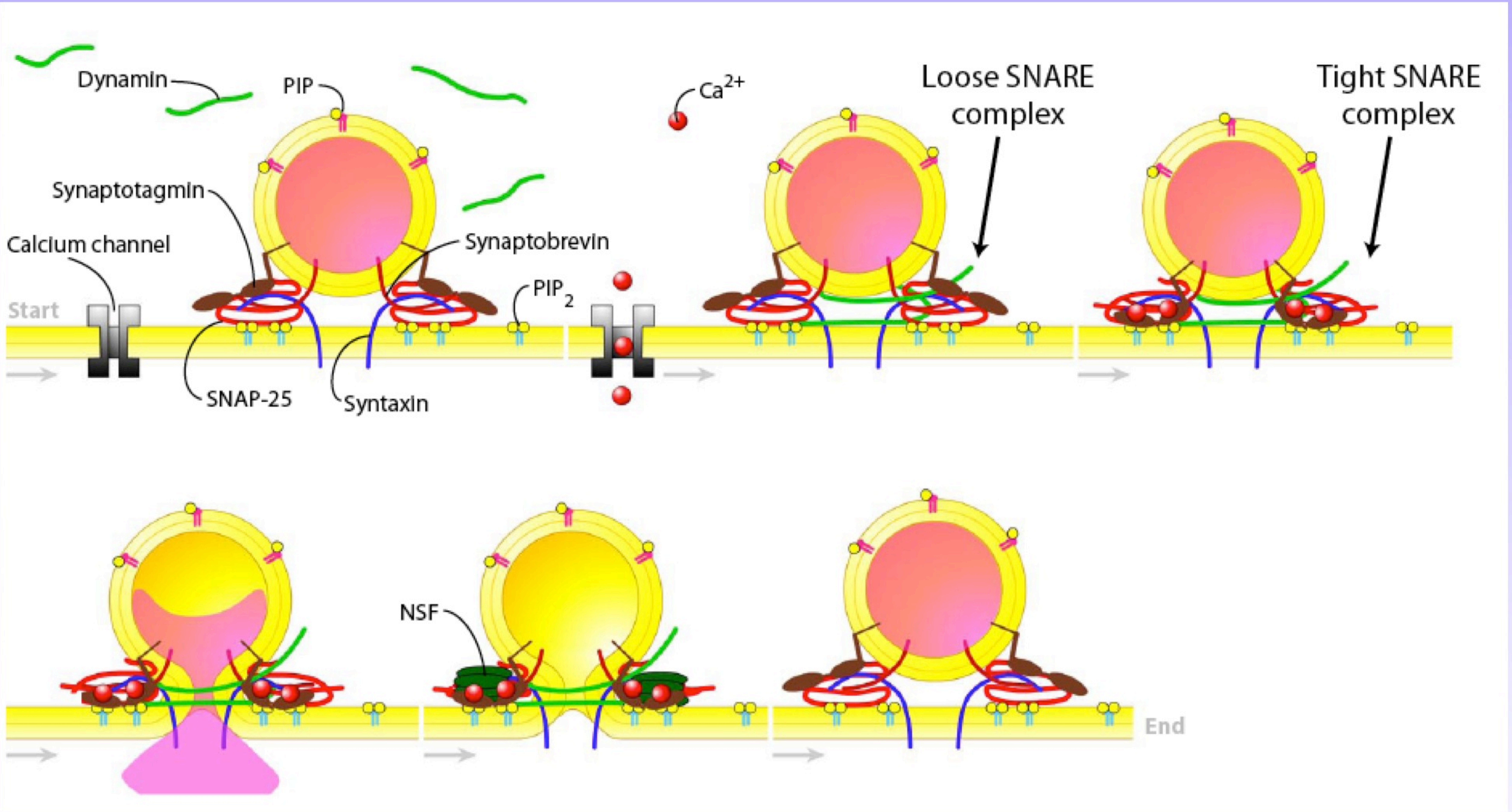
Recyclage des Vésicules synaptiques:

endocytose mediée par la clathrine (la voie lente)



Thierry Galli1 and Volker Haucke
www.stke.org/cgi/content/full/sigtrans;2004/264/re19

Kiss and Run ?

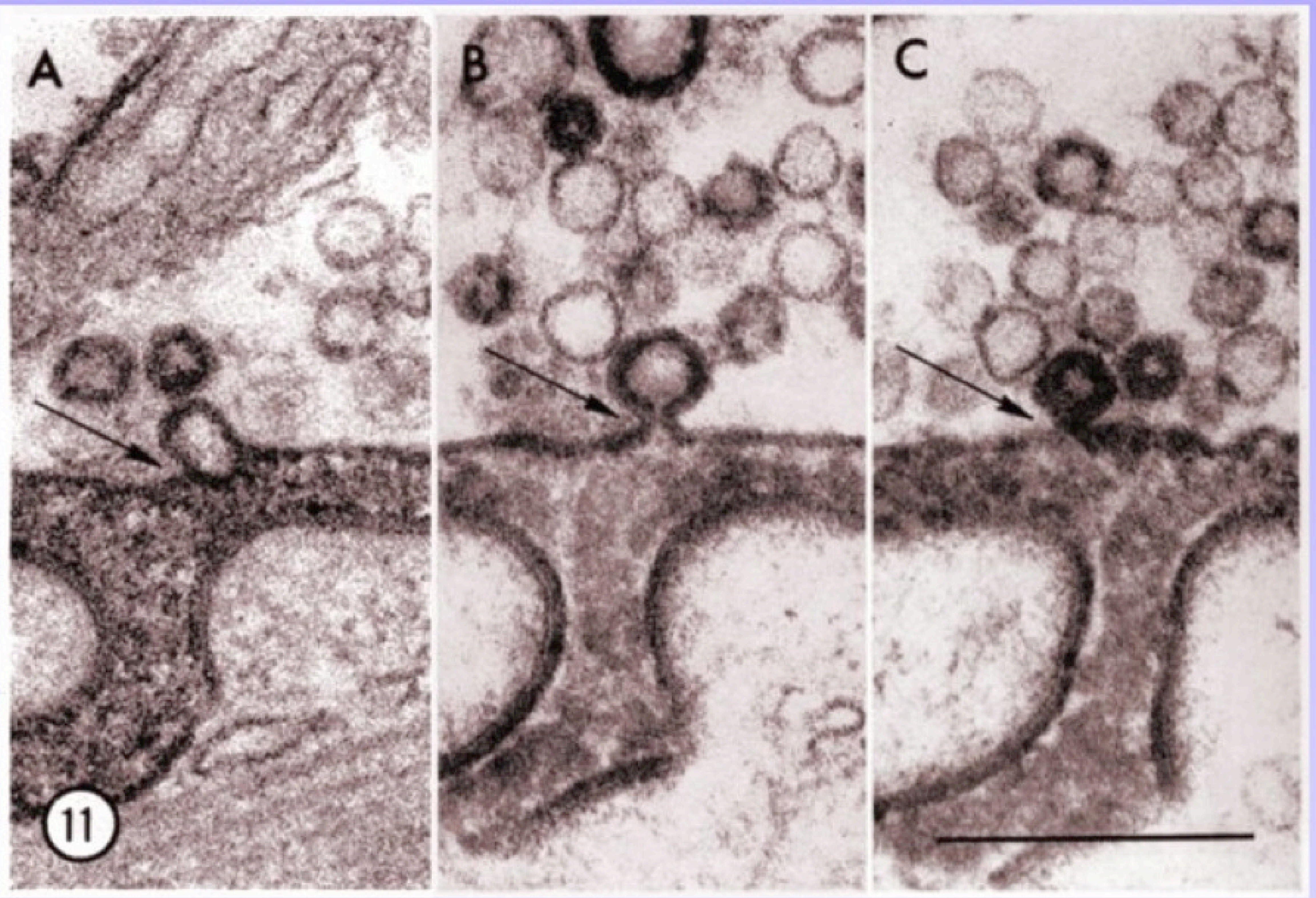


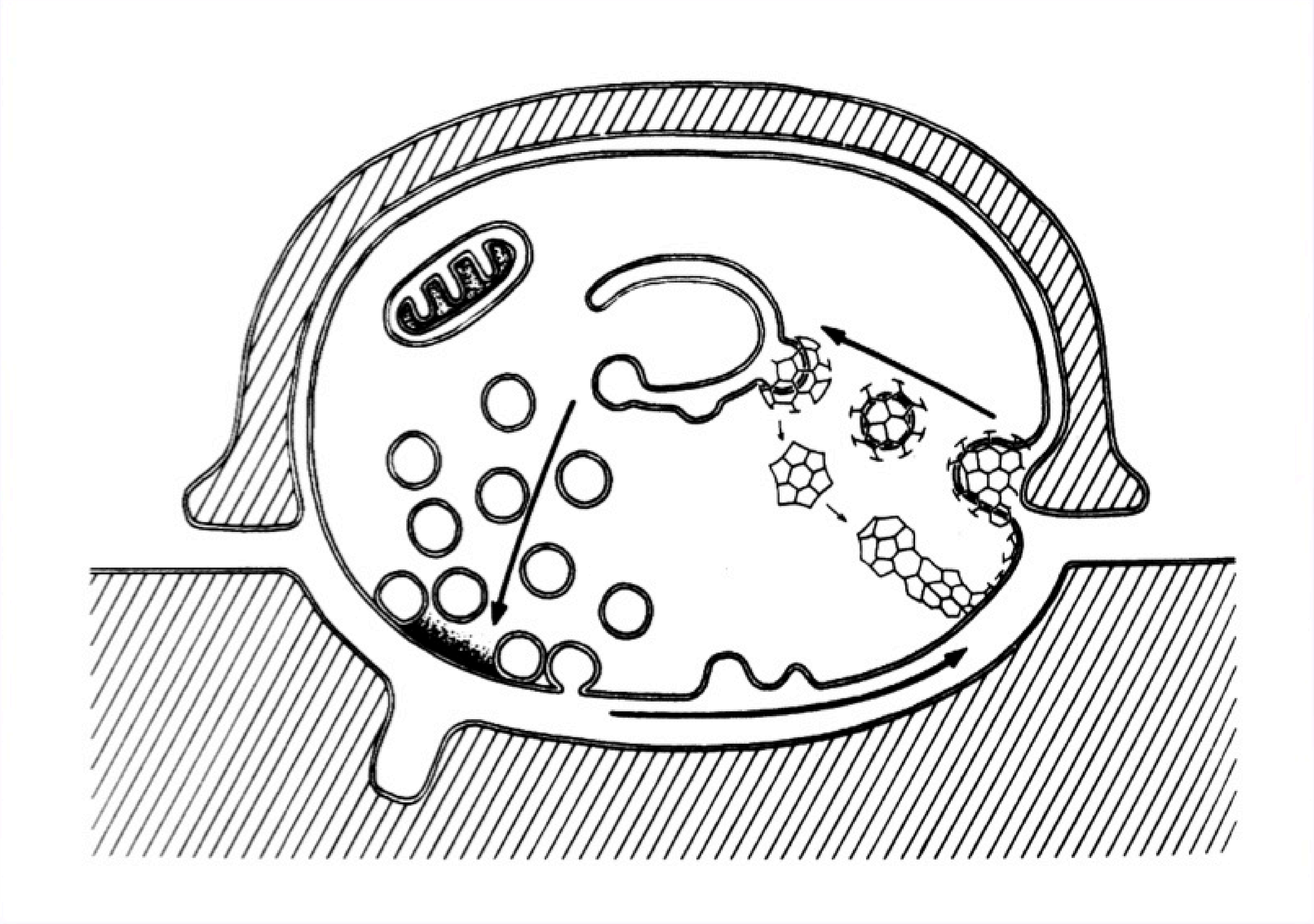
Thierry Galli1 and Volker Haucke

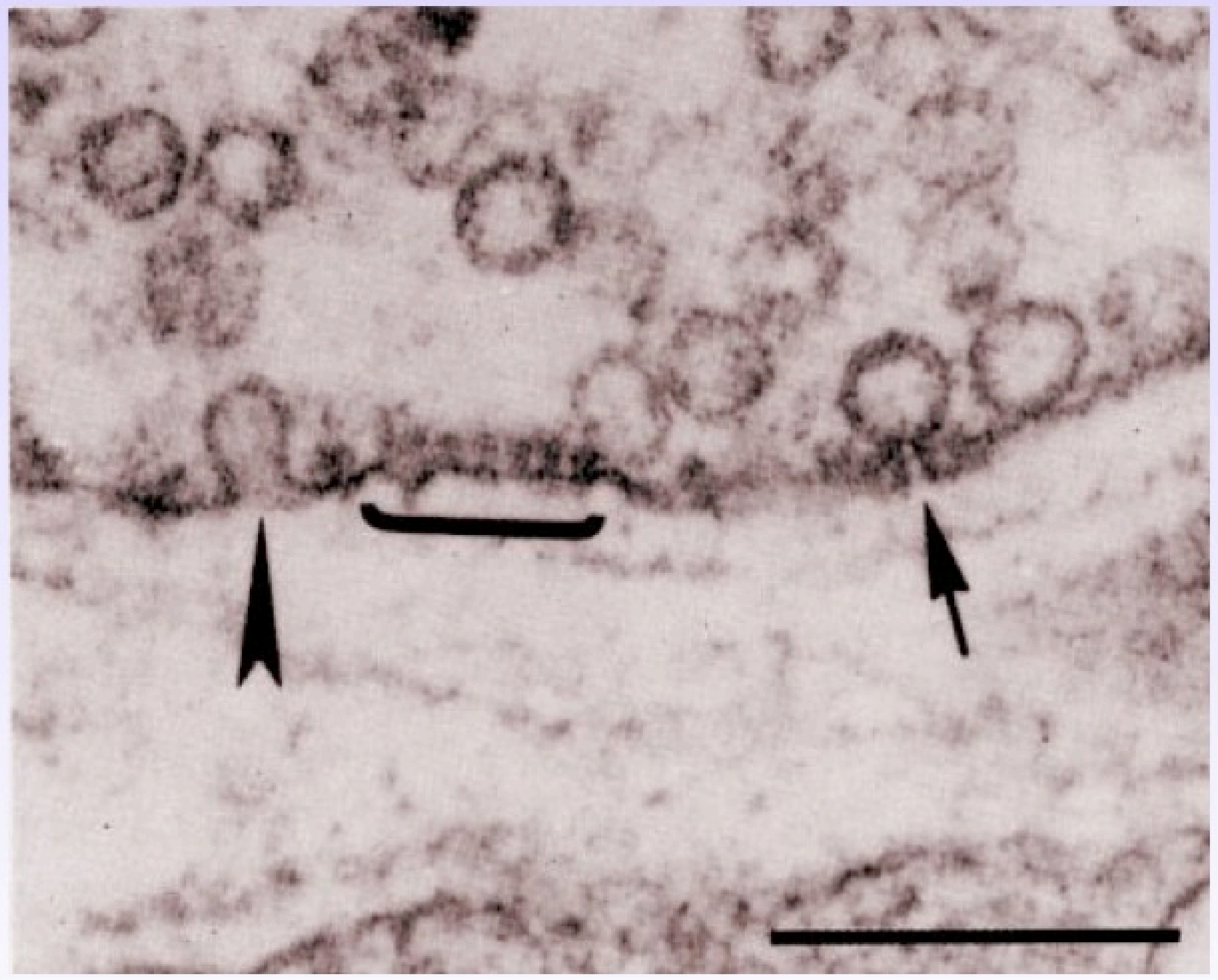
www.stke.org/cgi/content/full/sigtrans;2004/264/re19

Science's STKE

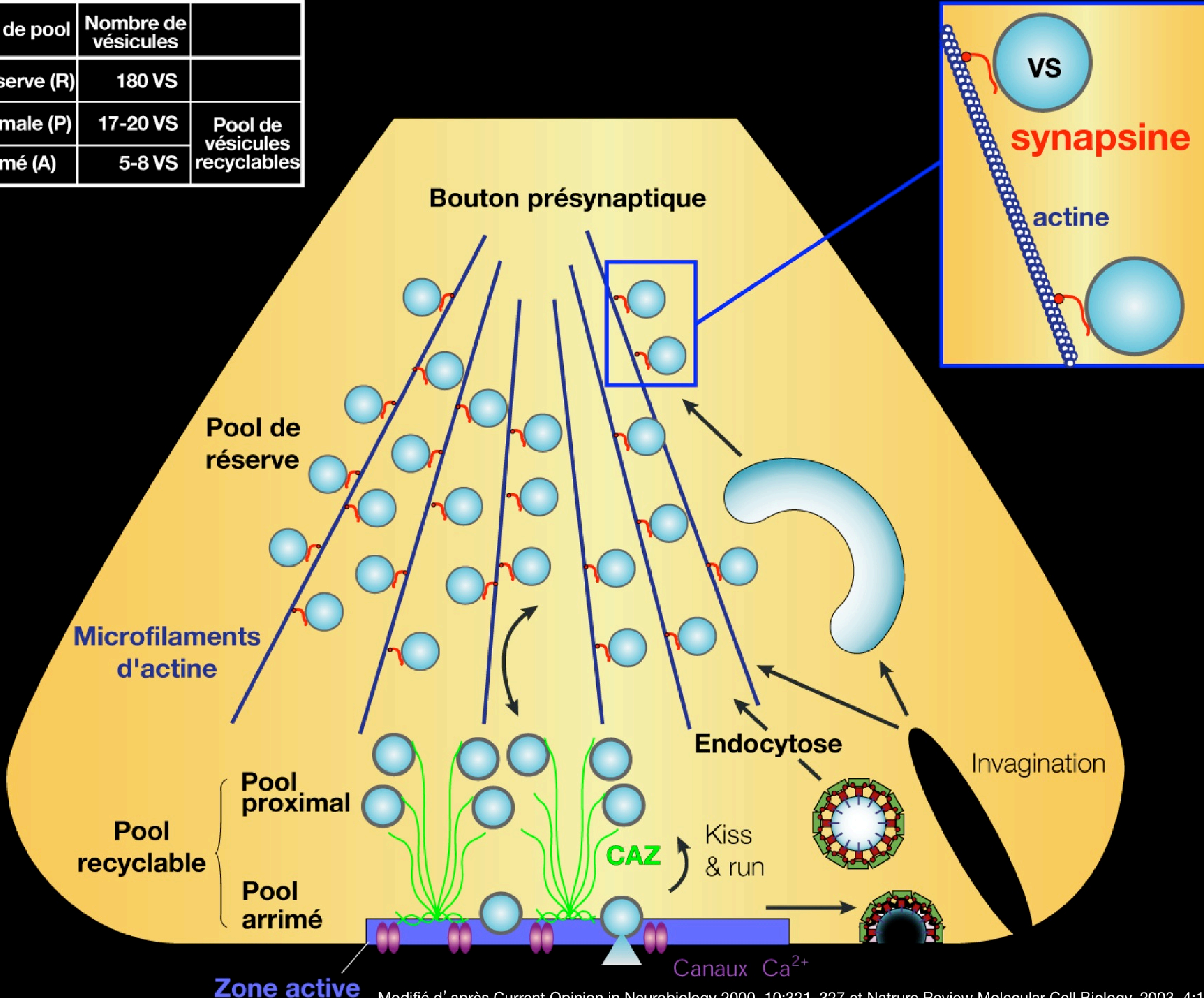
Et en microscopie électronique ?







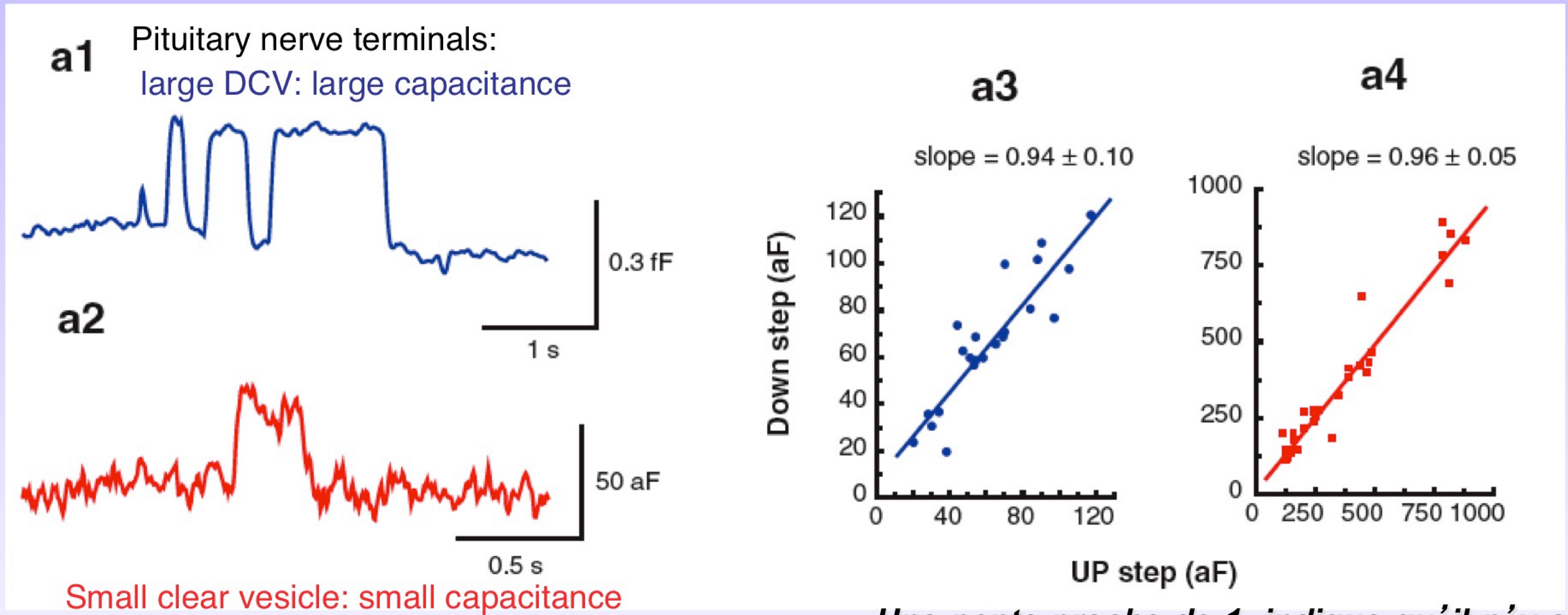
Type de pool	Nombre de vésicules	
de réserve (R)	180 VS	
proximale (P)	17-20 VS	Pool de vésicules recyclables
arrimé (A)	5-8 VS	



Modifié d' après Current Opinion in Neurobiology 2000, 10:321–327 et Natrure Review Molecular Cell Biology, 2003, 4:127-139.

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 evènement 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 vesicules + larges: augmentation de 16fF

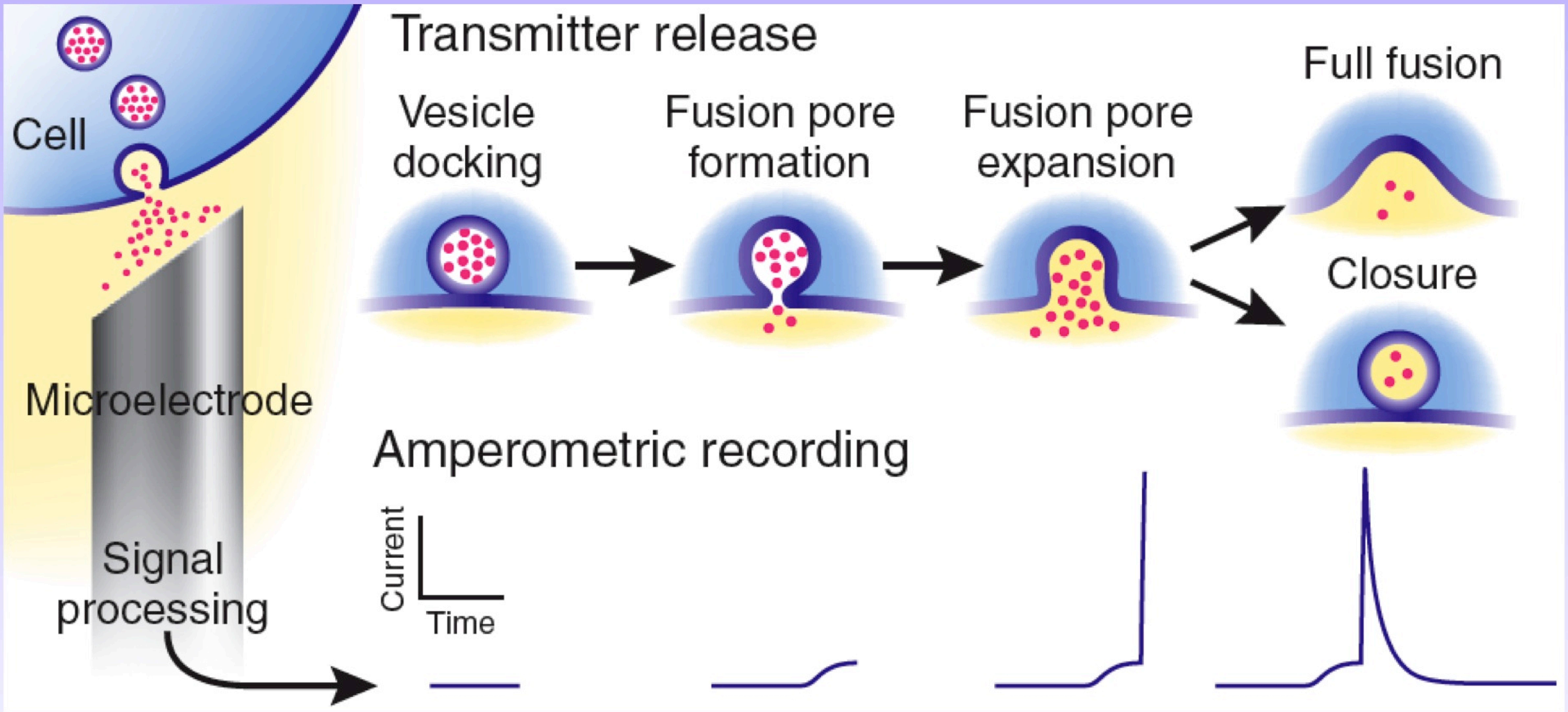


Une pente proche de 1, indique qu'il n'y a pas de transfert de membrane et indique plutot un evenement de type kiss-and-run.

Jackson & Chapman, Annual Rev. Biophys. Biomol. Struct. 2006

Comment mesurer l'exocytose ?

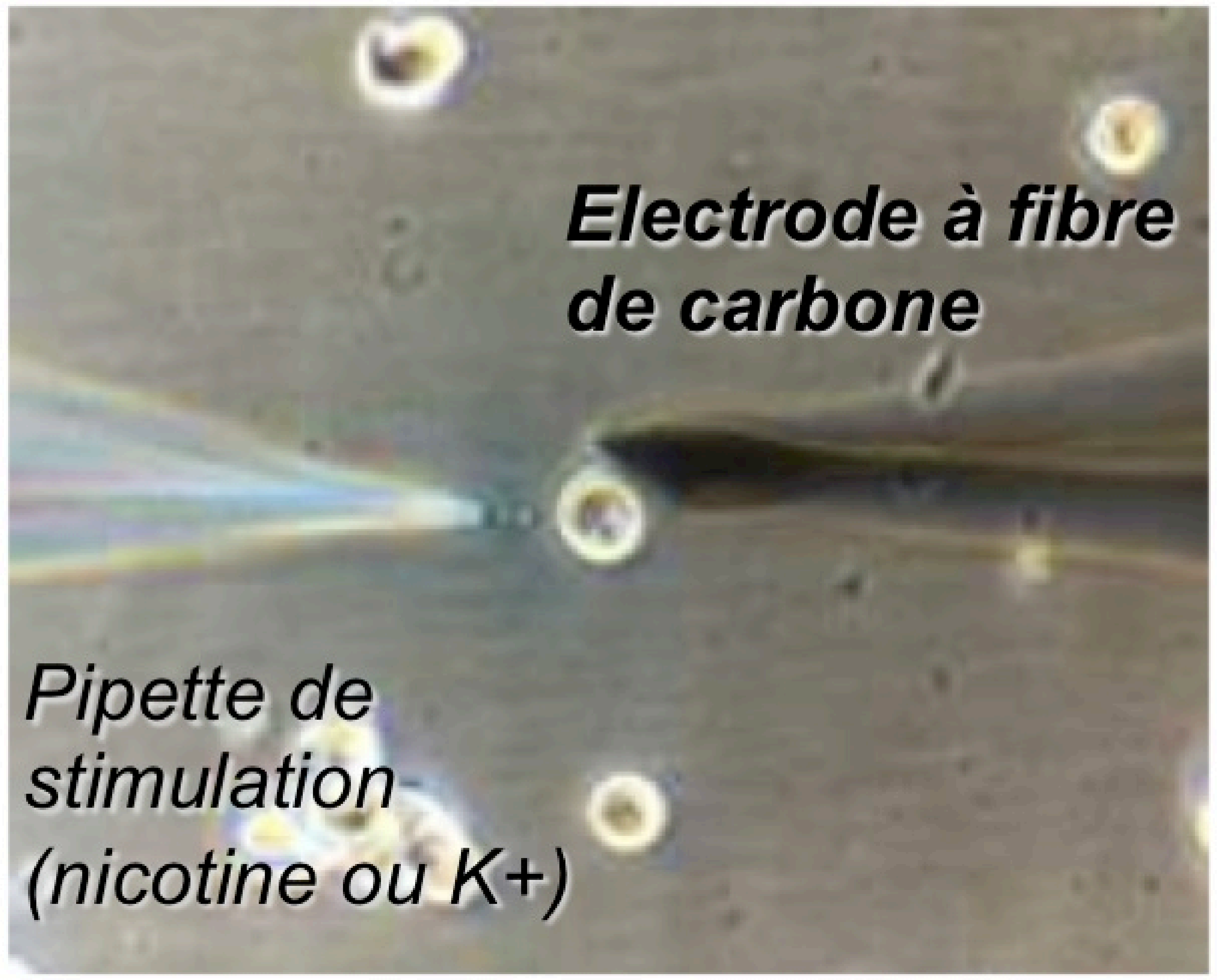
2) L'ampérométrie à fibre de carbone (5-10 microns de diamètre):
On stimule les cellules par une dépolarisation. La cellules sécrète alors des molécules. En presence d'un potentiel approprié, les molécules (catécholamines, indolamines) sécrétées s'oxydent et libèrent des électrons. La mesure du courant d'oxydation donne accès à la quantité de molecules sécrétées par événement unitaire d'exocytose.



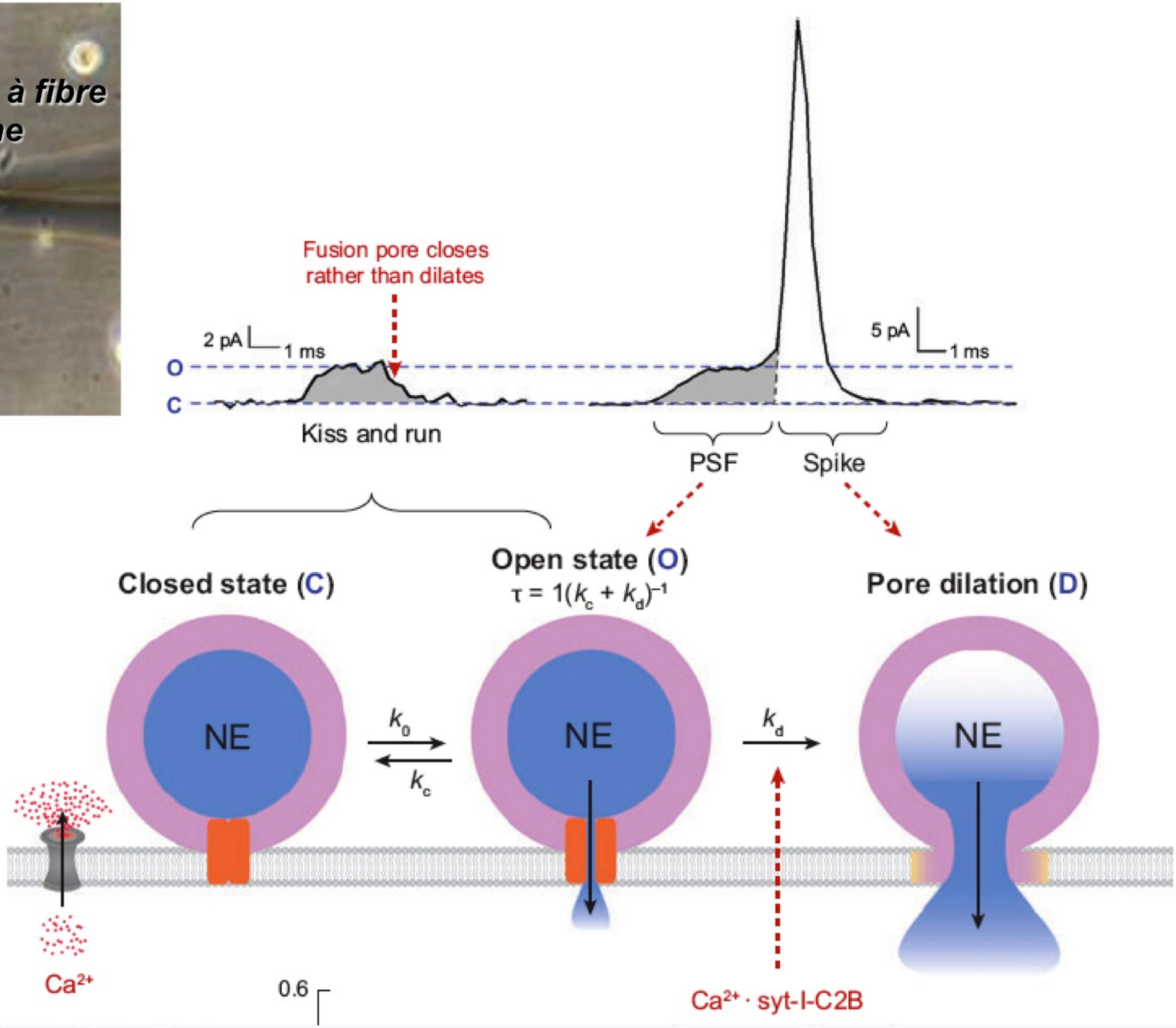
Cellules mesurées: *cellules chromaffines (noradrénaline, adrénaline), mastocytes (histamines, serotonines), et cellules ß du pancreas (insuline).*
Résolution temporelle: *<1ms*
Sensibilité de détection: *quelques milliers de molecules (Chen et al., 1994)*

Comment mesurer l'exocytose ?

2) L'ampérométrie à fibre de carbone:



F. Darchen (UPR1929)

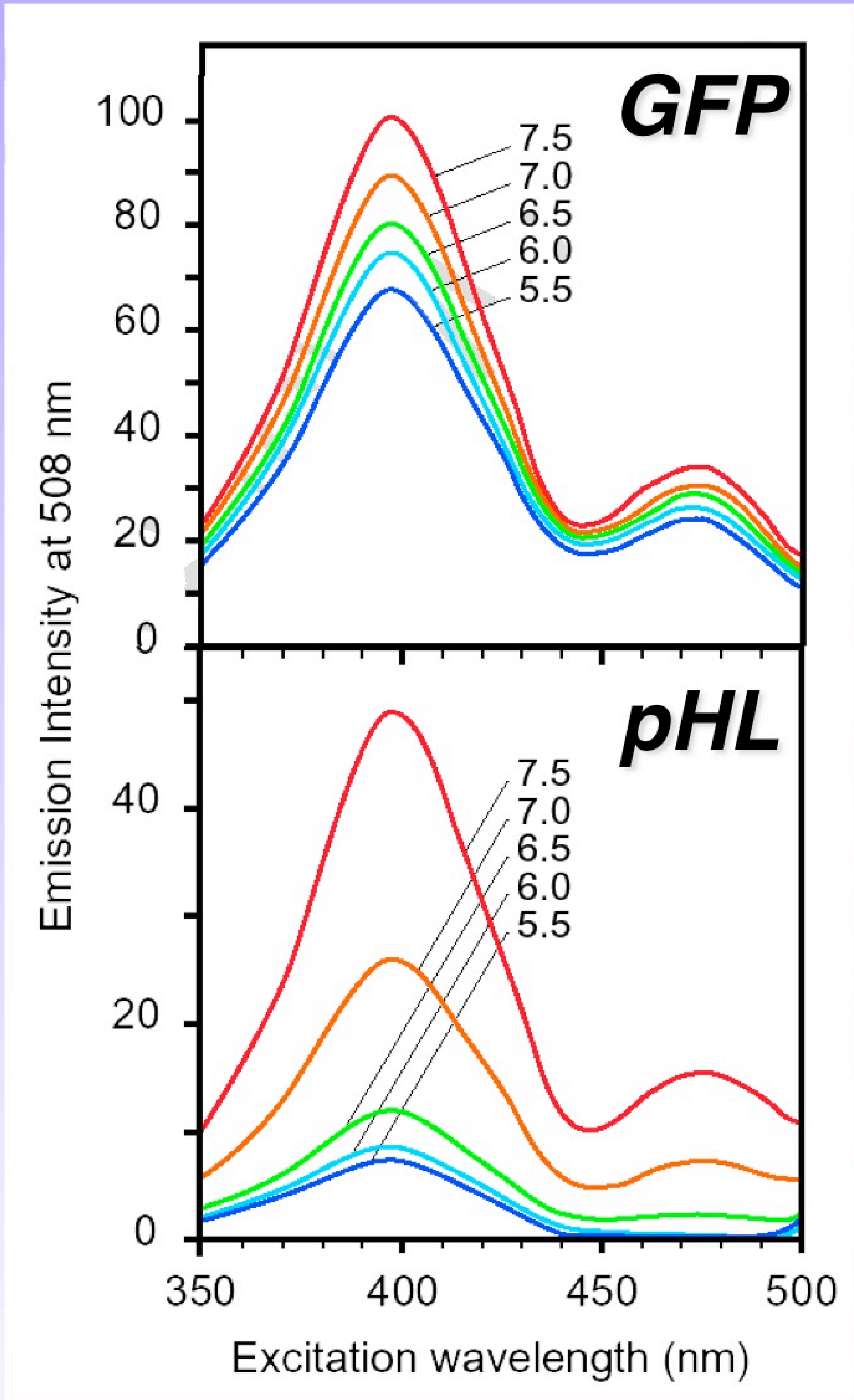


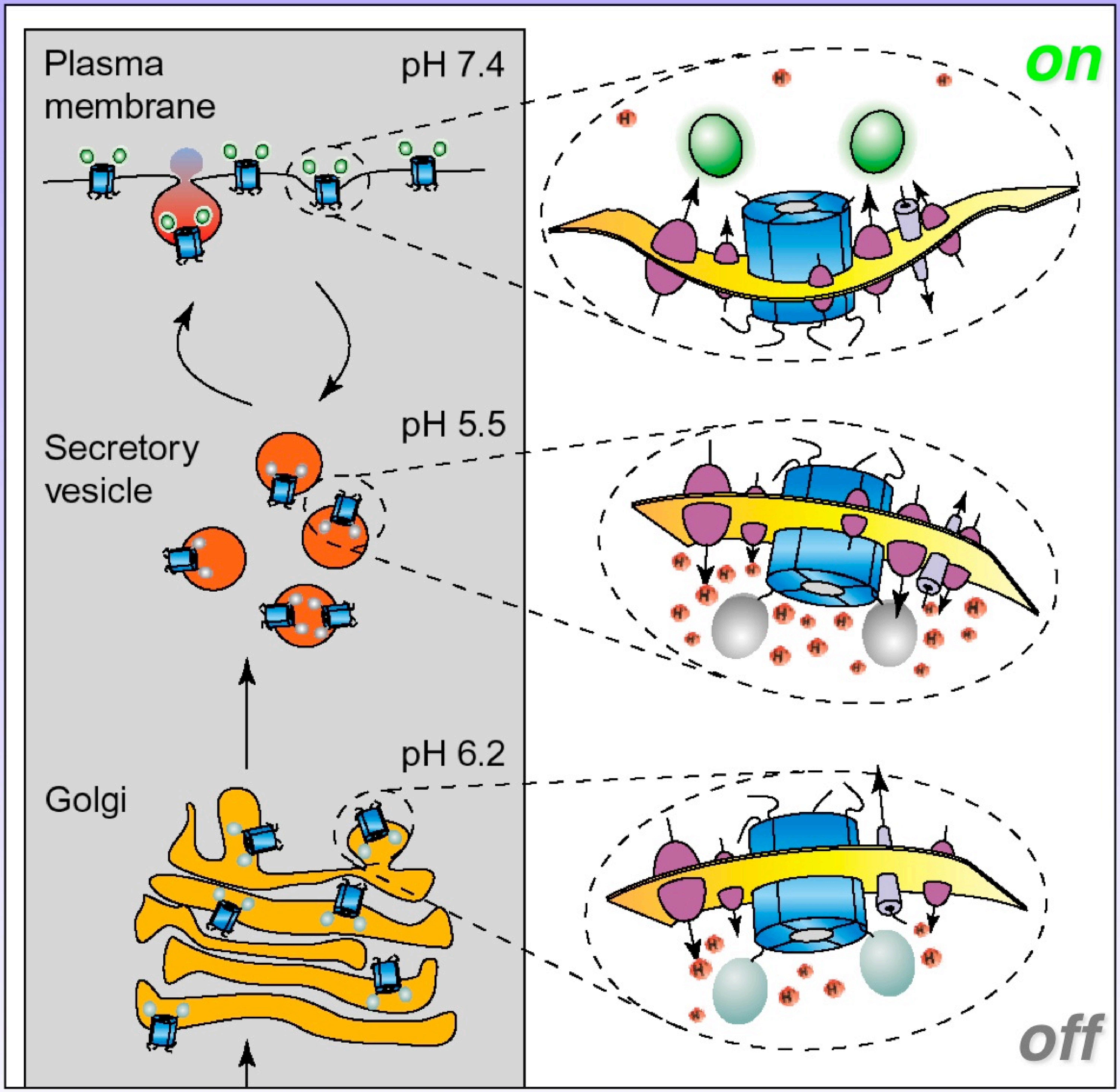
Ex: Syt1 prolonge le PSF, Syt4 le diminue.

Chapman, Annu. Rev. Biochem. 2008.77:615-641

Comment mesurer l'exocytose ?

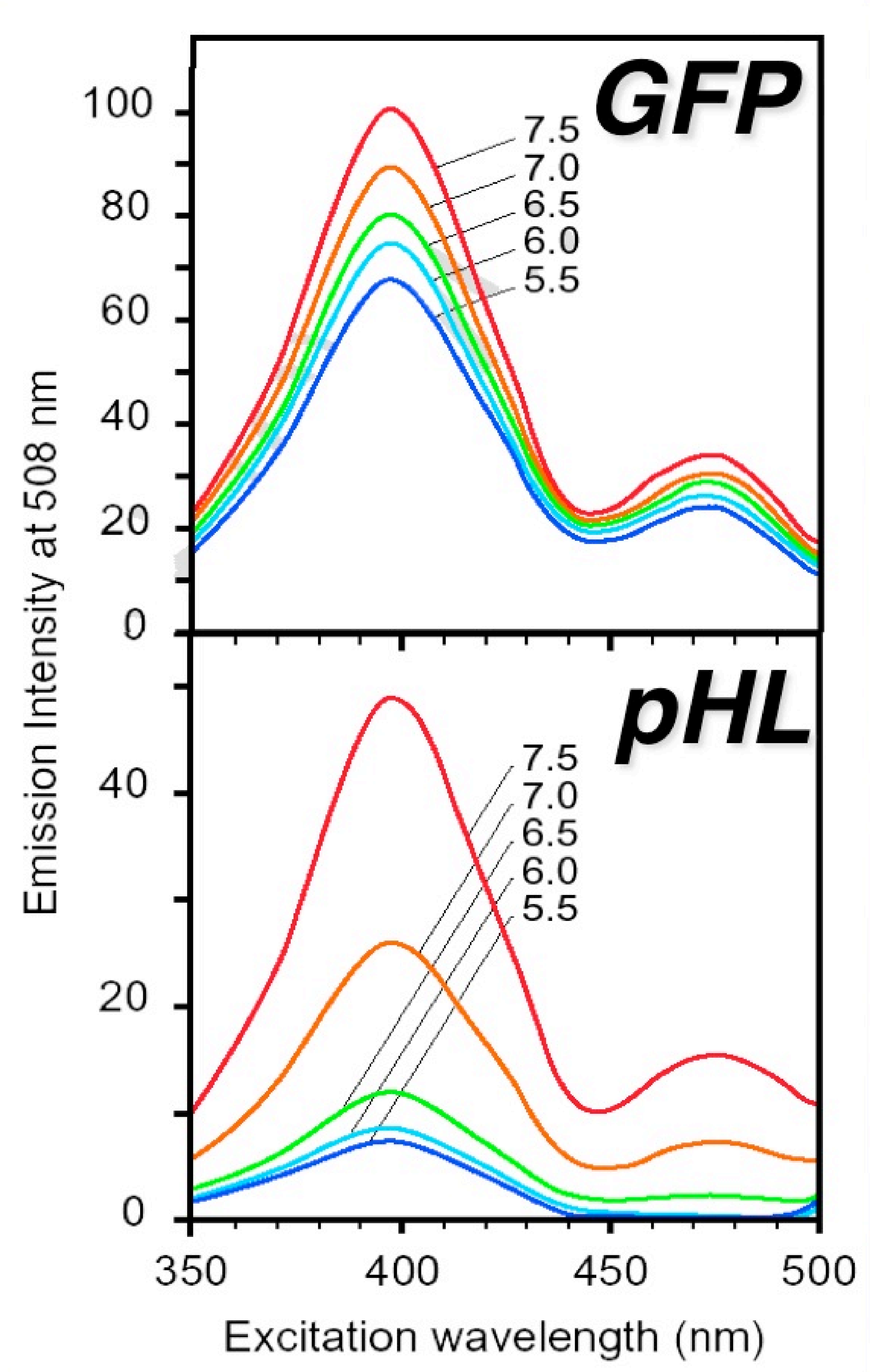
3) Mesure de fluorescence avec la GFP sensible au pH : la pHluorin

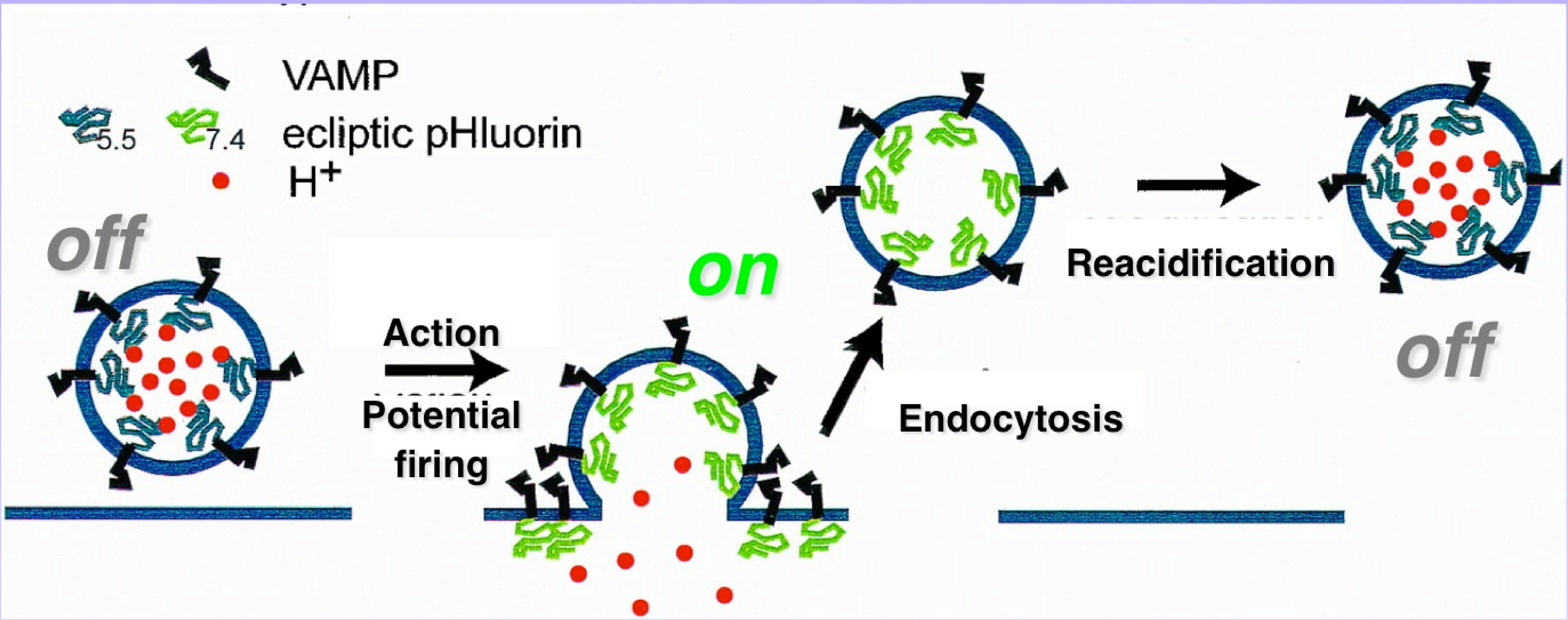


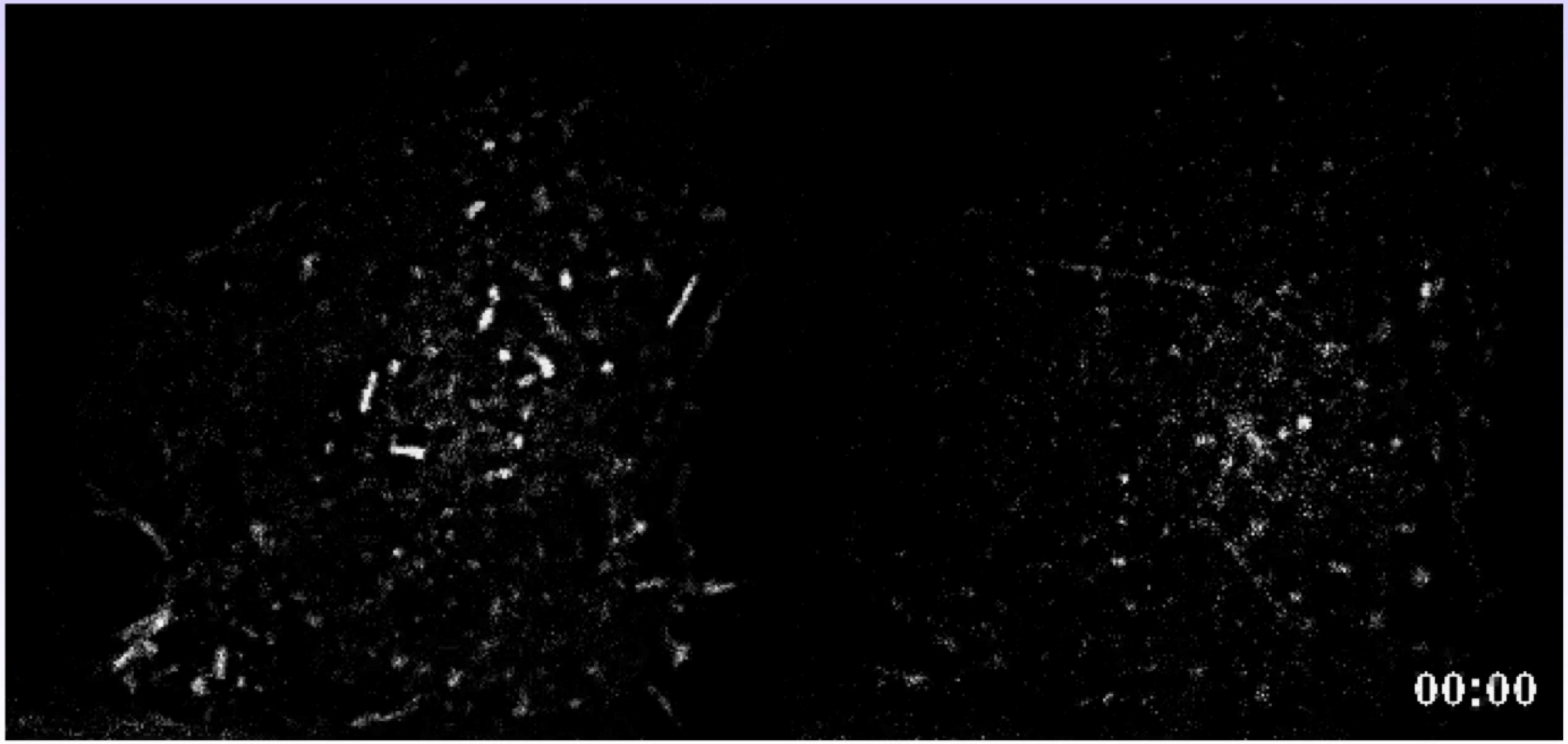


Comment mesurer l'exocytose ?

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



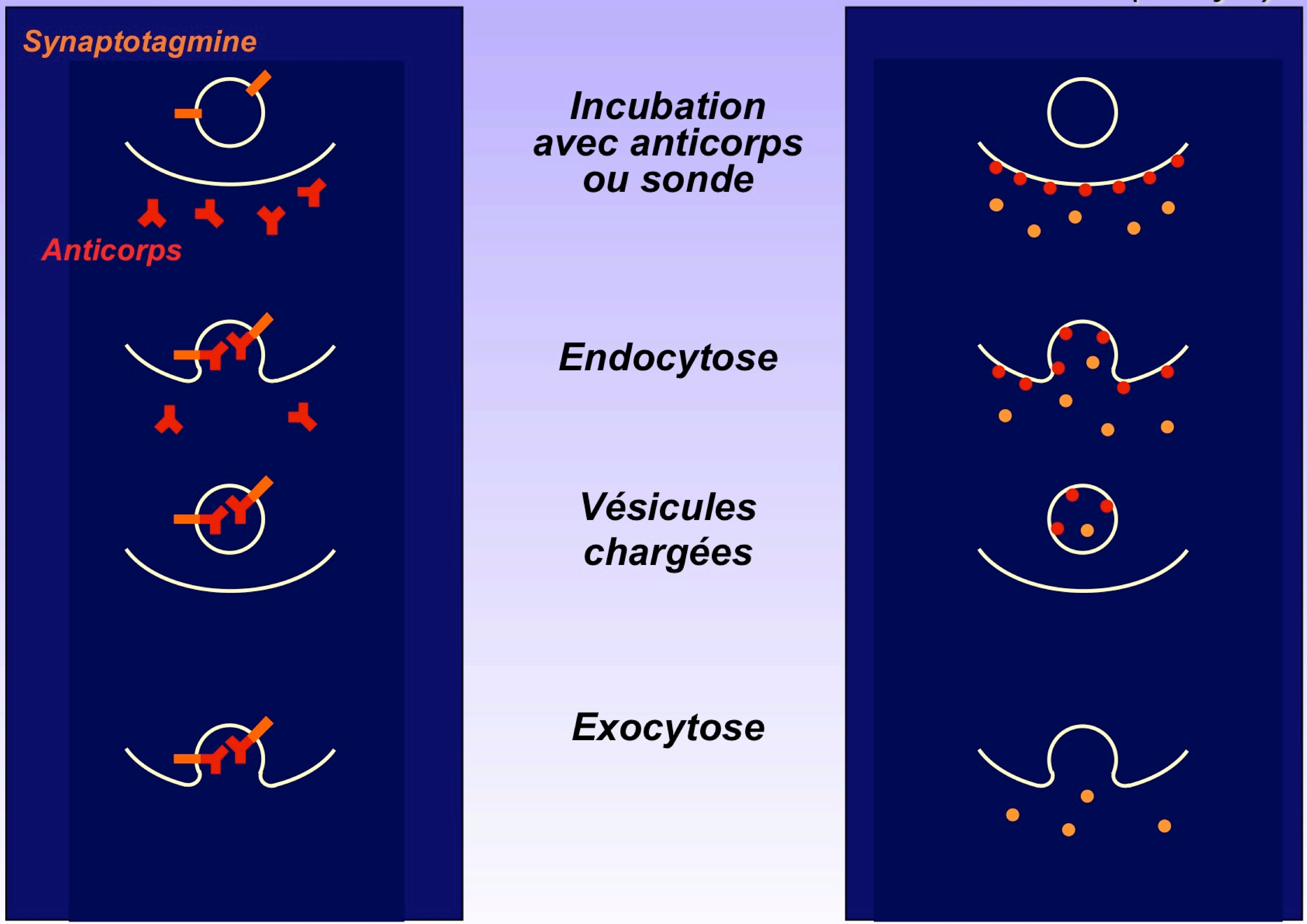




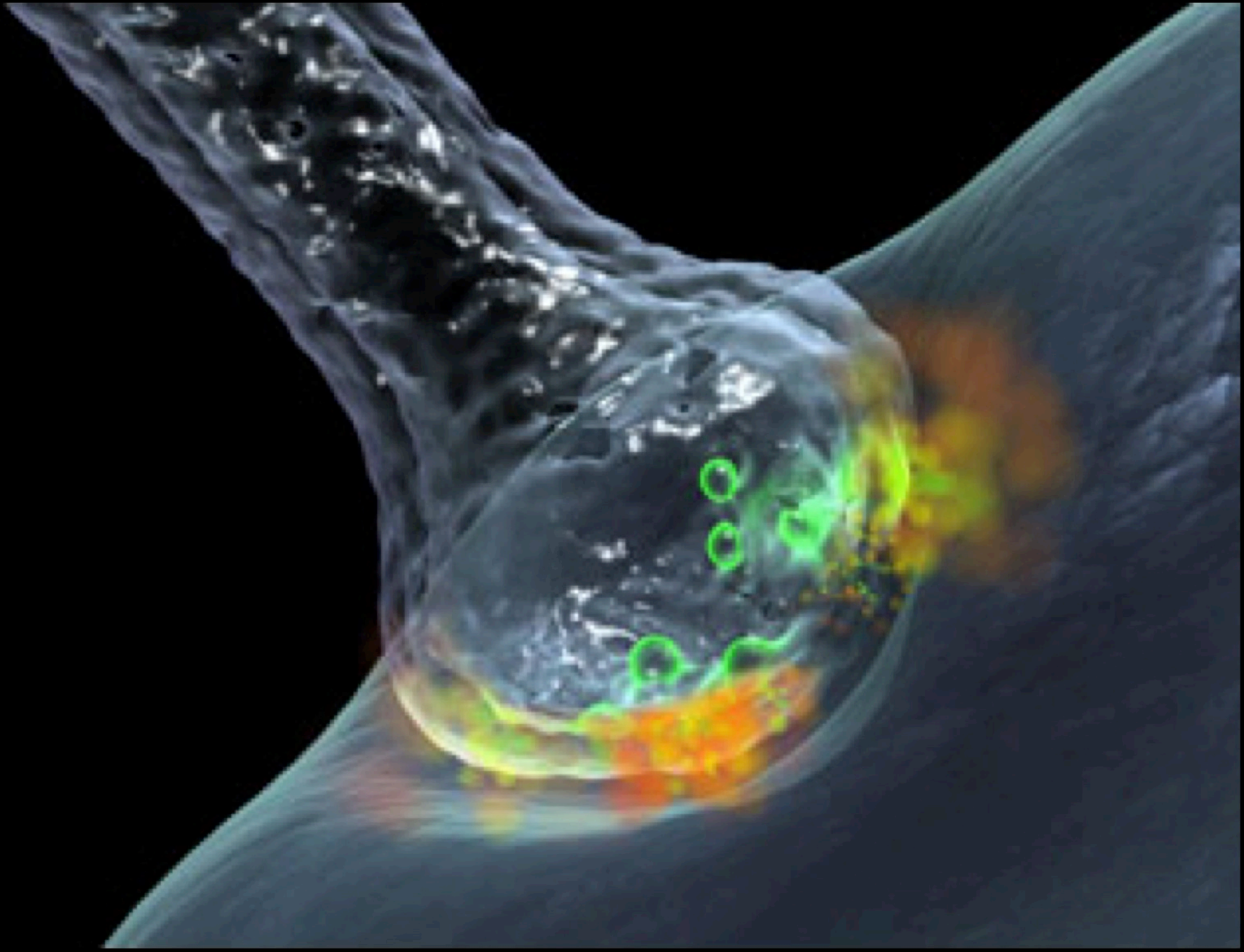
Comment mesurer le recyclage ?

1) L'utilisation d'anticorps anti-synaptotagmine

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

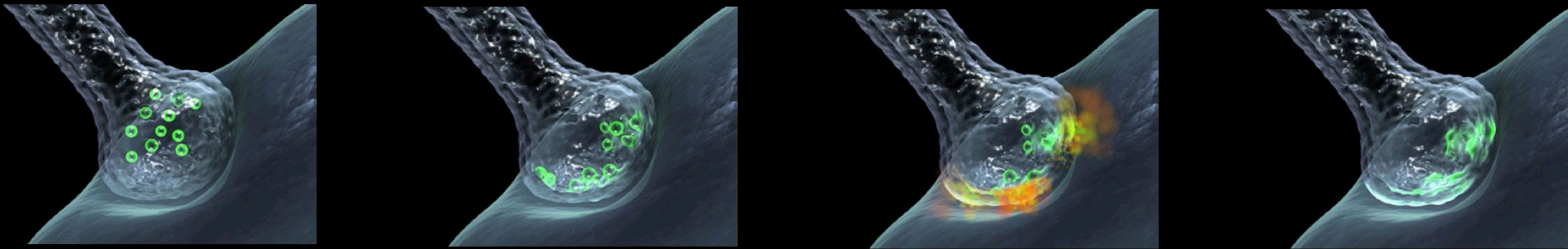


Neuronal transmission: exocytosis



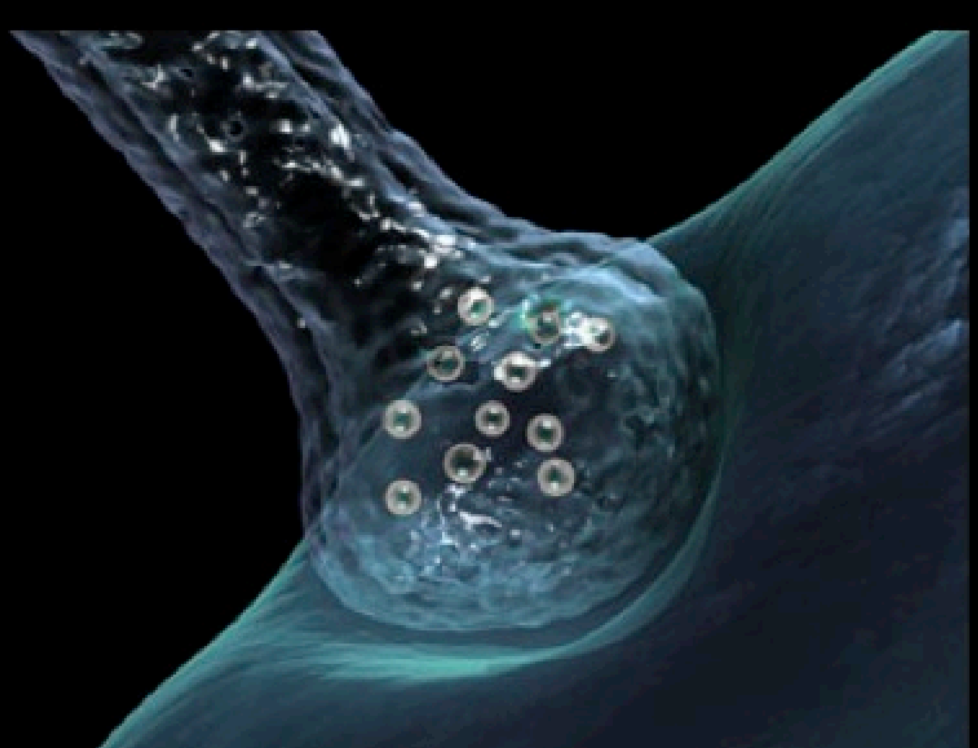
Master2 BCPP
Lydia Danglot – Développement de la Synapse

Neuronal transmission: exocytosis



FM experiments

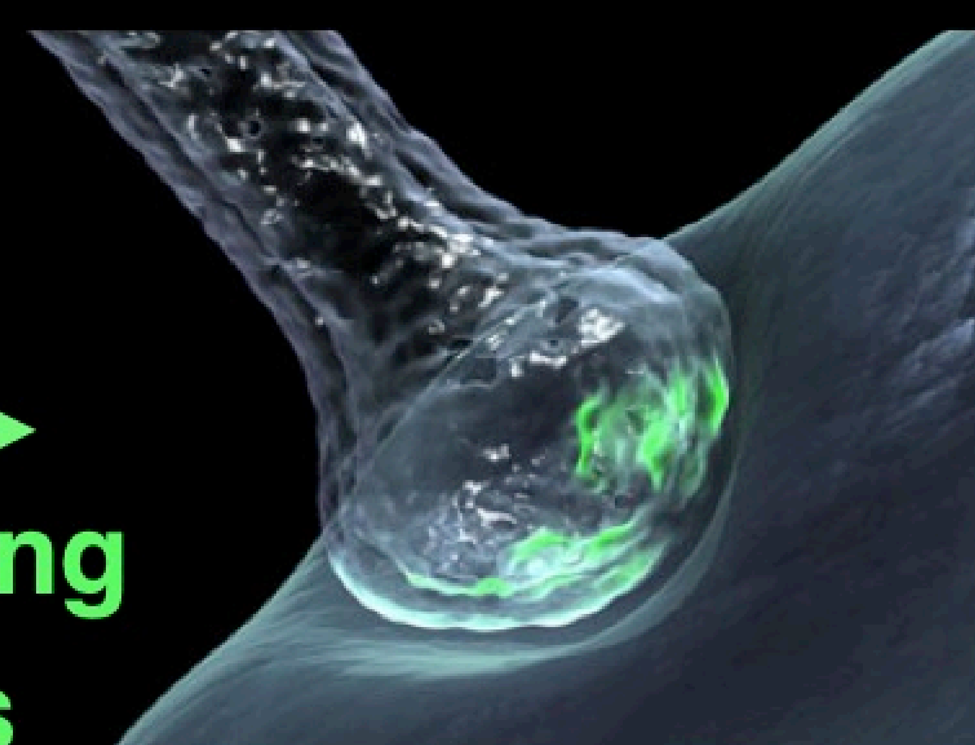
1. Loading of fluorescent dyes

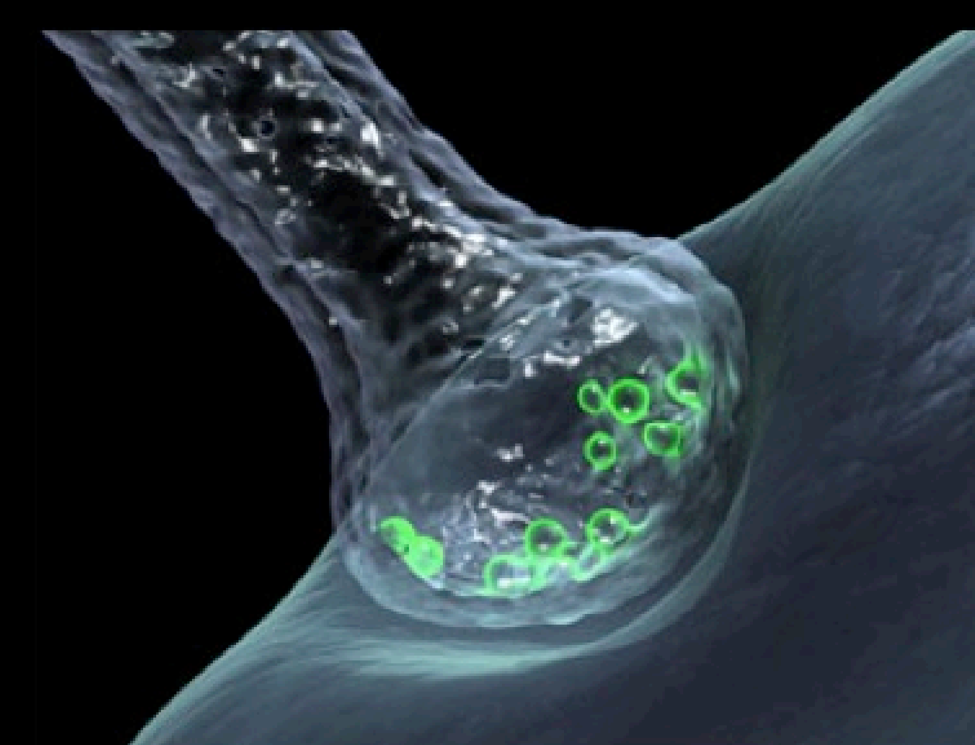


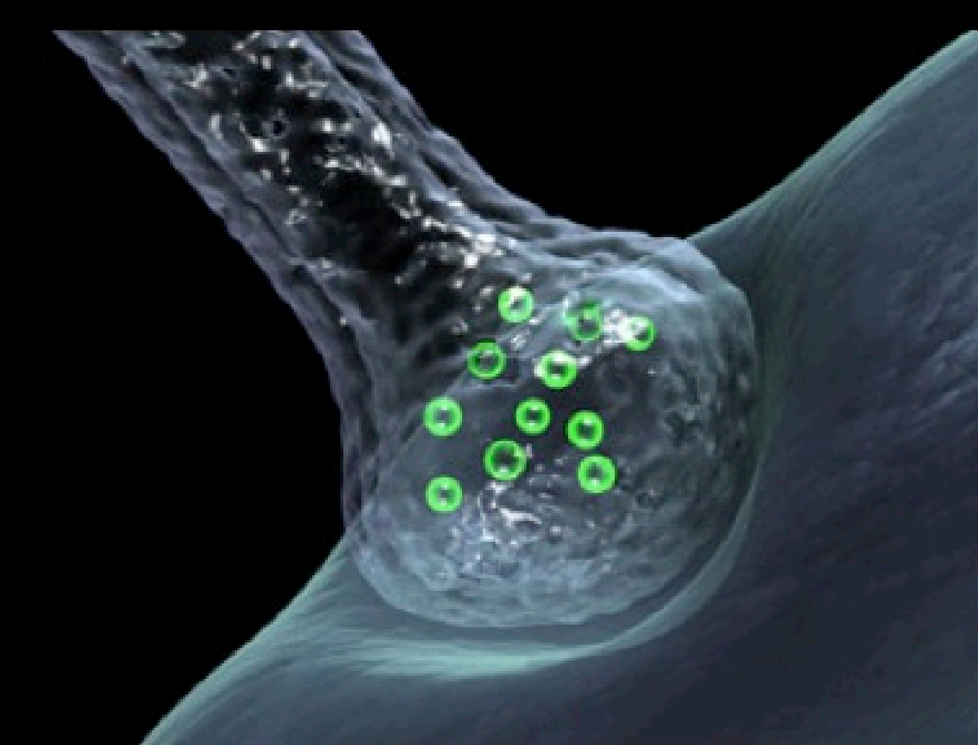
FM

Loading

90 s

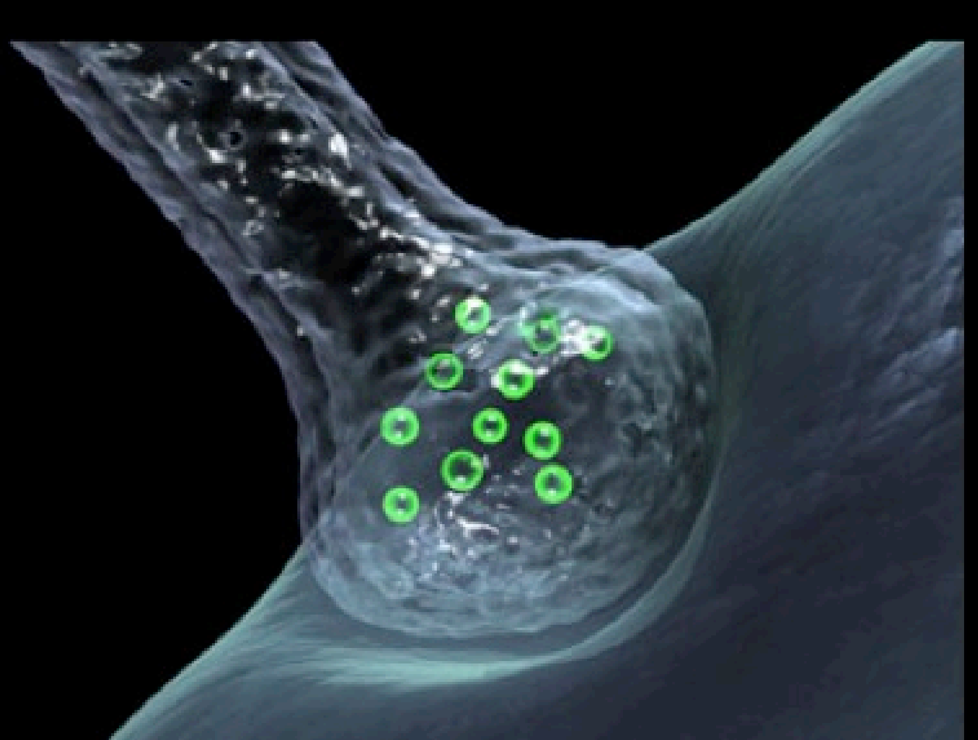






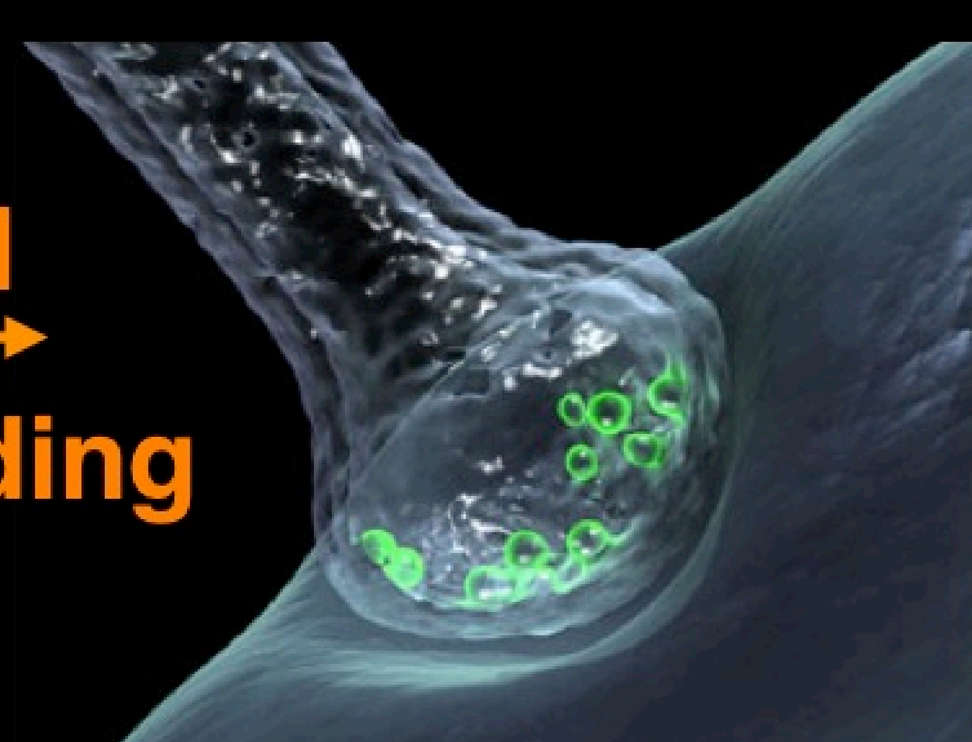
Zero Ca $^{2+}$: block ExoC

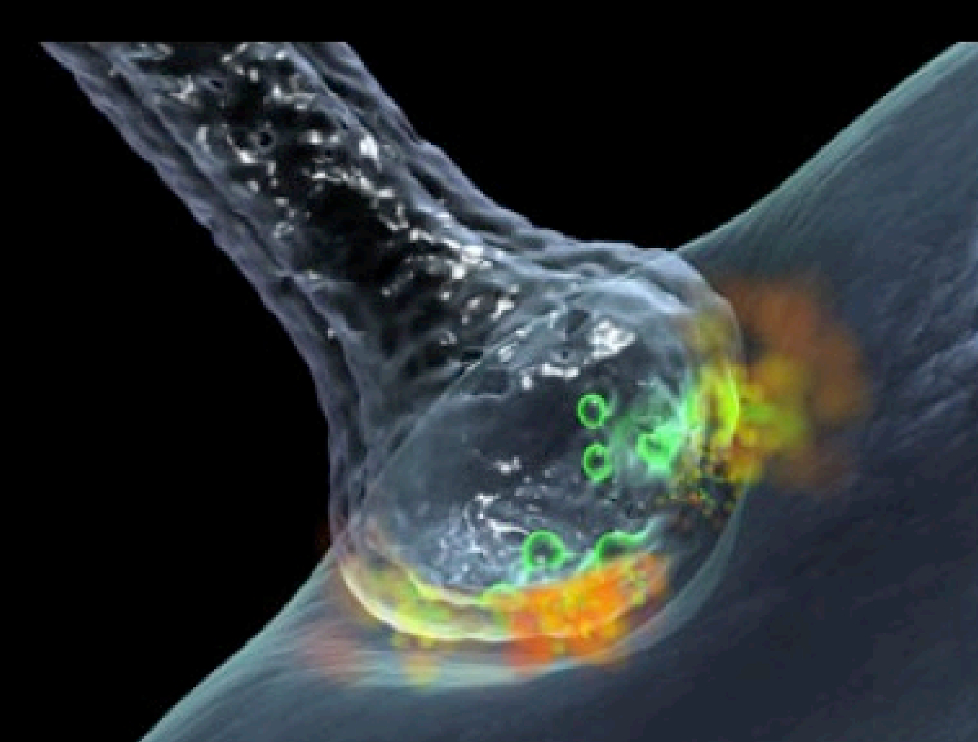
2. Mesurement of the unloading of FM dyes

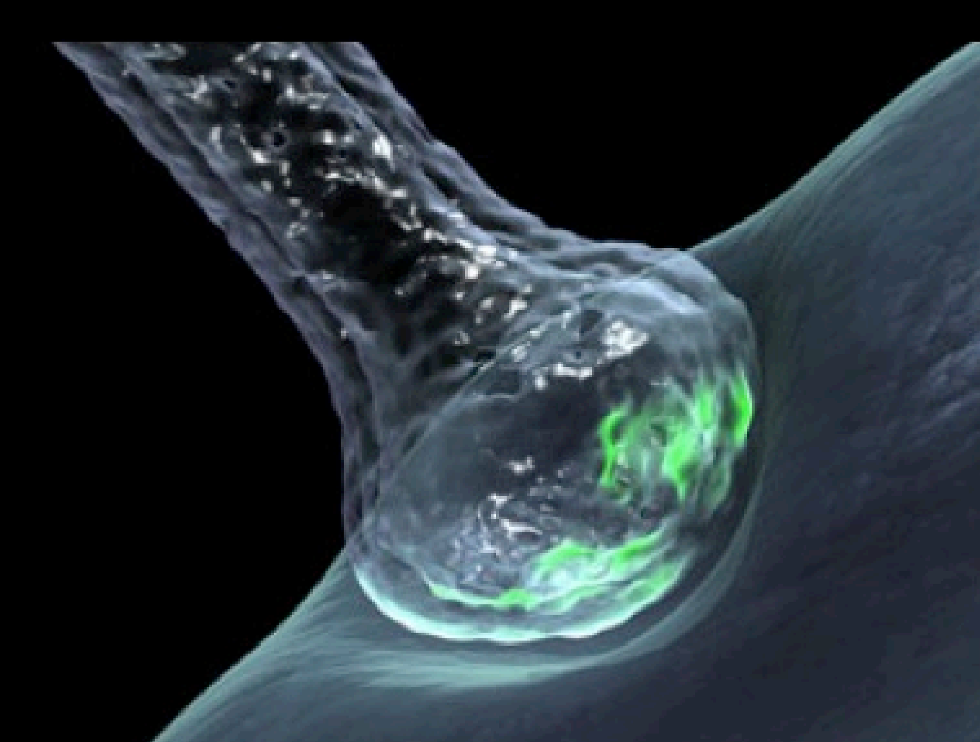


KCl

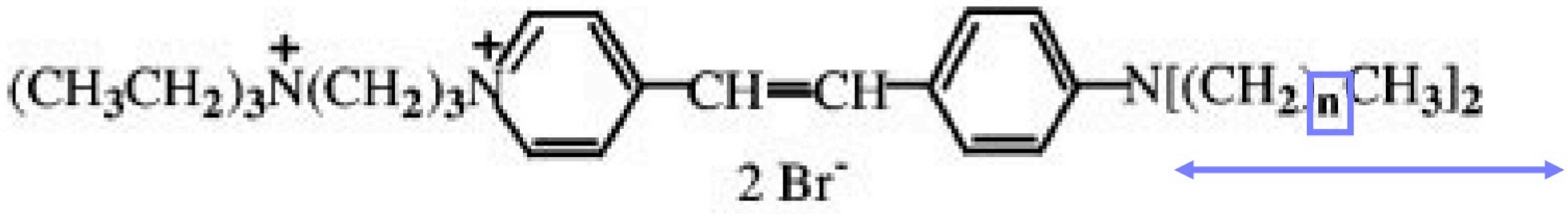
Unloading







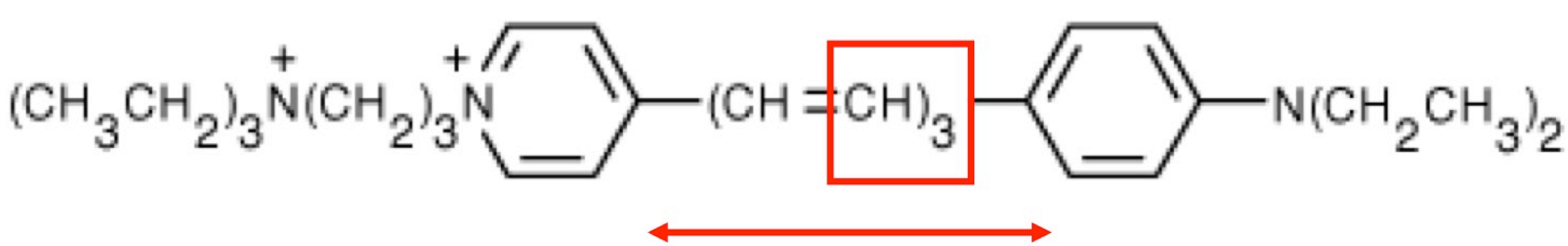
Basic properties of the FM dyes



Hydrophobic tail :
dissociation rate

n=1 FM 2-10
n=3 FM 1-43
n=4 FM 1-84

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



Carbon spacer :

Red-shiftes fluorescence

FM 4-64

Basic properties of the FM dyes

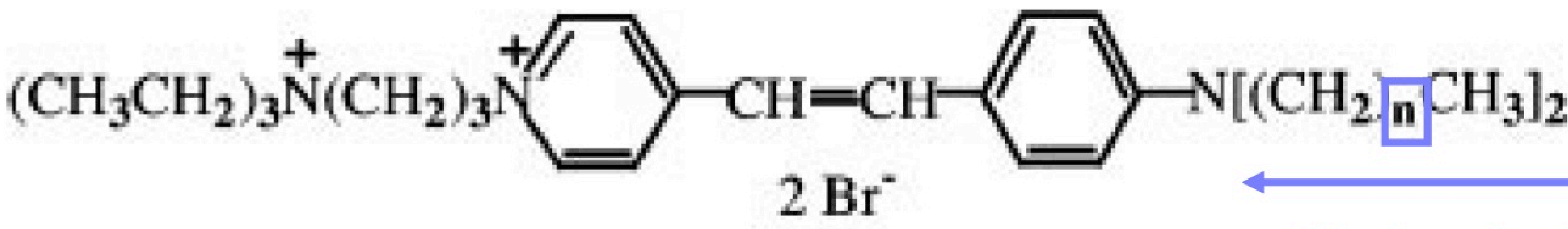


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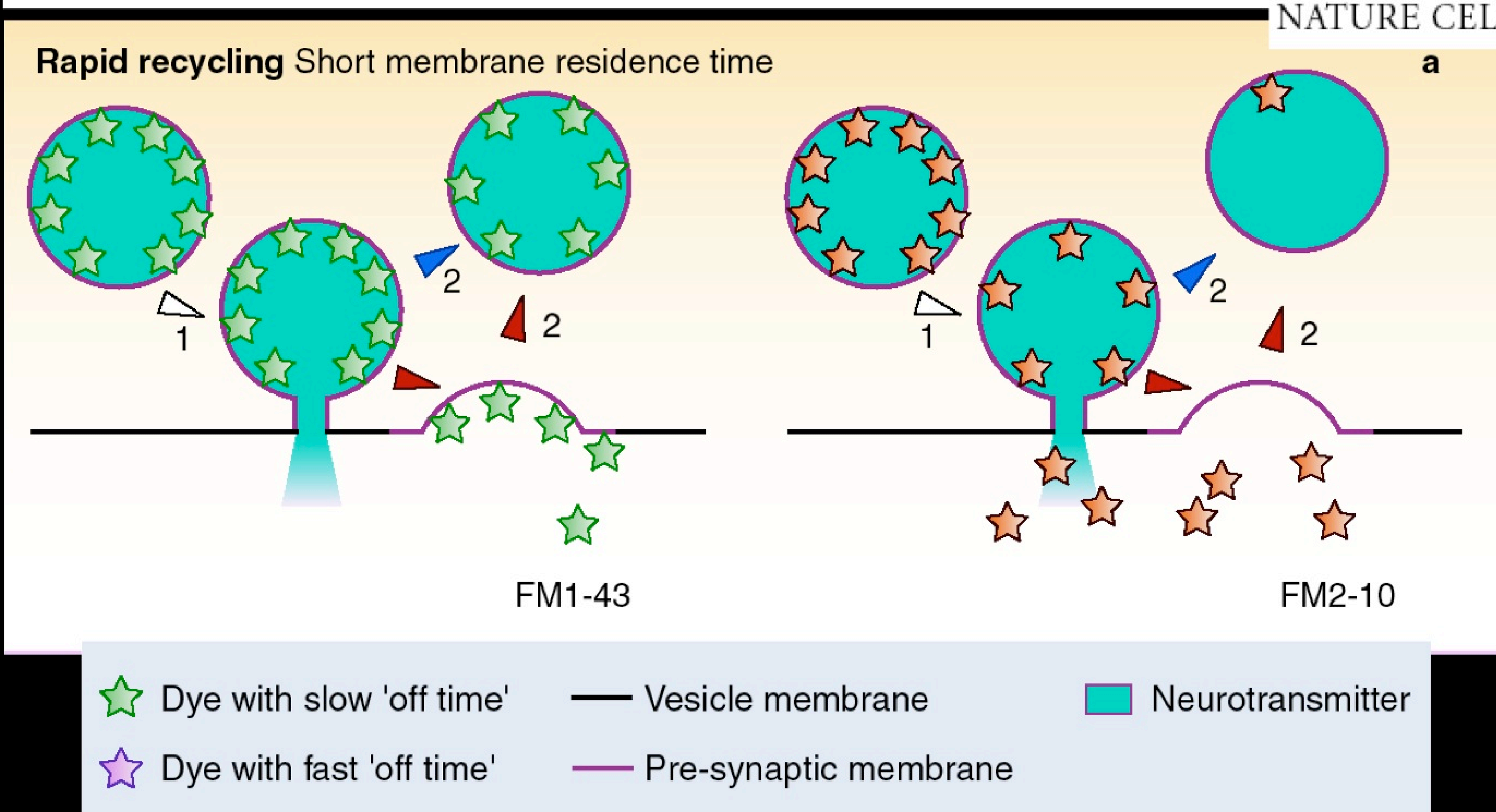
FIG. 1. **Chemical structures of FM 2–10, FM 1–43, and FM 1–84.**



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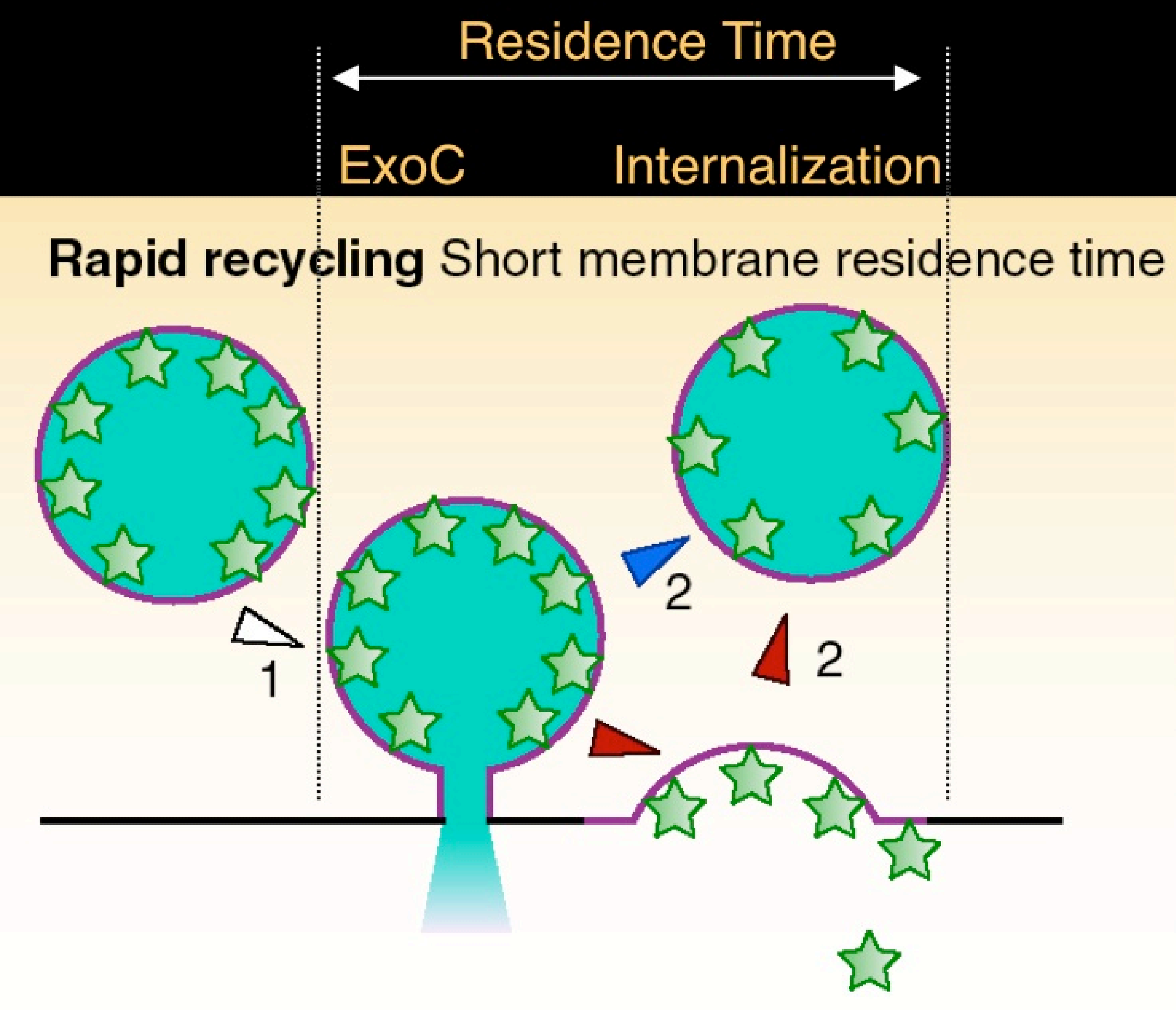
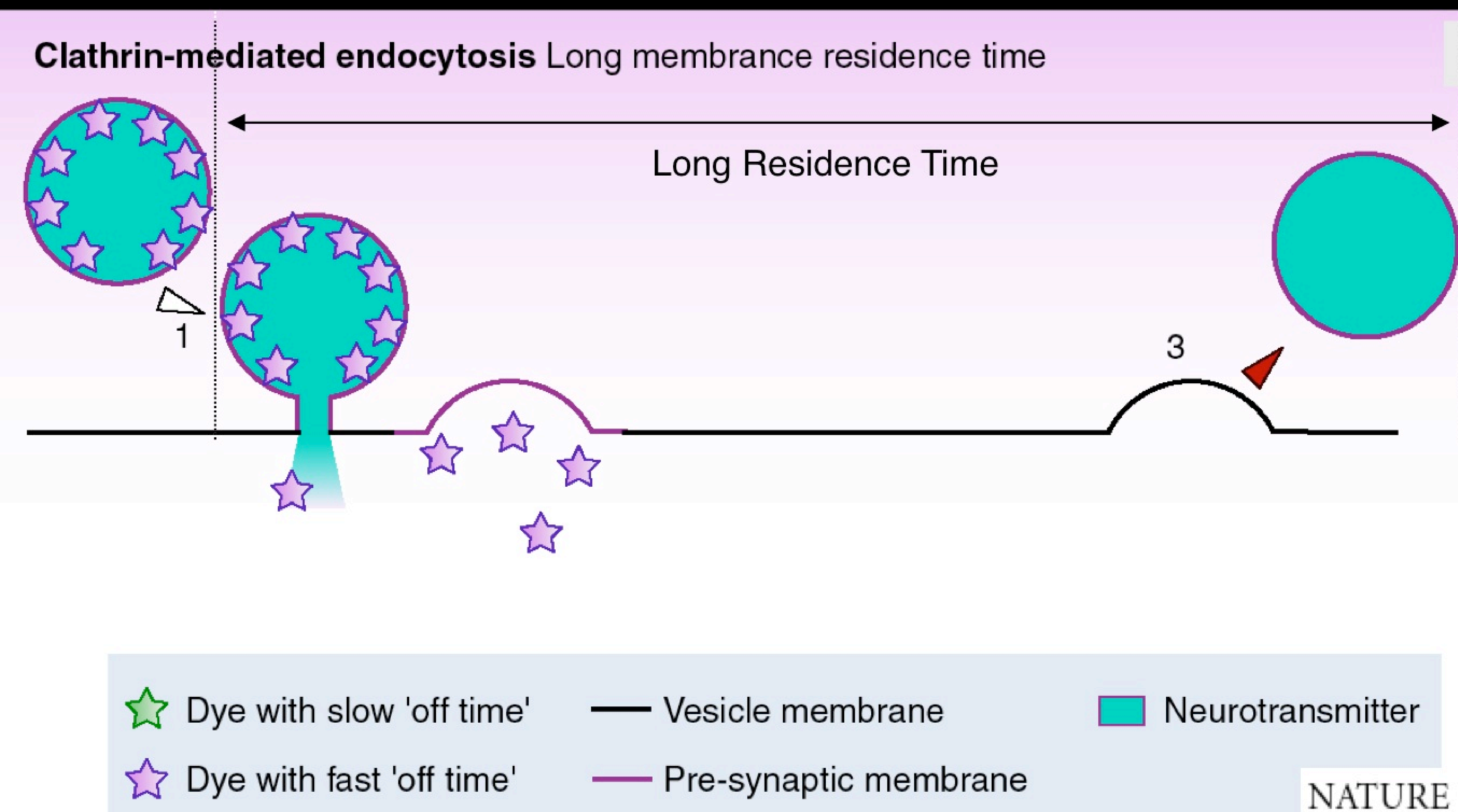
FM2-10 is faster than FM1-43

How to discriminate between Kiss & run and Endocytosis



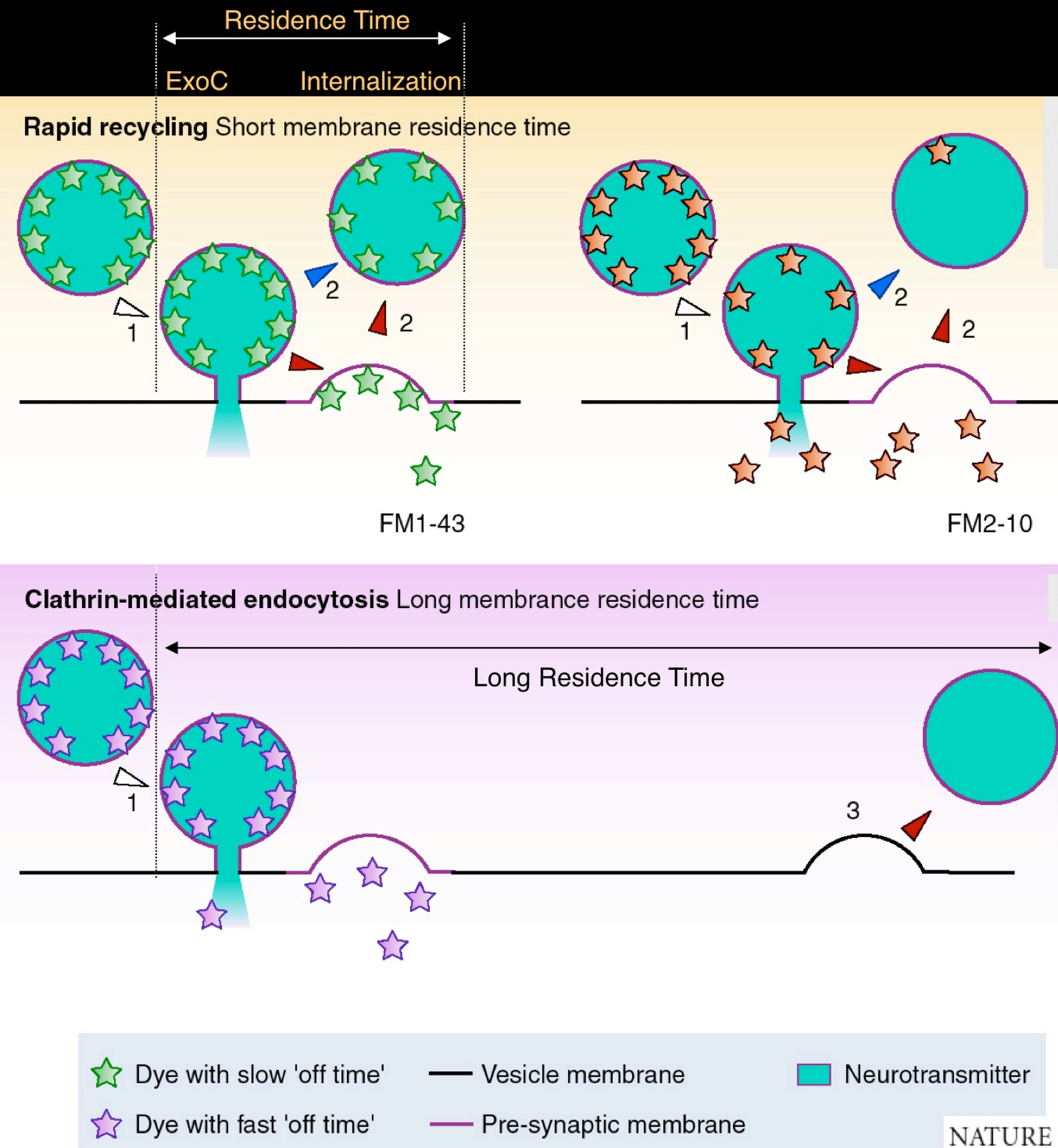
Kiss and run :

Short Residence time (Res.T)



Clathrin mediated endocytosis:

Long 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 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

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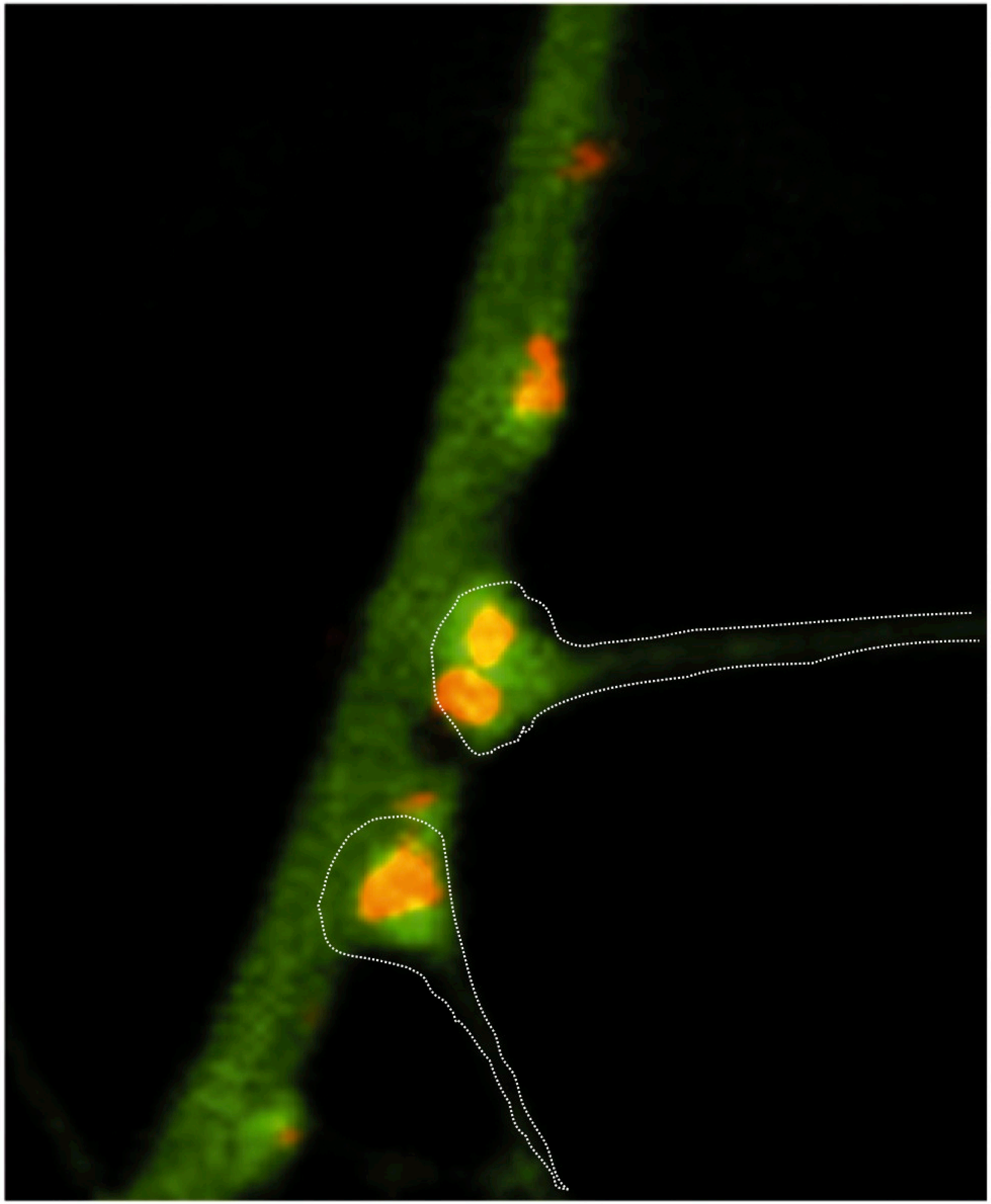


Current Opinion in Neurobiology

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.

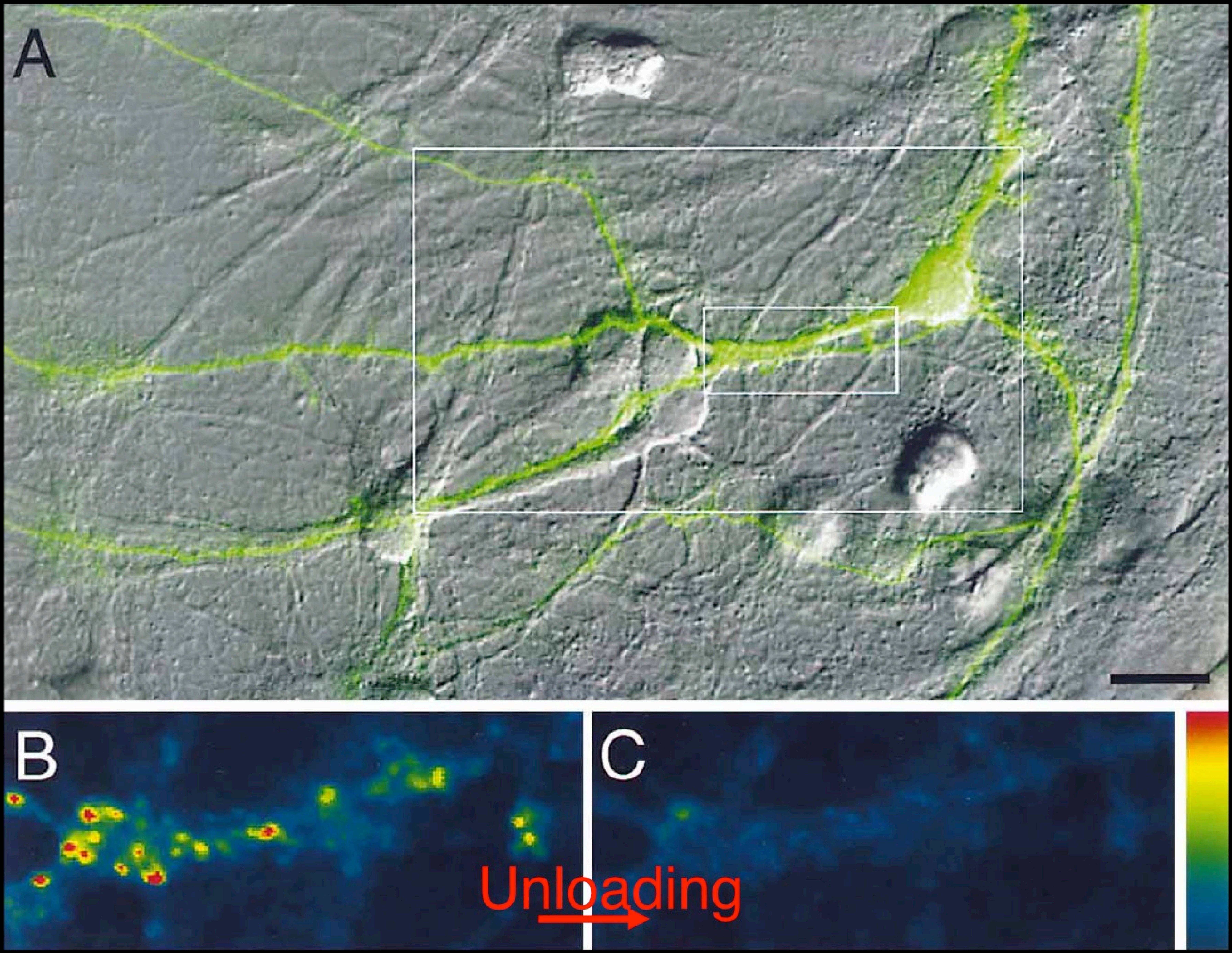
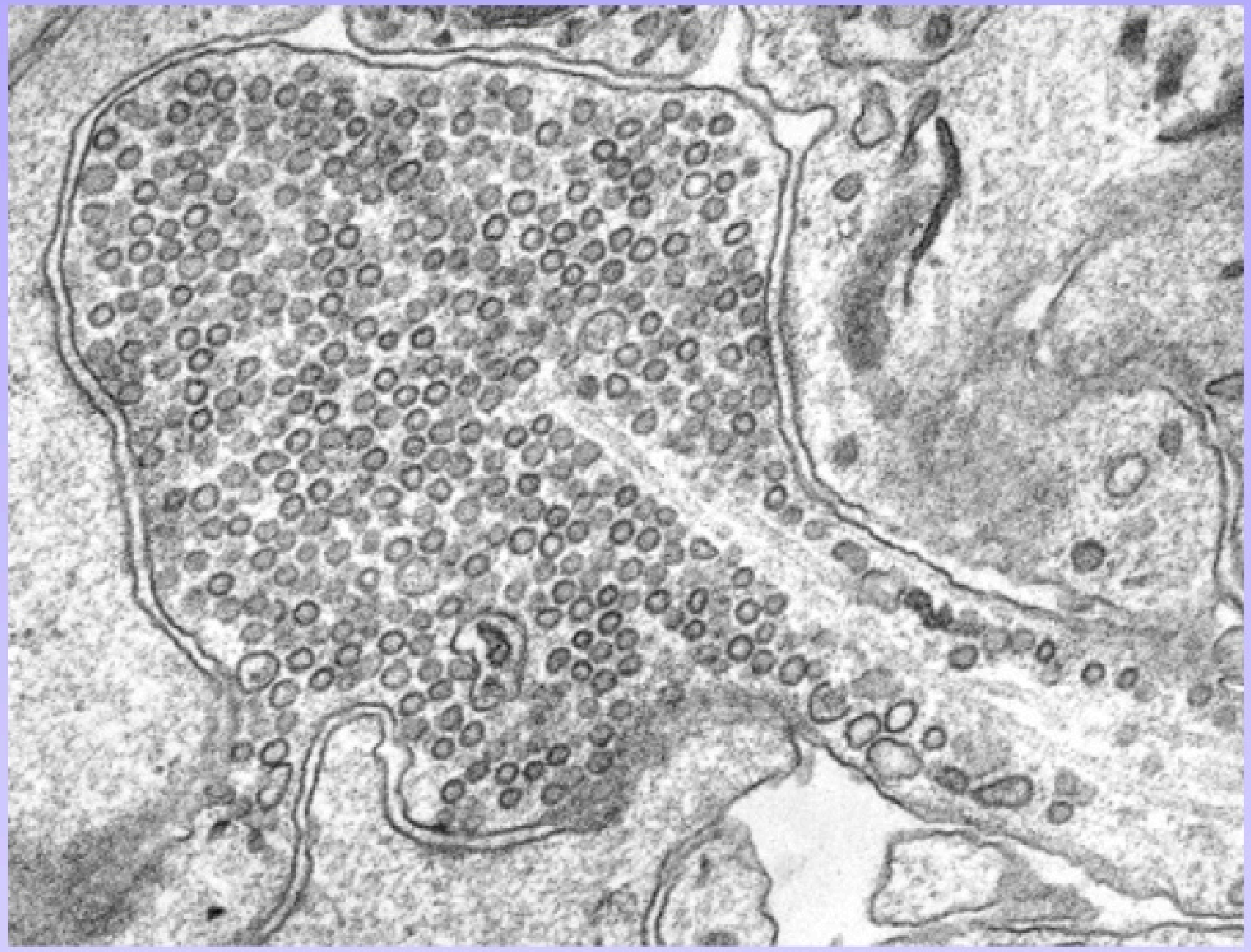
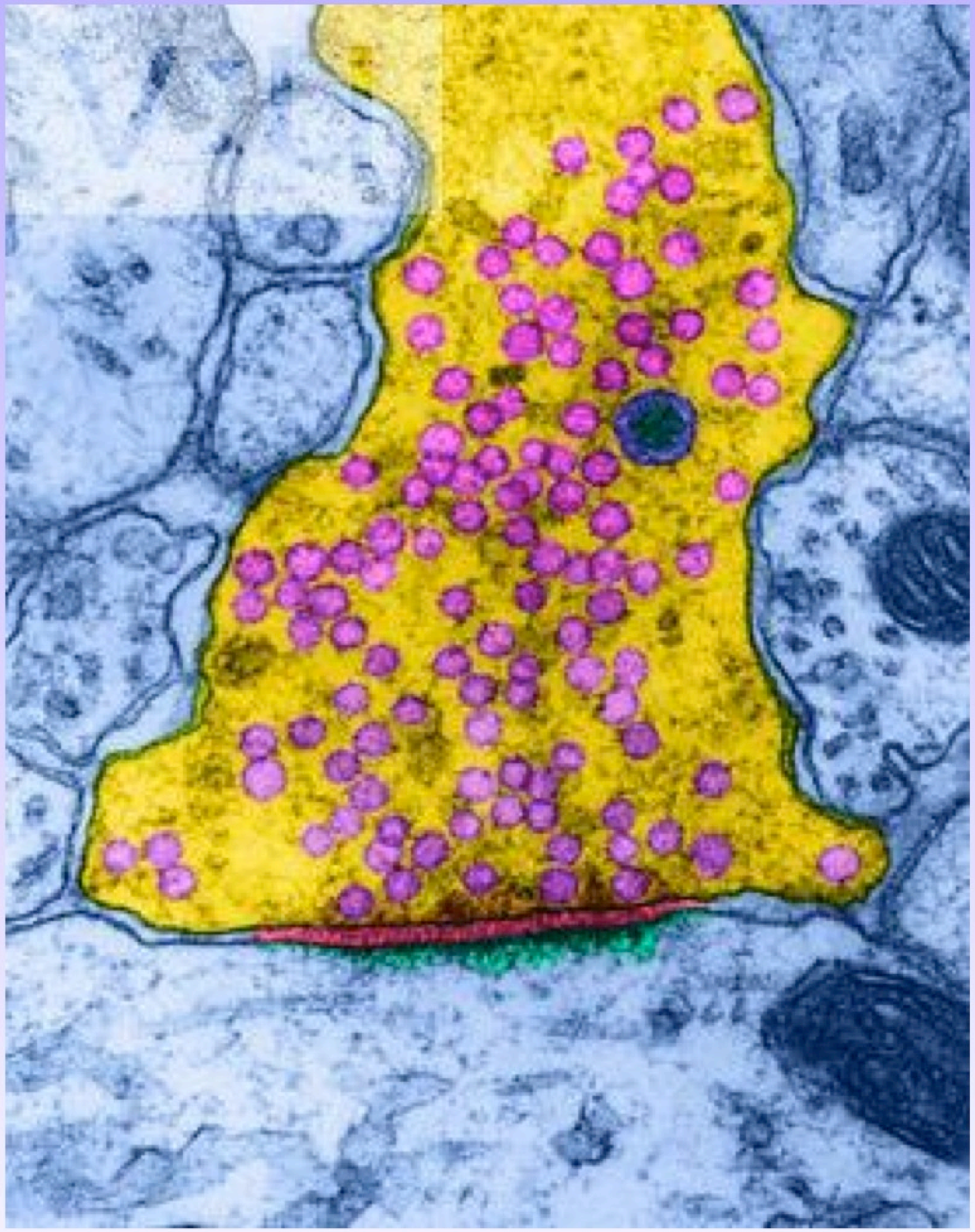


Figure 1. Imaging of Dendritic Structure and Presynaptic Boutons in Live Cultured Hippocampal Neurons

(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).

Les vésicules synaptiques





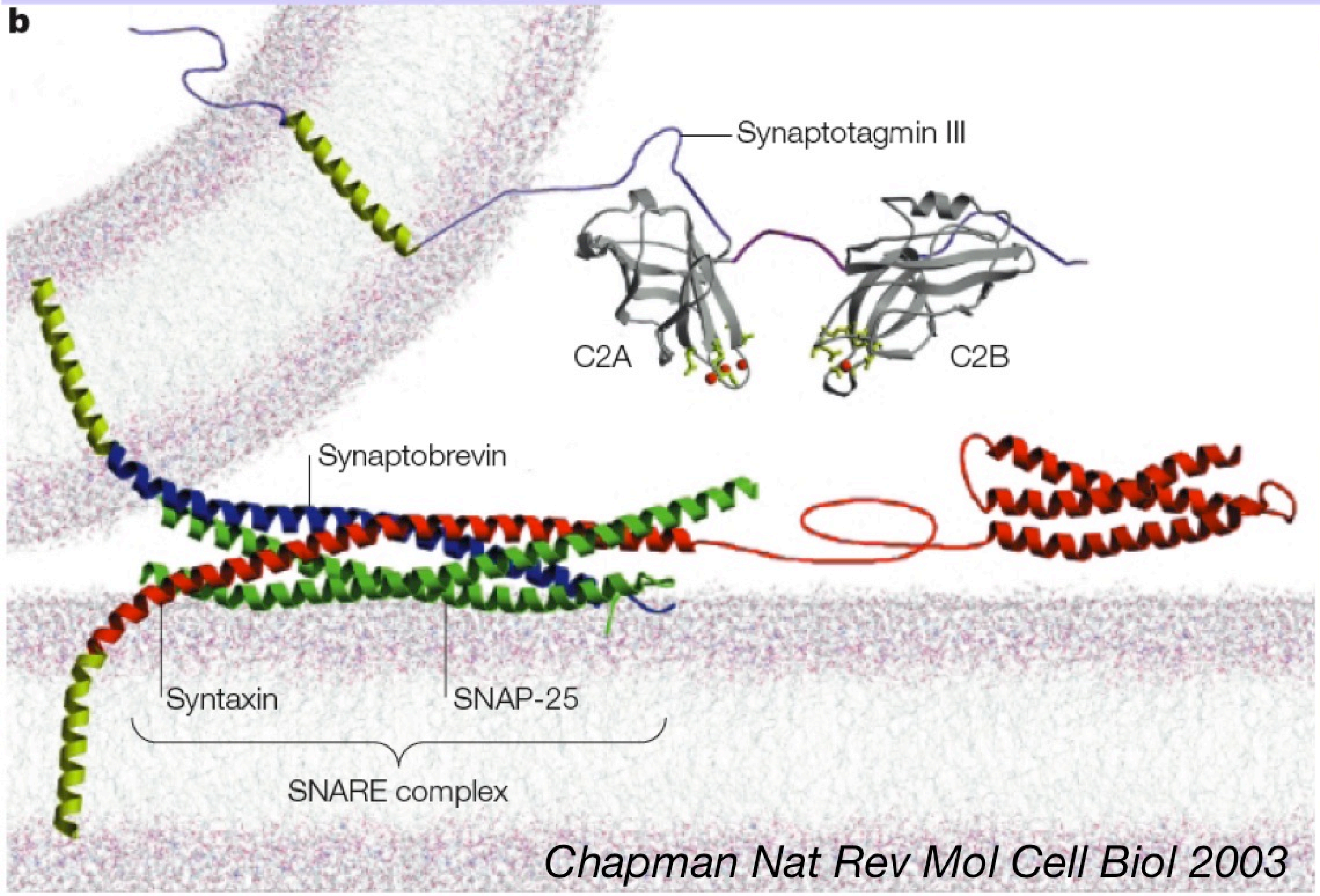
- 50 nm de diamètre
- 500 VS/terminaison

  x10 000 contacts = 10^6 à 10^7 VS/neurone

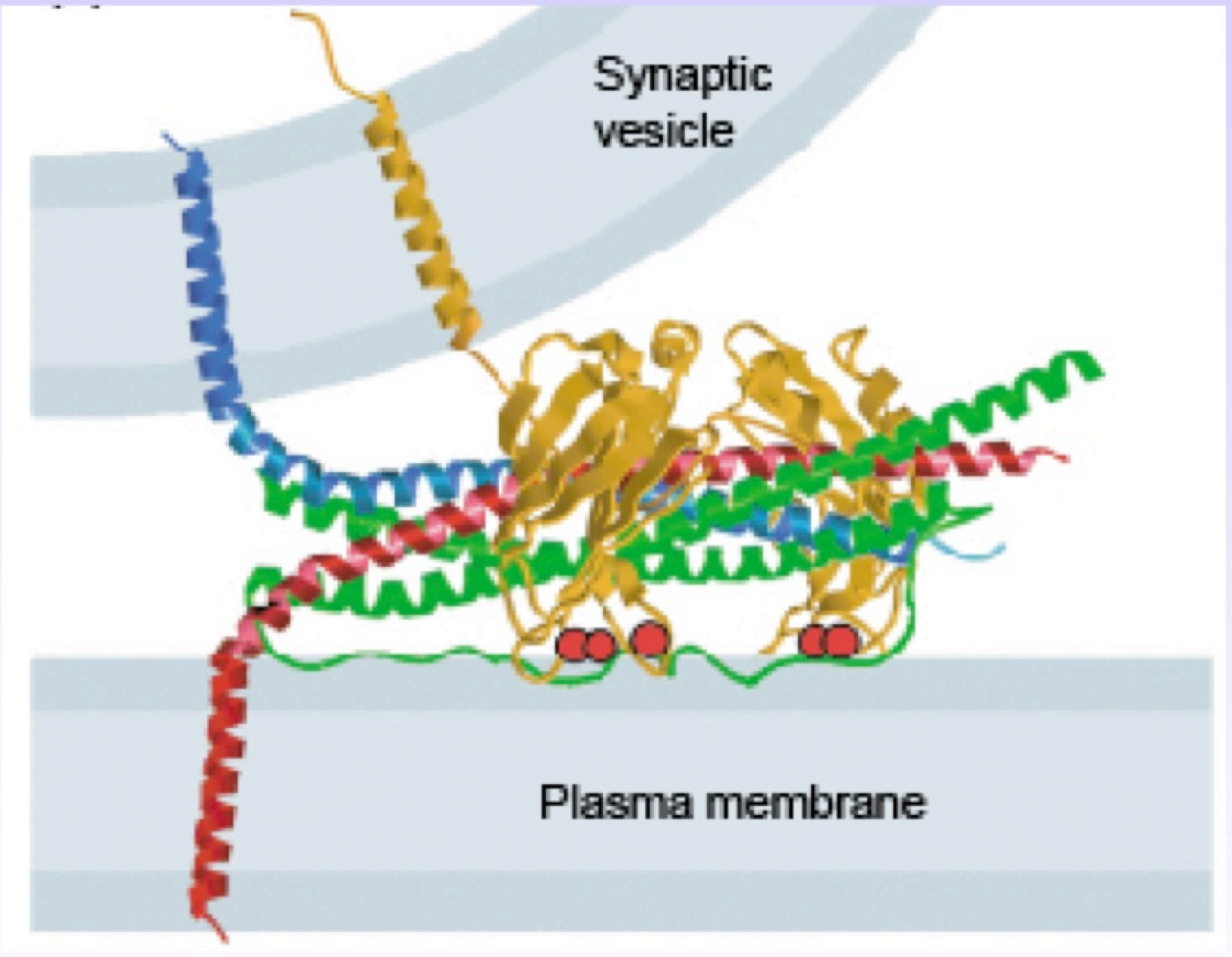
  x 10^{11} neurones = 10^{17} VS/SNC
- cholestérol:phospholipides = 1:2
- 12000 molécules de phospholipides / VS
- phospholipides:protéines = 1:1
- 20 à 40 protéines différentes / VS
- synaptophysine= 7% protéine VS = 0,3% protéines totales

Les protéines SNARE et l'exocytose

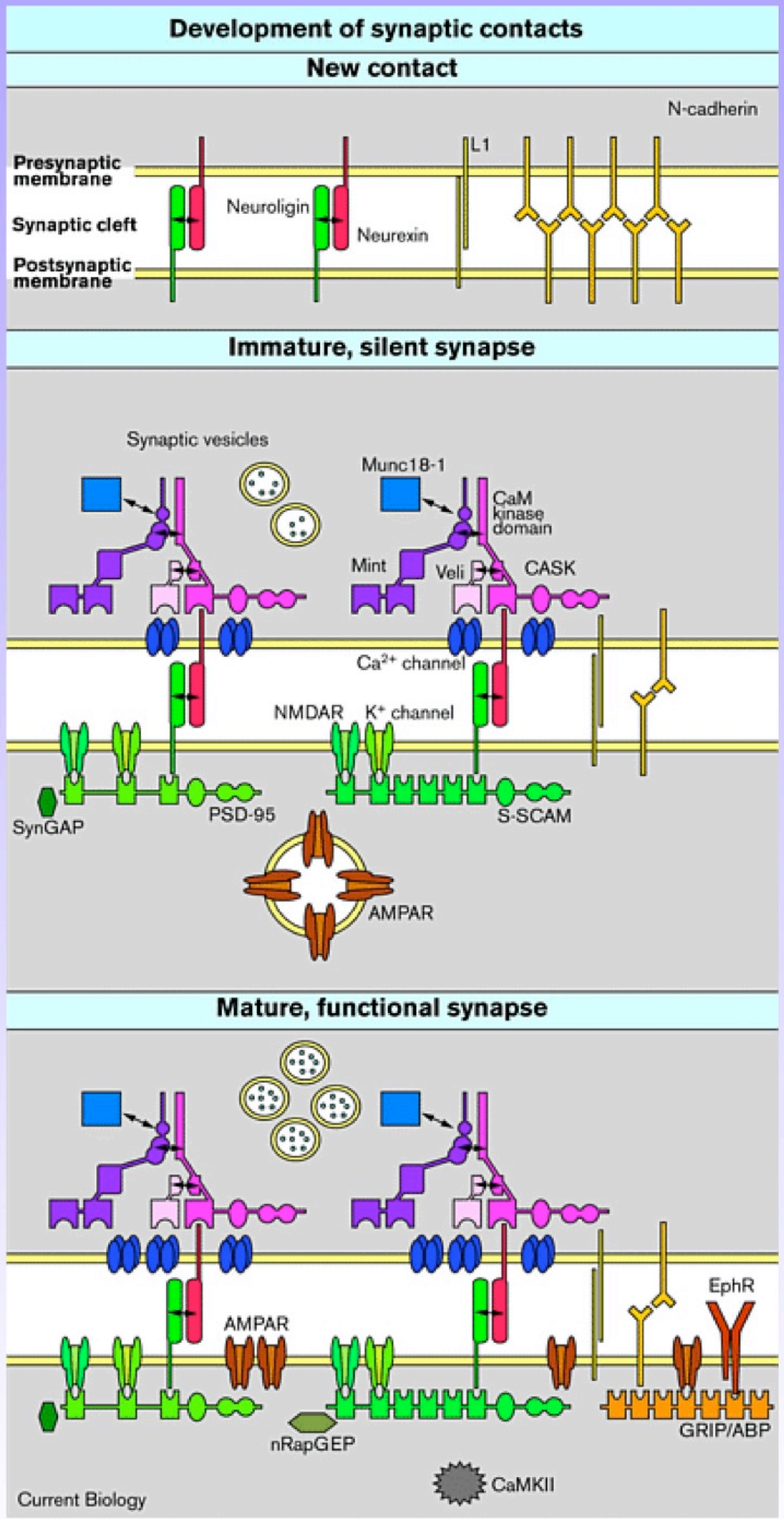
Adapted From E.R. Chapman, NATURE REVIEWS I MOLECULAR CELL BIOLOGY VOLUME 3 I JULY 2002 I 1



Chapman Nat Rev Mol Cell Biol 2003



9. Formation de la synapse en culture



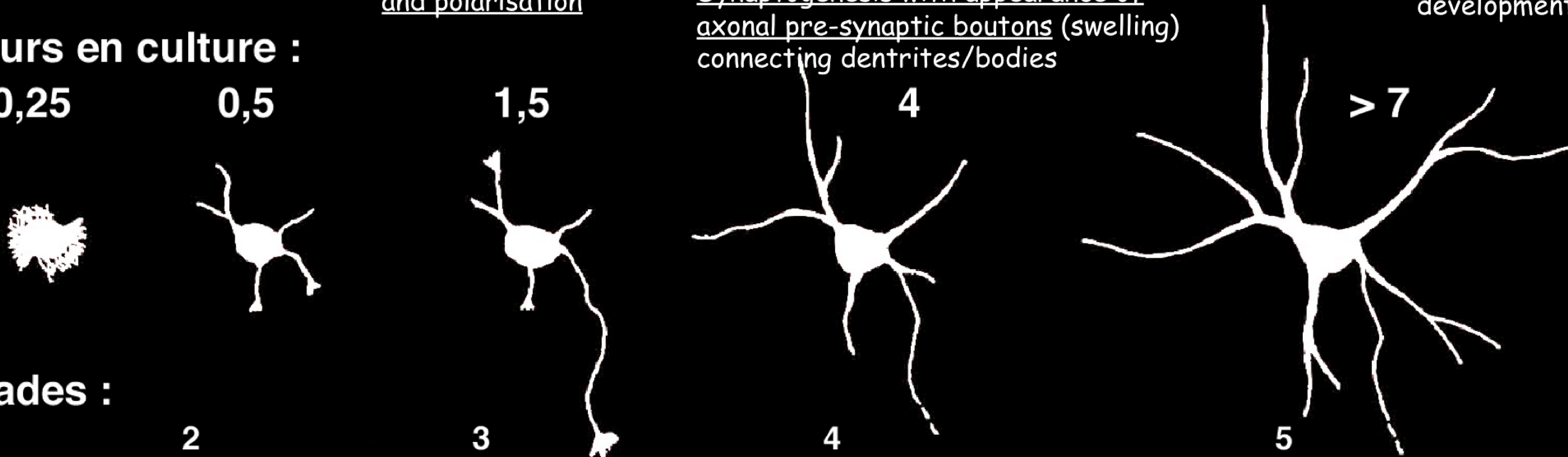
Axonal outgrowth and polarisation

DIV 4
Dentrites outgrowth
Synaptogenesis with appearance of axonal pre-synaptic boutons (swelling) connecting dentrites/bodies

DIV 9-10
Onset of dentritic spines development

Jours en culture :

0,25 | **0,5** | **1,5** | **4** | **> 7**



Stades :

1 Lamelipodes

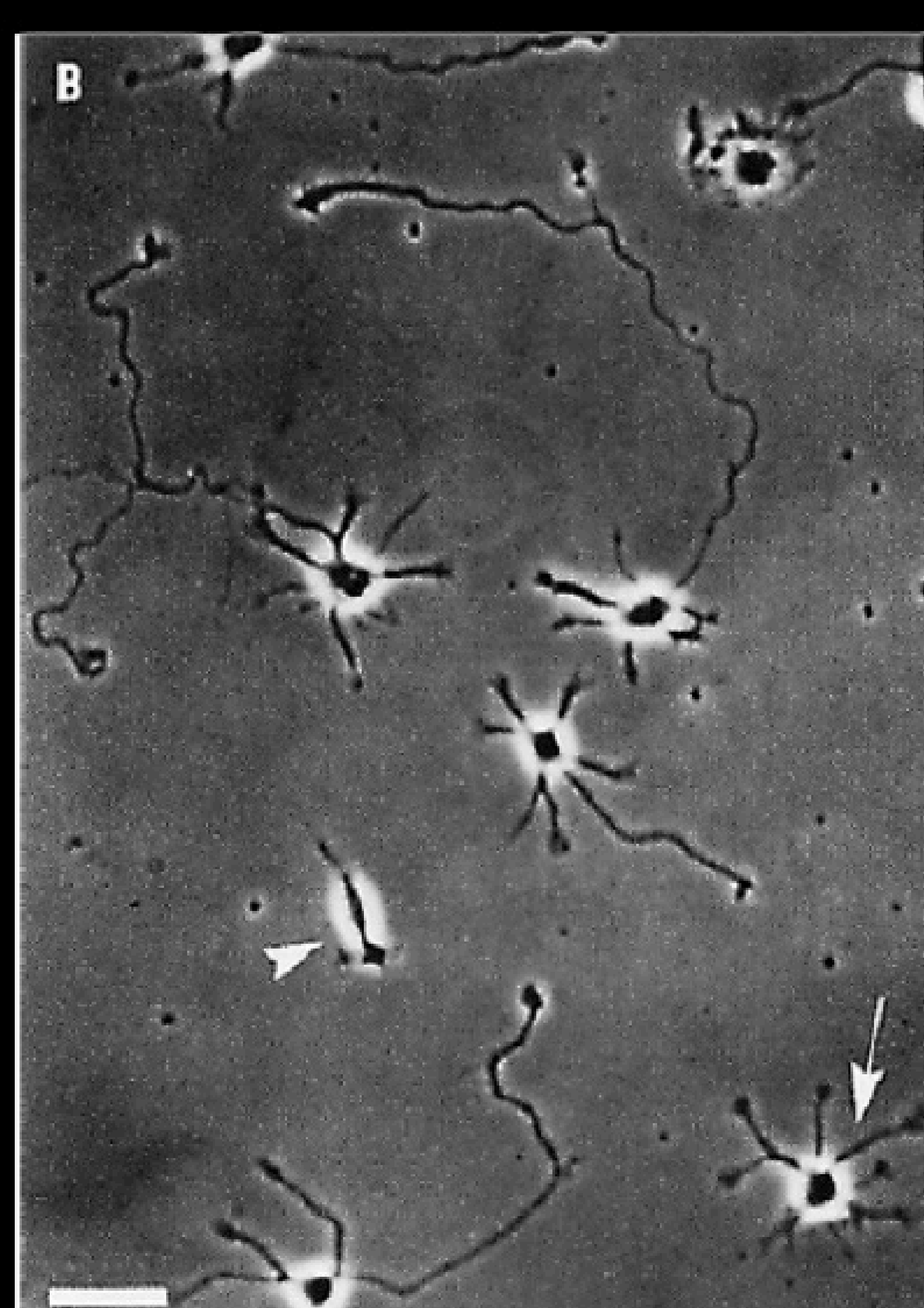
2 Neurites mineurs

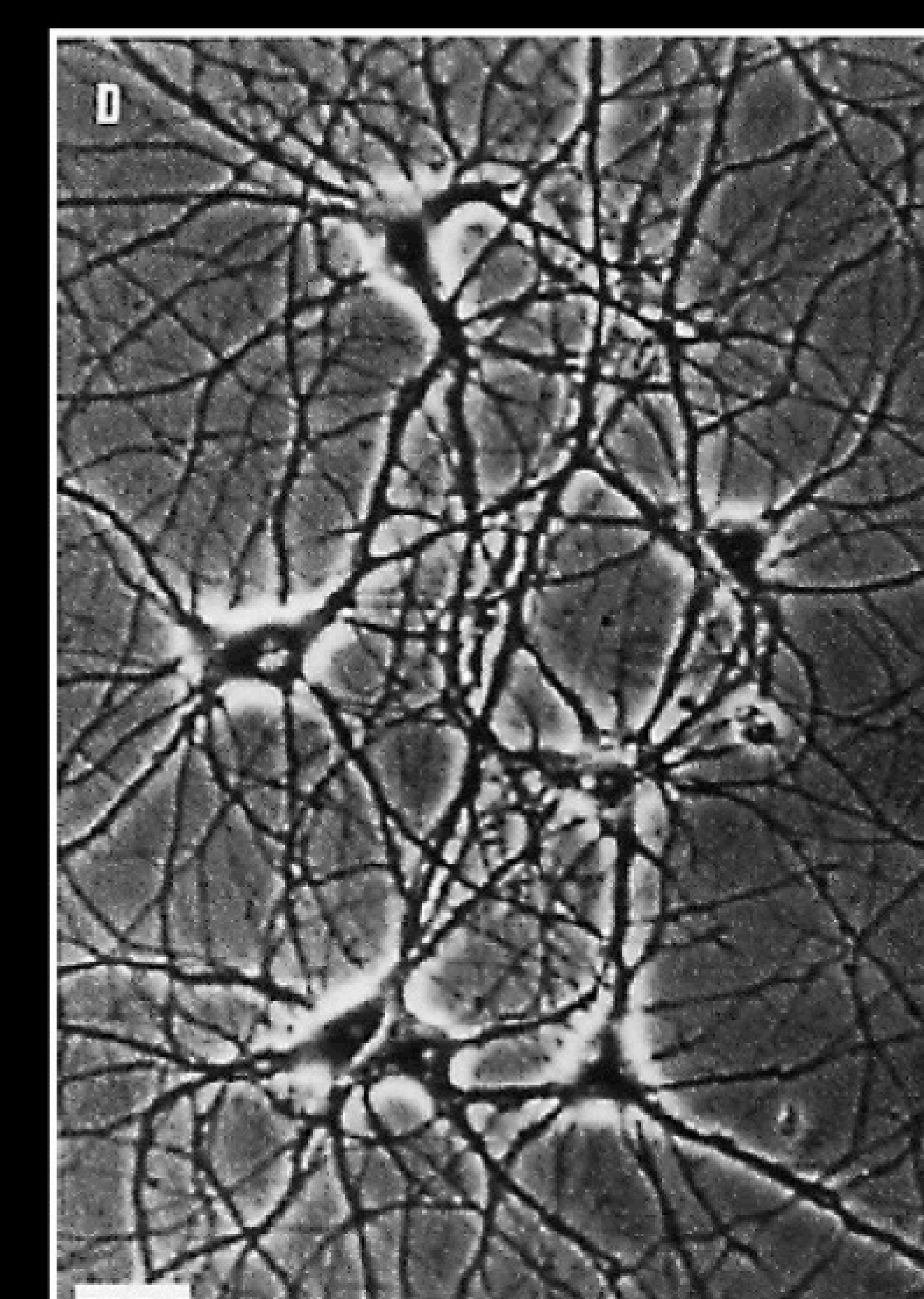
3 Croissance axonale

4 Croissance dendritique

5 Maturation

DIV 18-21



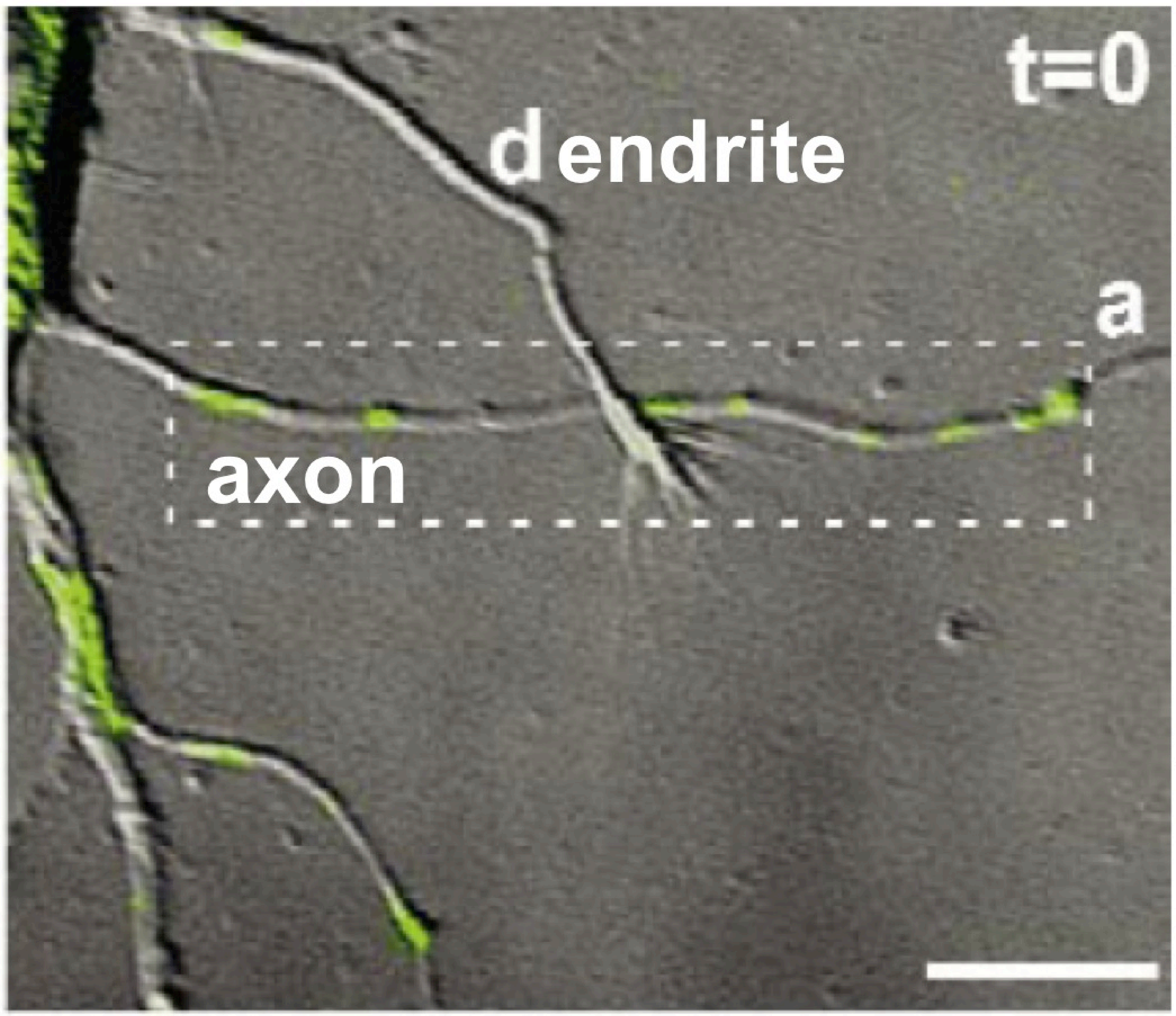


Phase contrast microscopy (Goslin et al., 1977)

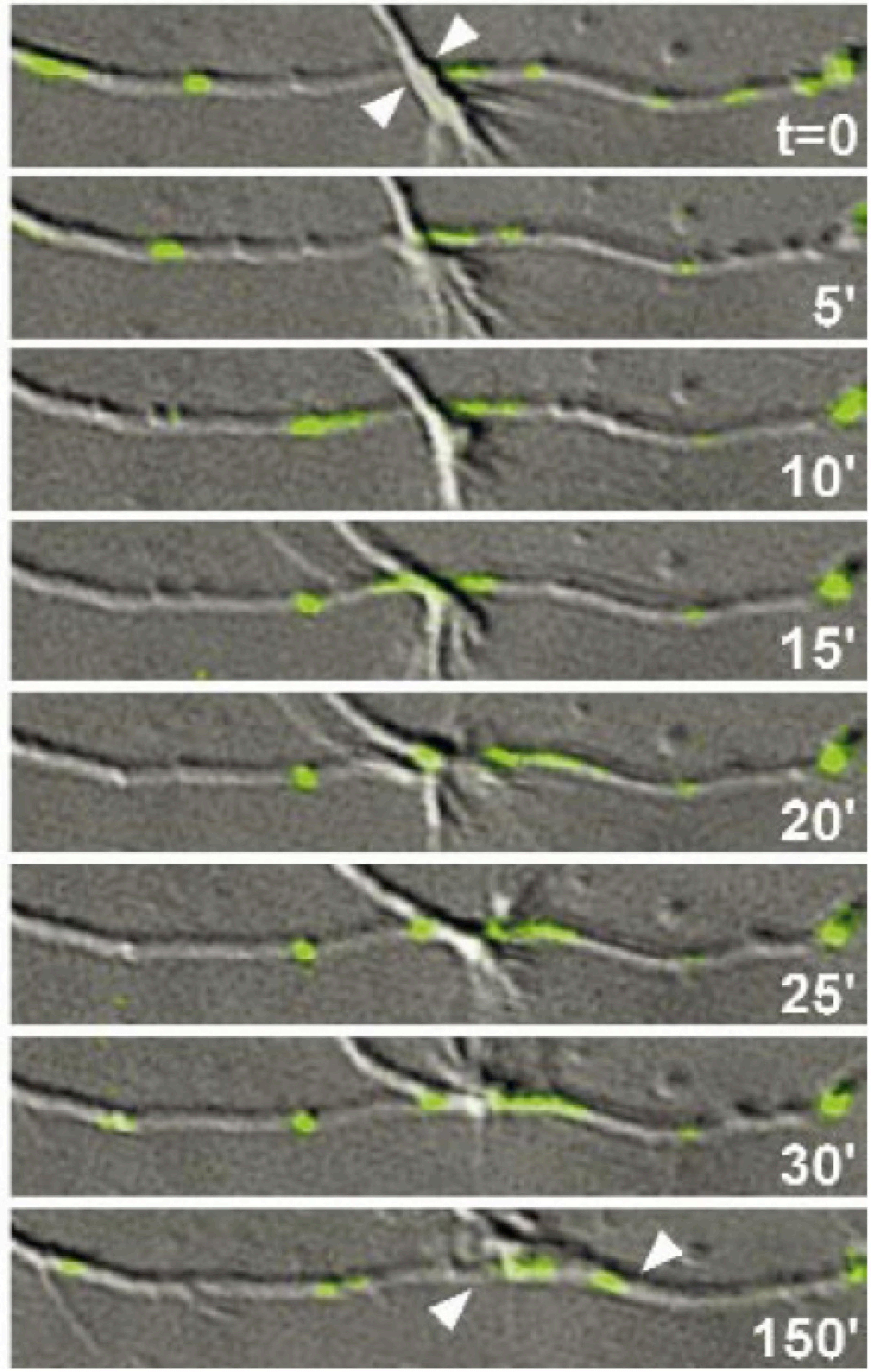
Assembly of presynaptic active zones from cytoplasmic transport packets

Susanne E. Ahmari, JoAnn Buchanan and Stephen J Smith

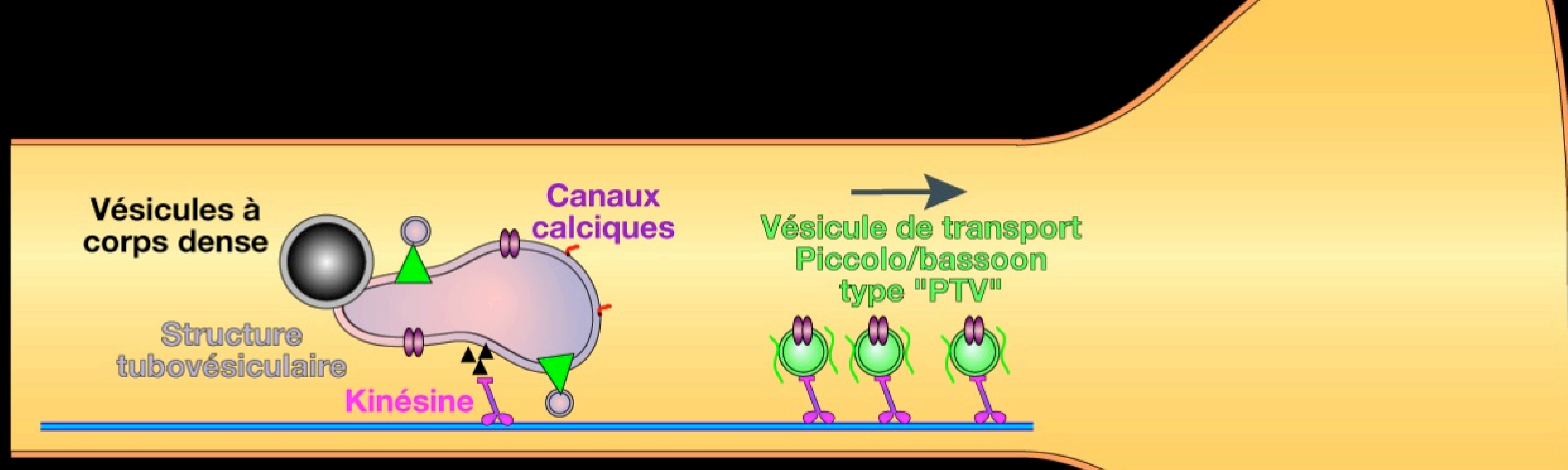
Hippocampal neuron – 10 DIV- VAMP-GFP



Autaptic contact event is followed by an accumulation of VAMP–GFP at the site of contact



Transport axonal des protéines présynaptiques



Structures tubovésiculaires

500-1500 nm de diametre

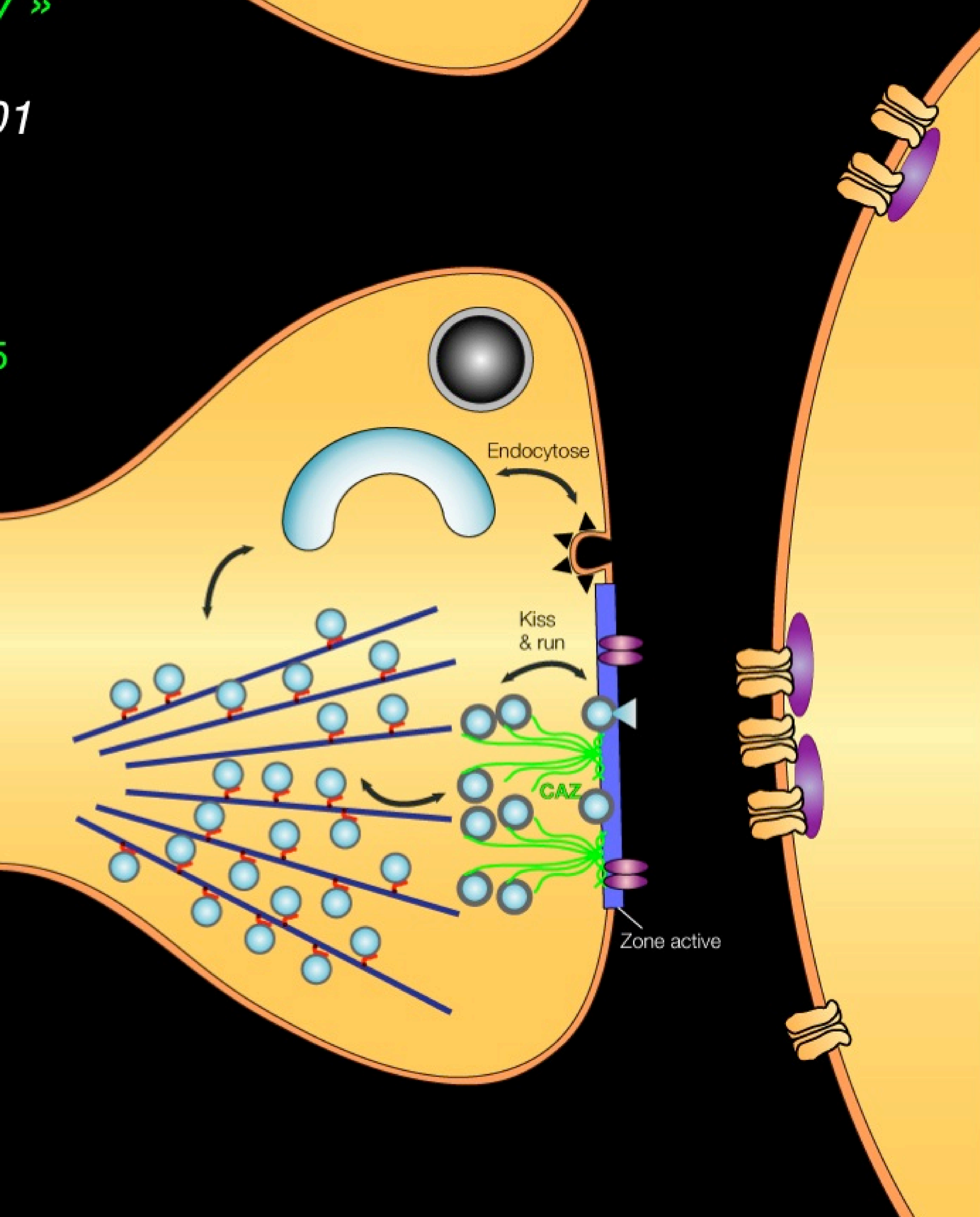
Ahmari et coll., Nat Neur, 2000.

VAMP
SV2
VDCC
Synapsine
amphiphysine

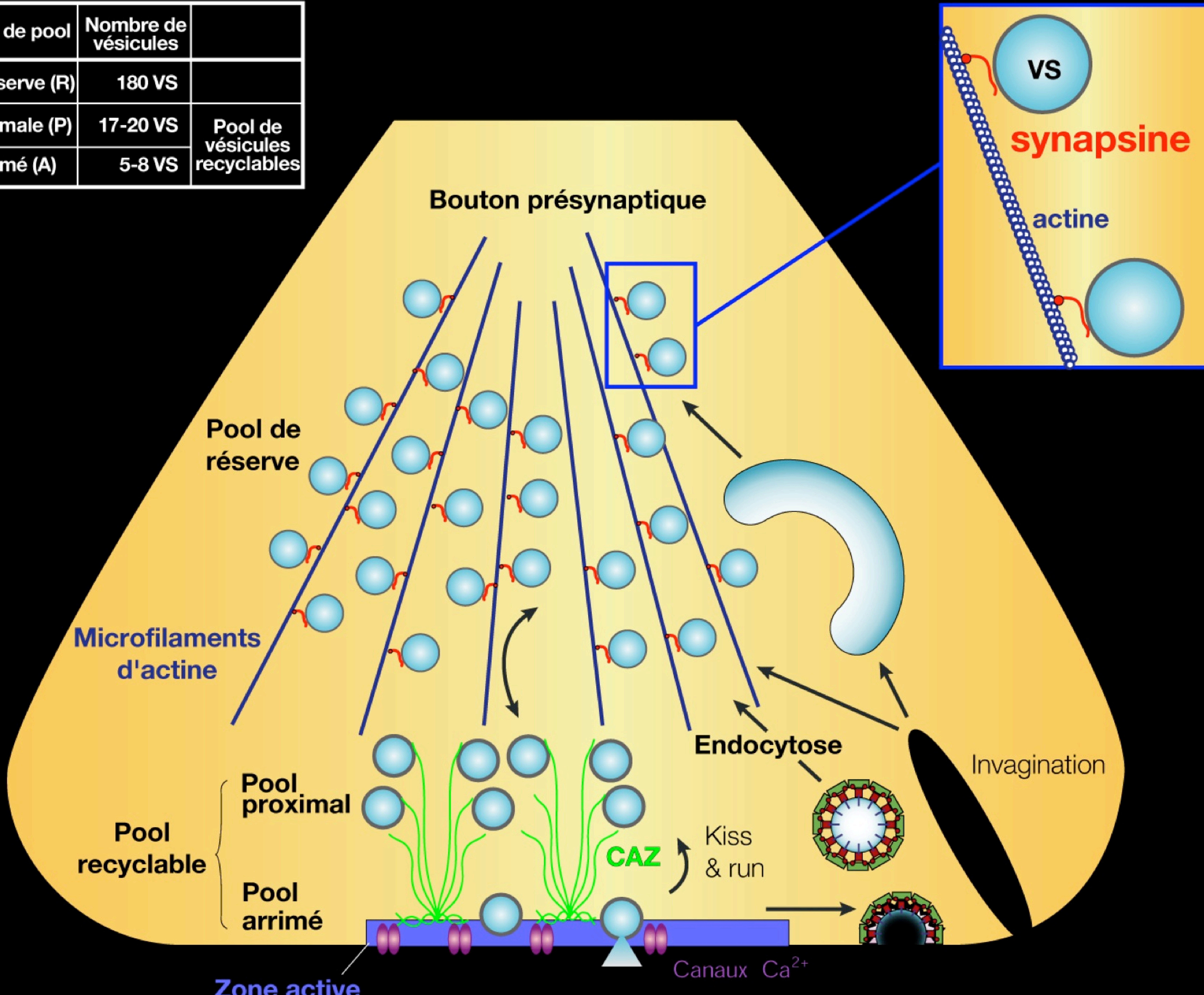
Vésicules de transport
Piccolo/ Bassoon: « PTV »
80 nm de diamètre

Zhai et al., Neuron, 2001

Piccolo
Bassoon
RIM, Munc13, VDCC
Munc18, Syntaxine, SNP25
N-Cadhérine, …



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	

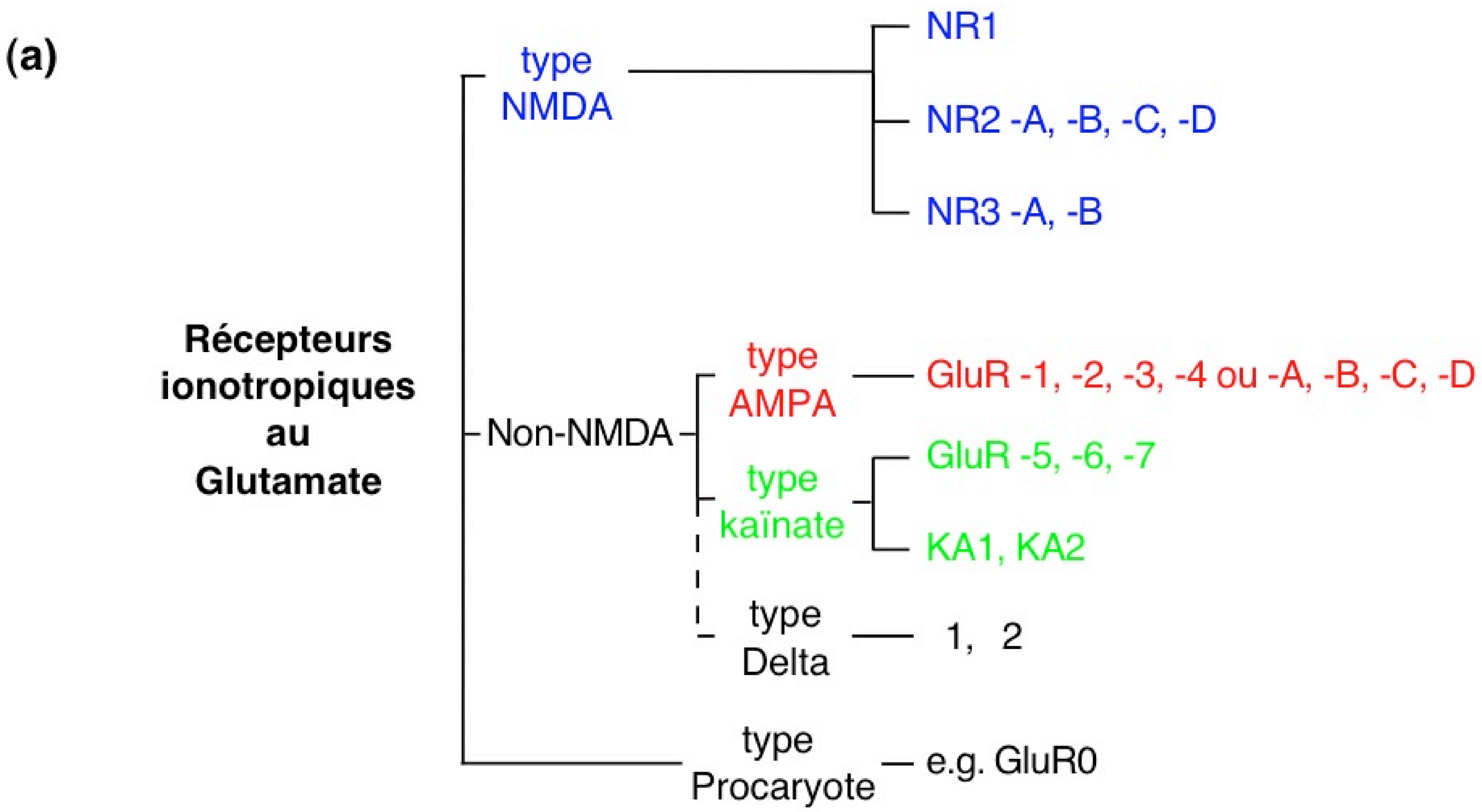


Modifié d’ après Current Opinion in Neurobiology 2000, 10:321–327 et Natrure Review Molecular Cell Biology, 2003, 4:127-139.

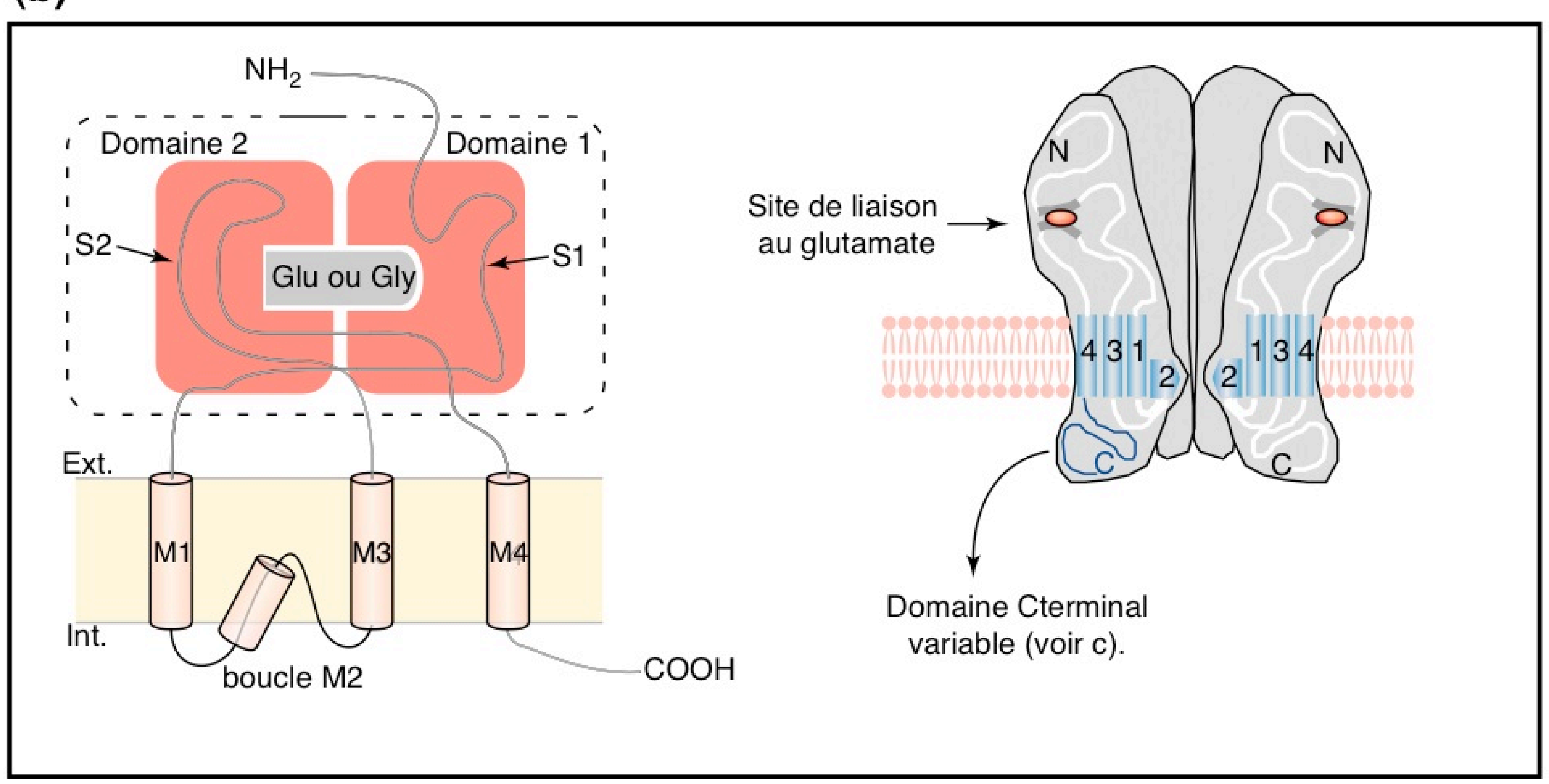
Heterogeneity in the Molecular Composition of Excitatory Postsynaptic Sites during Development of Hippocampal Neurons in Culture

Anuradha Rao,[1] Eunjoon Kim,[2] Morgan Sheng,[2] and Ann Marie Craig[1]

(a)



(b)



1220 J. Neurosci., February 15, 1998, 18(4):1217–1229

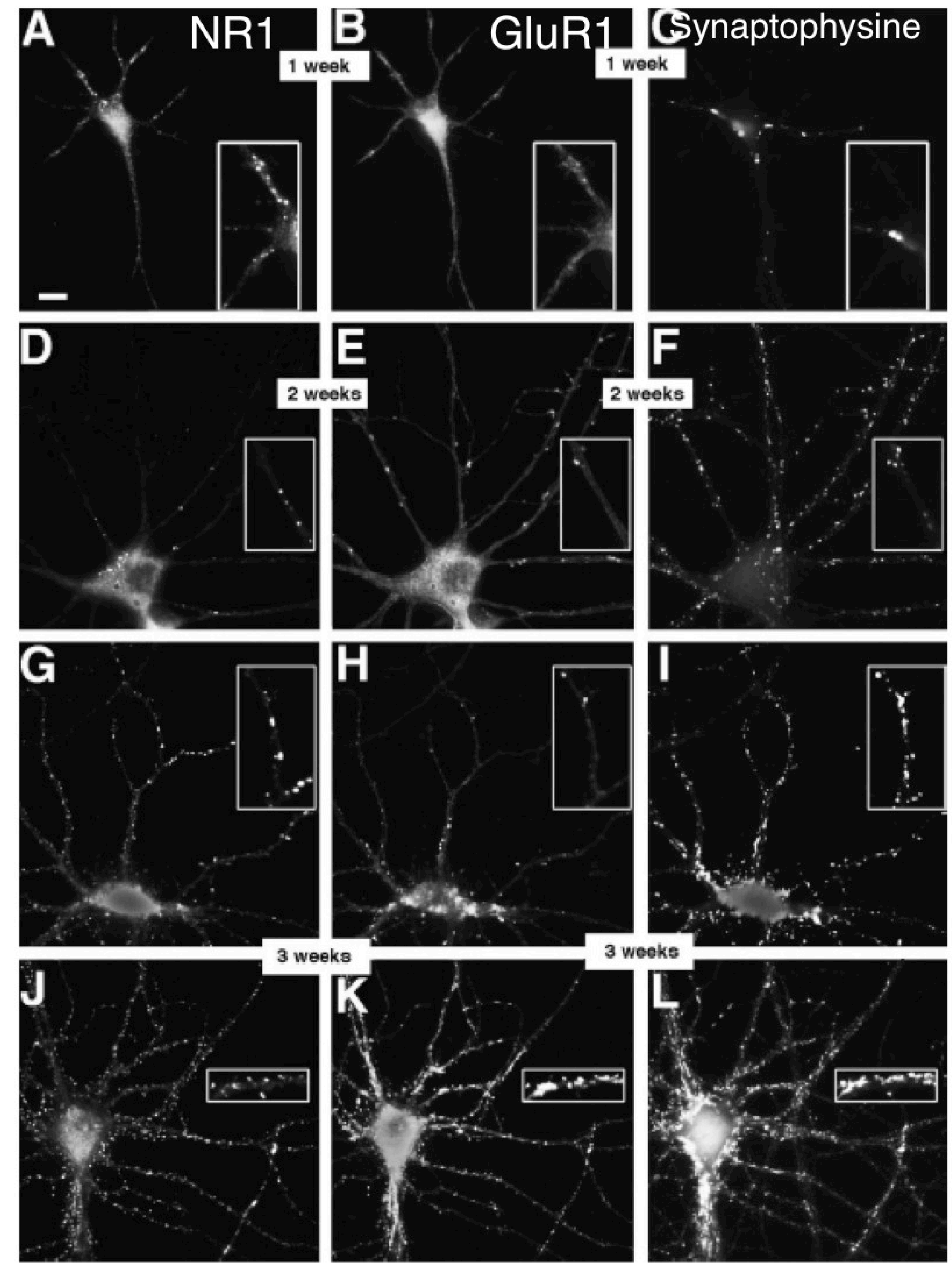


Figure 2. NMDA- and AMPA-type receptors cluster separately until late in development. Cultures fixed at 5 (*A–C*), 14 (*D–F*), and 21 (*G–L*) d *in vitro* were immunostained for NR1 (*A, D, G, J*), GluR1 (*B, E, H, K*), and the presynaptic marker synaptophysin (*C, F, I, L*). In the 5 d cell, NR1 (*A*) forms clusters at sites distinct from the synaptic sites indicated by synaptophysin clusters (*C*). GluR1 staining is detectable but diffuse (*B*). At 14 d, NR1 clusters have moved out into the proximal dendrite (*D*) and are still distinct from presynaptic sites (*F*). GluR1 forms aggregates at spiny sites along the full length of the dendrites (*E*), always apposed to presynaptic sites (*F*). By 21 d, two additional patterns of NR1 staining are apparent. *Figure legend continues.*

Heterogeneity in the Molecular Composition of Excitatory Postsynaptic Sites during Development of Hippocampal Neurons in Culture

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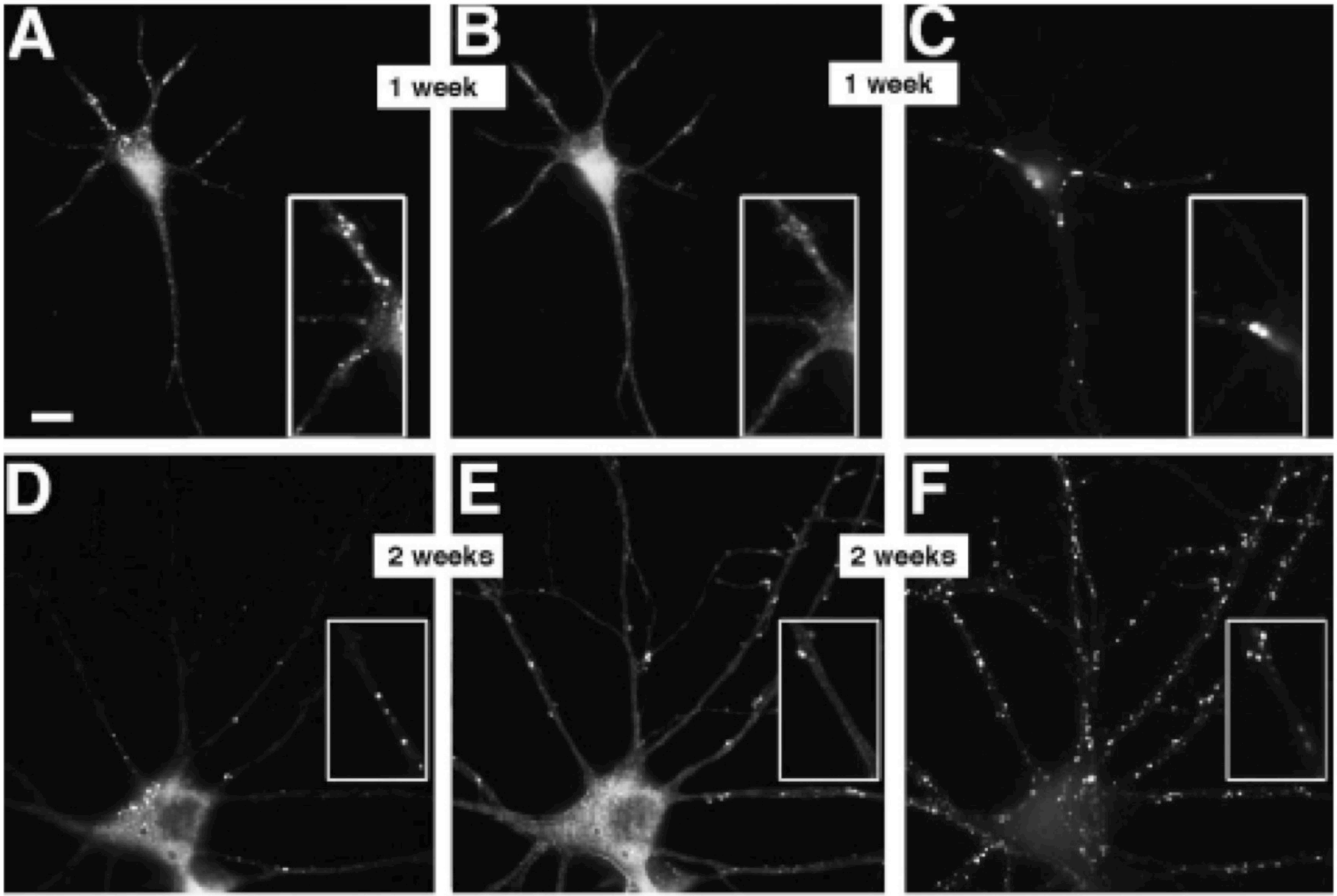
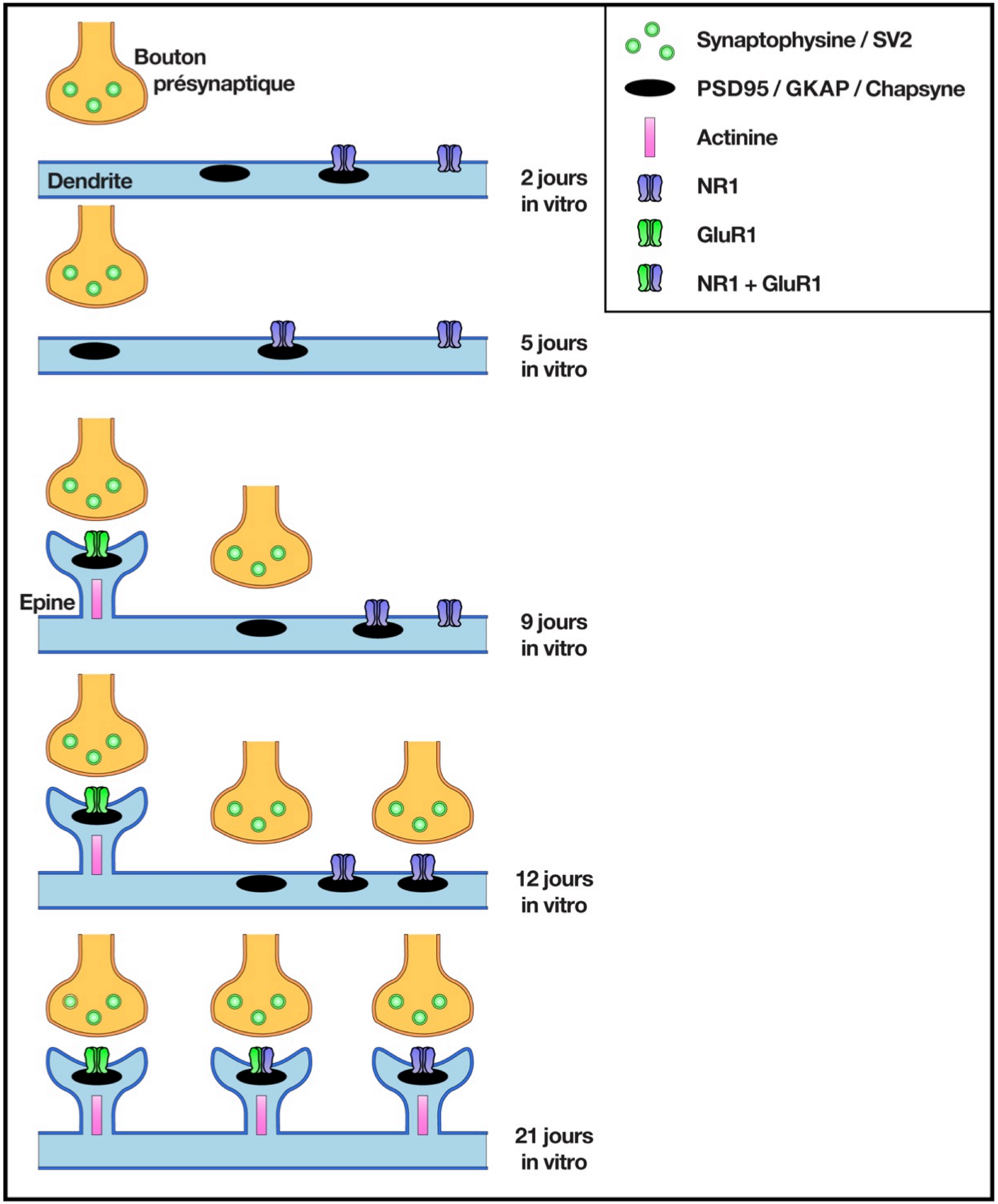


Heterogeneity in the Molecular Composition of Excitatory Postsynaptic Sites during Development of Hippocampal Neurons in Culture

Anuradha Rao,[1] Eunjoon Kim,[2] Morgan Sheng,[2] and Ann Marie Craig[1]

Figure 4. PSD-95 is present at excitatory synaptic sites, but not at inhibitory synapses. Hippocampal neurons fixed at 21 d in culture were immunostained for PSD-95 (*A*, *C*) and either GluR1 (*B*) or the $GABA_A$ receptor $\beta 2/3$ subunit (*D*). PSD-95 clusters colocalized with GluR1-labeled excitatory synapses (indicated by *arrows* in *A*, *B*), but not with the $GABA_A$ receptor-labeled inhibitory synapses (*arrows* in *C*, *D*). Scale bar, 10 μm.

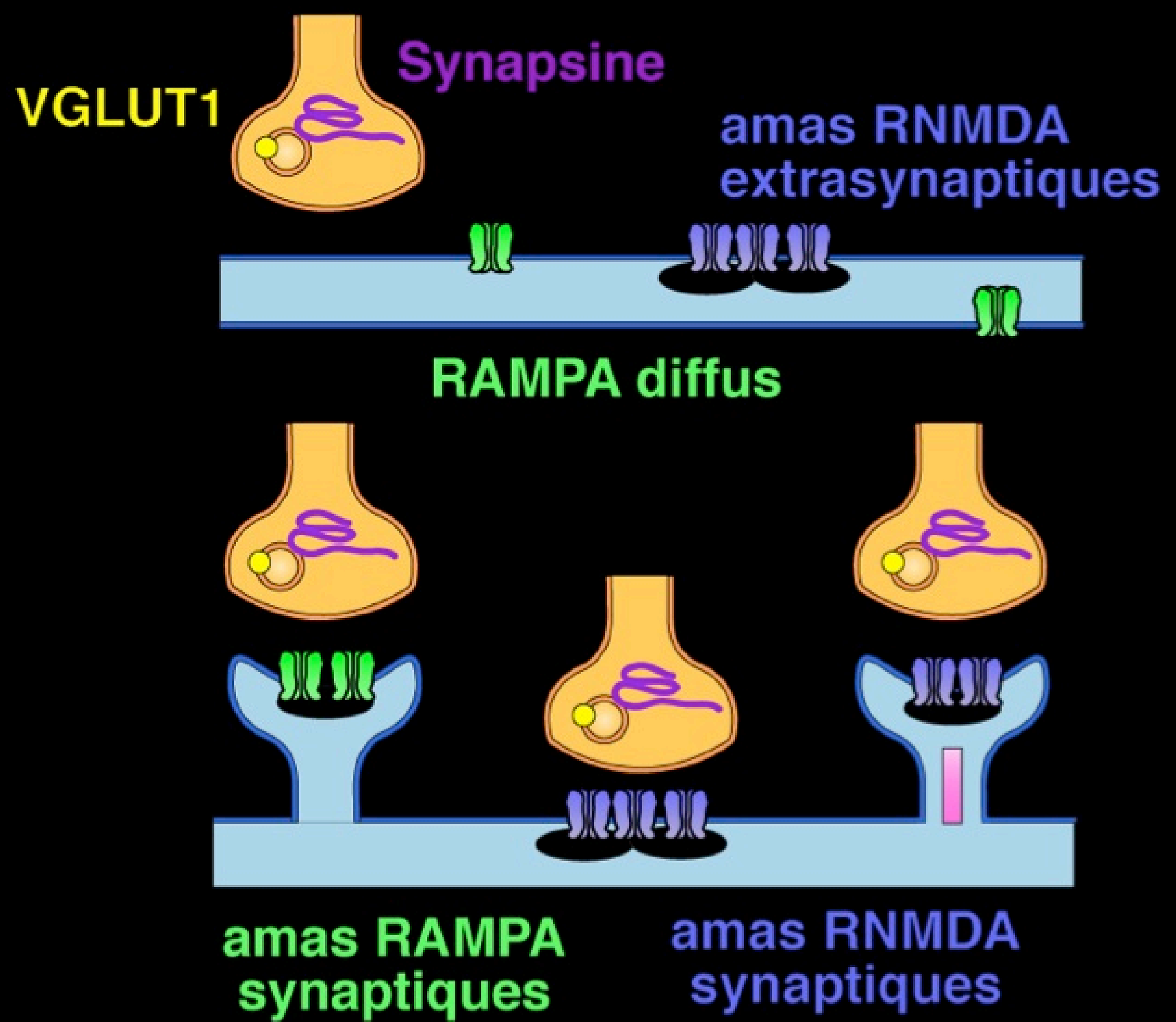

Synaptophysine / SV2
PSD95 / GKAP / Chapsyne
Actinine
NR1
GluR1
NR1 + GluR1
Bouton présynaptique
Dendrite
2 jours in vitro
5 jours in vitro
Epine
9 jours in vitro
12 jours in vitro
21 jours in vitro

Ciblage des protéines post-synaptiques

Synapse excitatrice hippocampe

Laboratoire A.M. Craig

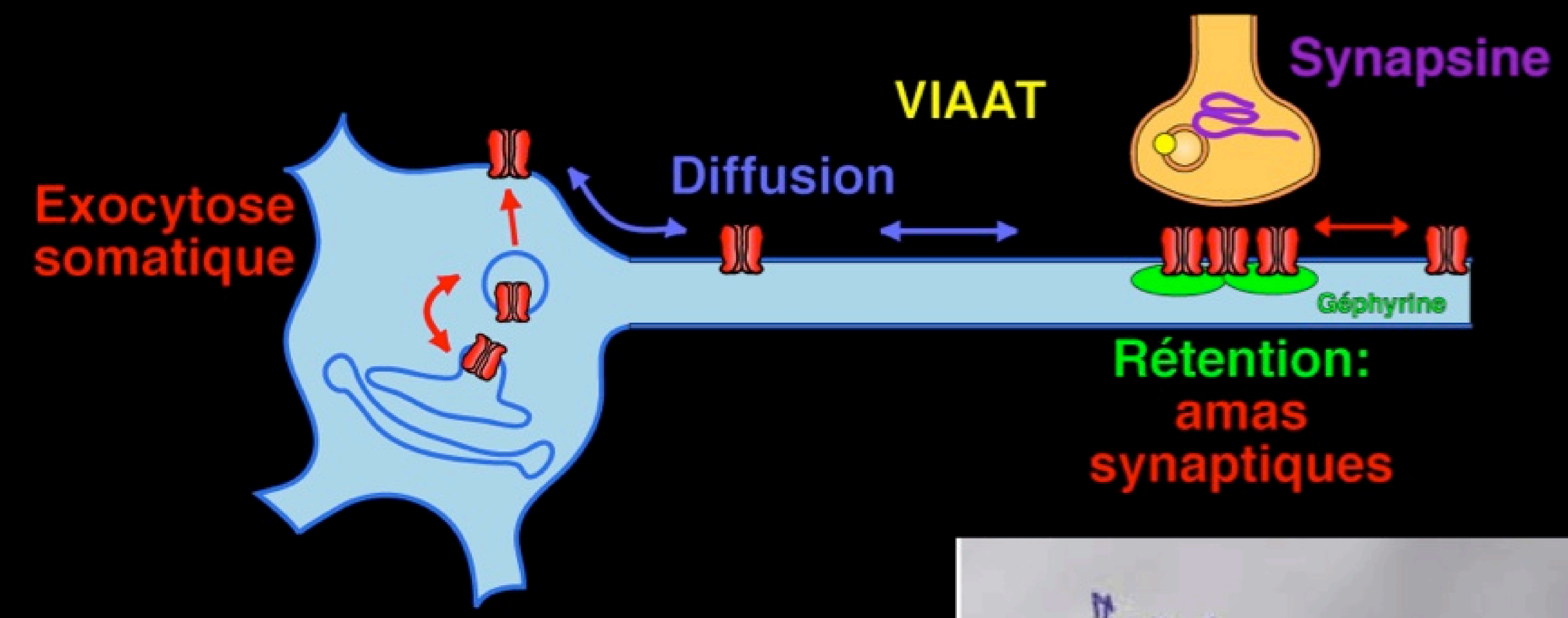
Rao et al., J. Neurosci., 2000
Rao et al., J. Neurosci., 2000

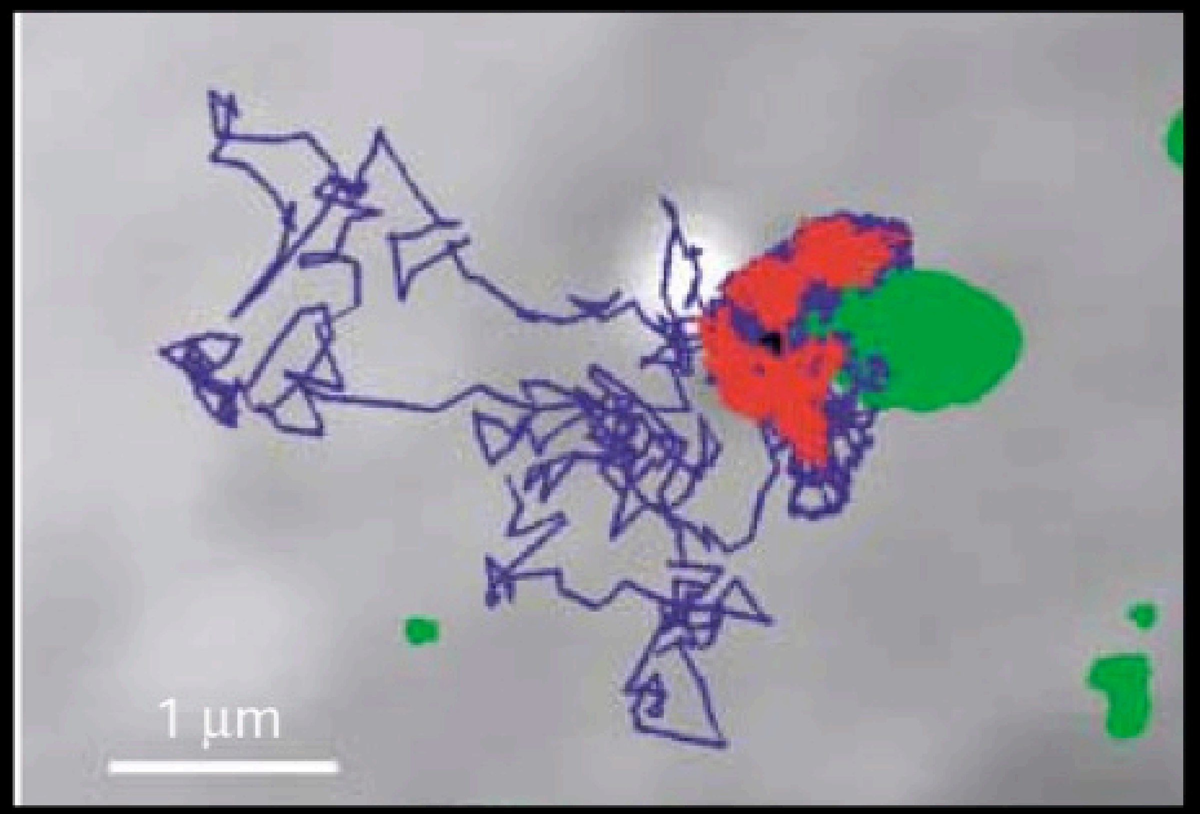


Synapse inhibitrice moelle épinière

Laboratoire A.Triller

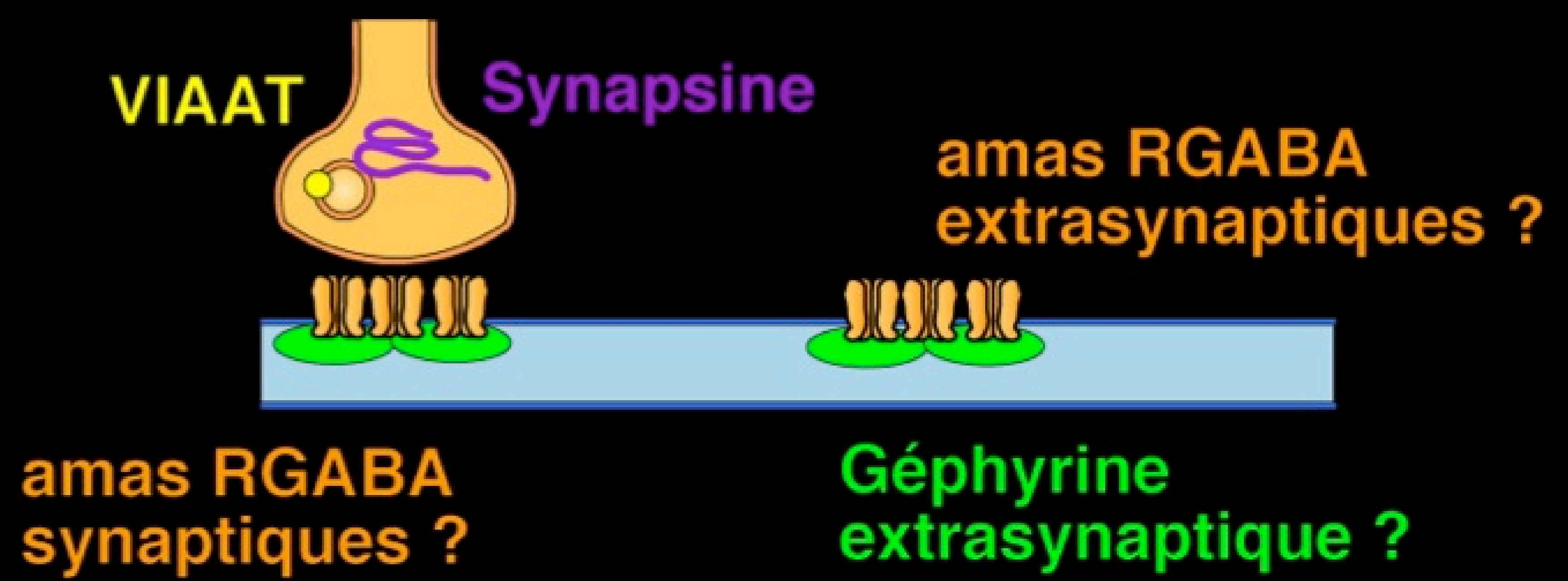
Rosenberg et al., J. Neurosci, 2000
Meier et al, JCS, 2000
Meier et al., Nat. Neur., 2001





Synapse inhibitrice hippocampe

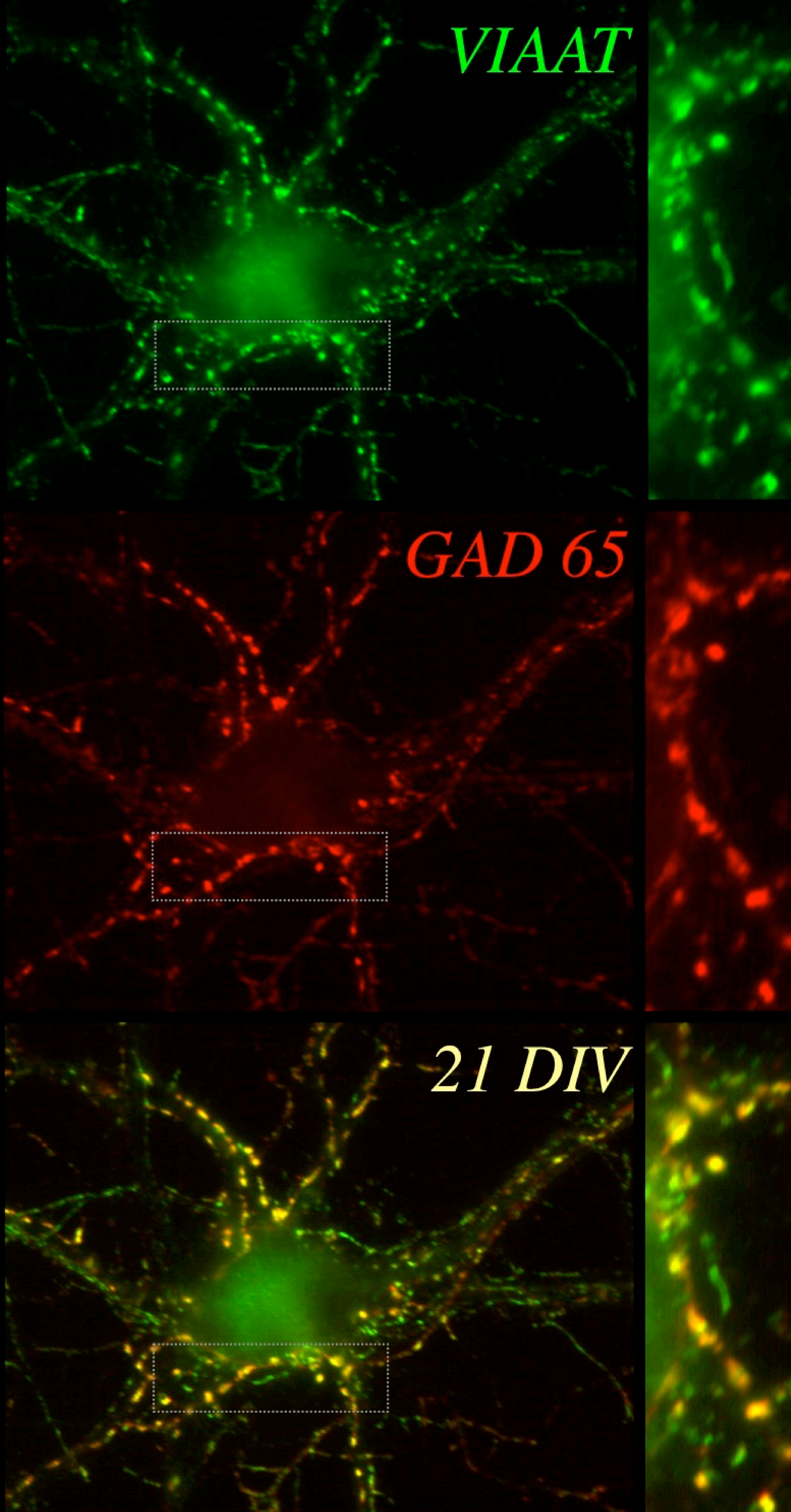
?



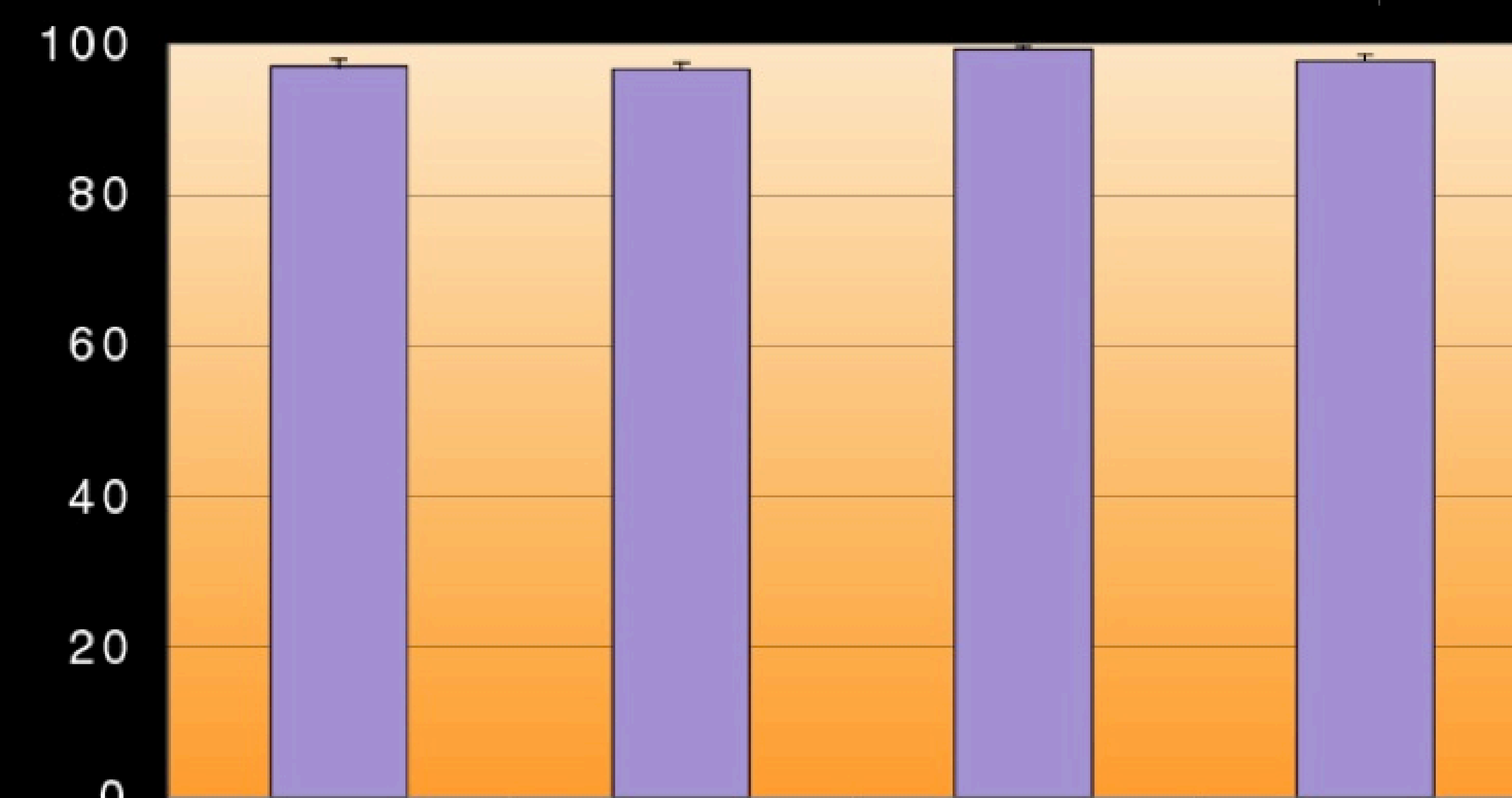
VIAAT: Expression diffuse dans l'axone puisamas dès 10 j

Danglot et al. Mol Cell Neurosci 2003

Quantification des colocalisations de VIAAT avec la Synapsine et GAD65



	10 div	14 div	17 div	21 div
VIAAT colocalisé / VIAAT total	84%	89%	89%	95%

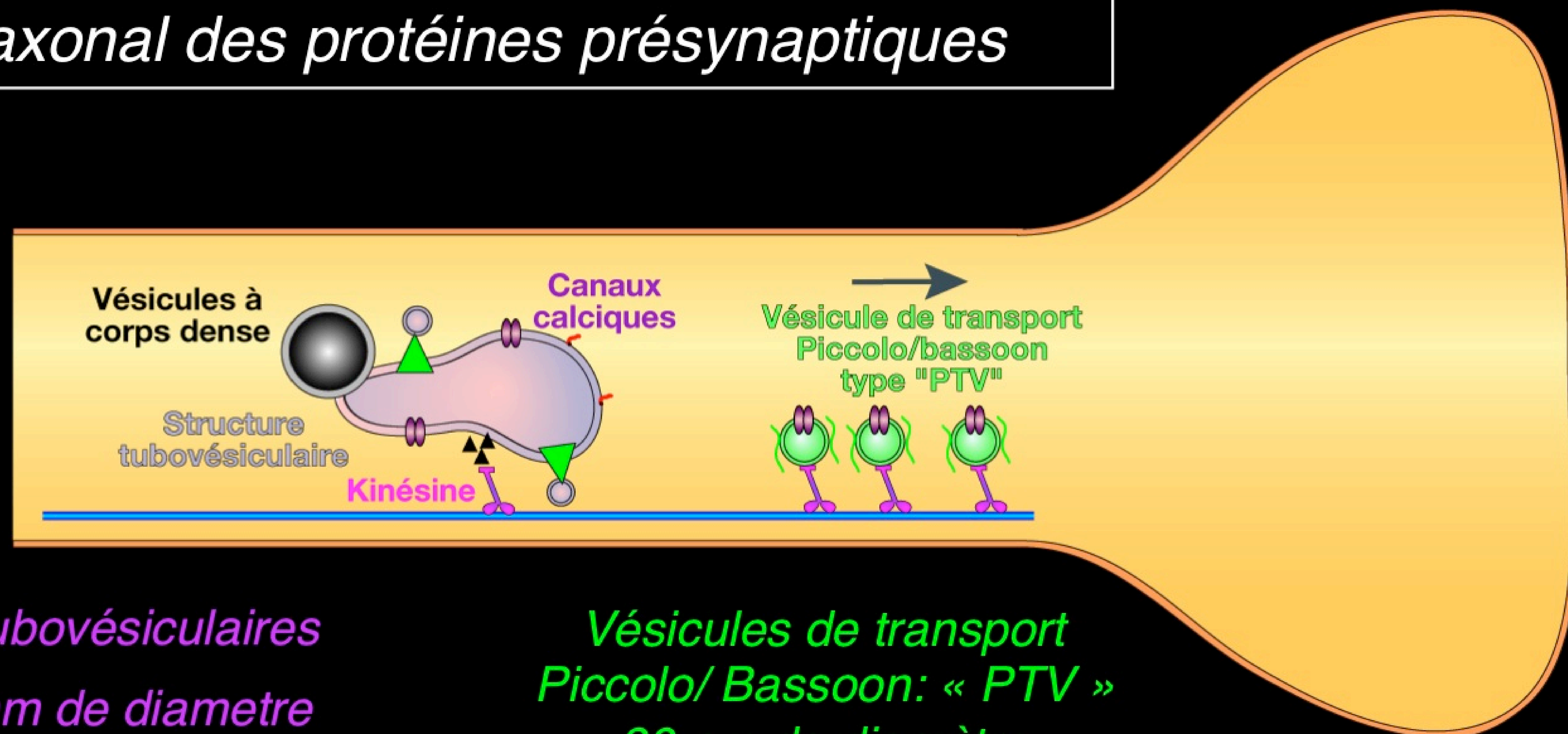


	10div	14 div	17 div	21 div
GAD65 colocalisée / GAD totale	97,0%	96,6%	99,3%	97,7%

VIAAT et GAD65 suivent le même profil d'expression.

Synapsin
VIAAT & GAD 65
Plating
3 DIV
7 DIV
11DIV
14DIV
17DIV
21DIV

Transport axonal des protéines présynaptiques



Structures tubovésiculaires

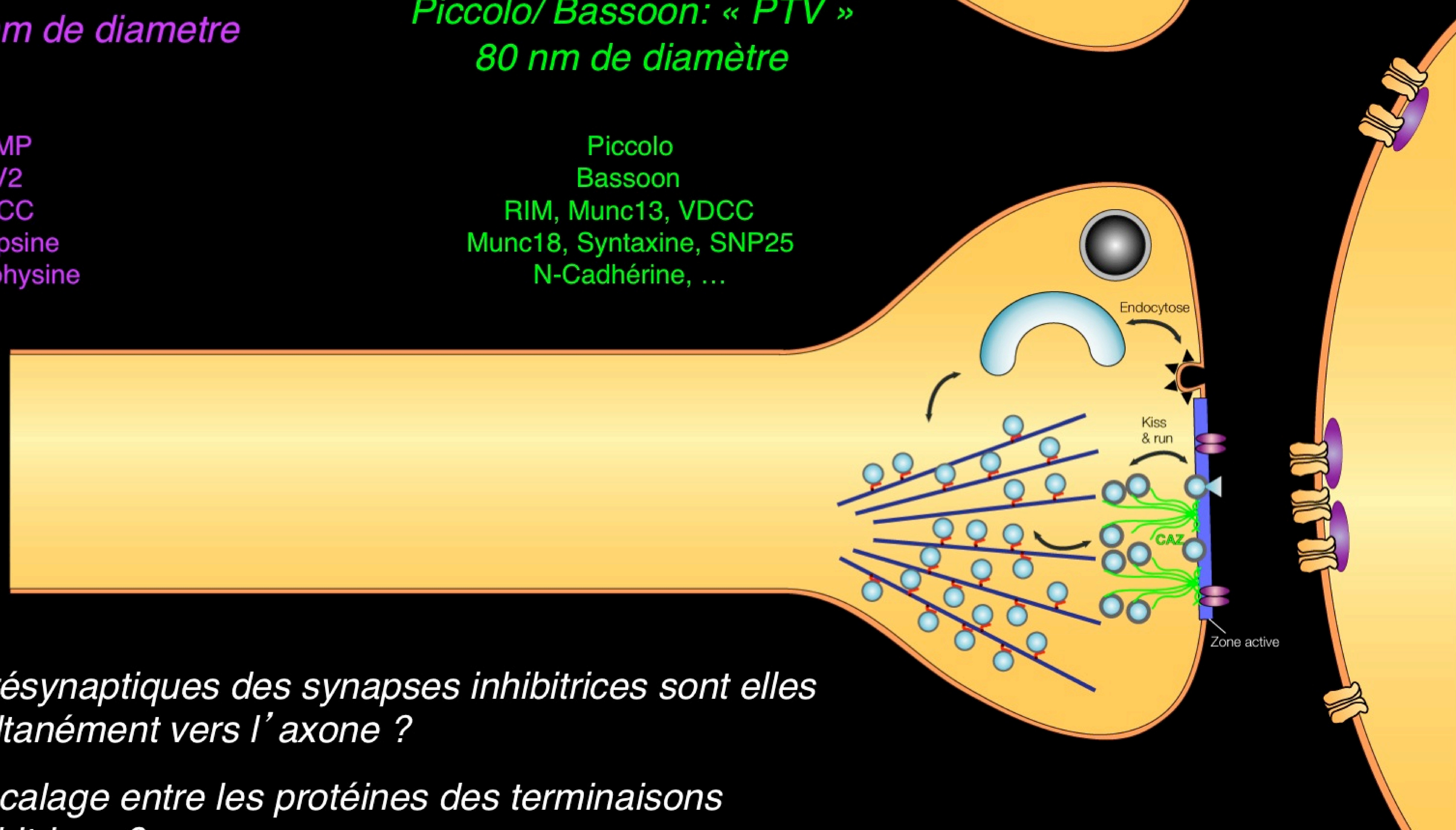
500-1500 nm de diametre

VAMP
SV2
VDCC
Synapsine
amphiphysine

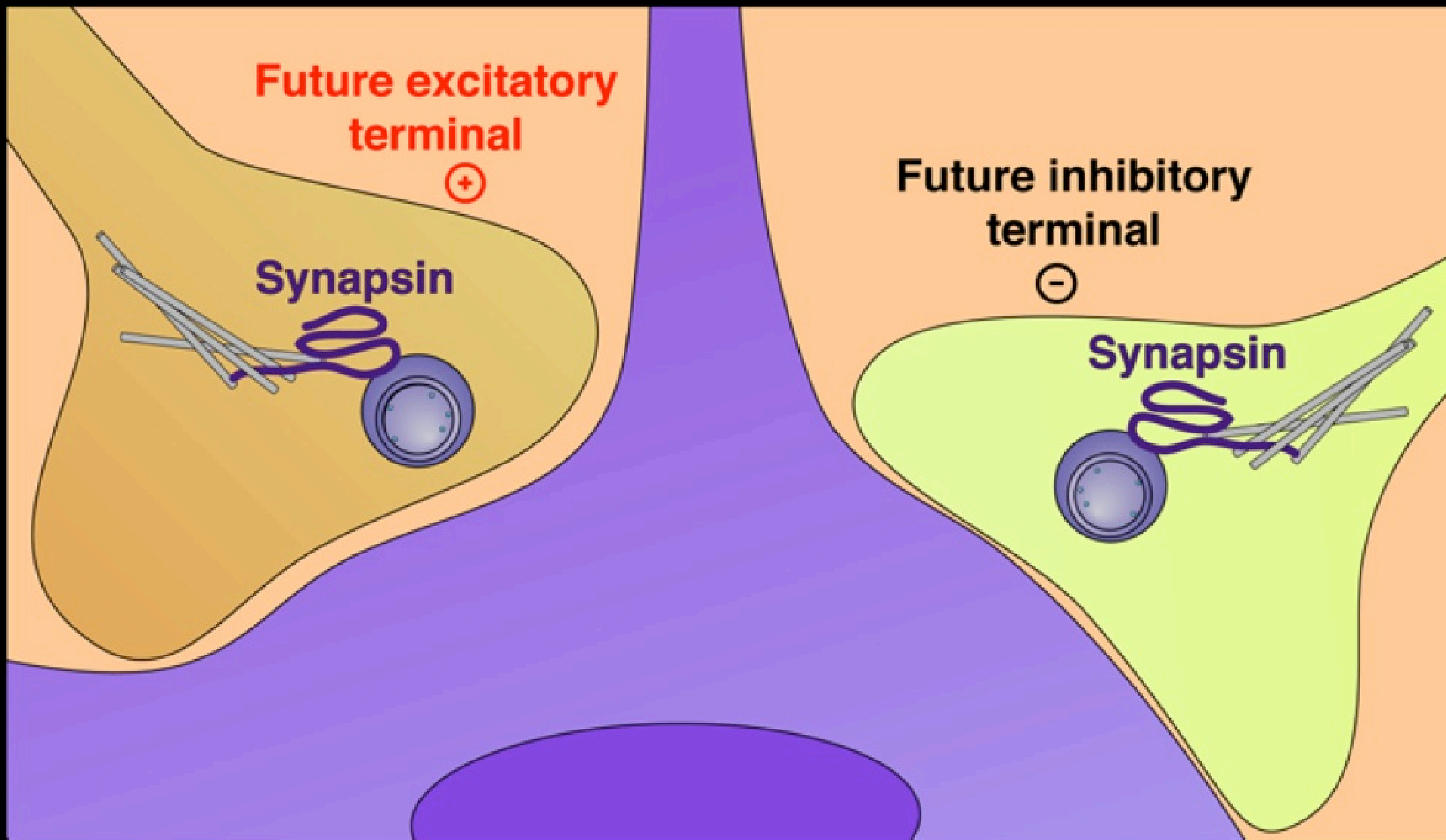
Vésicules de transport Piccolo/ Bassoon: « PTV »

80 nm de diamètre

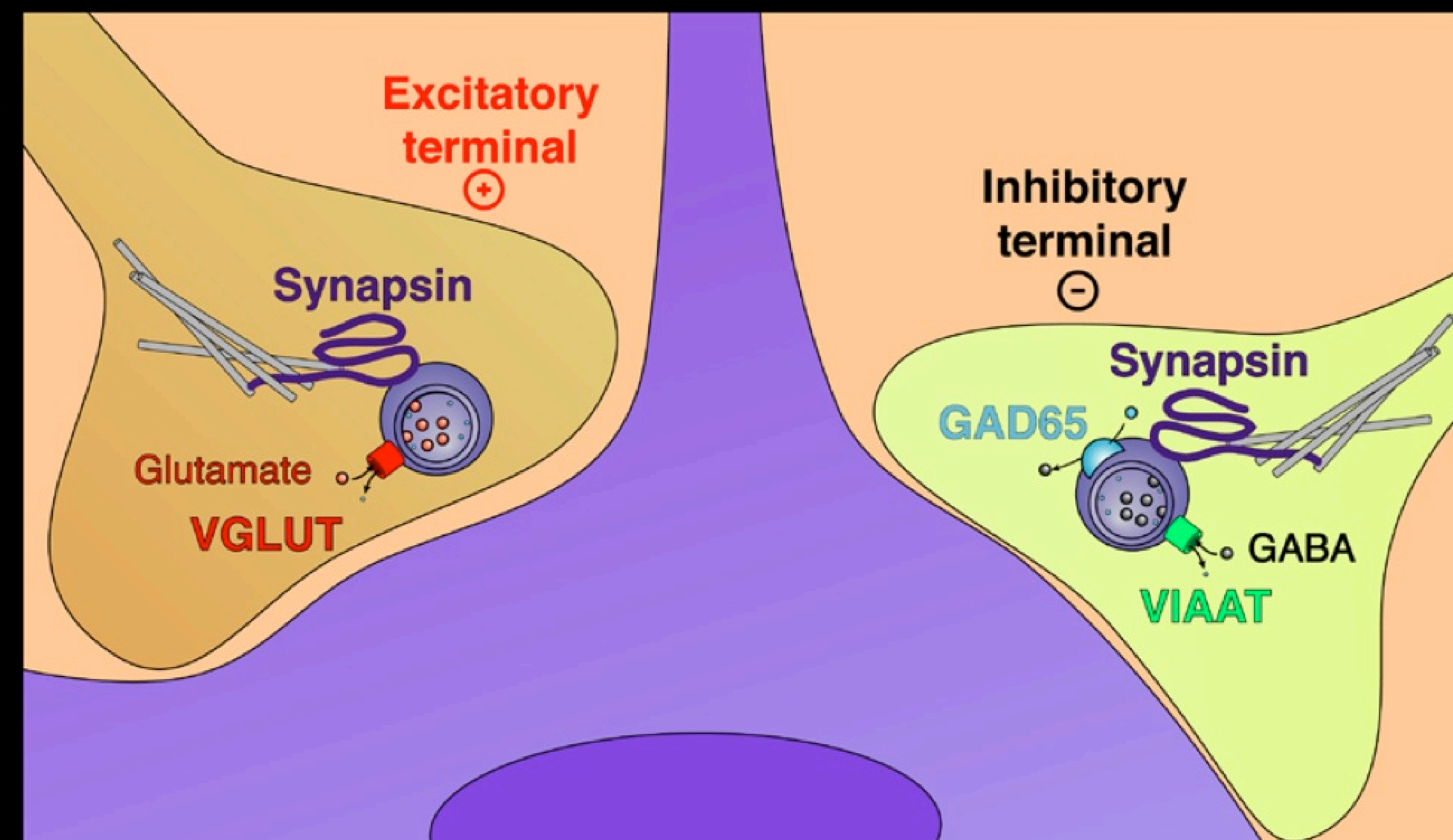
Piccolo
Bassoon
RIM, Munc13, VDCC
Munc18, Syntaxine, SNP25
N-Cadhérine, …

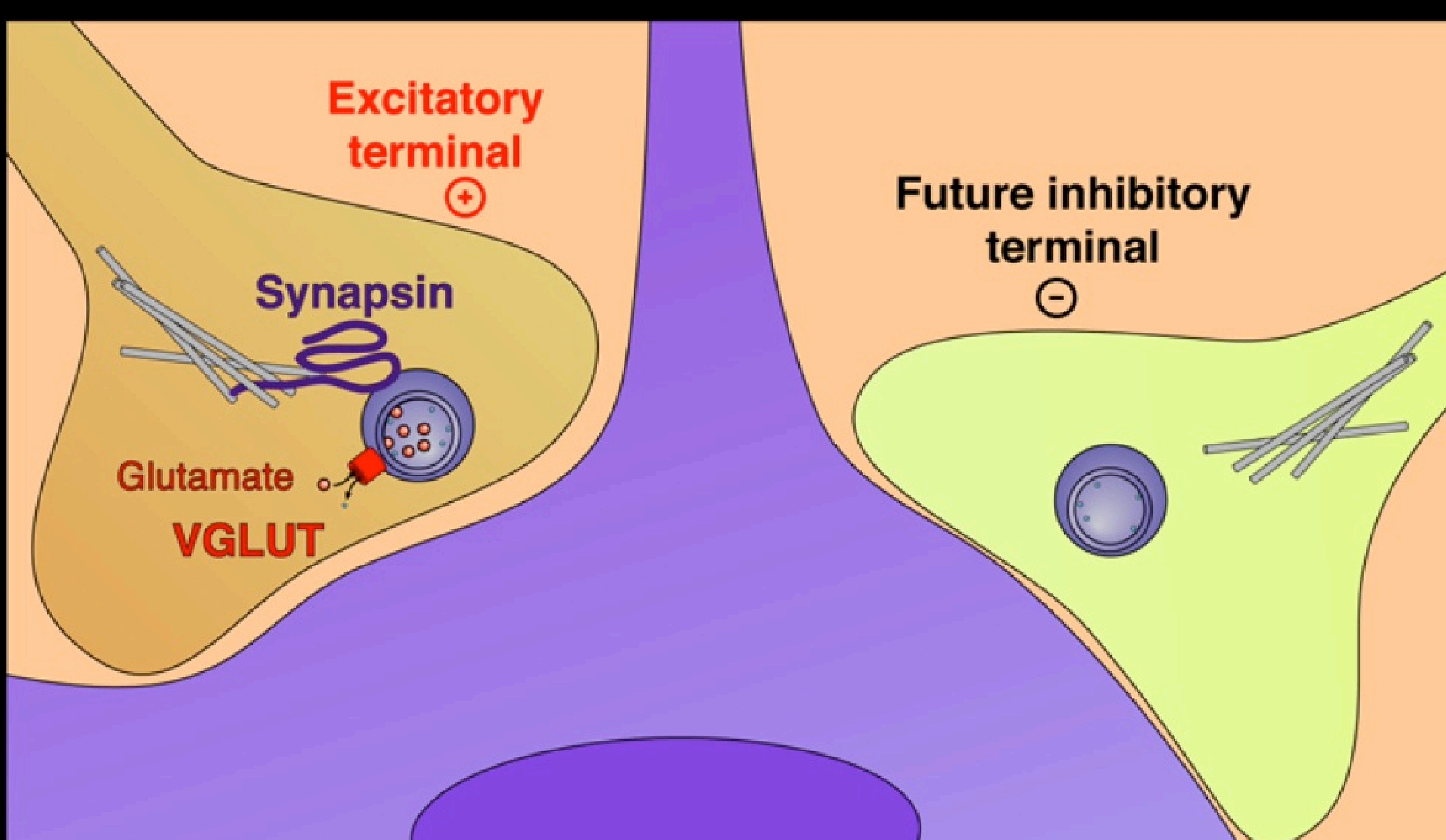


- *Les protéines présynaptiques des synapses inhibitrices sont elles transportées simultanément vers l’axone ?*
- *Existe -t-il un décalage entre les protéines des terminaisons excitatrices et inhibitrices ?*



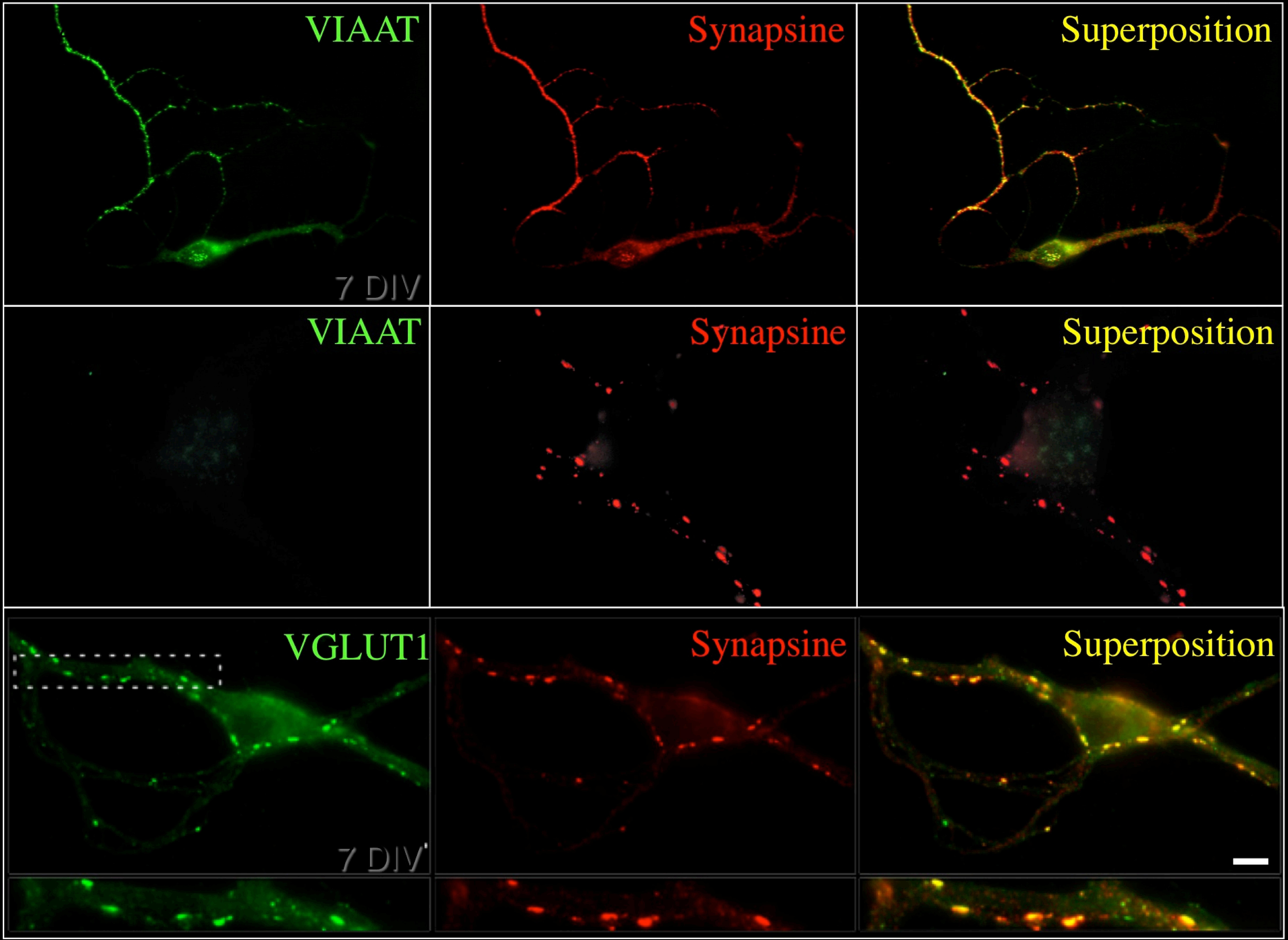
Hypothese A:
La Synapsine est
exprimé précocément.

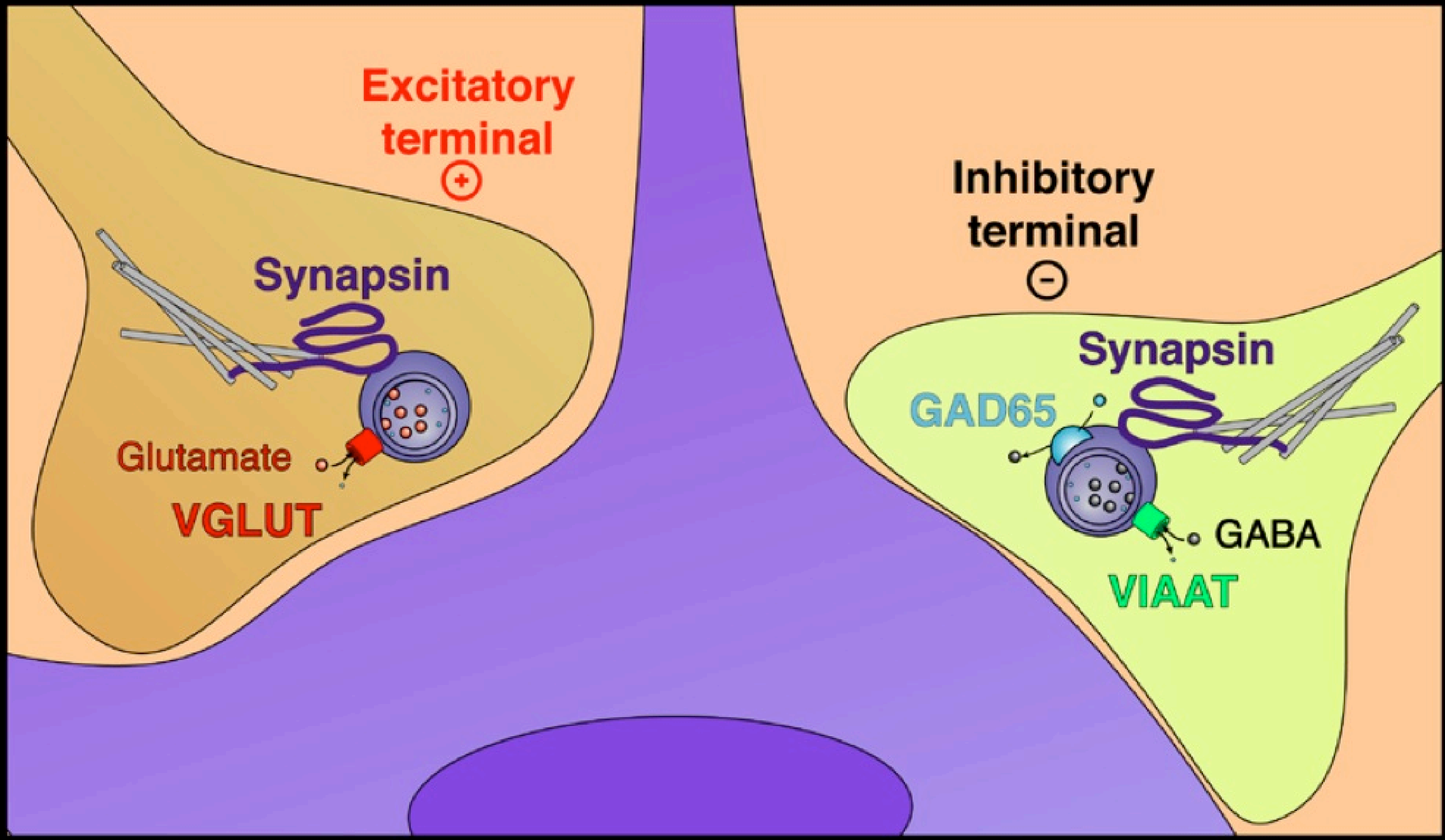


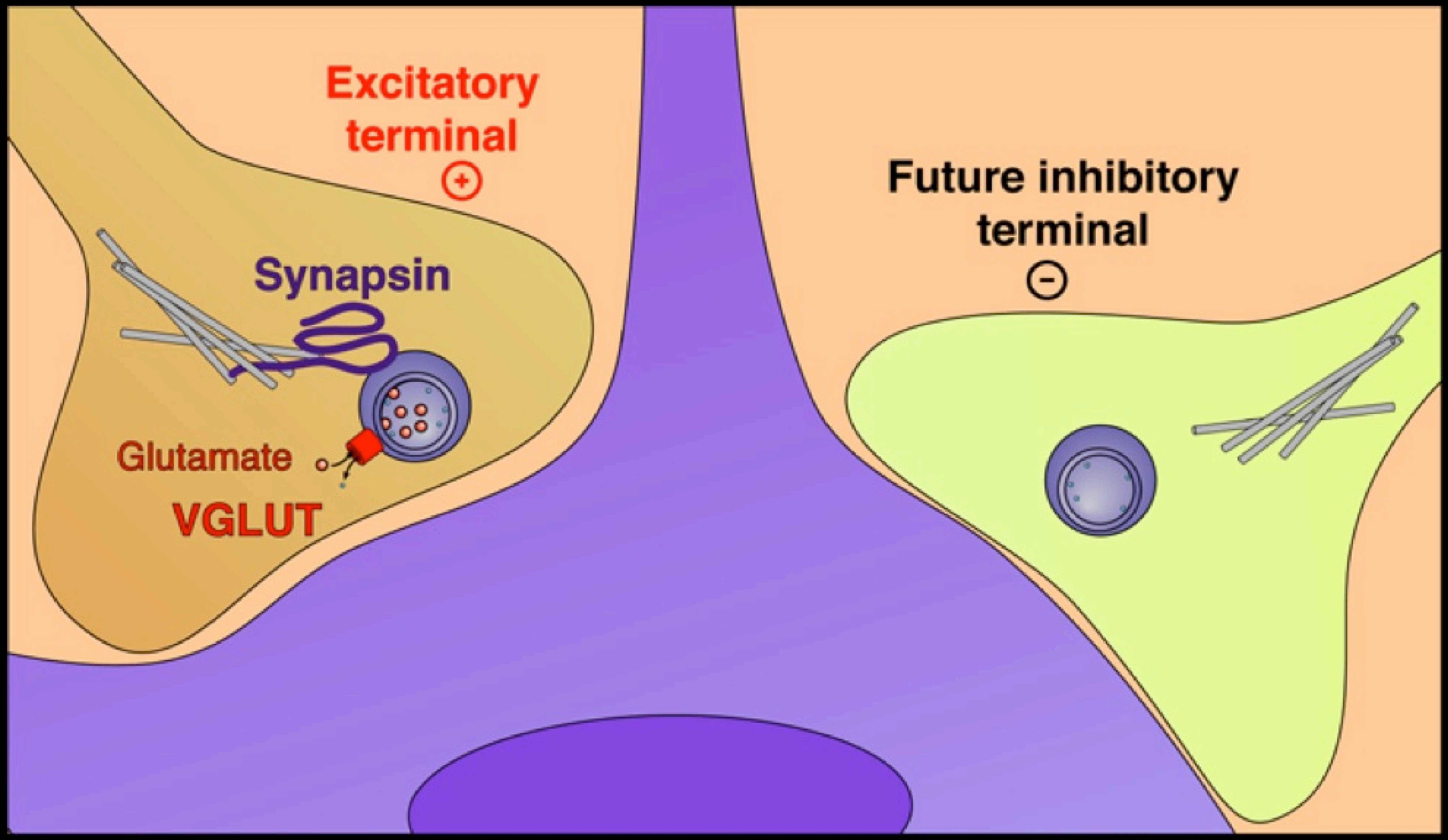


Hypothesis B:
Les protéines des terminaisons
Excitatrices sont exprimées précocément.

Expression séquentielle des protéines présynaptiques ?



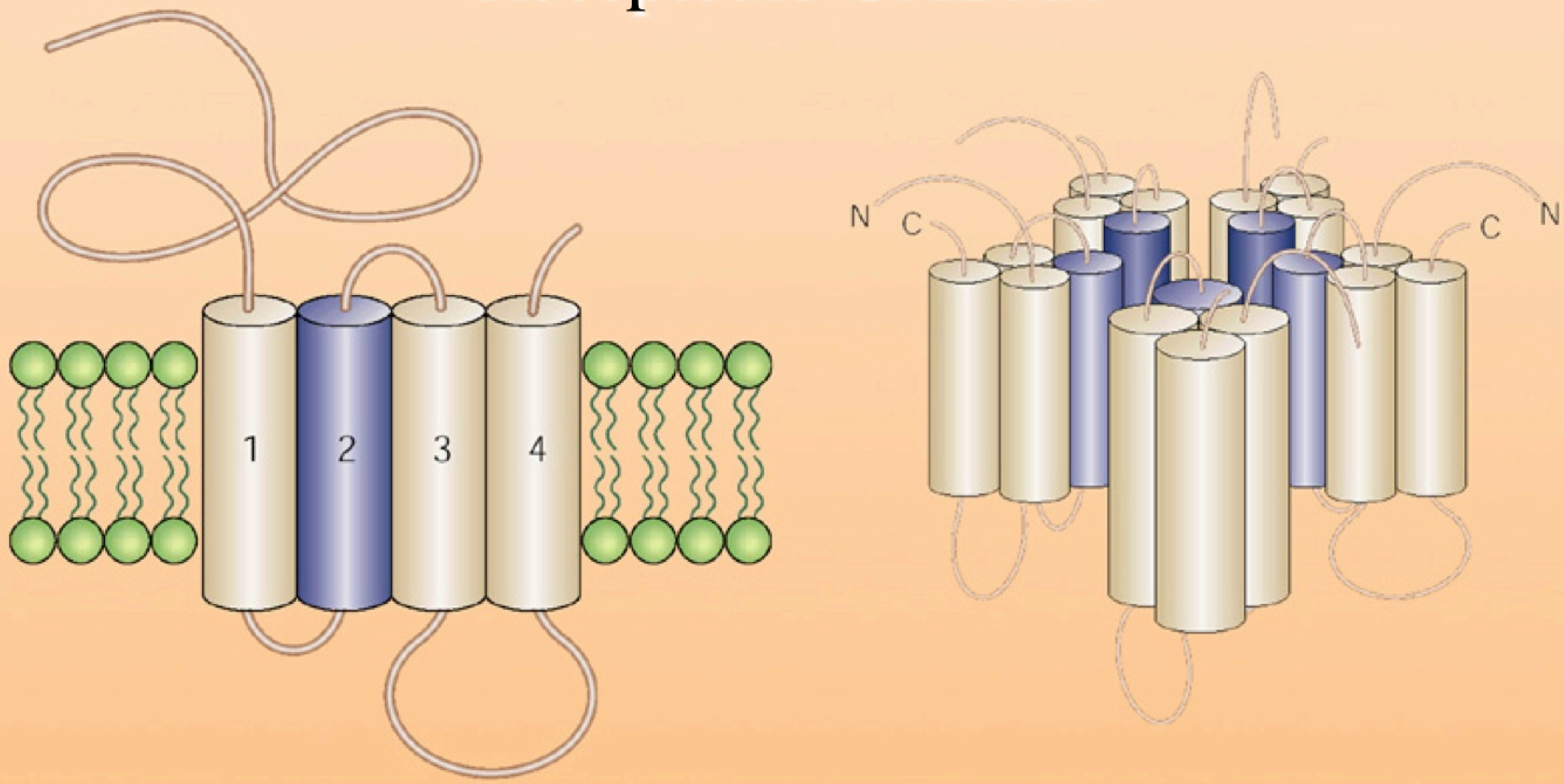


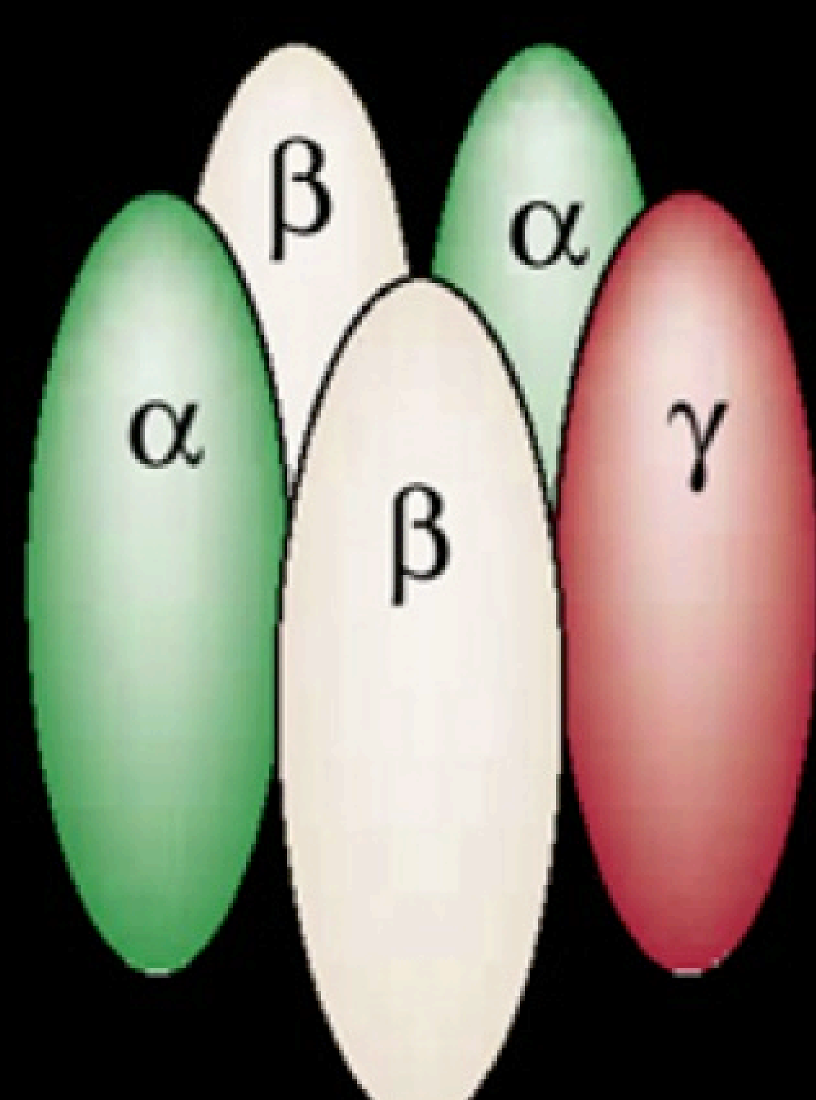


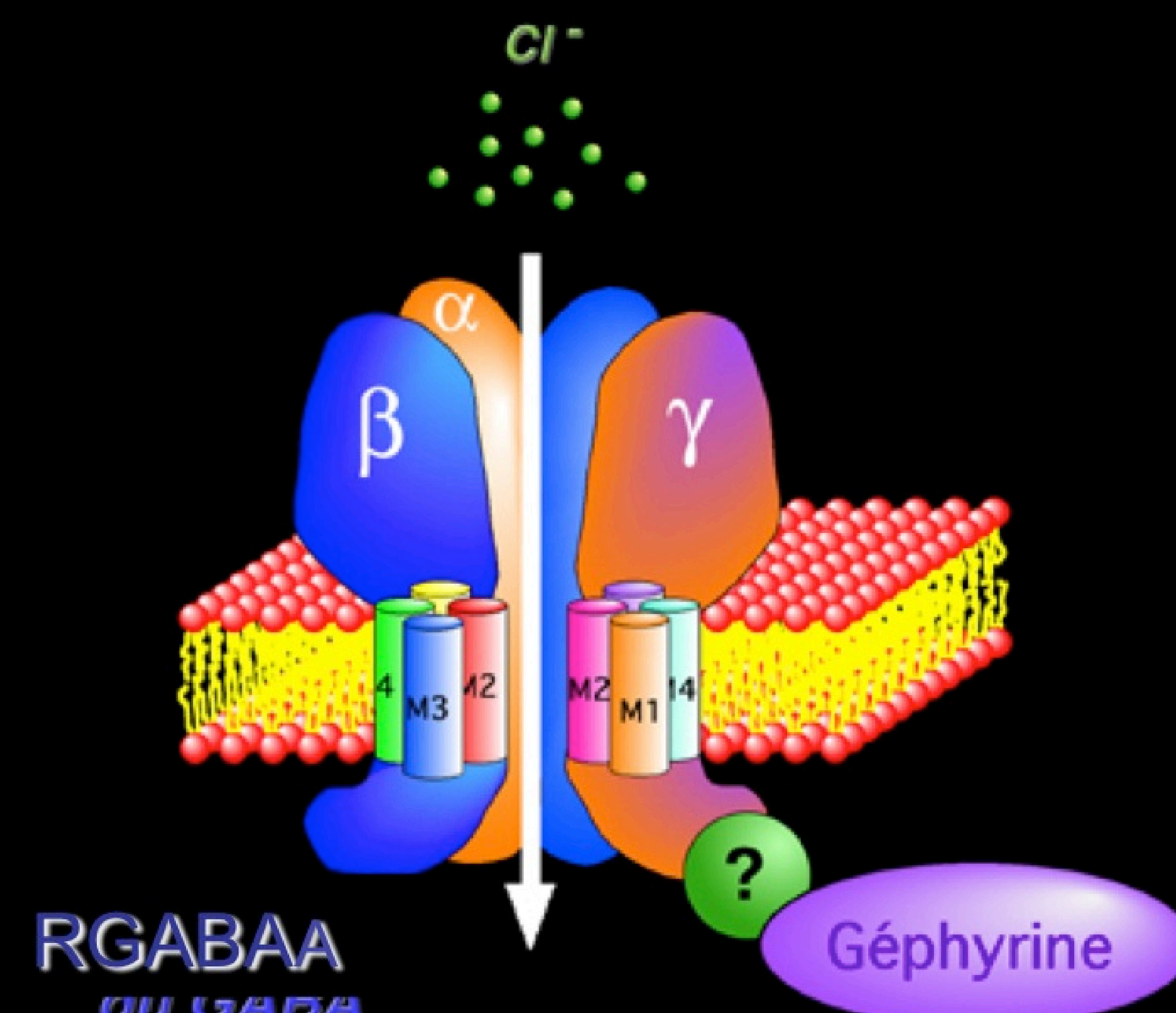
Hypothesis B:

Excitatory synapses are precocious.

Récepteurs GABAA





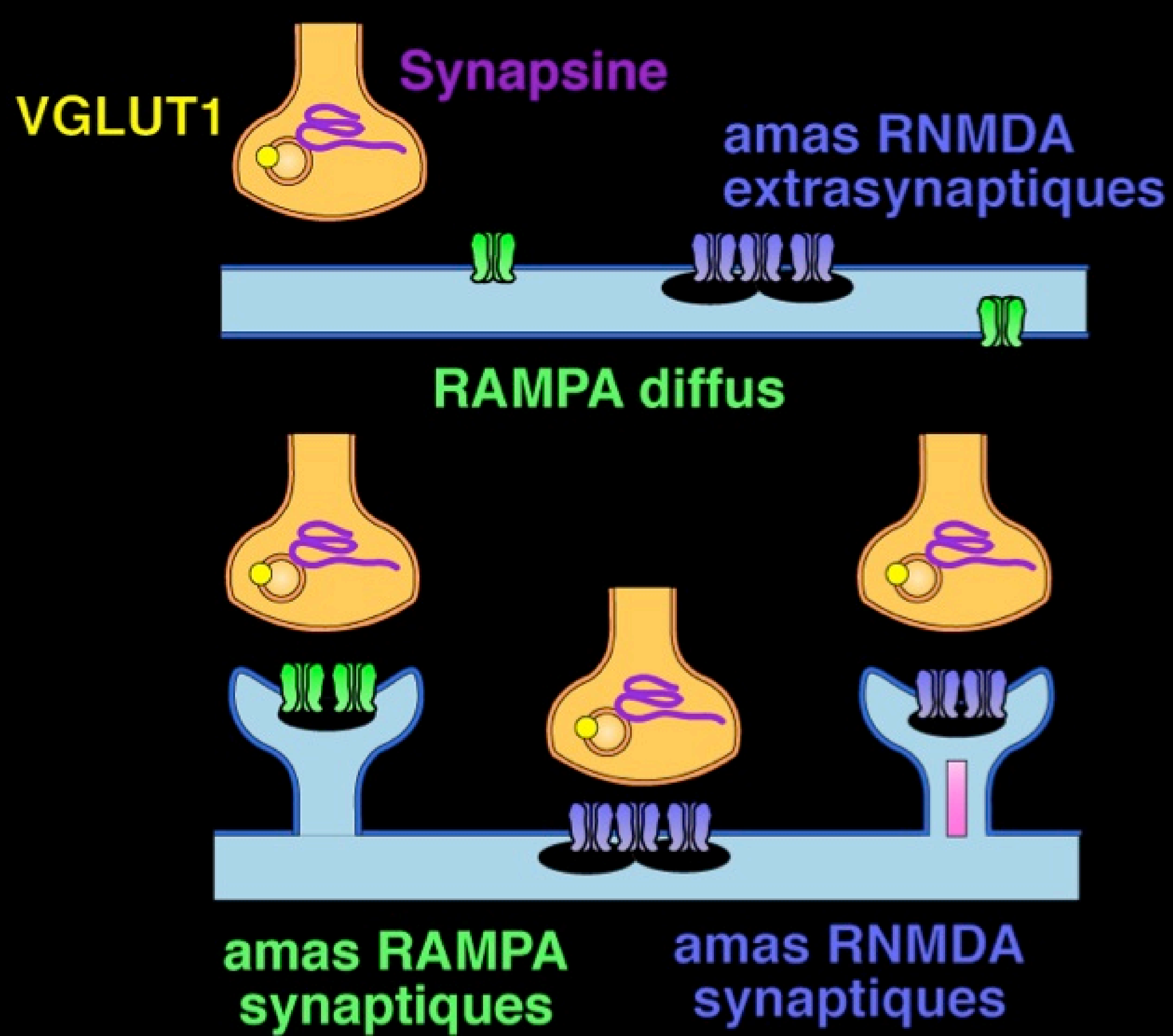


Ciblage des protéines post-synaptiques

Synapse excitatrice hippocampe

Laboratoire A.M. Craig

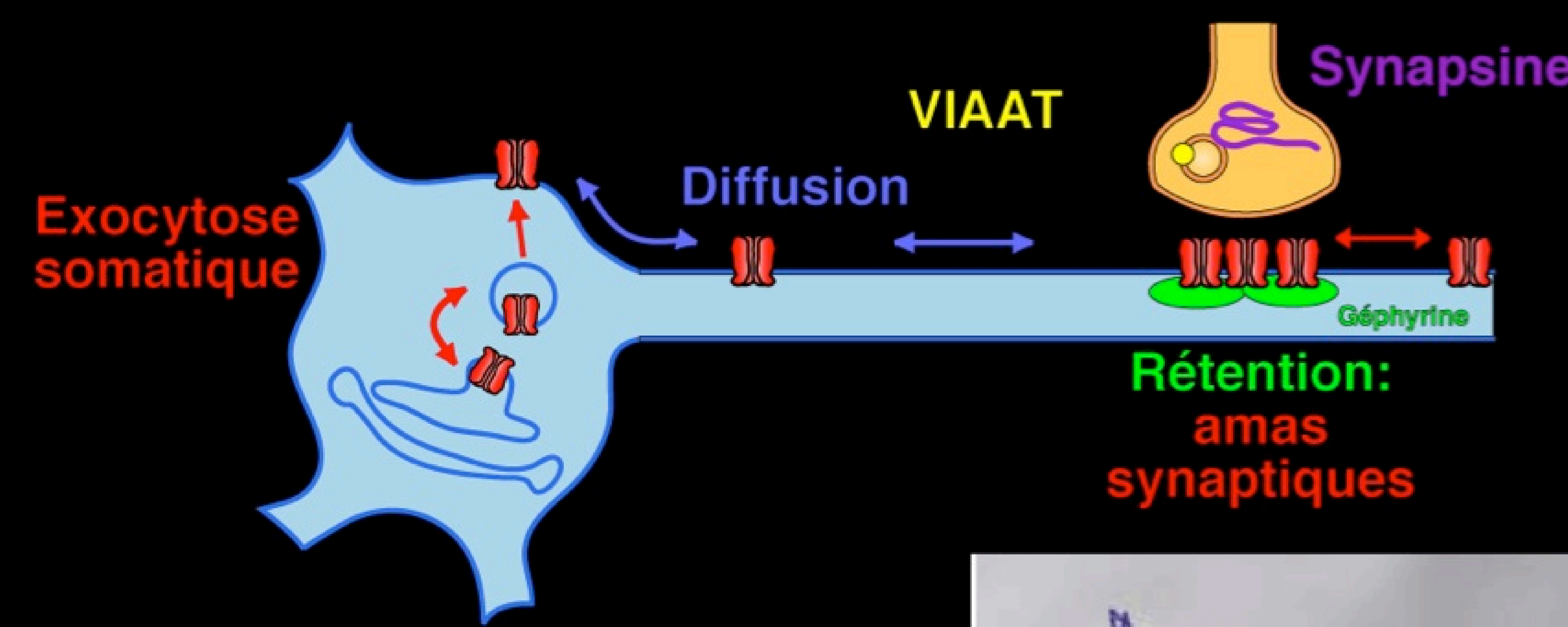
Rao et al., J. Neurosci., 2000
Rao et al., J. Neurosci., 2000

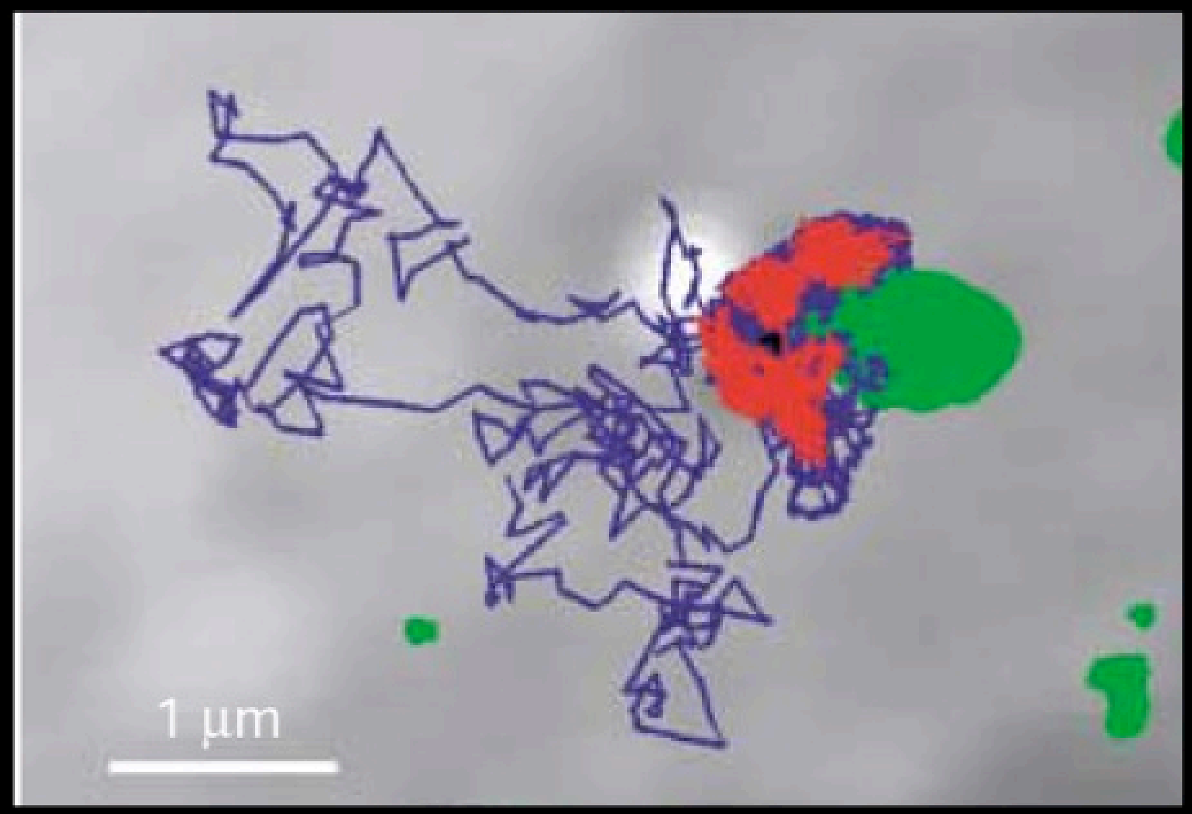


Synapse inhibitrice moelle épinière

Laboratoire A.Triller

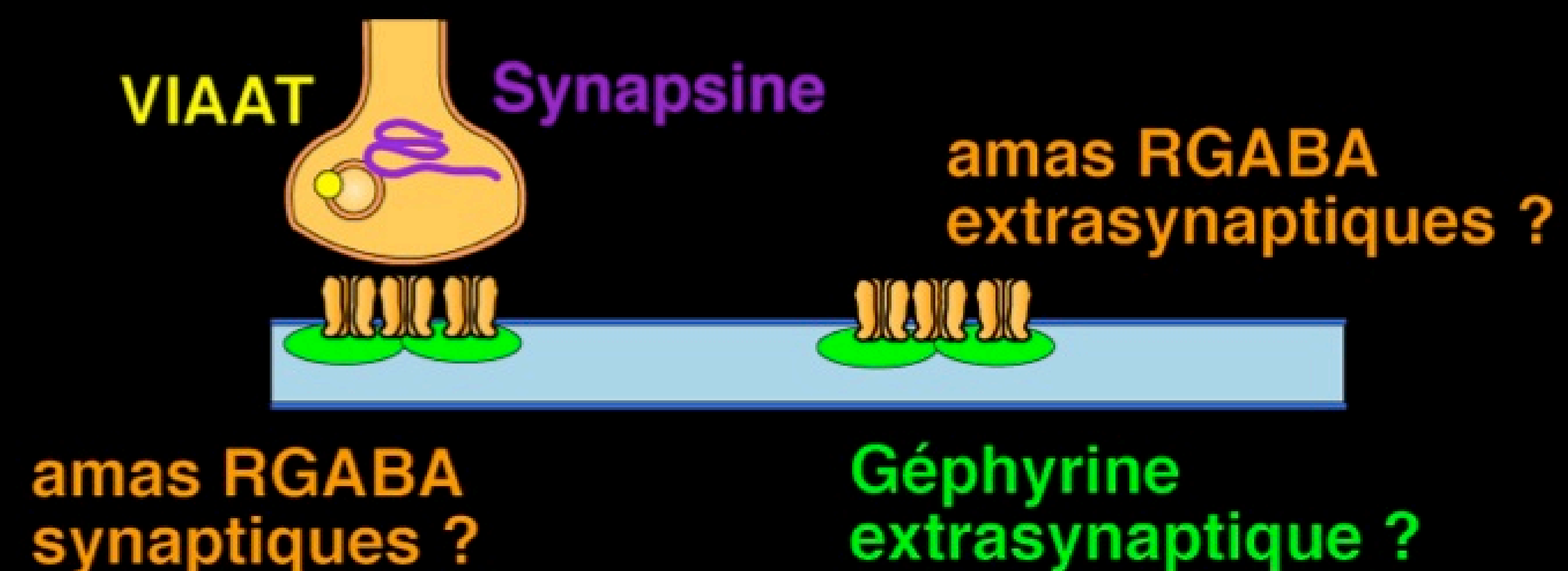
Rosenberg et al., J. Neurosci, 2000
Meier et al, JCS, 2000
Meier et al., Nat. Neur., 2001



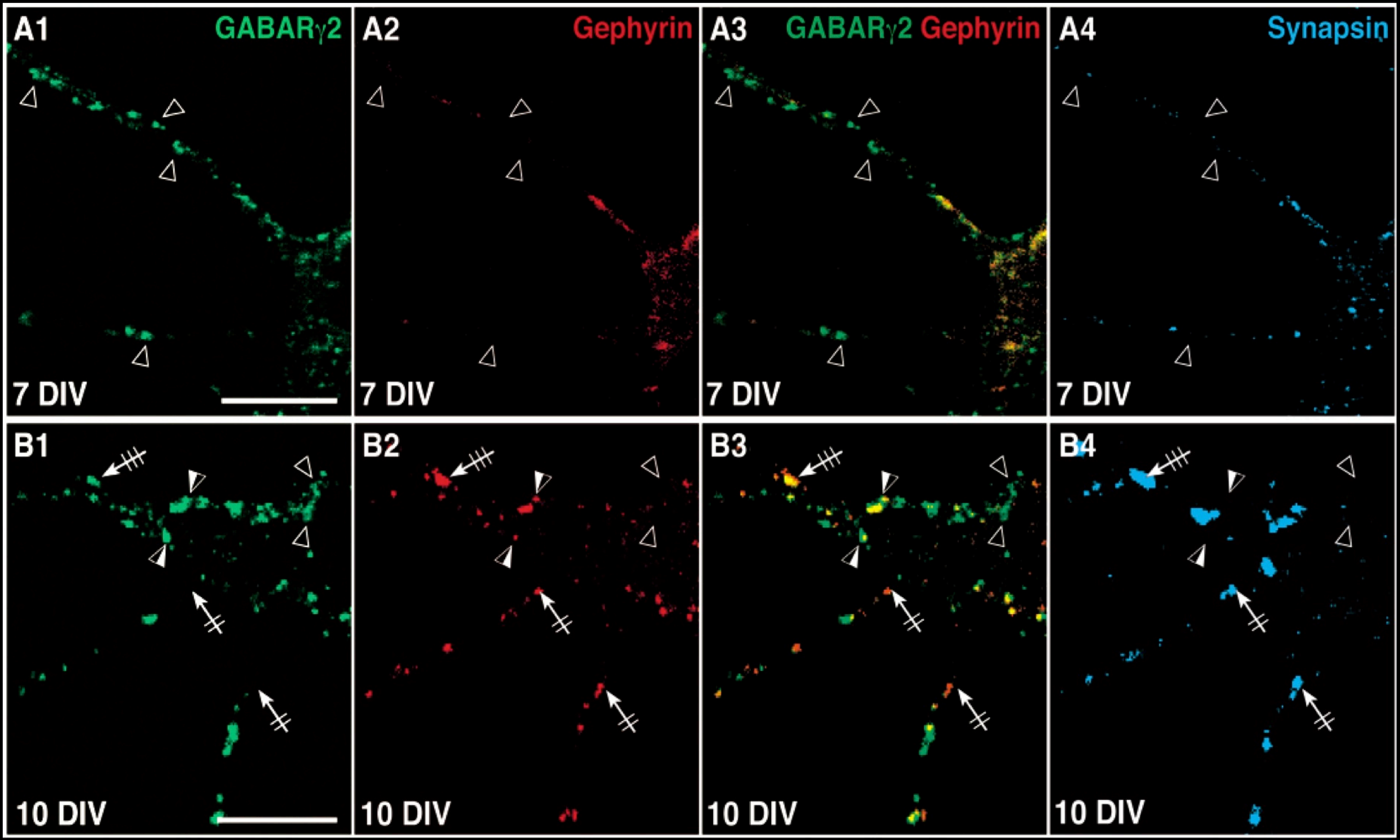


Synapse inhibitrice hippocampe

?

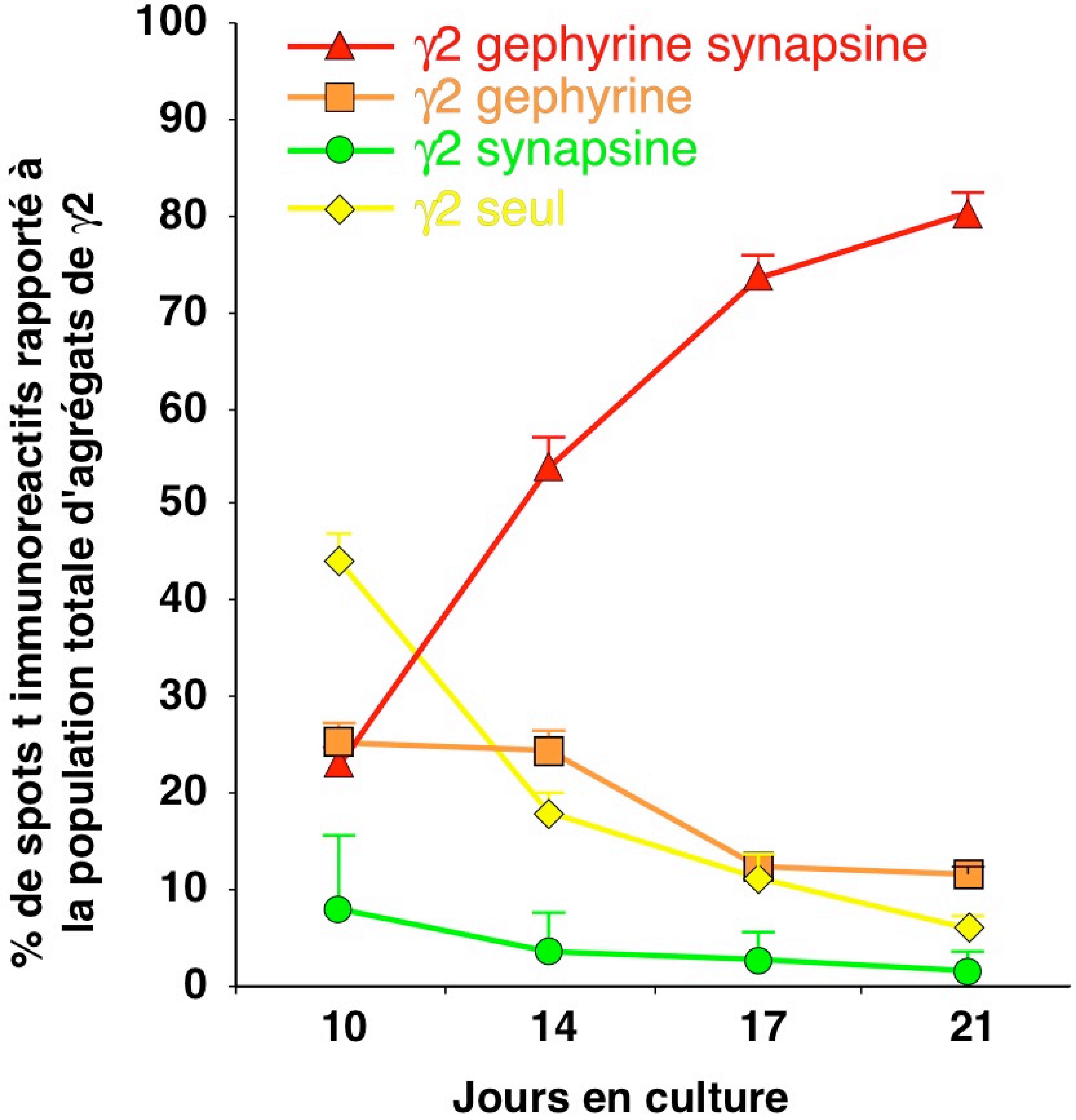


Colocalisation du GABAAR γ2 avec la géphyrine ?



GABAAR γ2 is first extrasynaptic, but the proportion of synaptic GABAA Rγ2 - gephyrin complexes increases during maturation.

Danglot et al. Mol Cell Neurosci 2003



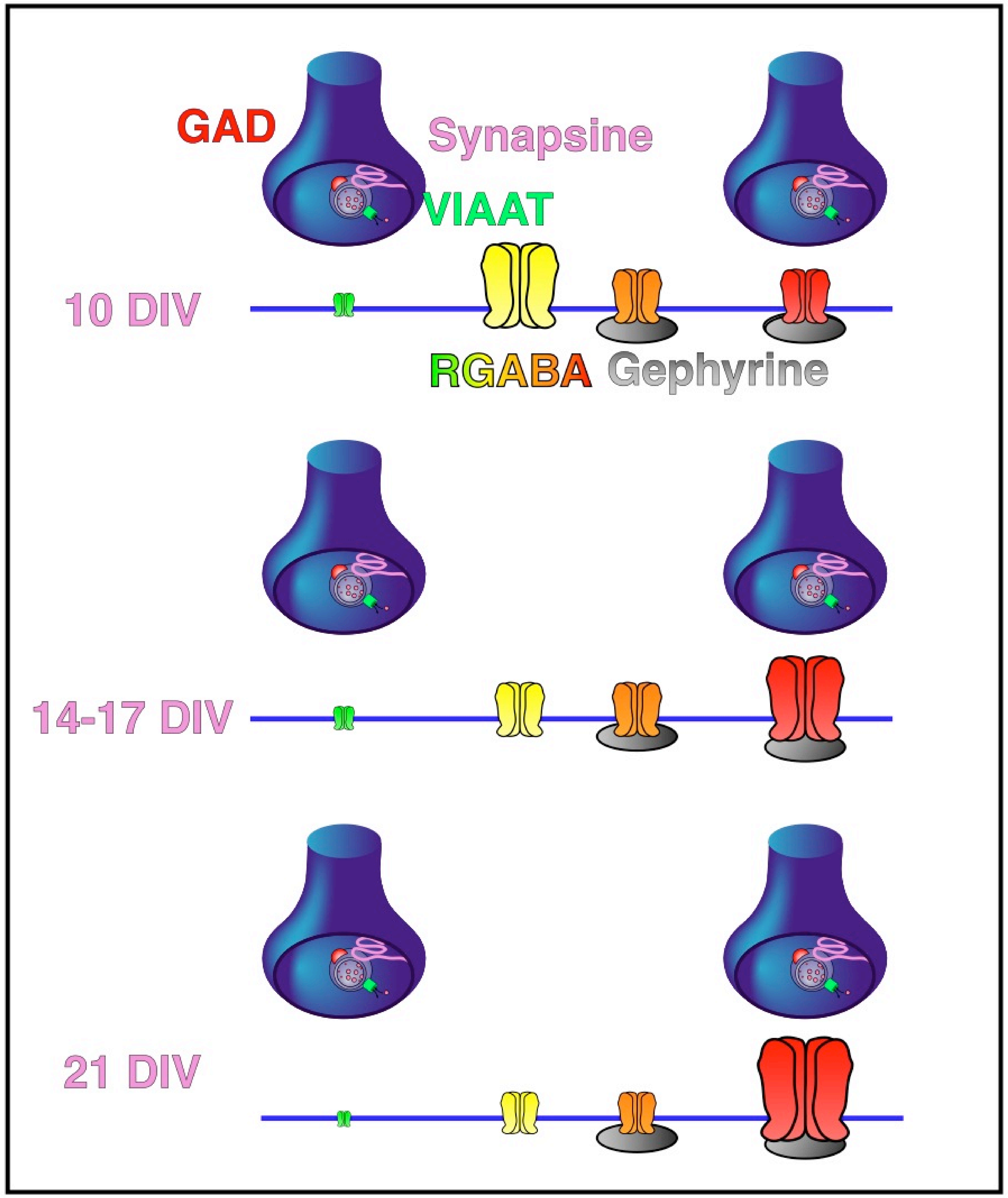
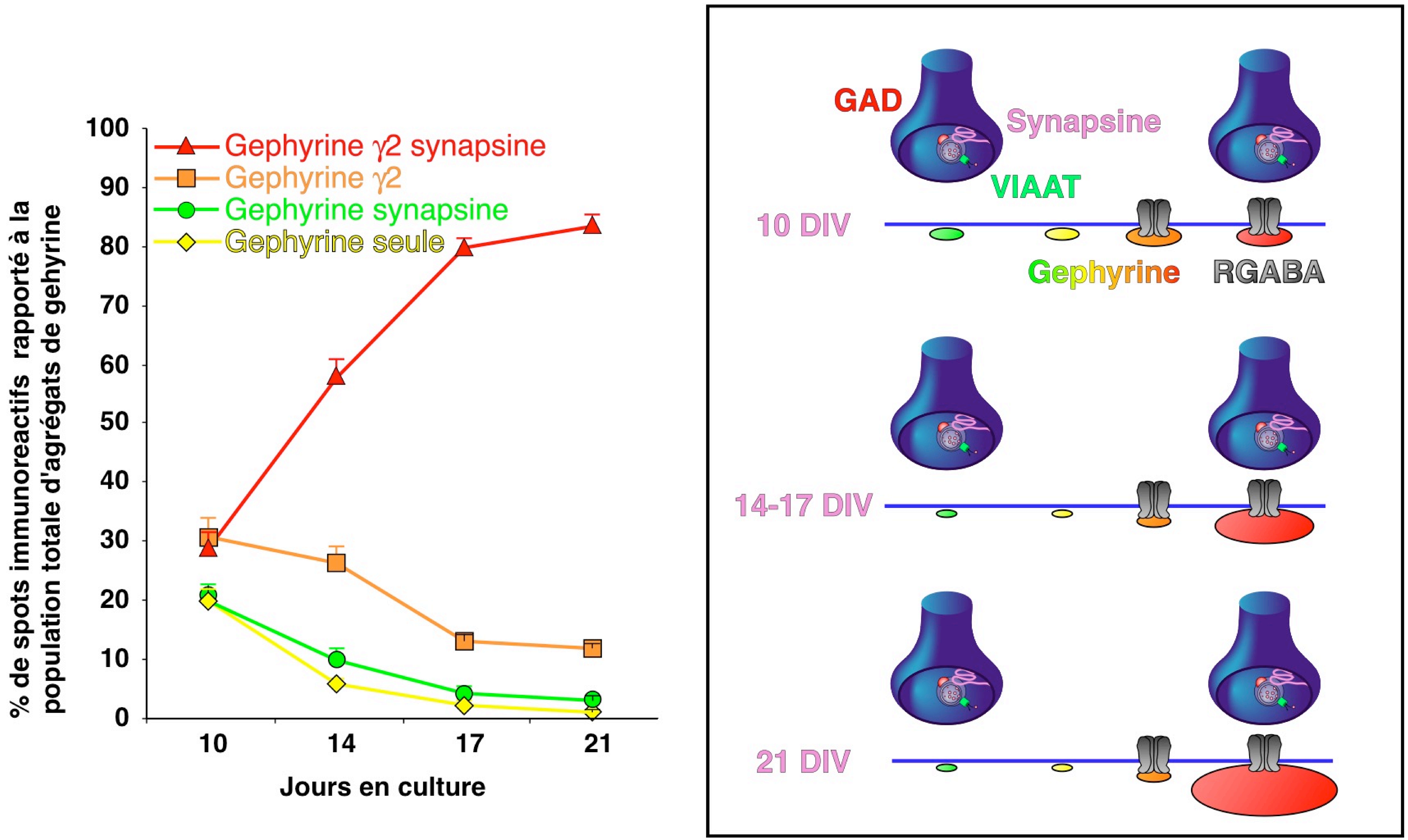


Figure 57 | **Mise en place des synapses GABAergiques dans les neurones d'hippocampe en culture.**
Les schémas ont été établis à partir des quantifications réalisées dans le premier article. La taille des symboles est proportionnelle à la fréquence des éléments étudiés.
A | Evaluation de la proportion de RGABAA gamma2 associés ou non à la géphyrine en position synaptique ou extrasynaptique.
B | Evaluation de la proportion de géphyrine associée ou non au RGABA en position synaptique ou extrasynaptique.
Au stade précoce (10 DIV), le RGABAgamma2 est majoritairement extrasynaptique, tandis que la géphyrine est également répartie entre les sites synaptiques et extrasynaptiques. Au cous de la maturation, la proportion de récepteurs colocalisant avec la géphyrine augmente en parallèle avec le nombre de RGABAgamma2 devenant synaptiques.



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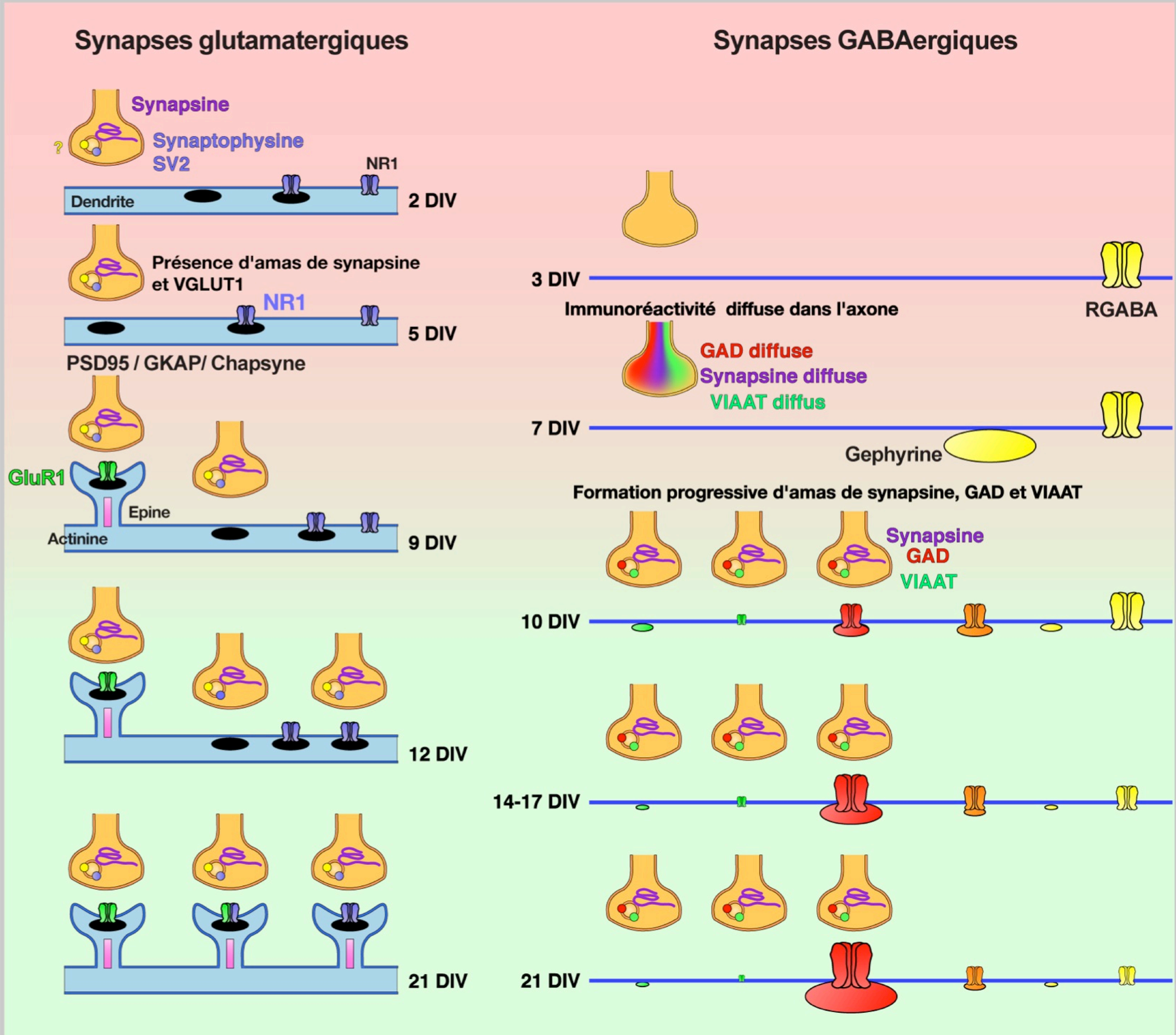

Synapses glutamatergiques
Synapses GABAergiques
Synapsine
?
Synaptophysine
SV2
NR1
Dendrite
2 DIV
Présence d'amas de synapsine
et VGLUT1
NR1
5 DIV
PSD95 / GKAP/ Chapsyne
GluR1
Epine
Actinine
9 DIV
12 DIV
21 DIV
3 DIV
RGABA
Immunoréactivité diffuse dans l'axone
GAD diffuse
Synapsine diffuse
VIAAT diffus
7 DIV
Gephyrine
Formation progressive d'amas de synapsine, GAD et VIAAT
Synapsine
GAD
VIAAT
10 DIV
14-17 DIV
21 DIV

NATURE REVIEWS I NEUROSCIENCE

CELLULAR AND MOLECULAR MECHANISMS OF PRESYNAPTIC ASSEMBLY

Noam E. Ziv* and Craig C. Garner‡

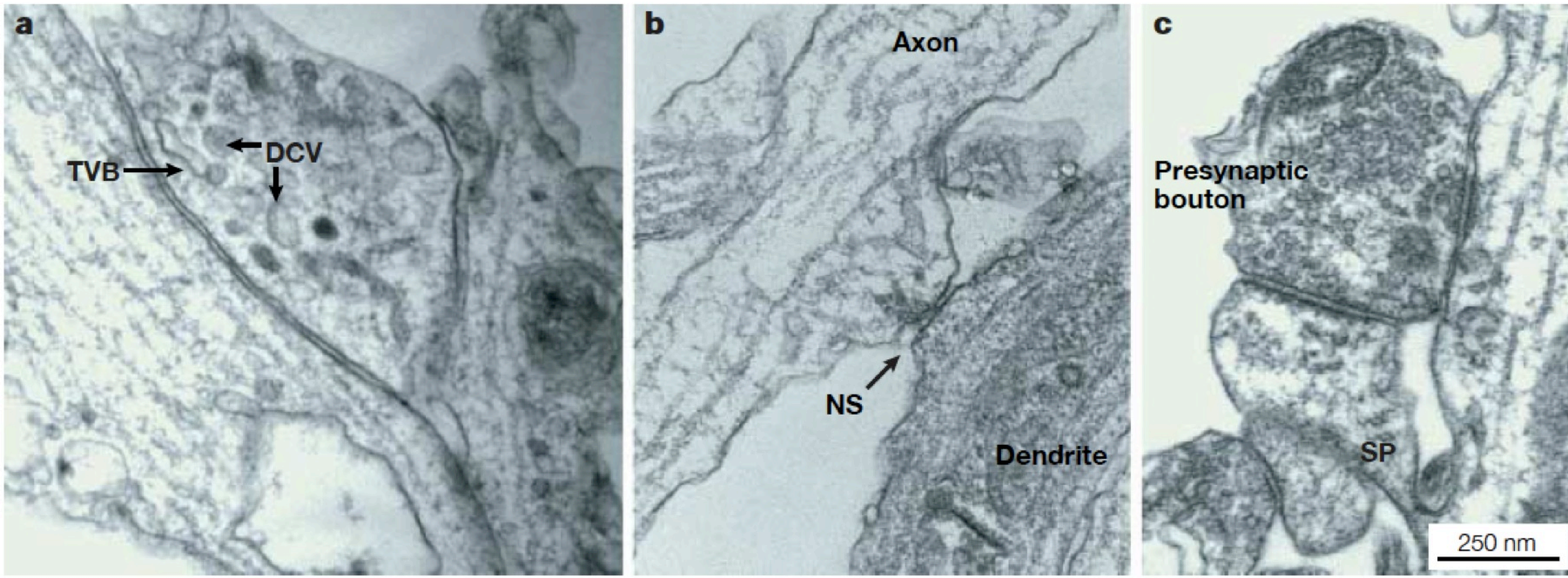
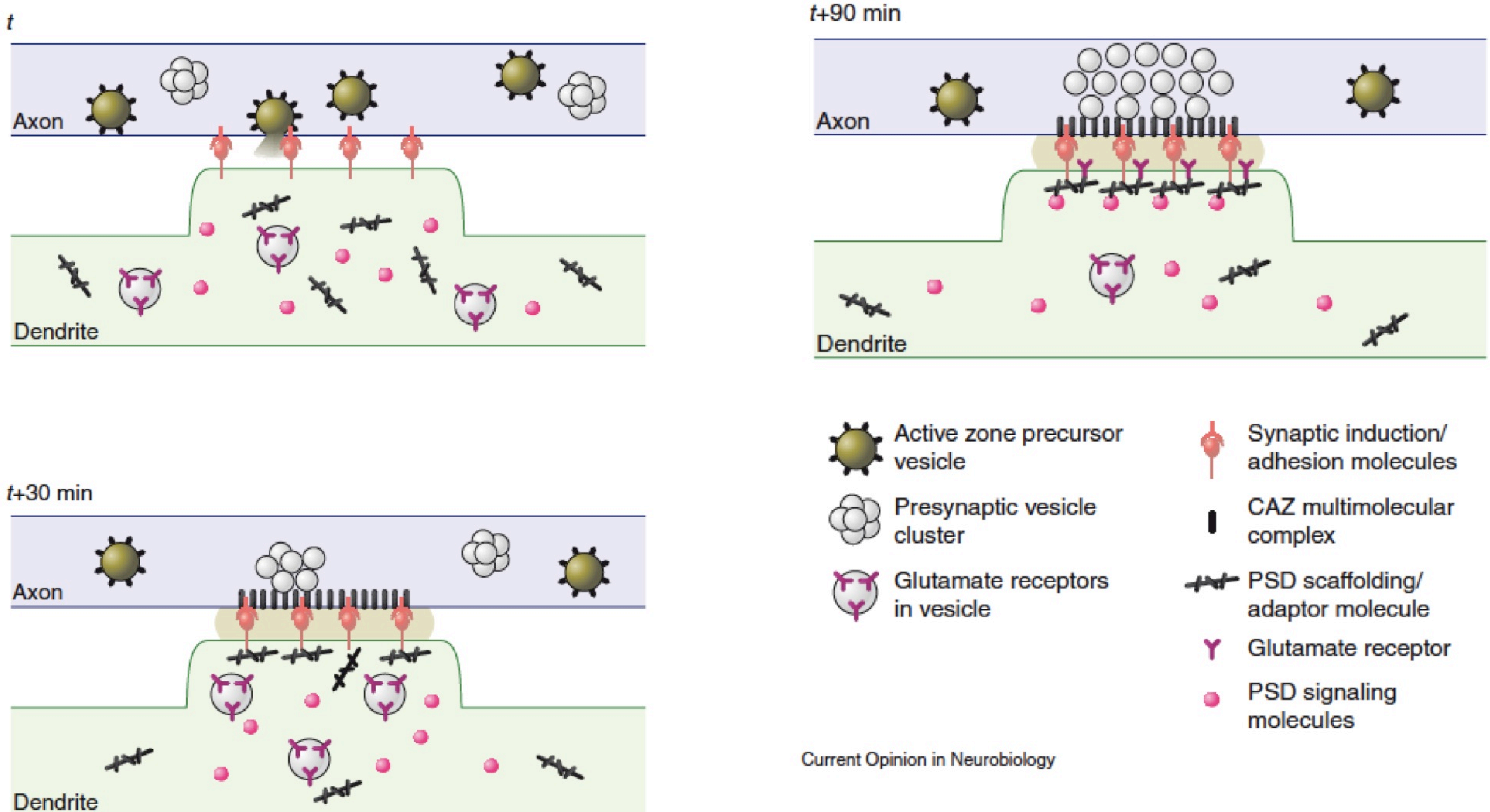


Figure 1 | **Ultrastructure of synapses at various stages of development.** Electron micrographs of an axonal varicosity (**a**), as well as a nascent synapse (**b**) and mature spiny synapse (**c**) formed between hippocampal neurons grown for 8 days (**a**, **b**) and 15 days (**c**) in culture. **a** | One characteristic of structures that seem to be involved in very early presynaptic formations is the aggregation of pleomorphic, tubulovesicular (TVB) and dense-core vesicles (DCV) in axonal varicosities. Certain DCVs have been shown to carry many structural proteins of the active zone, and they might represent active zone precursor vesicles that facilitate the rapid establishment of a functional neurotransmitter release site. However, as these formations lack obvious presynaptic ultrastructural features, there is some uncertainty as to their true identity. **b** | This state is thought to be followed by the appearance of flat parallel membranes, electron-dense membrane thickenings and a few synaptic vesicles at nascent synapses (NS). **c** | At more mature synapses, prominent active zones and postsynaptic densities are observed, with numerous synaptic vesicles clustered within the presynaptic bouton. As shown here, dendritic spines (SP) also appear at excitatory synapses. Micrographs were taken and kindly provided by JoAnn Buchanan, Stanford University.

Current Opinion in Neurobiology 2001, 11:536–543

Principles of glutamatergic synapse formation: seeing the forest for the trees

Noam E Ziv* and Craig C Garner†



Assembly of New Individual Excitatory Synapses: Time Course and Temporal Order of Synaptic Molecule Recruitment

Hagit Vardinon Friedman[1], Tal Bresler[1], Craig C Garner[2], Noam E Ziv

Figure 6. **Formation of a Presynaptic Bouton following New Axodendritic Contact**

(A) A hippocampal neuron expressing EGFP.

(B) Time-lapse sequence showing an axonal growth cone coming out of the top right corner, encountering the dendritic segment seen at the center of the image (t = 33′, yellow arrowhead) and crossing over to the left. This is an enlargement of the region enclosed in a yellow rectangle in (A). First images collected after a labeling episode are marked with asterisks.

(C) Recurrent FM 4-64 labeling revealed that a new bouton had appeared at the intersection site of the dendrite and the ingrowing axon (closed arrowhead). Regions shown correspond to respective areas enclosed in yellow rectangles in (B). Scale bar, 2 μm.

NATURE REVIEWS I NEUROSCIENCE

CELLULAR AND MOLECULAR MECHANISMS OF PRESYNAPTIC ASSEMBLY

Noam E. Ziv* and Craig C. Garner‡

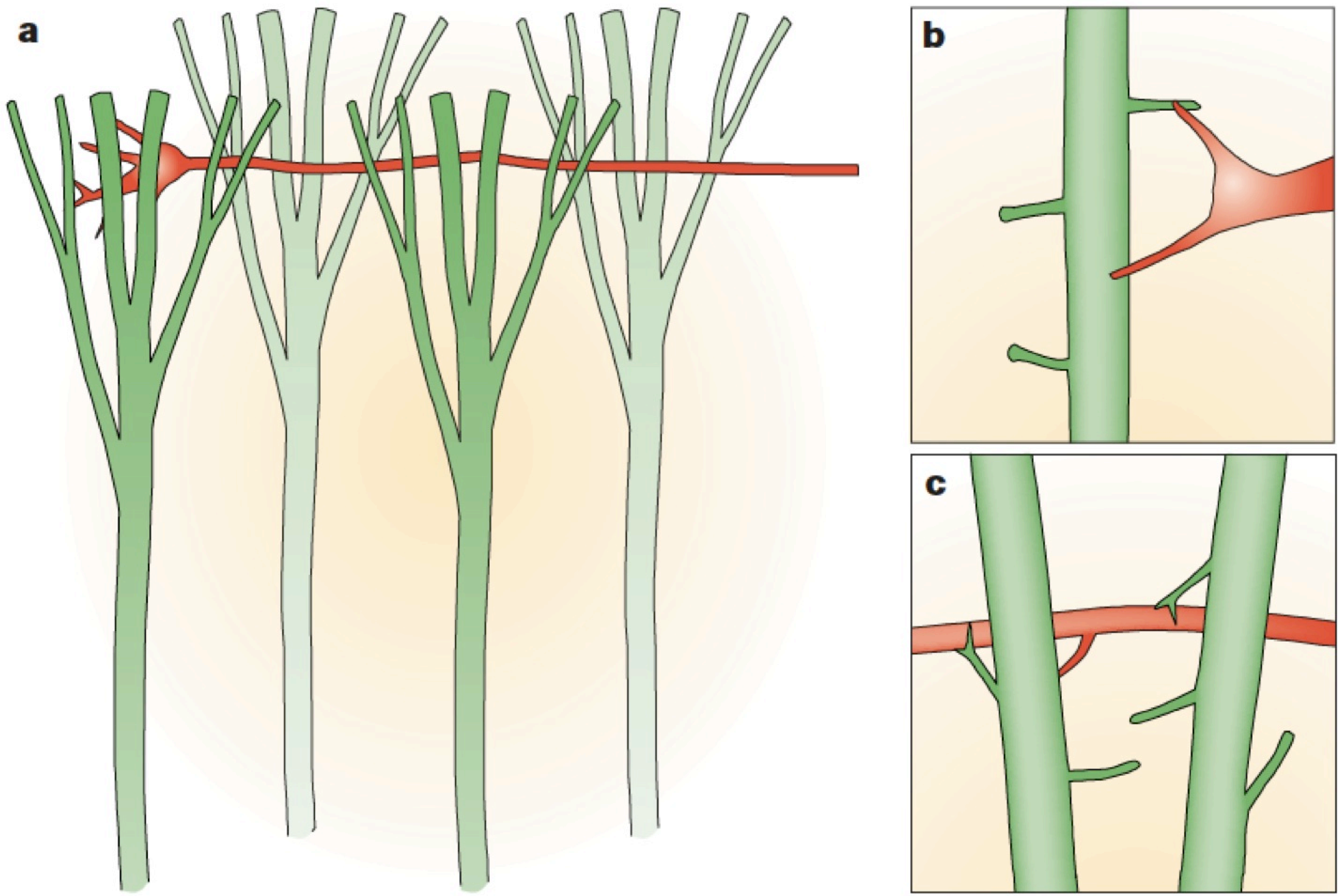
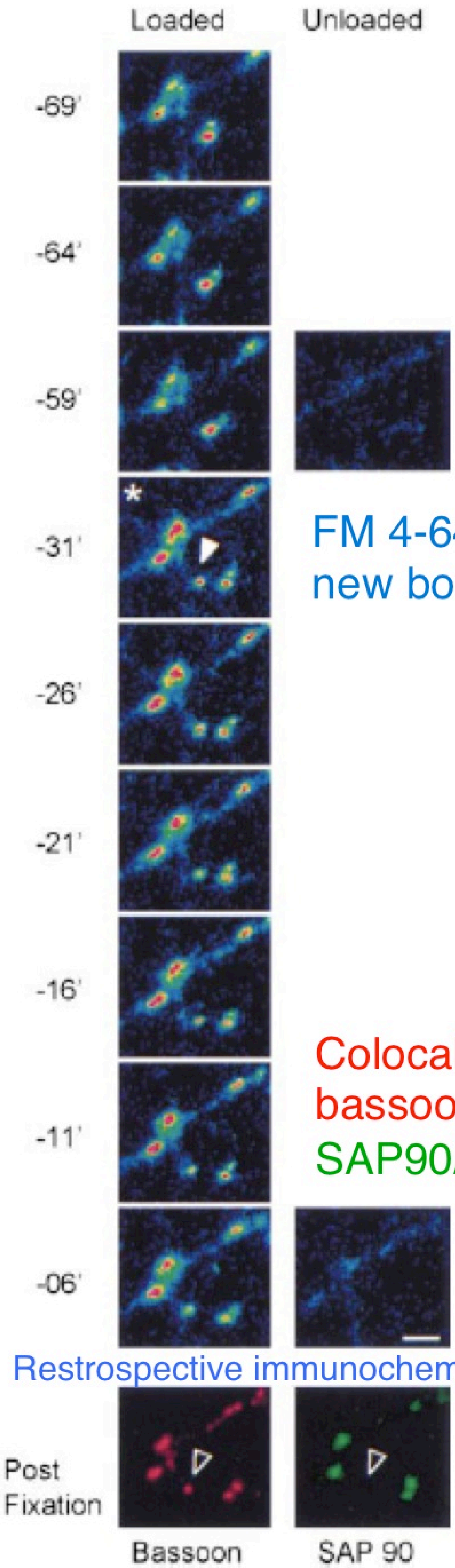


Figure 2 | **Formation of axodendritic contacts. a** | As elongating axons tipped by motile growth cones extend through the developing neuropil, presynaptic differentiation is induced at sites of contact with adjacent dendrites. **b**,**c** | Contacts with dendrites might occur at the leading edge of the elongating axon (**b**), or later on along well-formed axonal segments (**c**), by dendritic protrusive structures such as lateral filopodia and dendritic growth cones, and by lateral axonal filopodia.

Assembly of New Individual Excitatory Synapses: Time Course and Temporal Order of Synaptic Molecule Recruitment

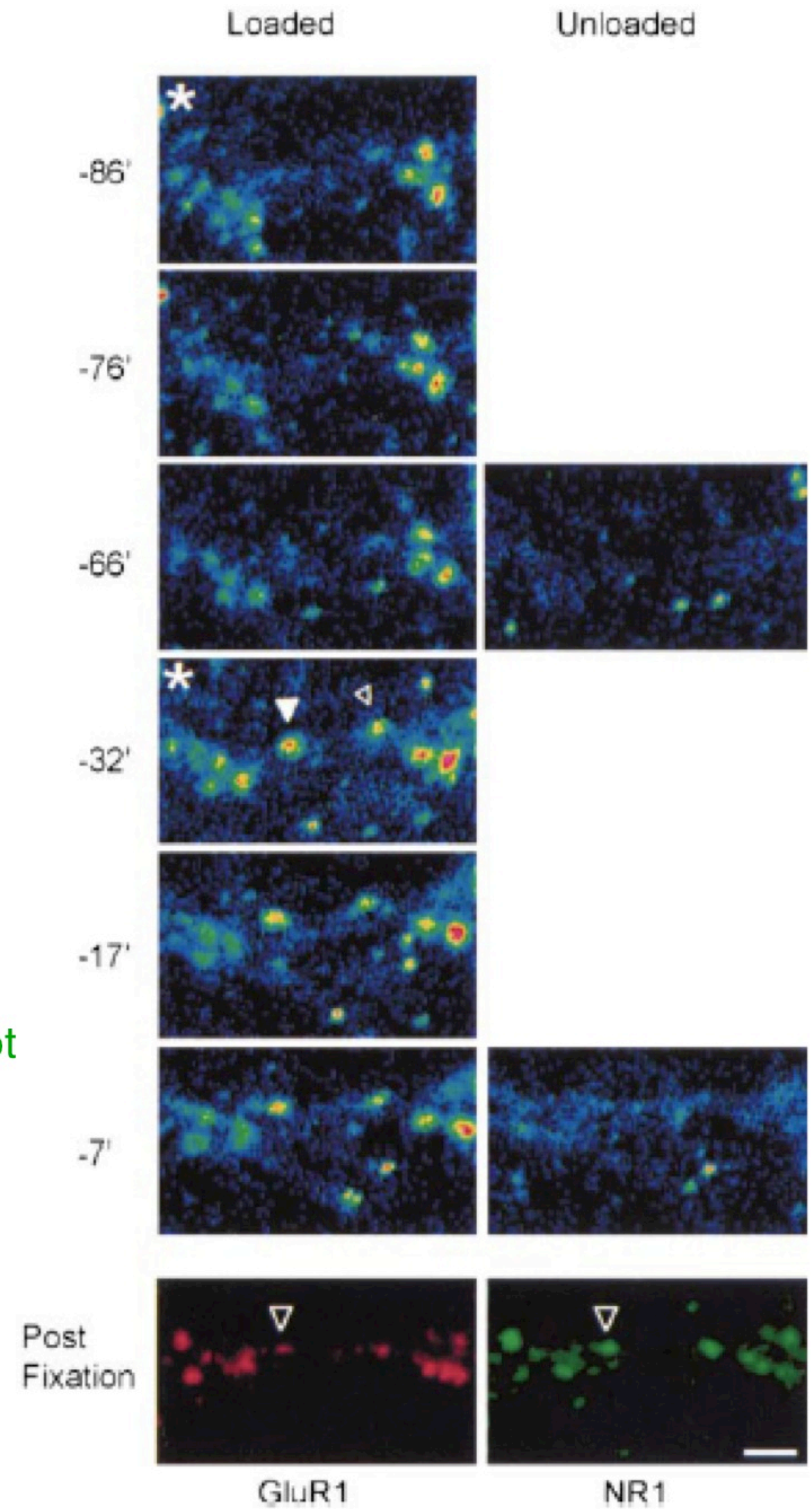
Hagit Vardinon Friedman[1], Tal Bresler[1], Craig C Garner[2], Noam E Ziv



FM 4-64: new bouton

Colocalize with bassoon but not SAP90/PSD95

Restrospective immunochemistry



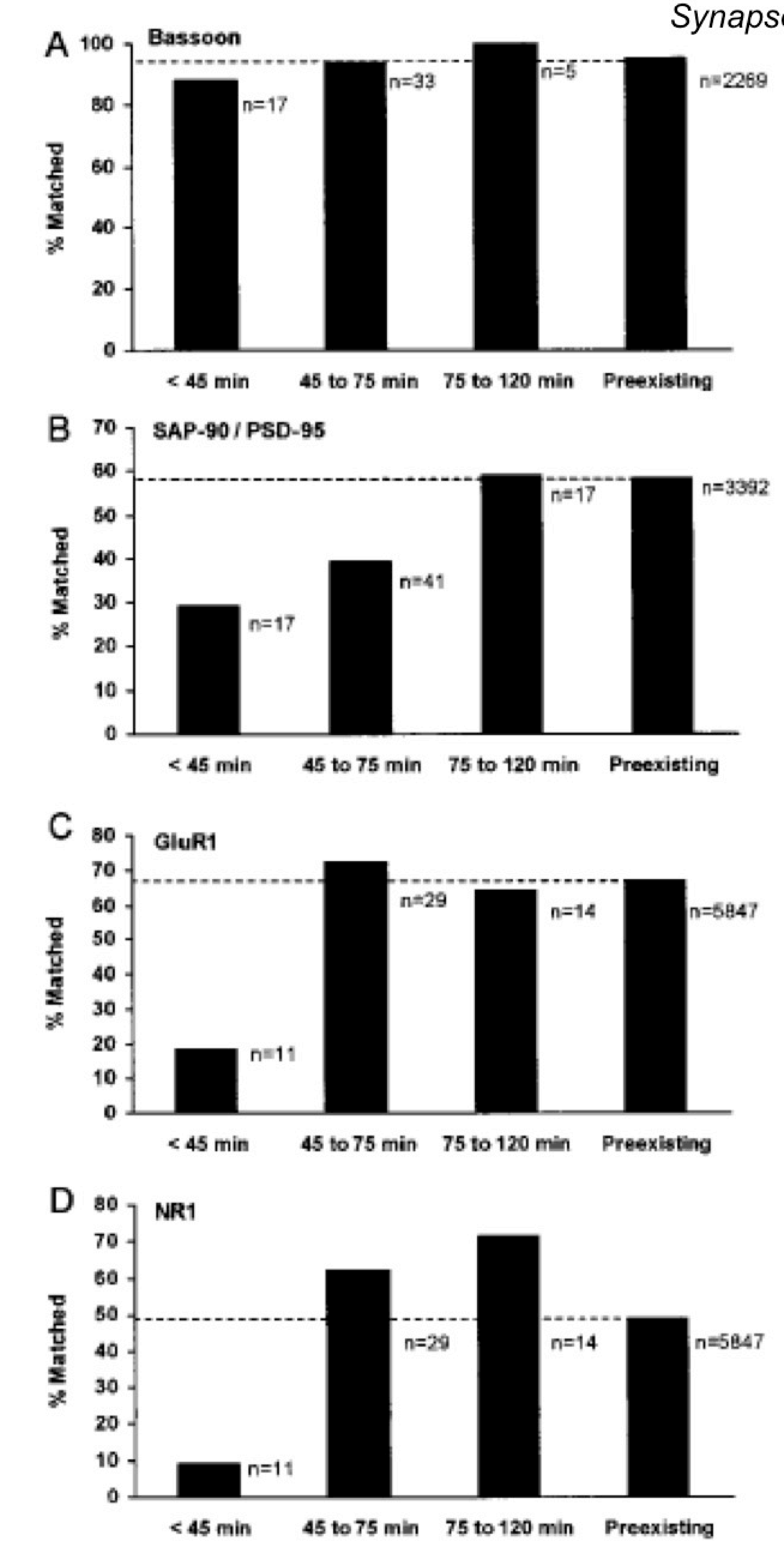


Figure 4. Accumulation Kinetics of Four Synaptic Proteins at Nascent Synapses

Assembly of New Individual Excitatory Synapses: Time Course and Temporal Order of Synaptic Molecule Recruitment

Hagit Vardinon Friedman[1], Tal Bresler[1], Craig C Garner[2], Noam E Ziv[1, ‡]

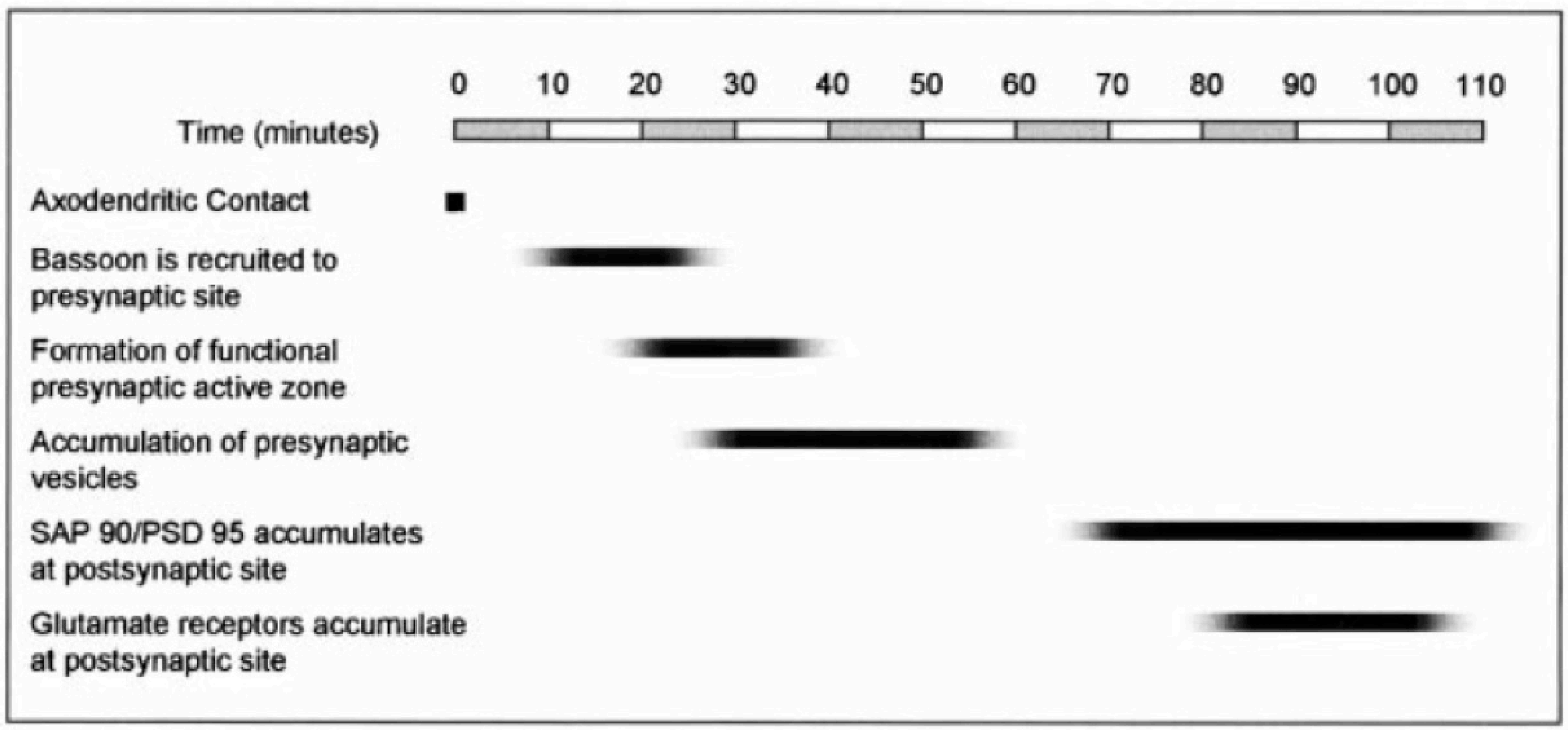


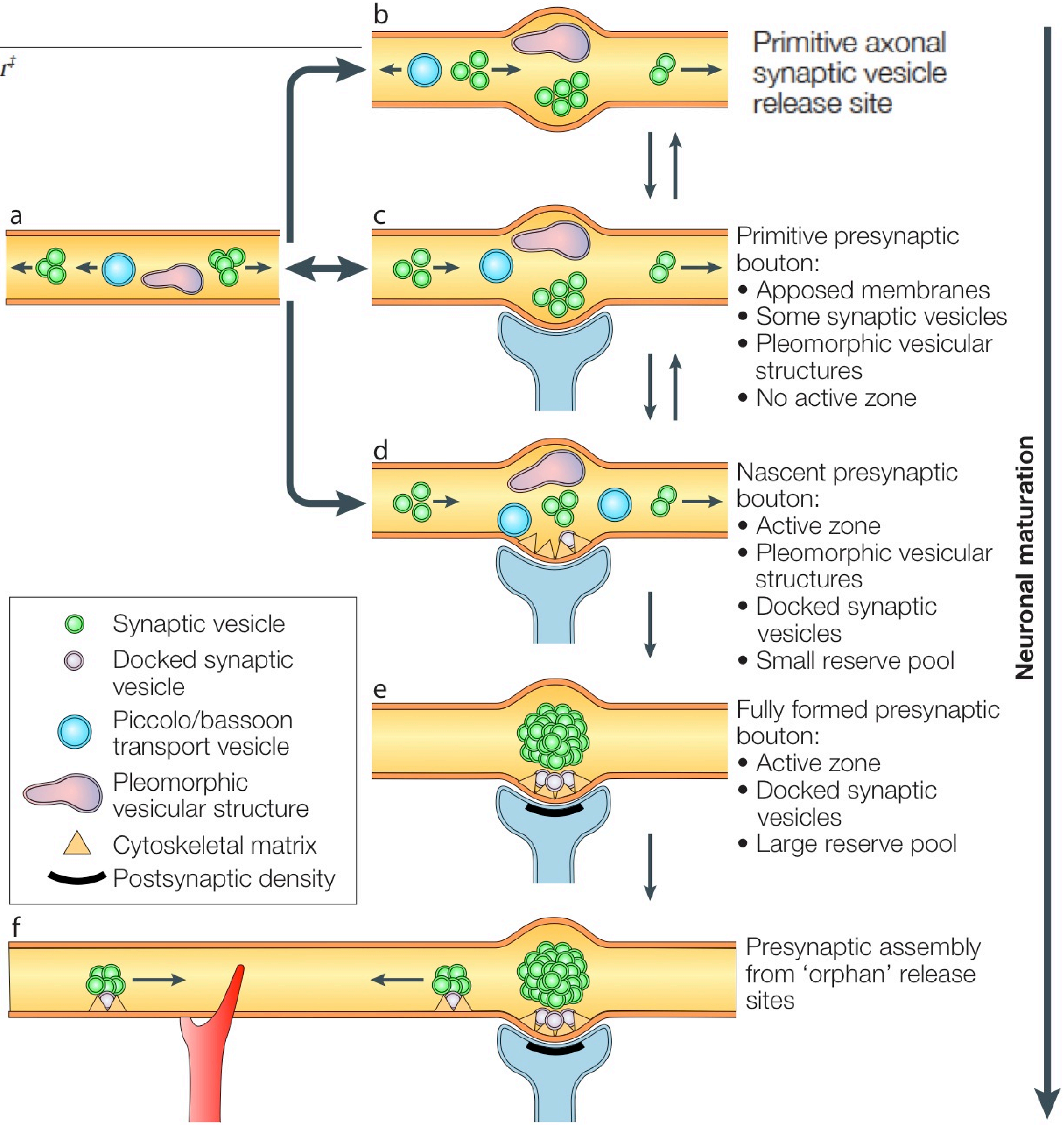
Figure 7. Proposed Time line of Glutamatergic Synapse Assembly

The putative timing of key events during the assembly of individual glutamatergic synapses as deduced from the findings described in this study. Note that this time line probably represents typical time frames for these events, but the actual timing may vary significantly between individual synapses, as suggested by the data in Figure 4 and discussed in the text.

CELLULAR AND MOLECULAR MECHANISMS OF PRESYNAPTIC ASSEMBLY

Noam E. Ziv* and Craig C. Garner‡

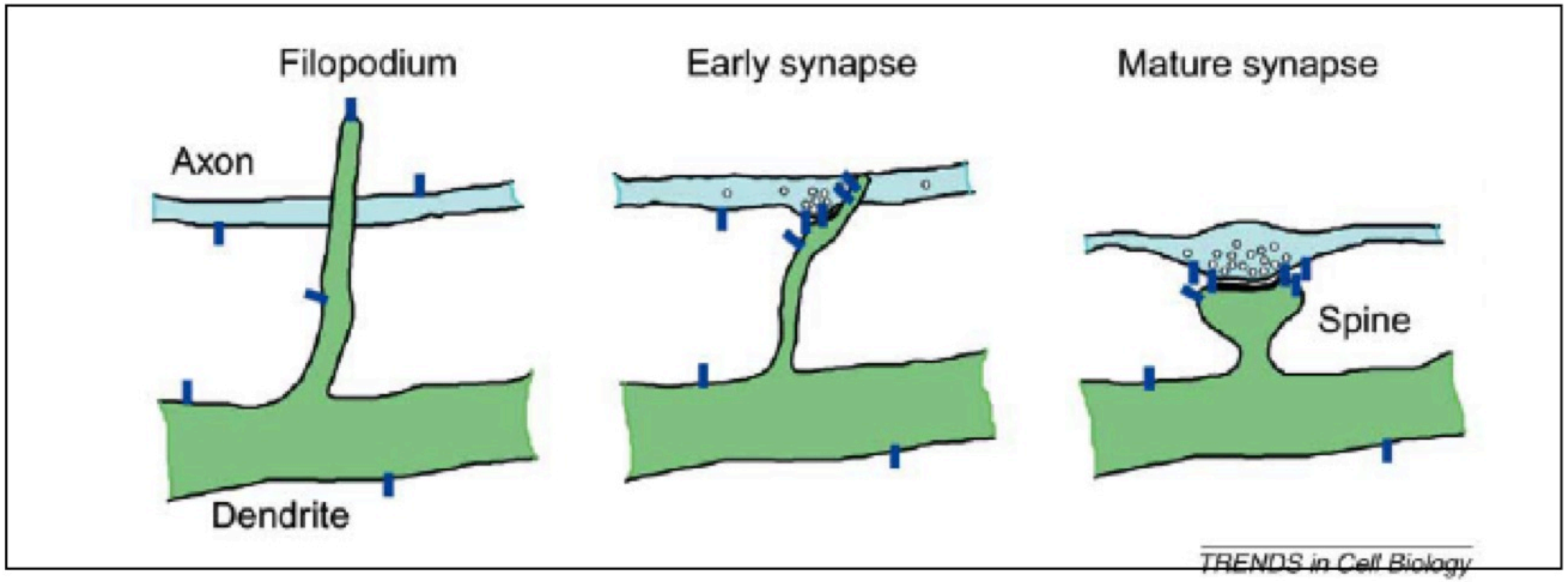
NATURE REVIEWS | NEUROSCIENCE



Synaptic spines are different from axonal varicosities

Actin-rich structures

Highly dynamic (shaping modification in msec to one hour)

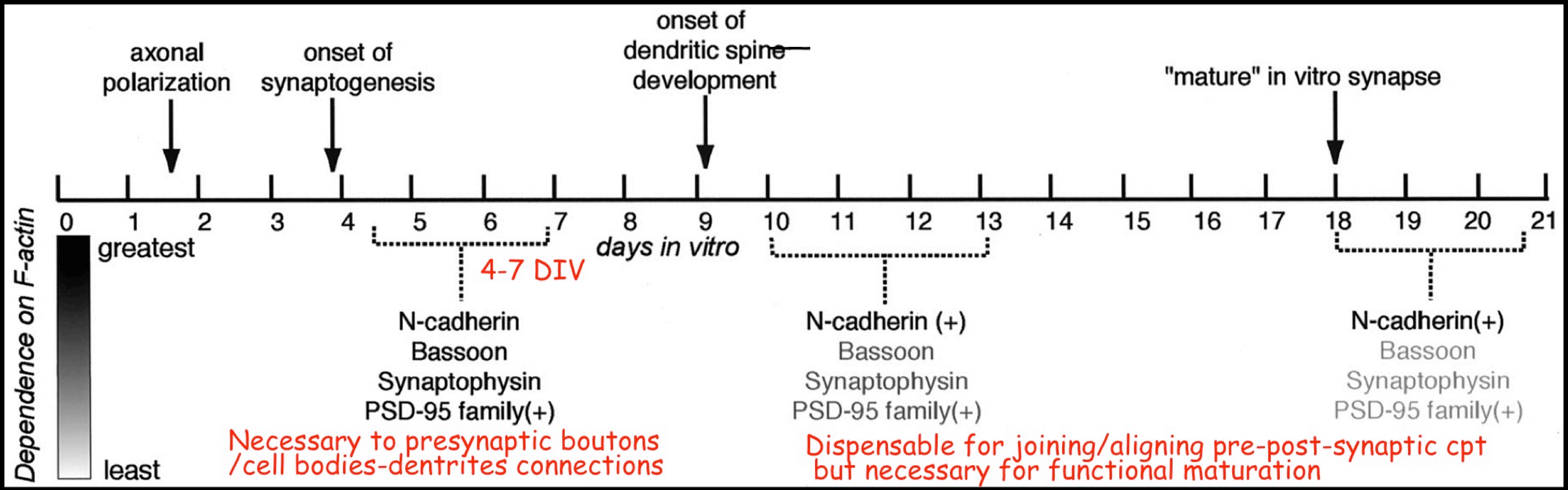


Migration
Filopodium

Contact and docking
= spine formation
cadherin-catenin
Dependent
(gain of function =
arborization)

Maturation
= mushroom
motility, strength, shaping
are cadherin-catenin
-dependent

Stages in the development of hippocampal neurons in culture (DIV = Days In Vitro)

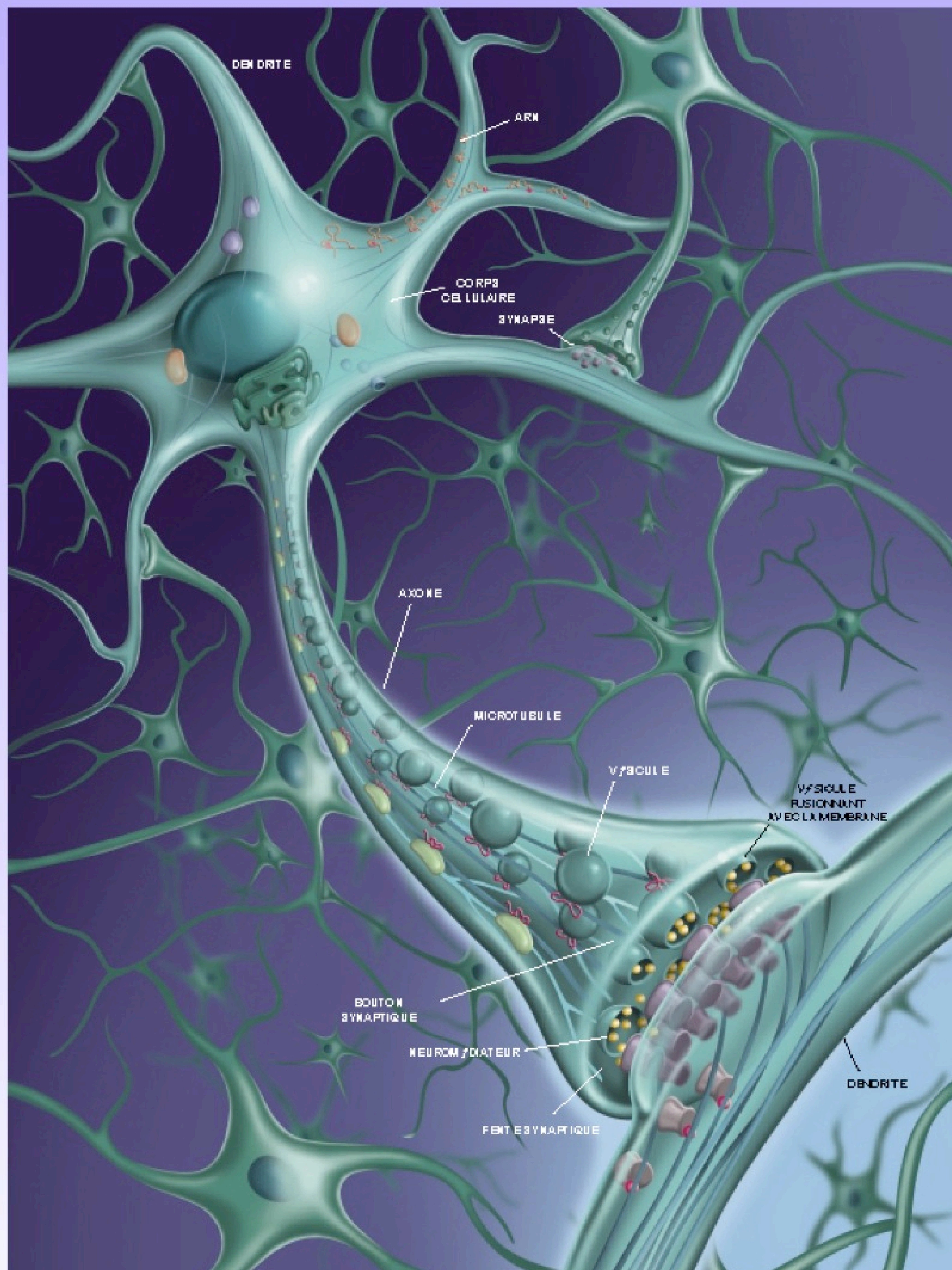


a role in directing pre- and postsynaptic specializations ?

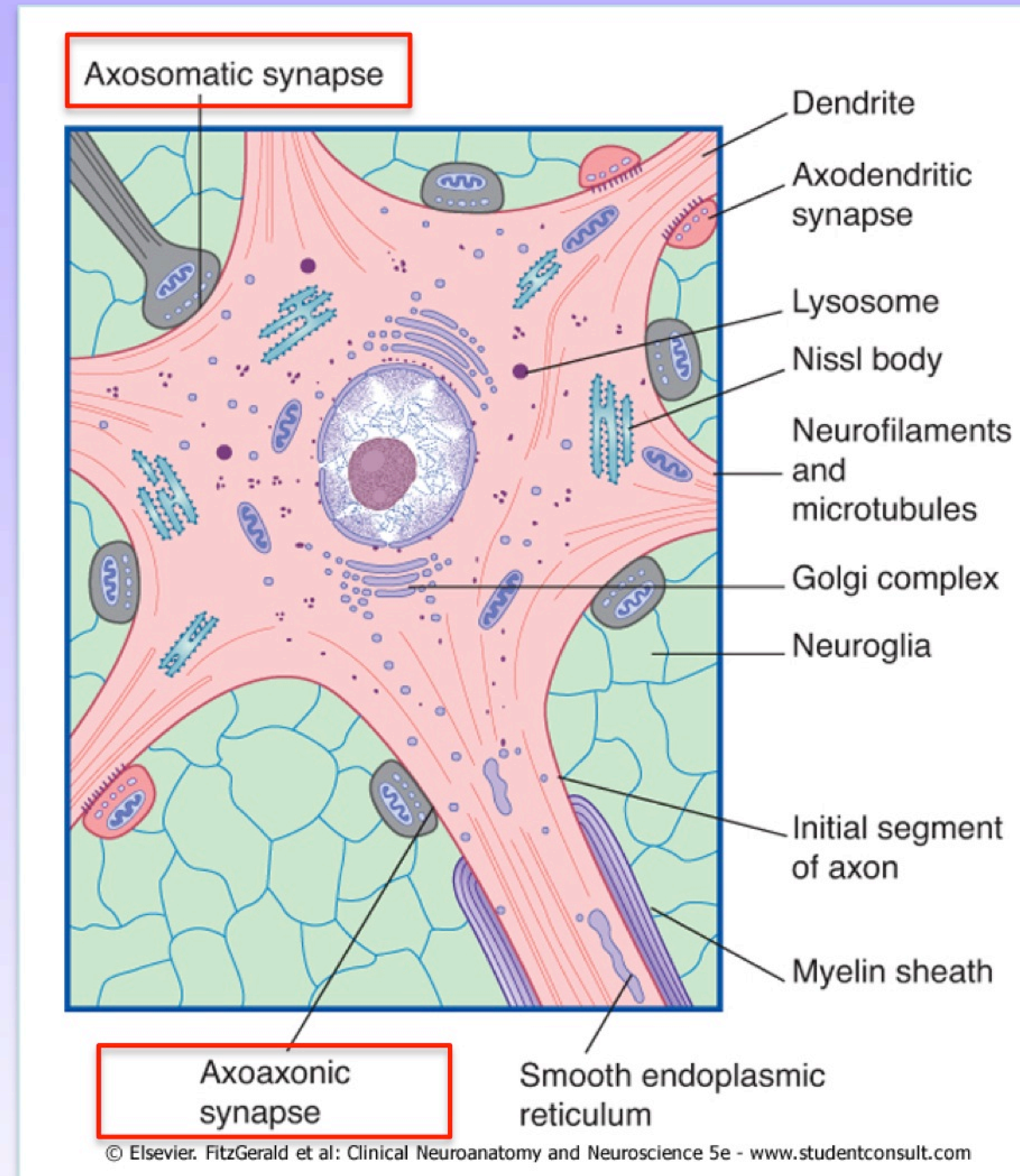
a role in dentritic spine morphology ?

J. Neurosci. 2001;21:6991

10. Structure des épines dendritiques



Thierry Galli & Fabienne Paumet, Pour la Science (2002)



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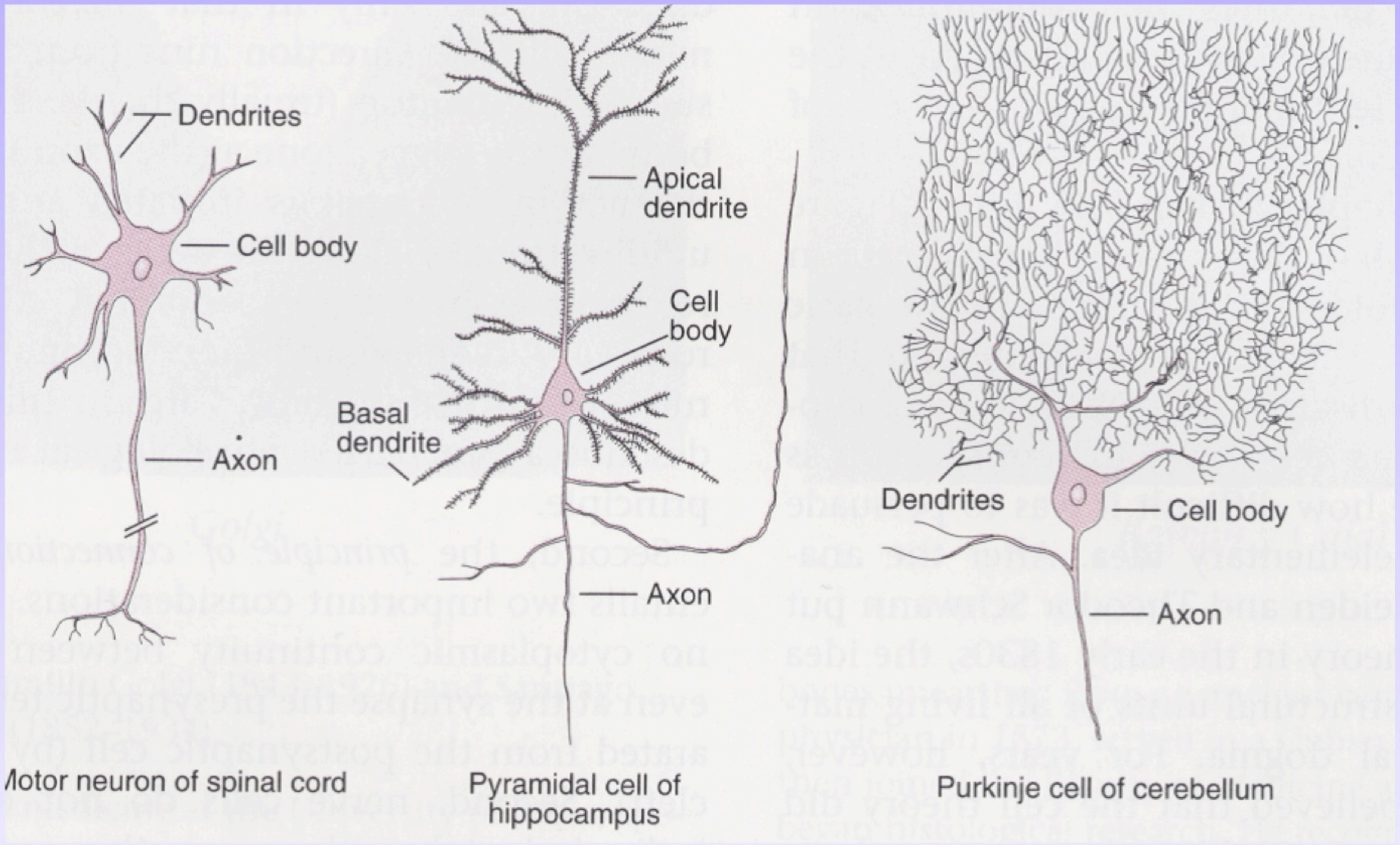
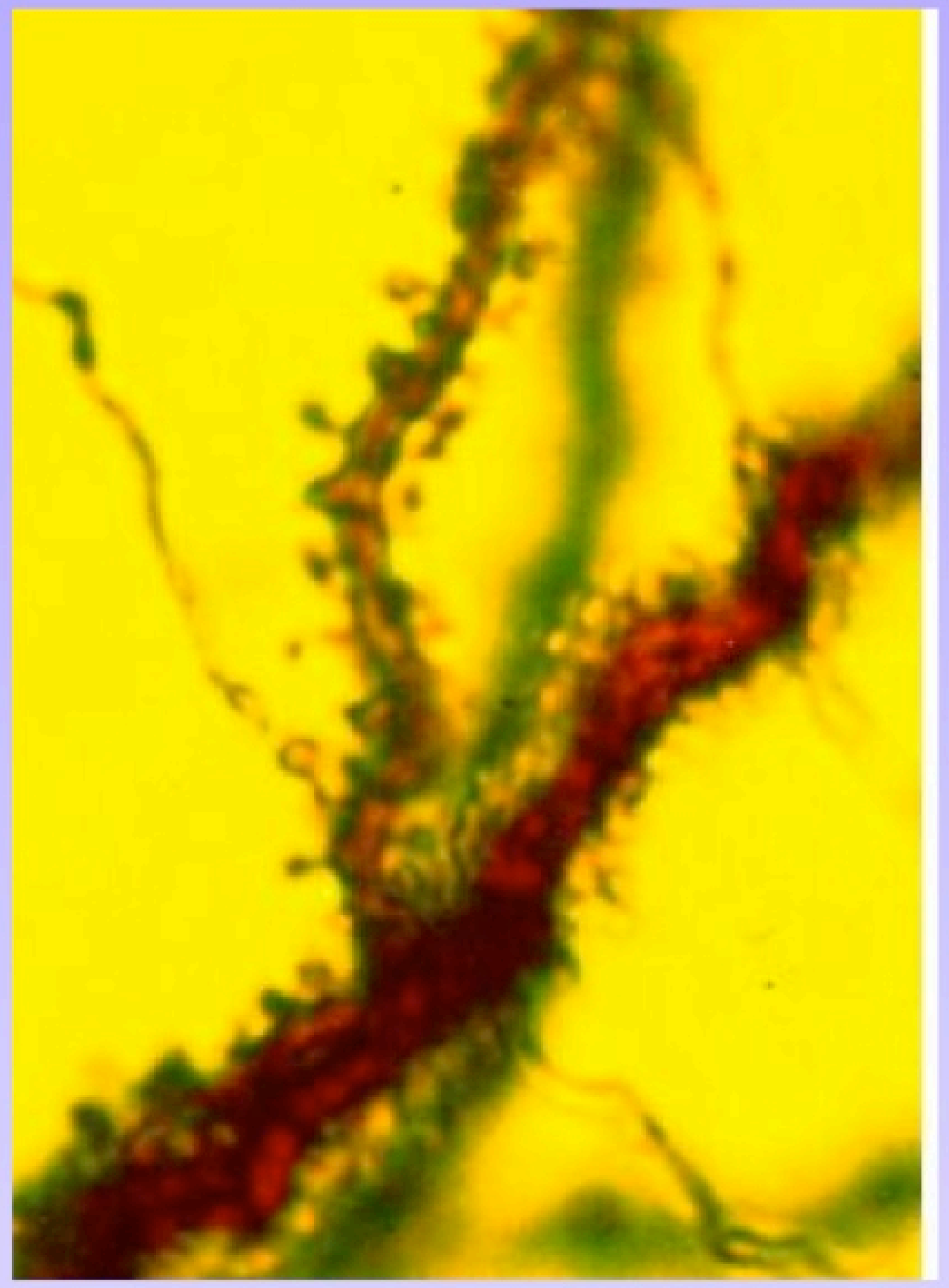
Clinical Neuronantomy and Neuroscience
www.studentconsult.com

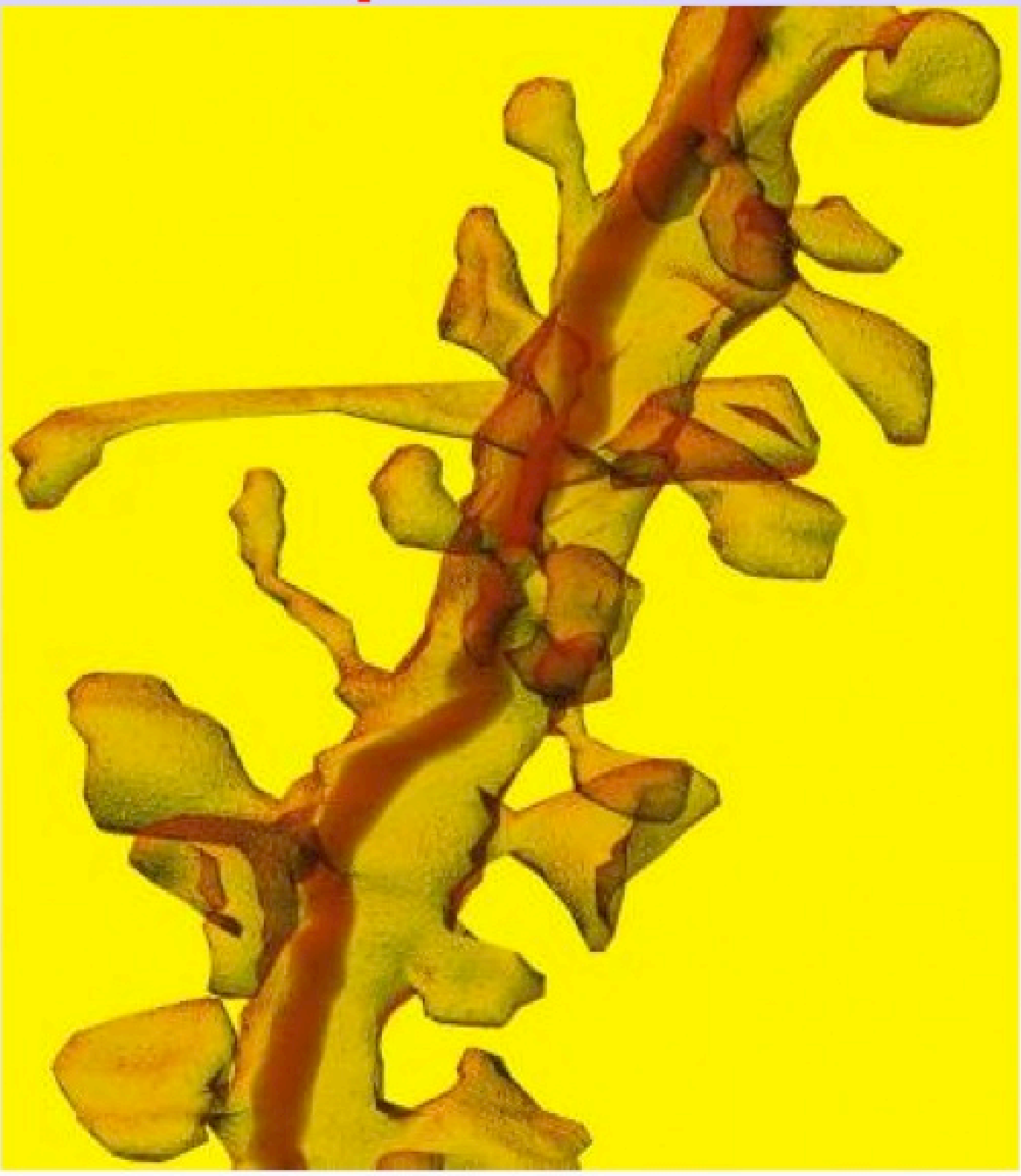
Different forms of dendrites



dendrite

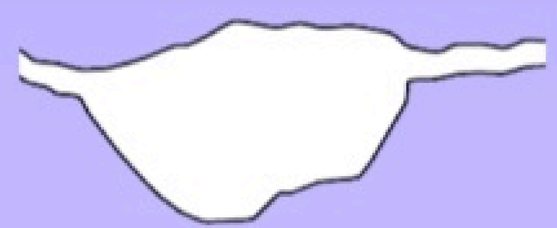
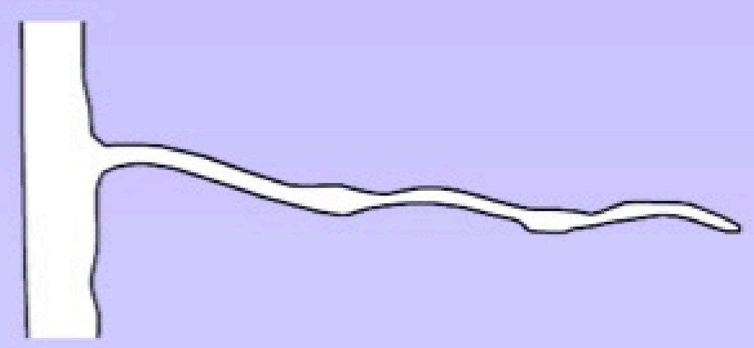
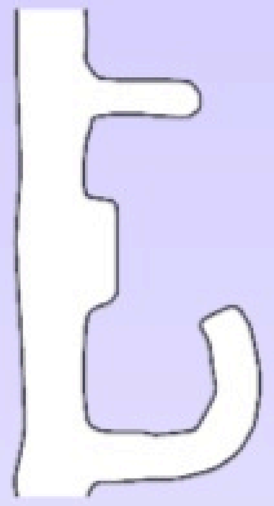
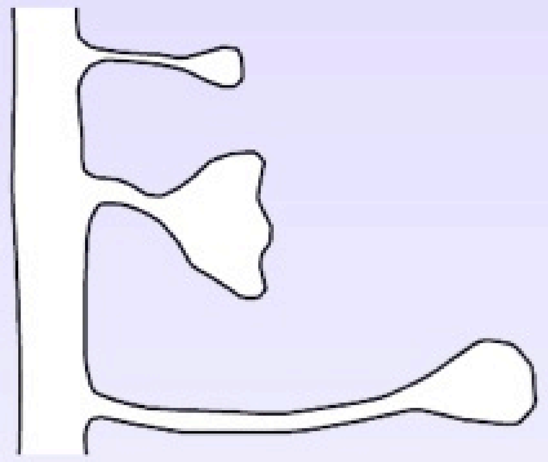
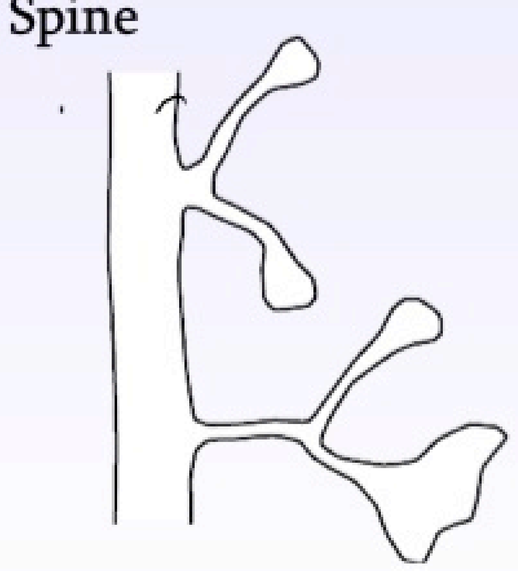


spine

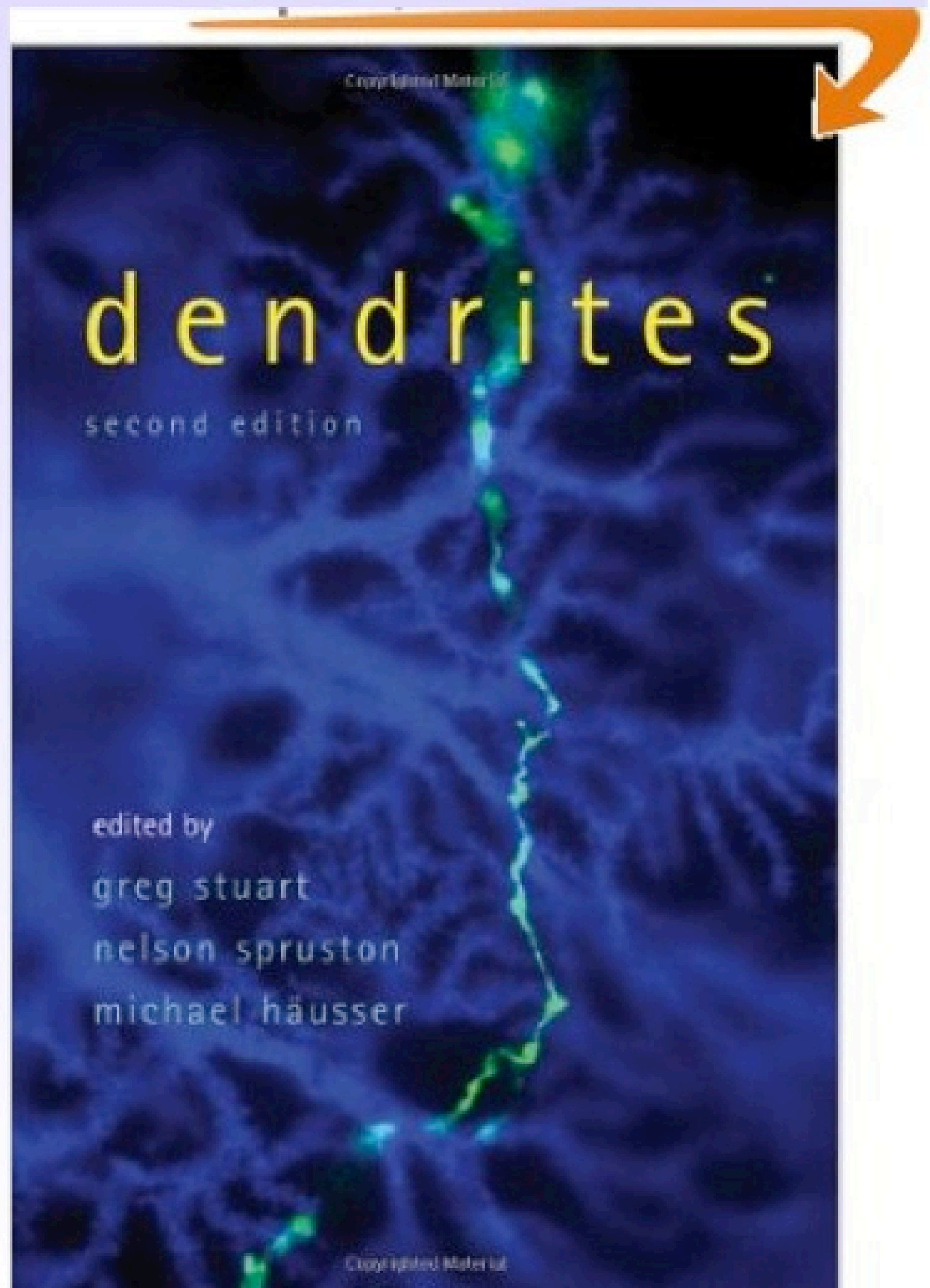


3D reconstruction from Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

Different forms of spines

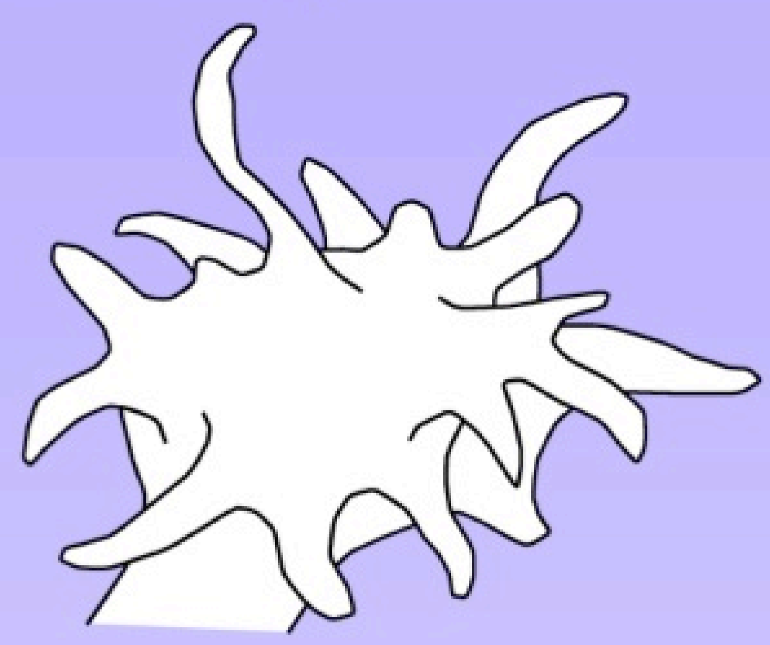
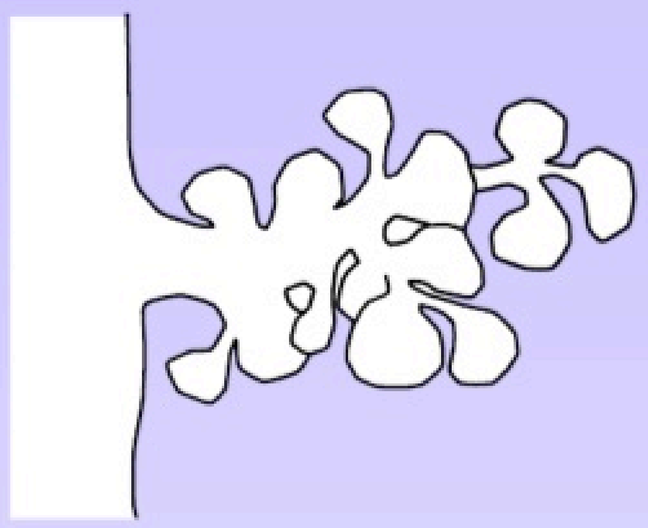
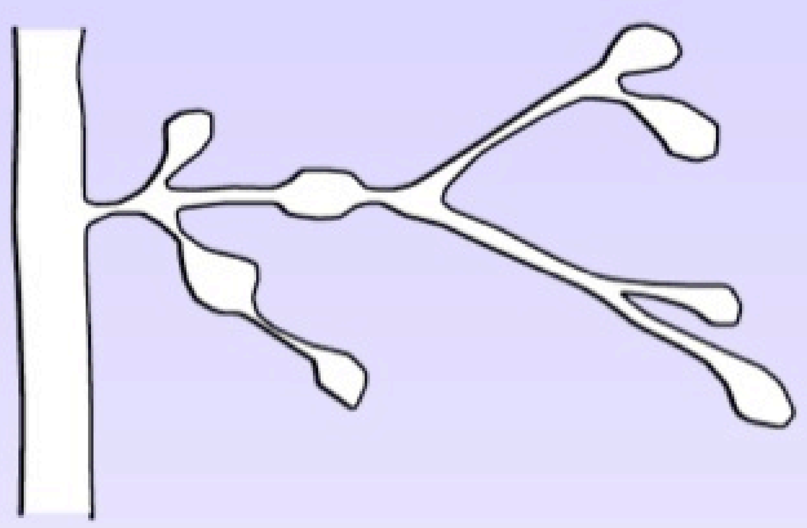
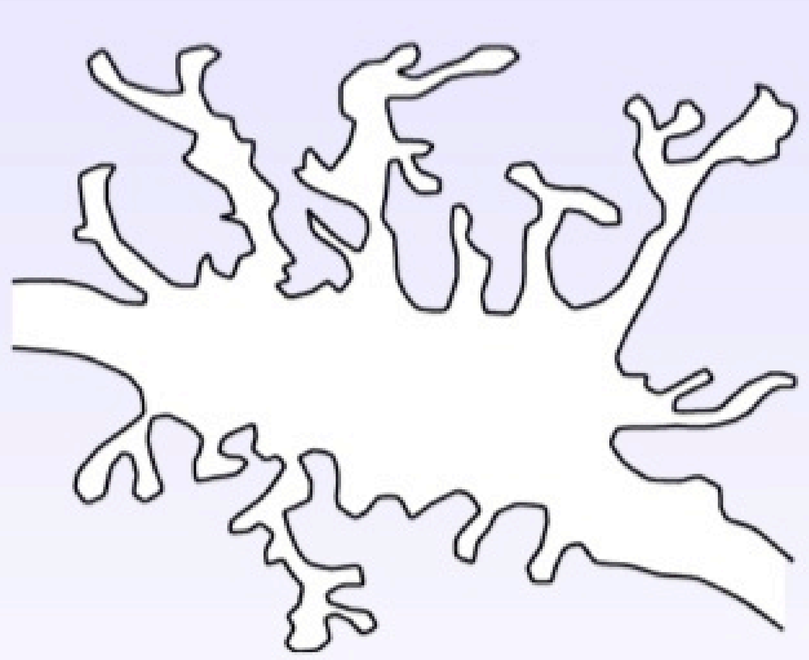
Pattern	Characteristics	Examples
Varicosity 	An enlargment in a thinner dendrite associated with synaptic contacts	Retinal amacrine cells
Filopodium 	A long, thin protrusion with a dense actin matrix and few internal organelles	Normally only seen during development
Simple Spine Sessile 	Synaptic protrusions without a neck constriction	
	Sessile spine	
	Stubby spine	Pyramidal cells of cortex
	Crook thorn	Cerebellar dentate nucleus
Pedunculated 	Bulbous enlargement at tip	
	Thin spine	Pyramidal cells of cortex
	Mushroom spine	Pyramidal cells of cortex
	Gemmule	Olfactory bulb granule cell
Branched Spine 	Each branch has a unique presynaptic partner and each branch has the shape characteristics of a simple spine	CA1 pyramidal cells Granule cells of dentate gyrus Cerebellar Purkinje cells

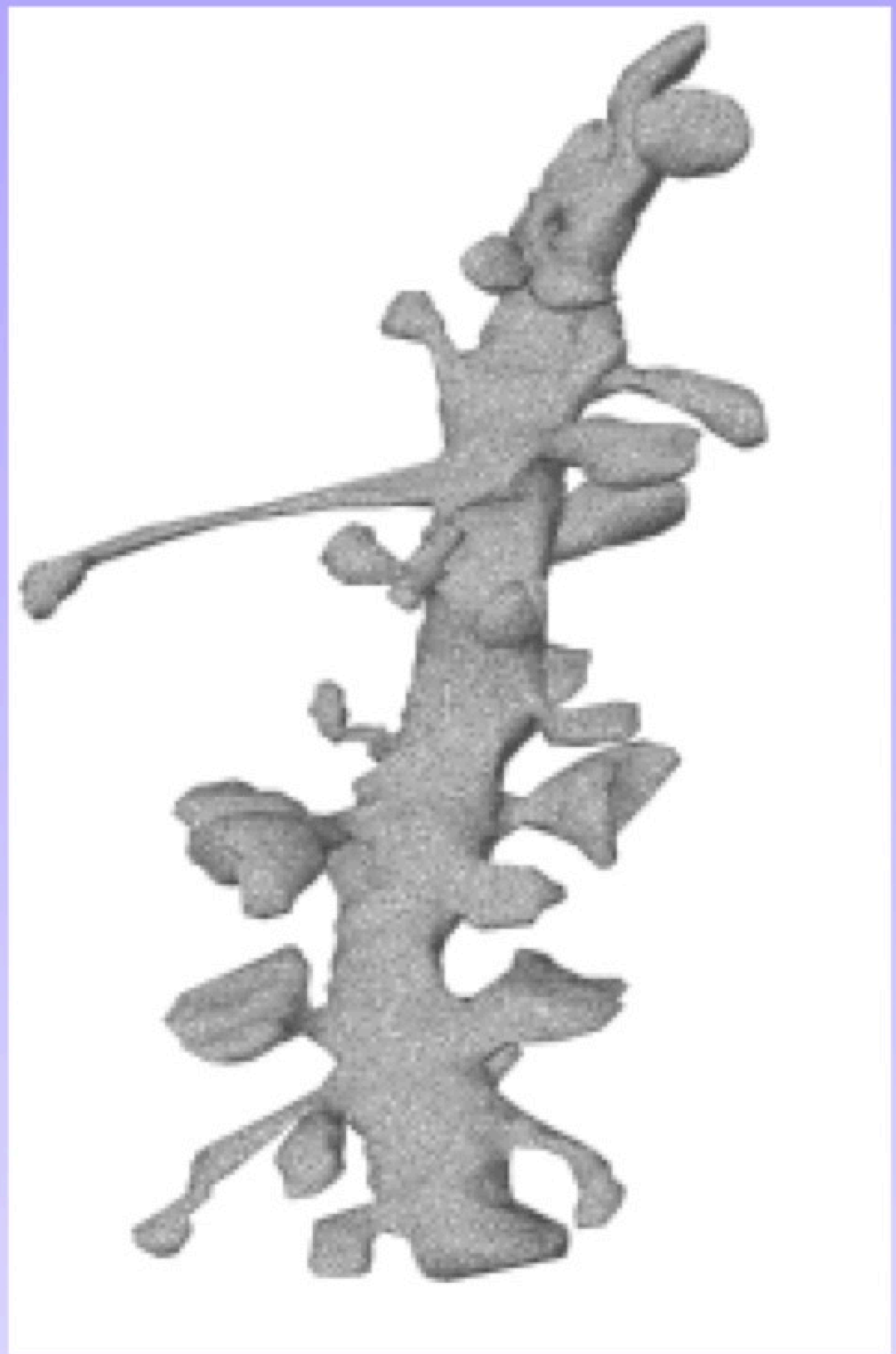
3D reconstruction from Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/



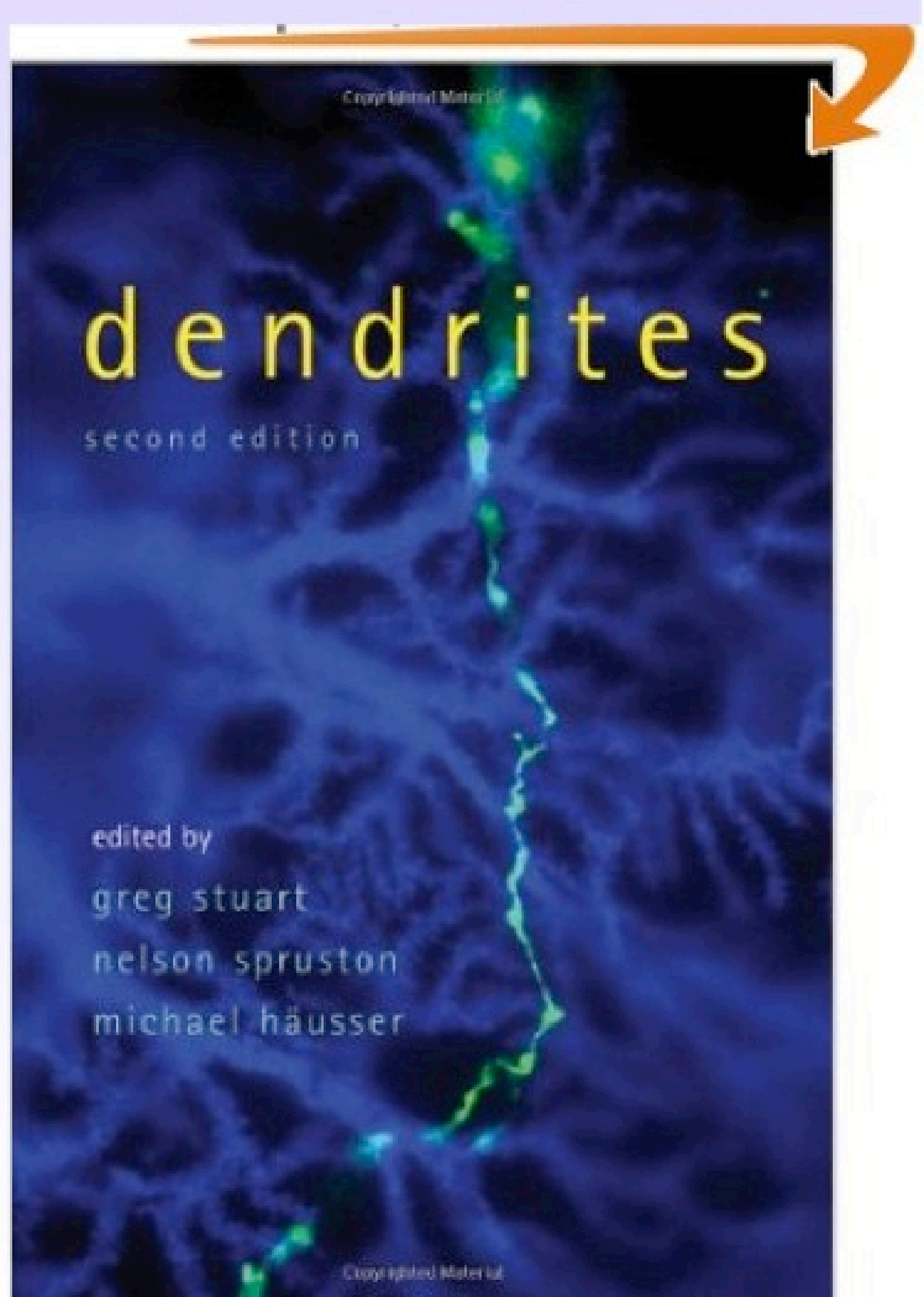
Adapted from Fiala JC, Harris KM (1999) Dendrite Structure. In: G Stuart, N Spruston, M Häusser, (eds.) Dendrites. Oxford University Press: Oxford UK.

Different forms of spines

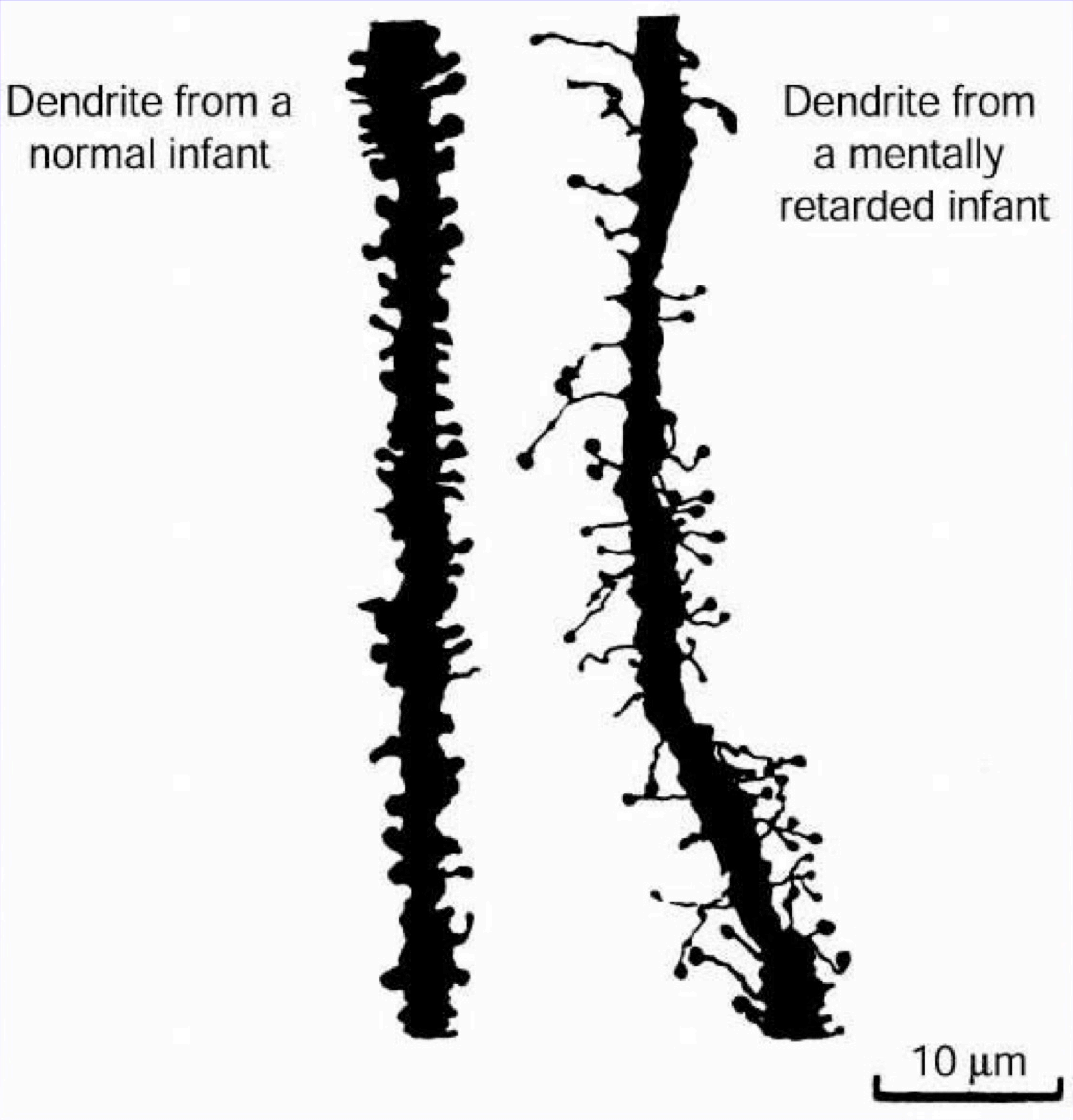
	Characteristics	Examples
Brush Ending 	Spray of complex dendritic protrusions at the end of dendrite that extends into glomerulus and contains presynaptic elements	Unipolar brush cells of cerebellar cortex and dorsal cochlear nucleus
Thorny Excrescence 	Densely lobed dendritic protrusion into a glomerulus	Proximal dendrites of CA3 pyramidal cells and dentate gyrus mossy cells
Racemose Appendage 	Twig-like branched dendritic appendages that contain synaptic varicosities and bulbous tips	Inferior olive Relay cells of lateral geniculate nucleus
Coralline Excrescence 	Dendritic varicosity extending numerous thin protrusions, velamentous expansions and tendrils	Cerebellar dentate nucleus Lateral vestibular nucleus



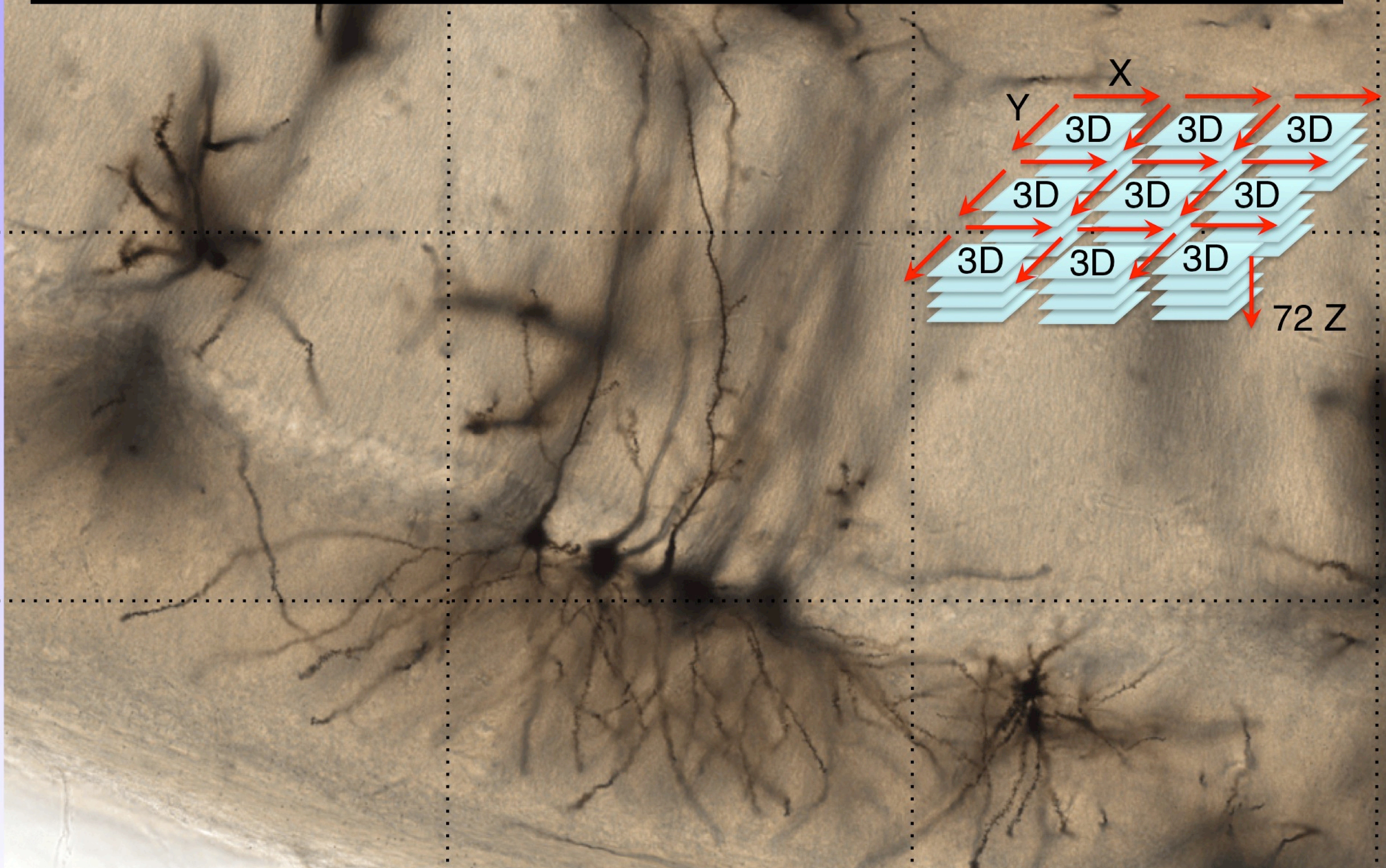
3D reconstruction from Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/



Fiala JC, Harris KM (1999) Dendrite Structure. In: G Stuart, N Spruston, M Häusser, (eds.) Dendrites. Oxford University Press: Oxford UK.


Dendrite from a normal infant
Dendrite from a mentally retarded infant
10 µm

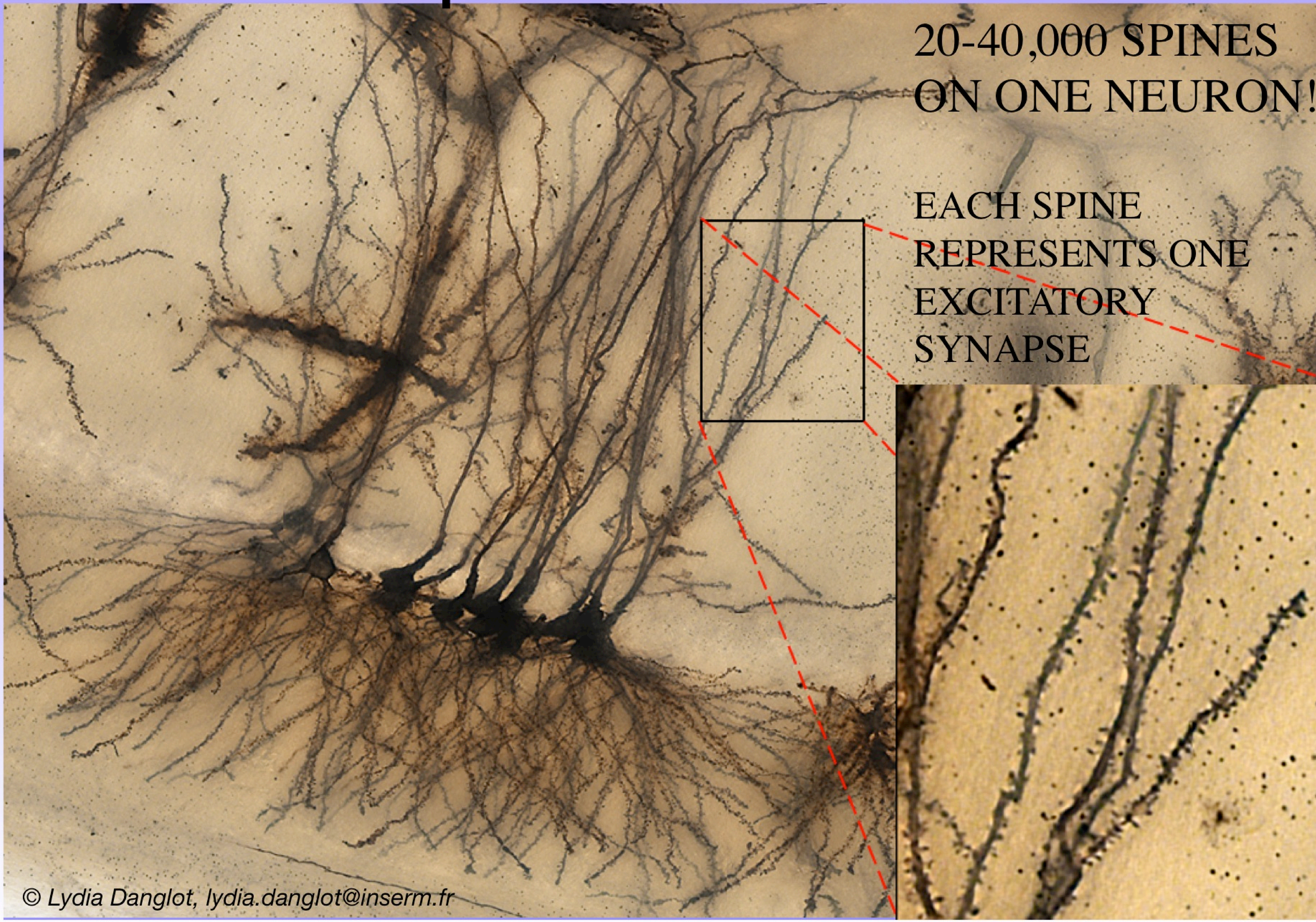
What about spine morphology in TI-VAMP KO mice ?



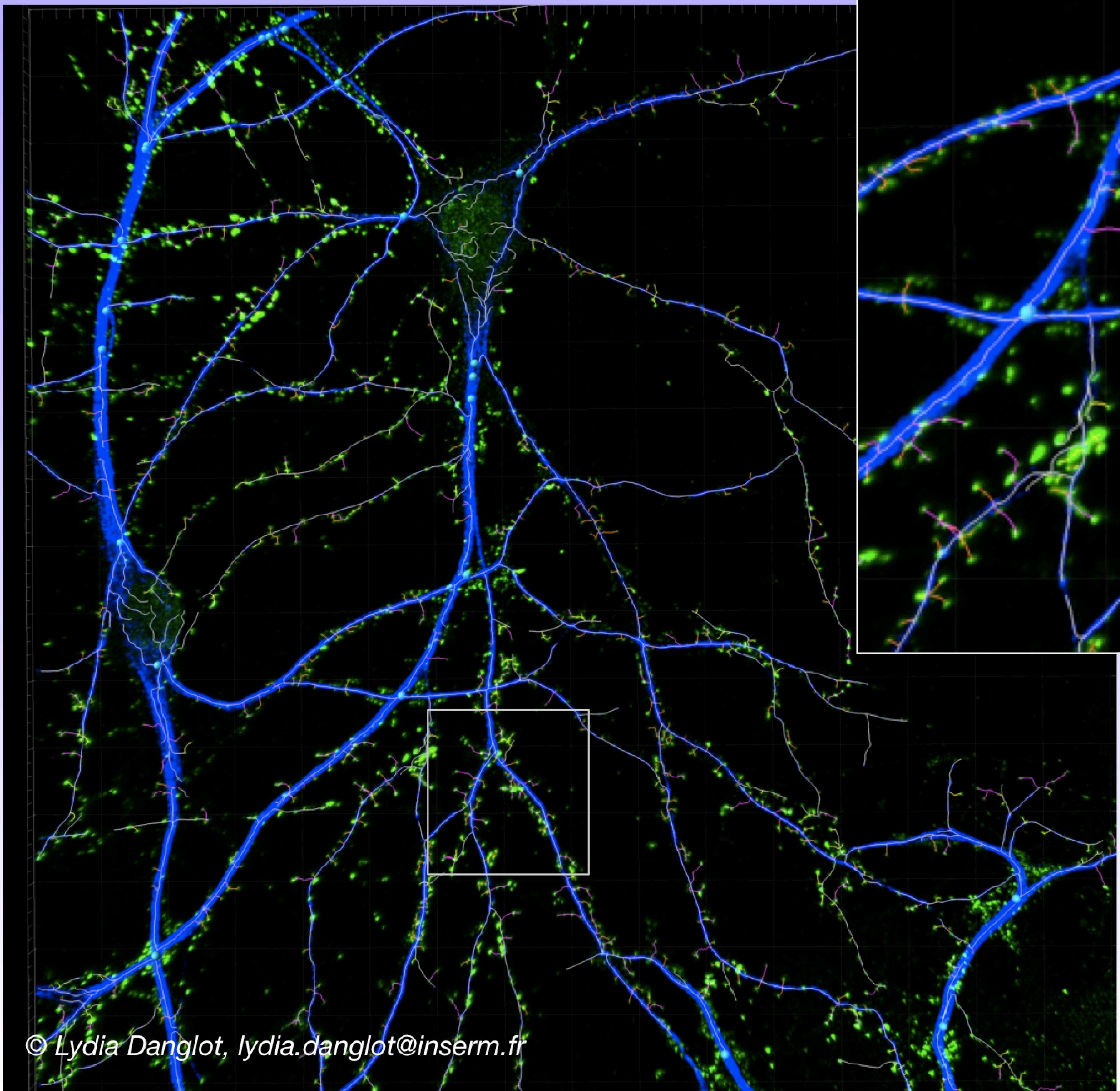
Movie

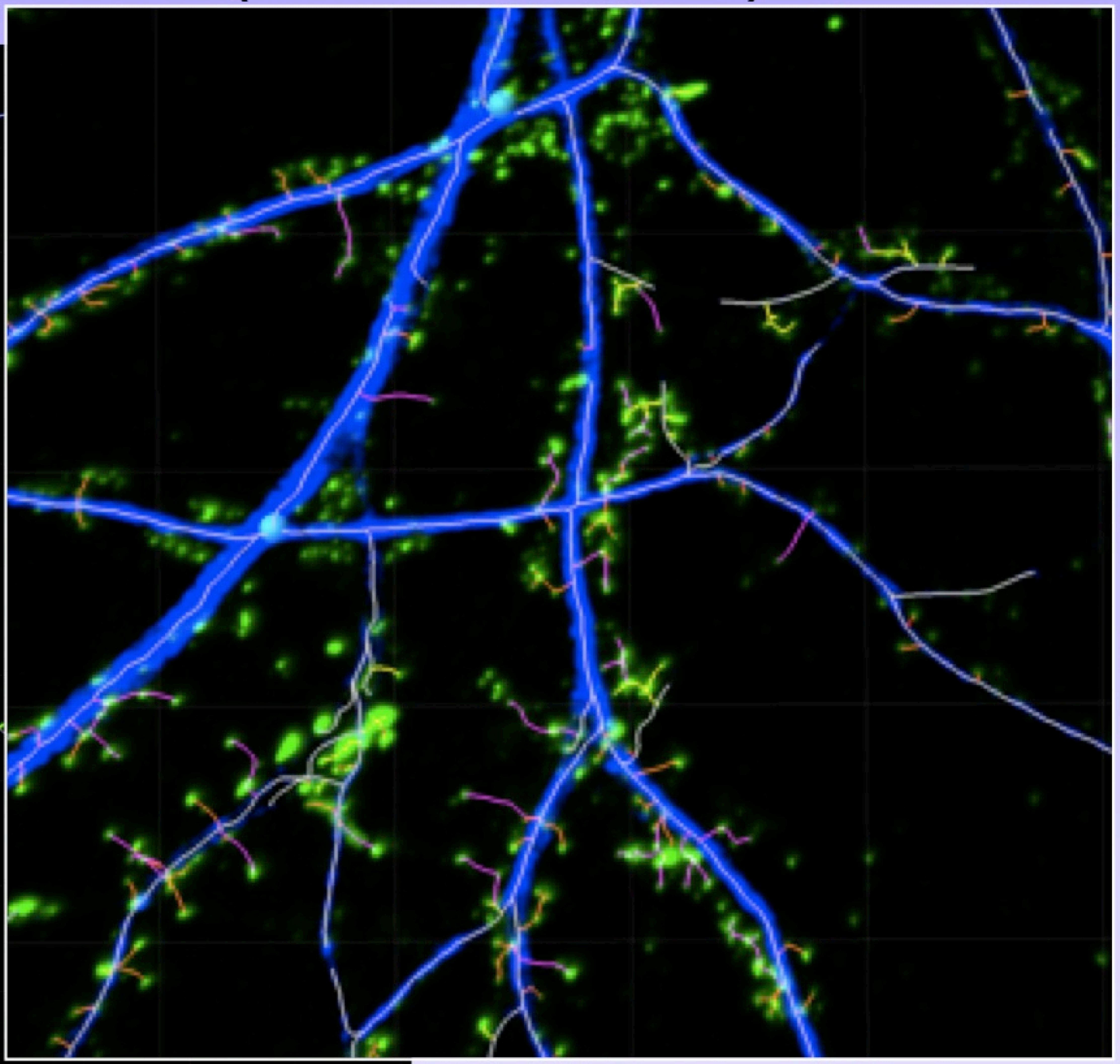
3D reconstruction of 9 Z-stacks of 72 tiled pictures taken at 40X

Dendritic Spines (golgi staining)



Dendritic Spines (fluorescence)





Spine morphology?

Tile confocal imaging,

3D reconstruction (Imaris)

and spine quantification (Matlab).

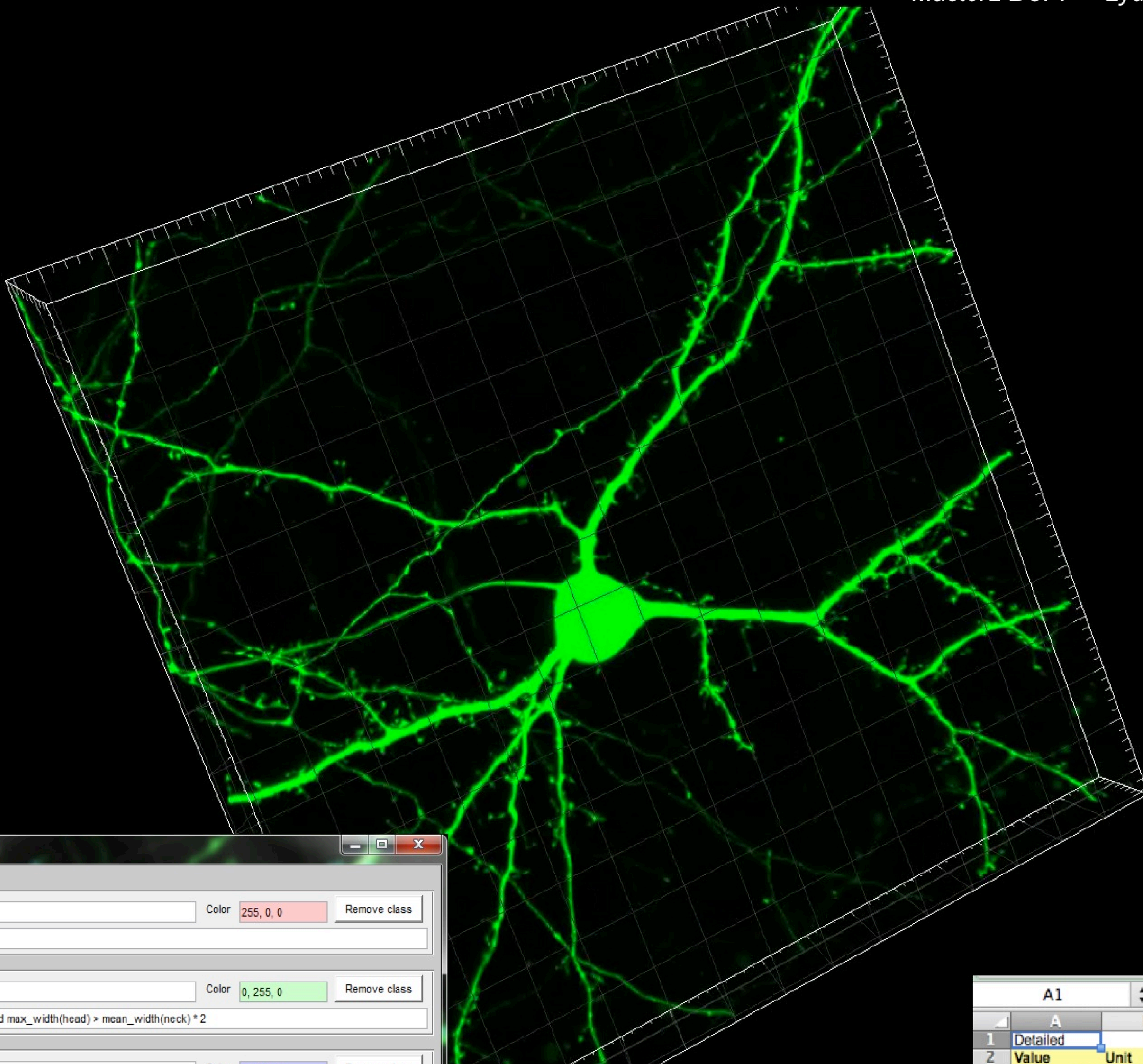

Epines dendritiques
STUBBY
MUSHROOM
FILOPODIA
Imaris Spines Classifier
Class 1
Name Stubby
Color 255, 0, 0
Remove class
Rule length(spine) < 1
Class 2
Name Mushroom
Color 0, 255, 0
Remove class
Rule length(spine) < 3 and max_width(head) > mean_width(neck) * 2
Class 3
Name Long Thin
Color 0, 0, 255
Remove class
Rule mean_width(head) >= mean_width(neck)
Class 4
Name Filopodia / Dendrite
Color 255, 0, 255
Remove class
Rule true
Variables
spine
head
neck
ground
volume
length
mean_width
max_width
min_width
Insert variable
Head length (%) 25
Ground length (%) 25
Create one Filament
... for each class
... with custom stats
Plot class statistics
Add new class
Classify spines
Excel file
A1
Detailed
Detailed
Value Unit Category Depth Level Surpass Obje Time
0,92 um^2 Spine 21 3 Stubby 1
1,41 um^2 Spine 21 3 Long Thin 1
0,49 um^2 Spine 21 3 Stubby 1
3,21 um^2 Spine 21 3 Long Thin 1
0,22 um^2 Spine 20 2 Stubby 1
3,24 um^2 Spine 21 3 Long Thin 1
0,94 um^2 Spine 20 3 Stubby 1
1,92 um^2 Spine 21 3 Long Thin 1
0,35 um^2 Spine 20 3 Stubby 1
1,16 um^2 Spine 21 3 Long Thin 1
1,02 um^2 Spine 20 3 Stubby 1
2,30 um^2 Spine 20 2 Long Thin 1

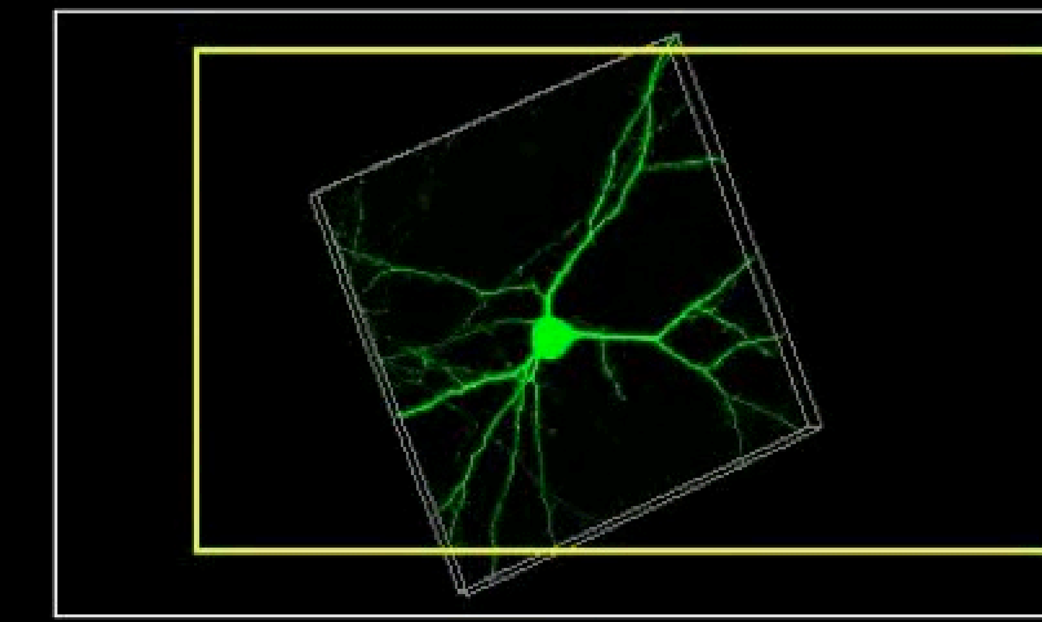
What about spine morphology in TI-VAMP KO mice ?

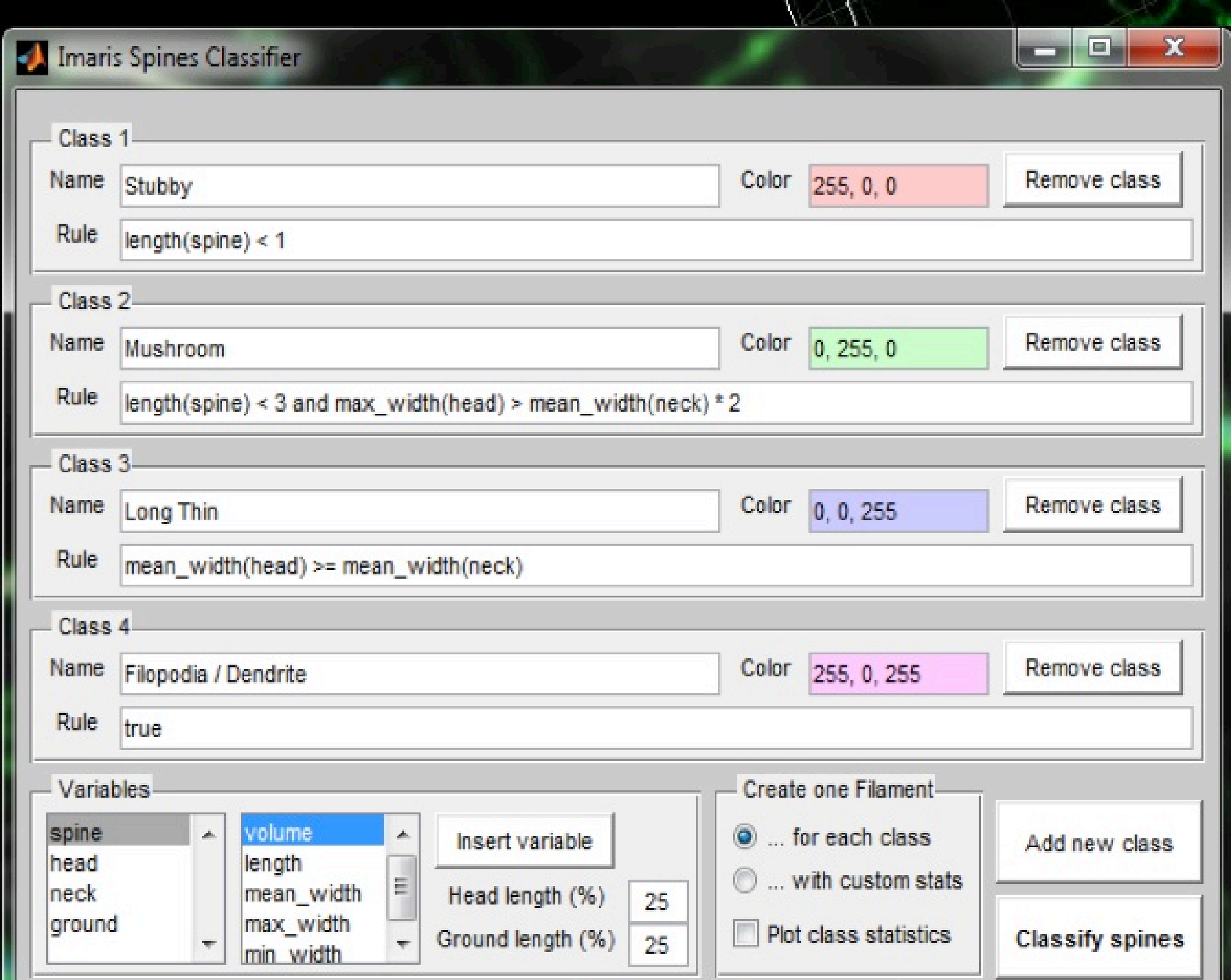
On going : tile confocal imaging,

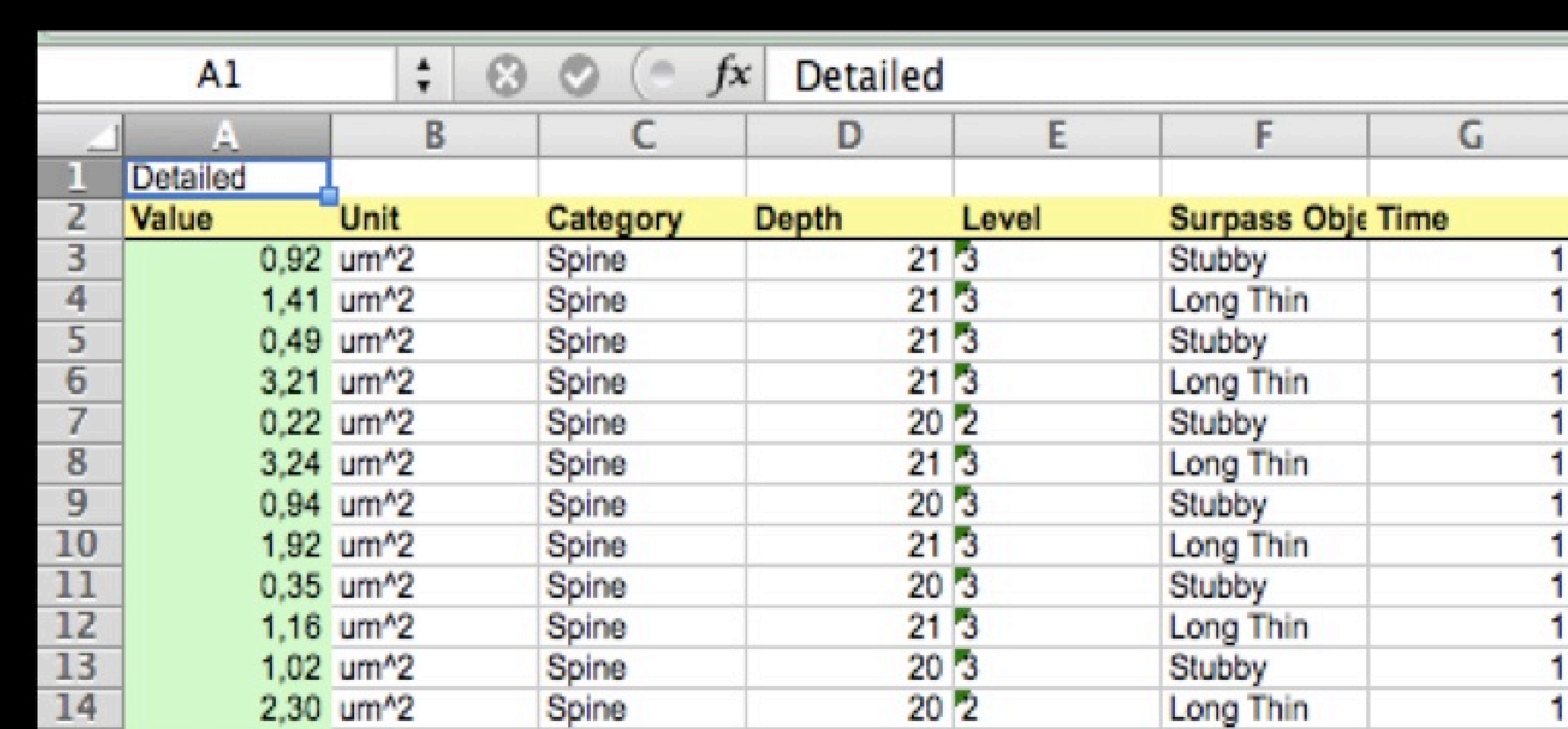
3D reconstruction (Imaris)

and spine quantification (Matlab).





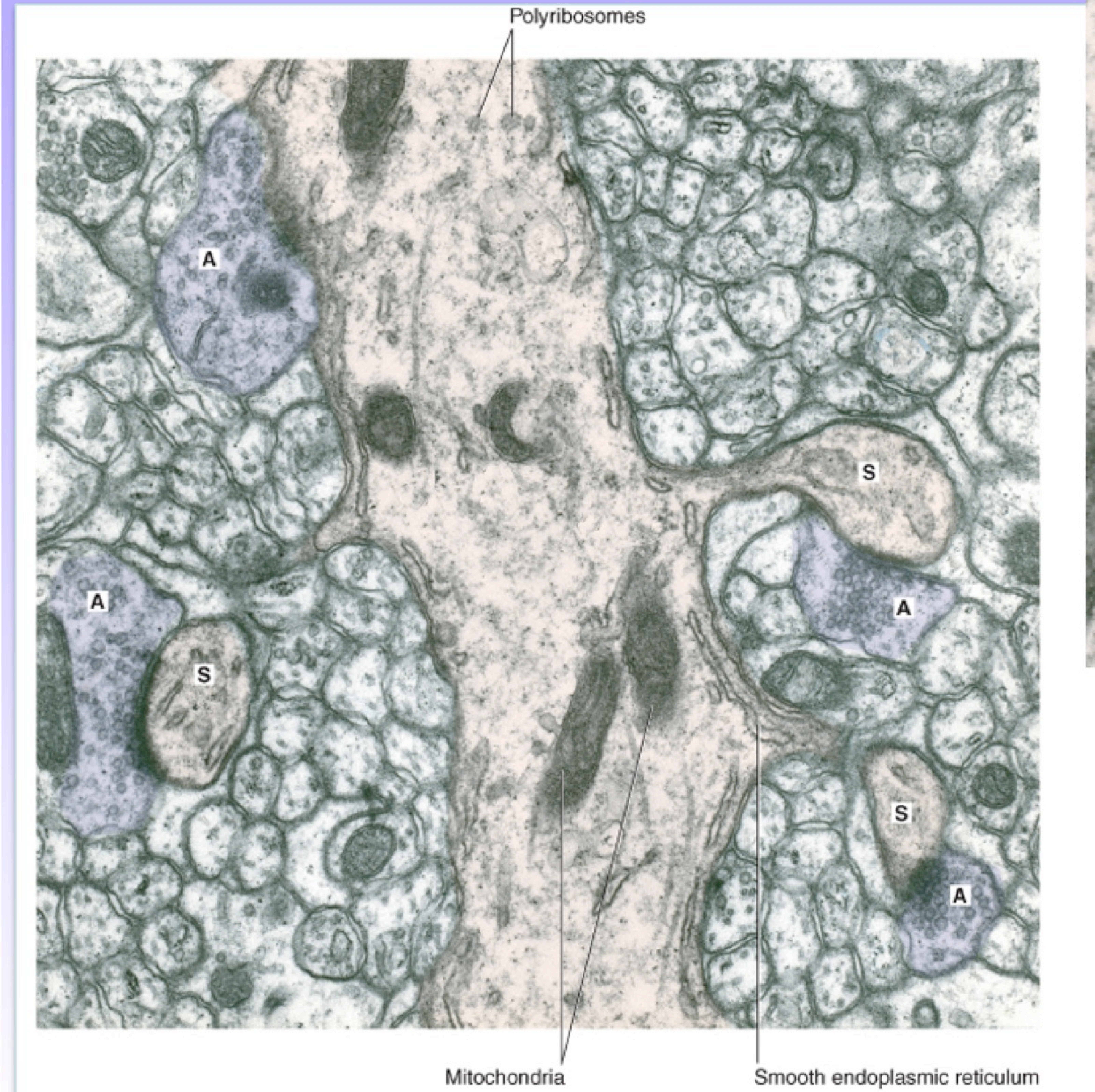




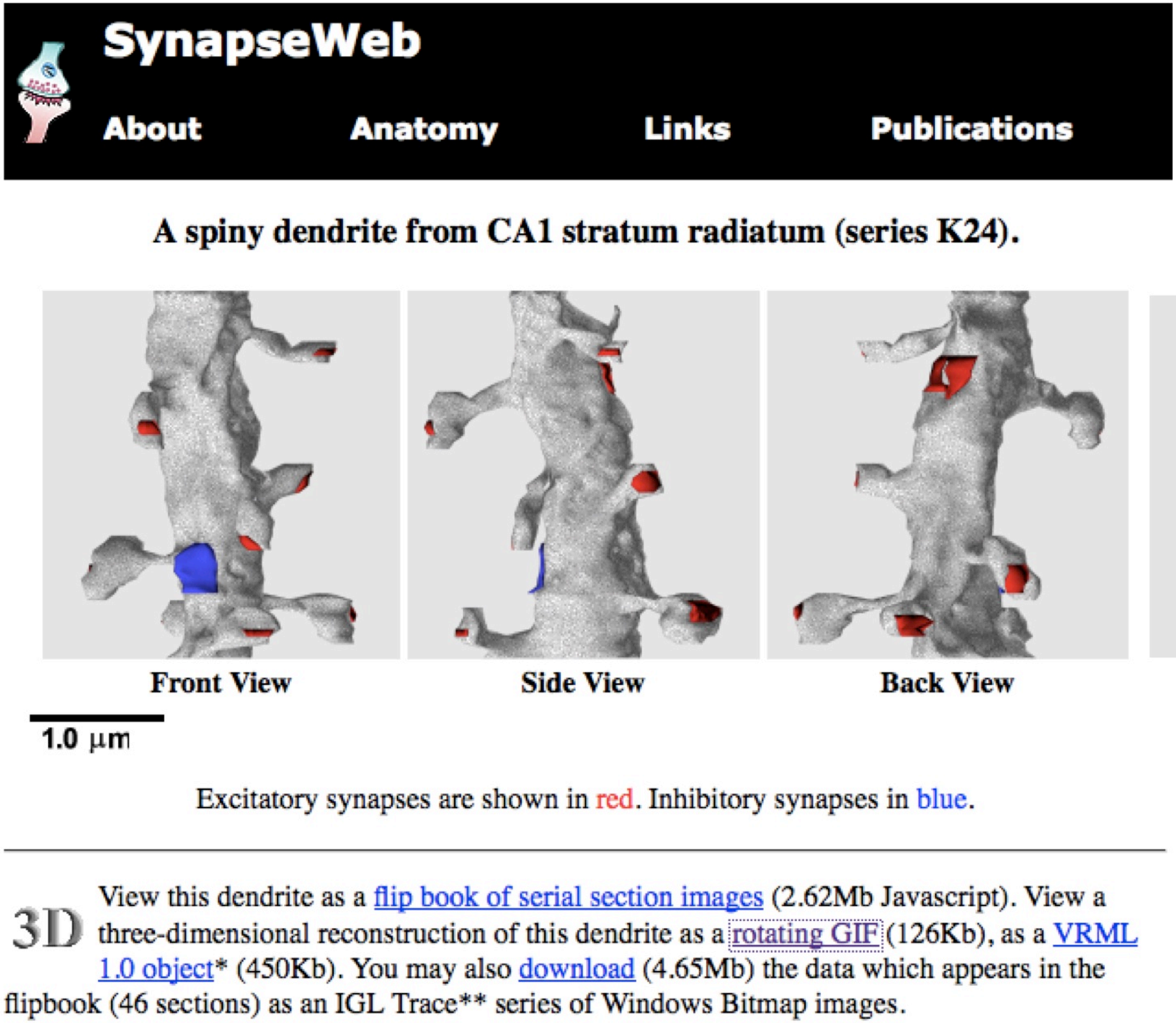
A1 | Detailed

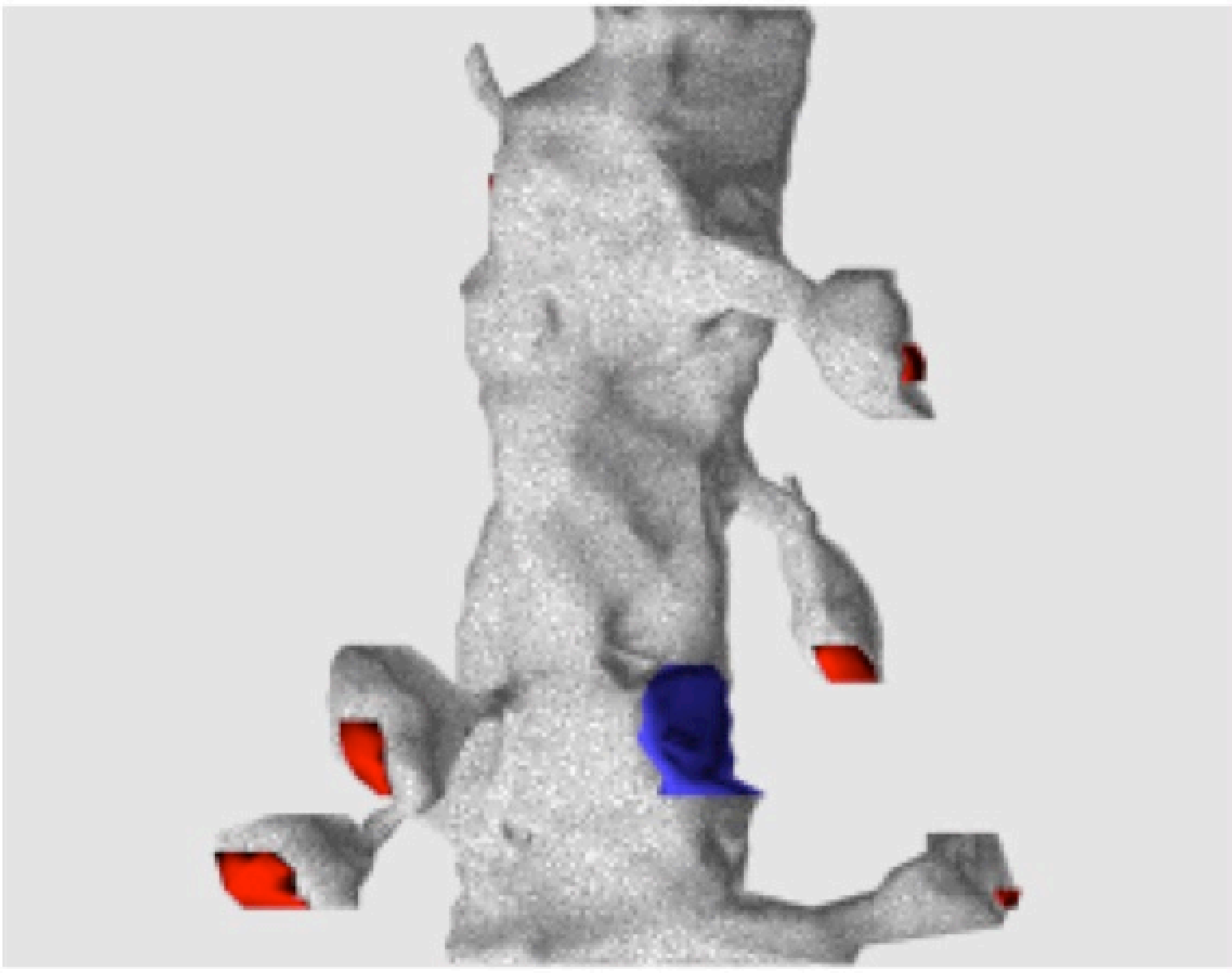
	A	B	C	D	E	F	G
1	Detailed						
2	Value	Unit	Category	Depth	Level	Surpass Obje	Time
3	0,92	um^2	Spine	21	3	Stubby	1
4	1,41	um^2	Spine	21	3	Long Thin	1
5	0,49	um^2	Spine	21	3	Stubby	1
6	3,21	um^2	Spine	21	3	Long Thin	1
7	0,22	um^2	Spine	20	2	Stubby	1
8	3,24	um^2	Spine	21	3	Long Thin	1
9	0,94	um^2	Spine	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
14	2,30	um^2	Spine	20	2	Long Thin	1

Dendritic Spines (electron microscopy)



Different forms of spines



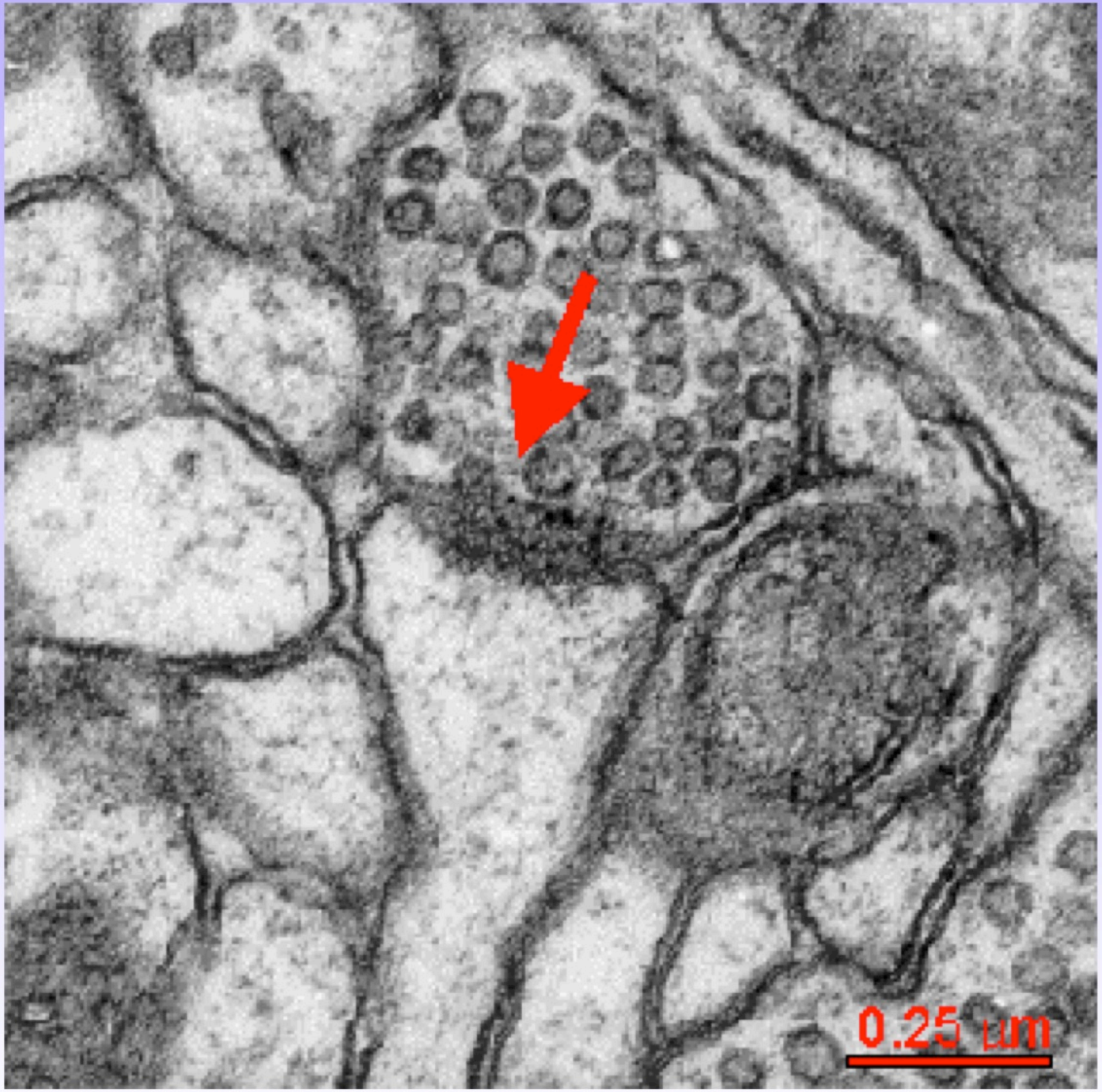


Excitatory synapse

Inhibitory synapse

from Synapse Web, Kristen M. Harris, PI, http://synapses.clm.utexas.edu/

Asymmetric synapse (GRAY'S TYPE I) excitatory



from Karin Sorra, Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

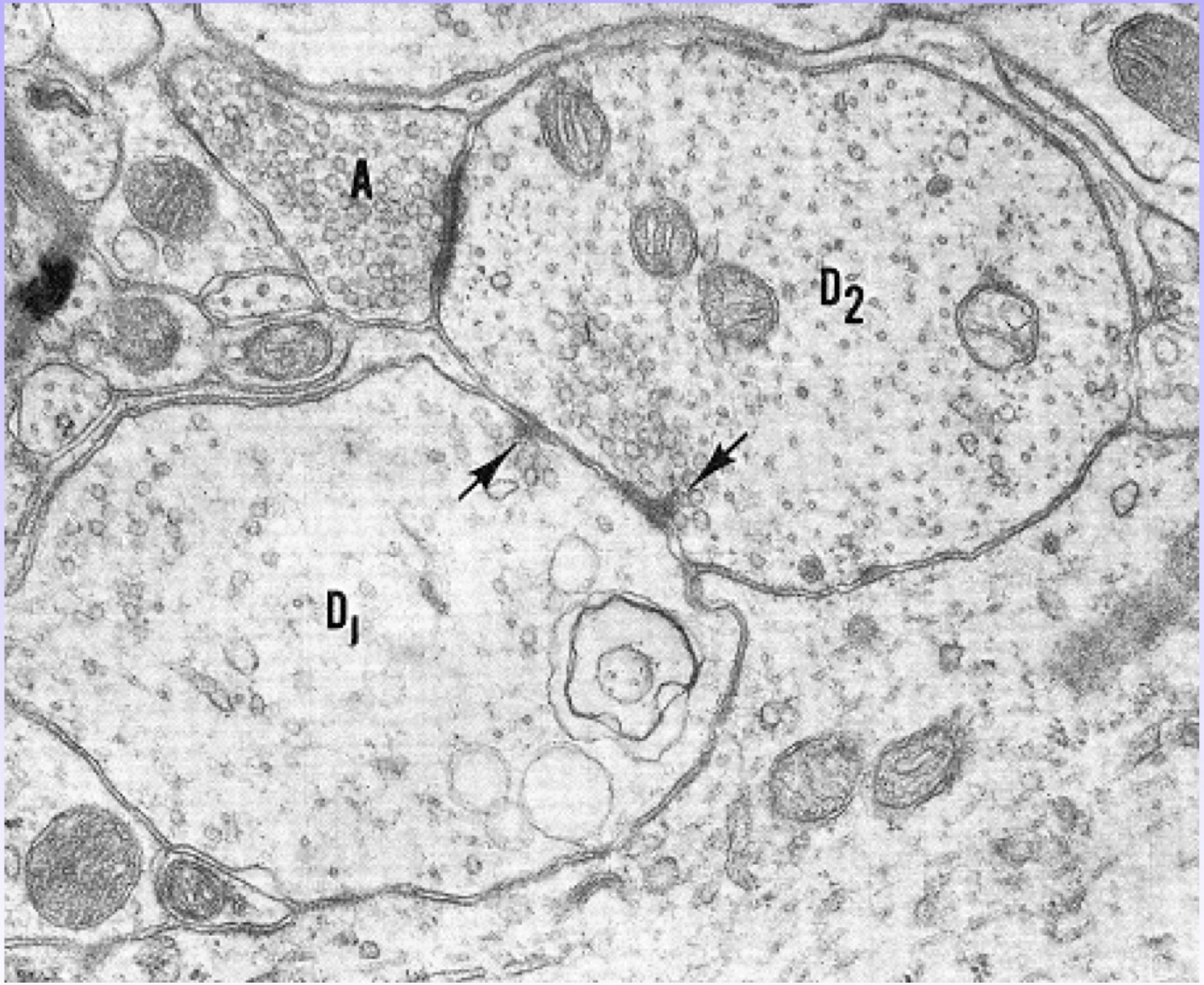
WHAT TYPE OF SYNAPSE?

from Karin Sorra, Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

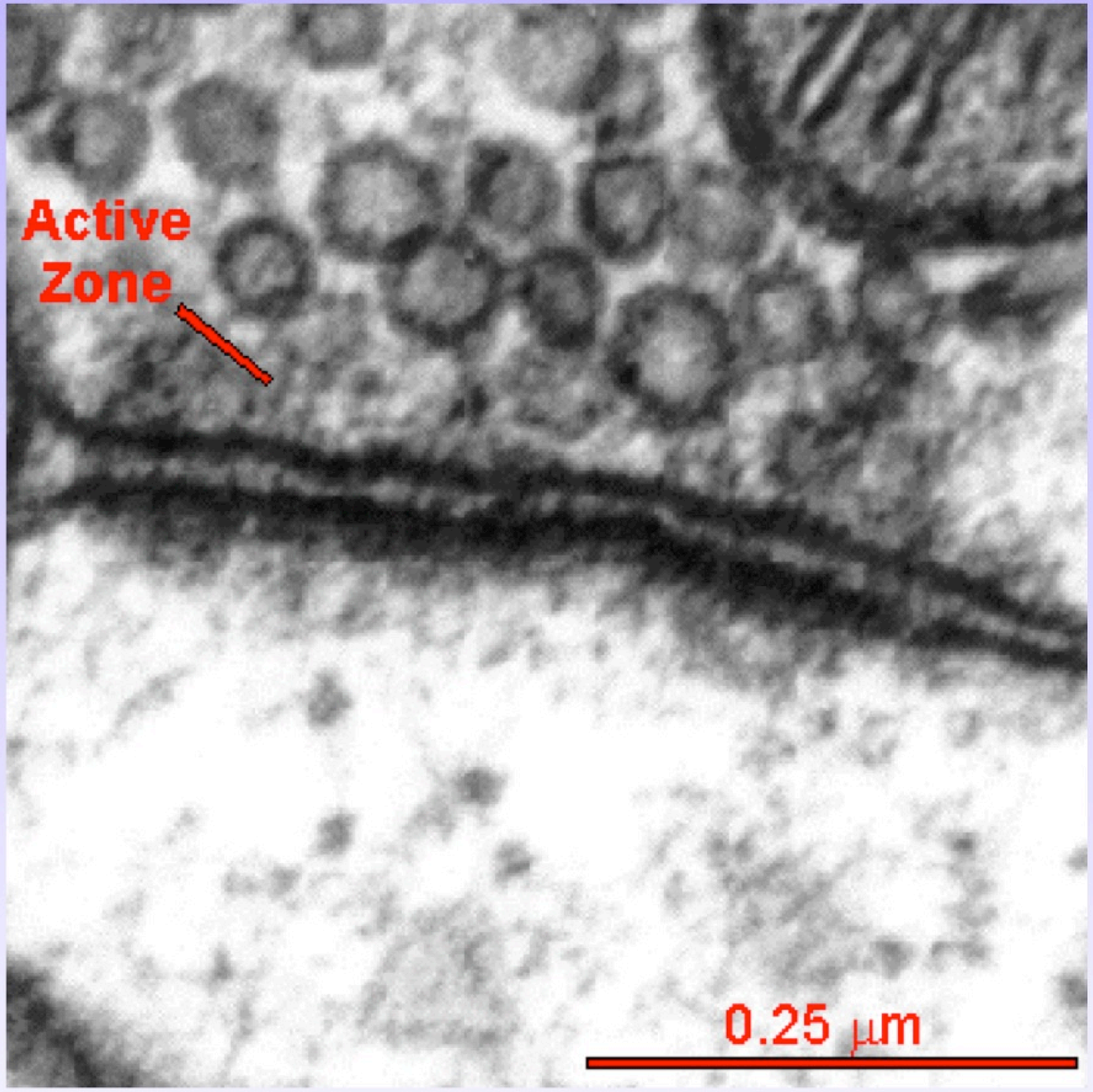
SYMMETRIC (GRAY'S II) : INHIBITORY

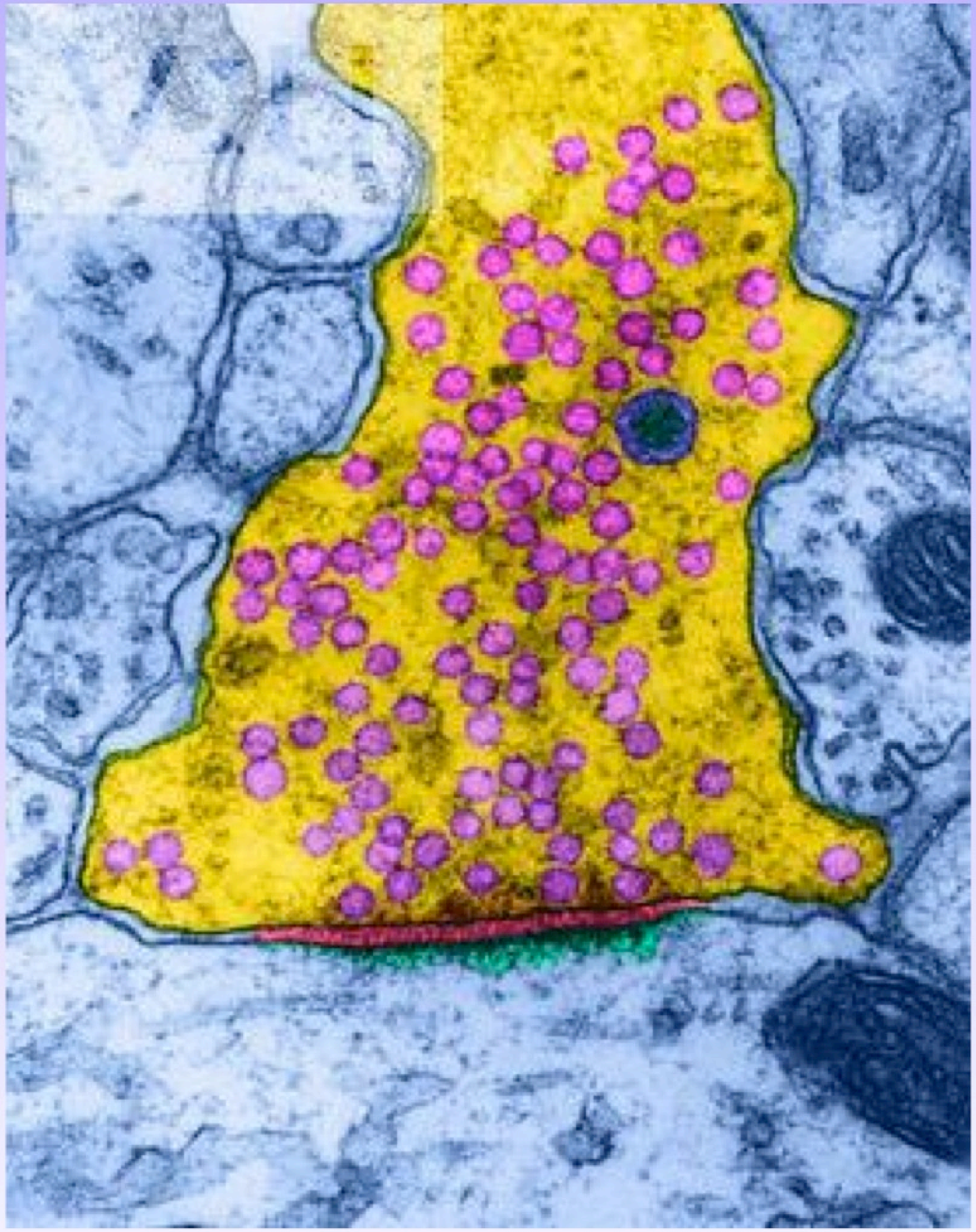
from Karin Sorra, Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

DENDRO-DENDRITIC SYNAPSE

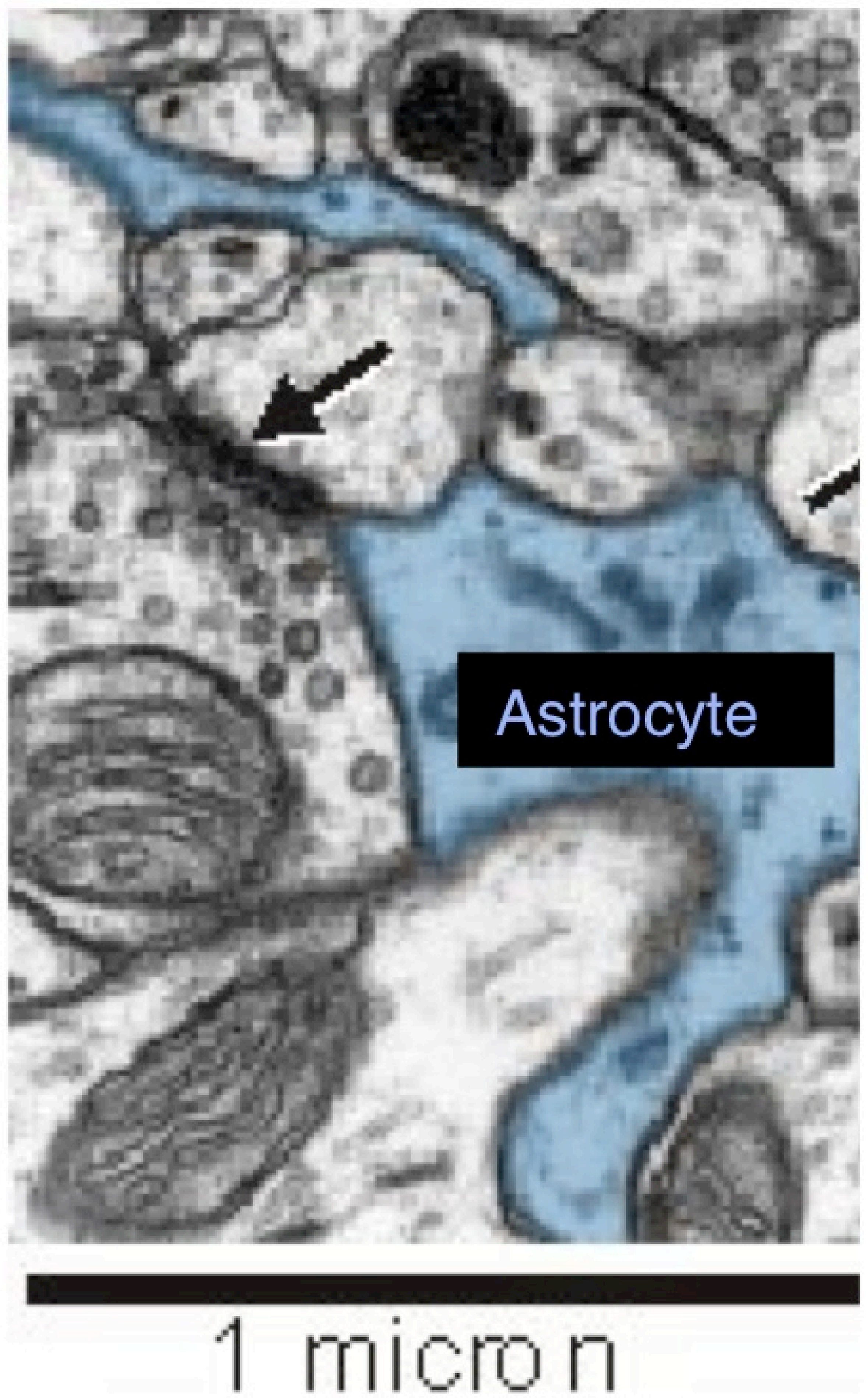


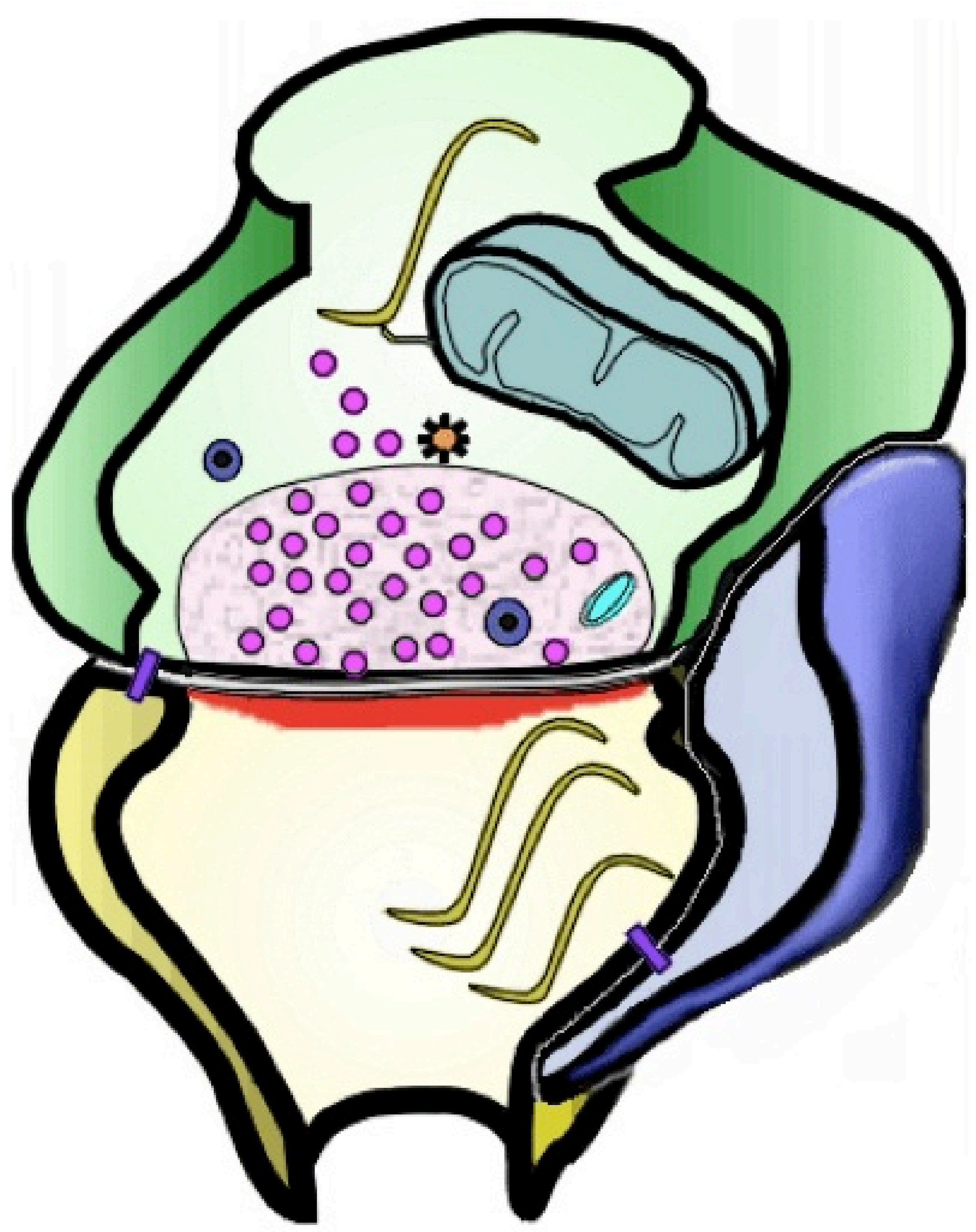
Presynaptic Active Zone





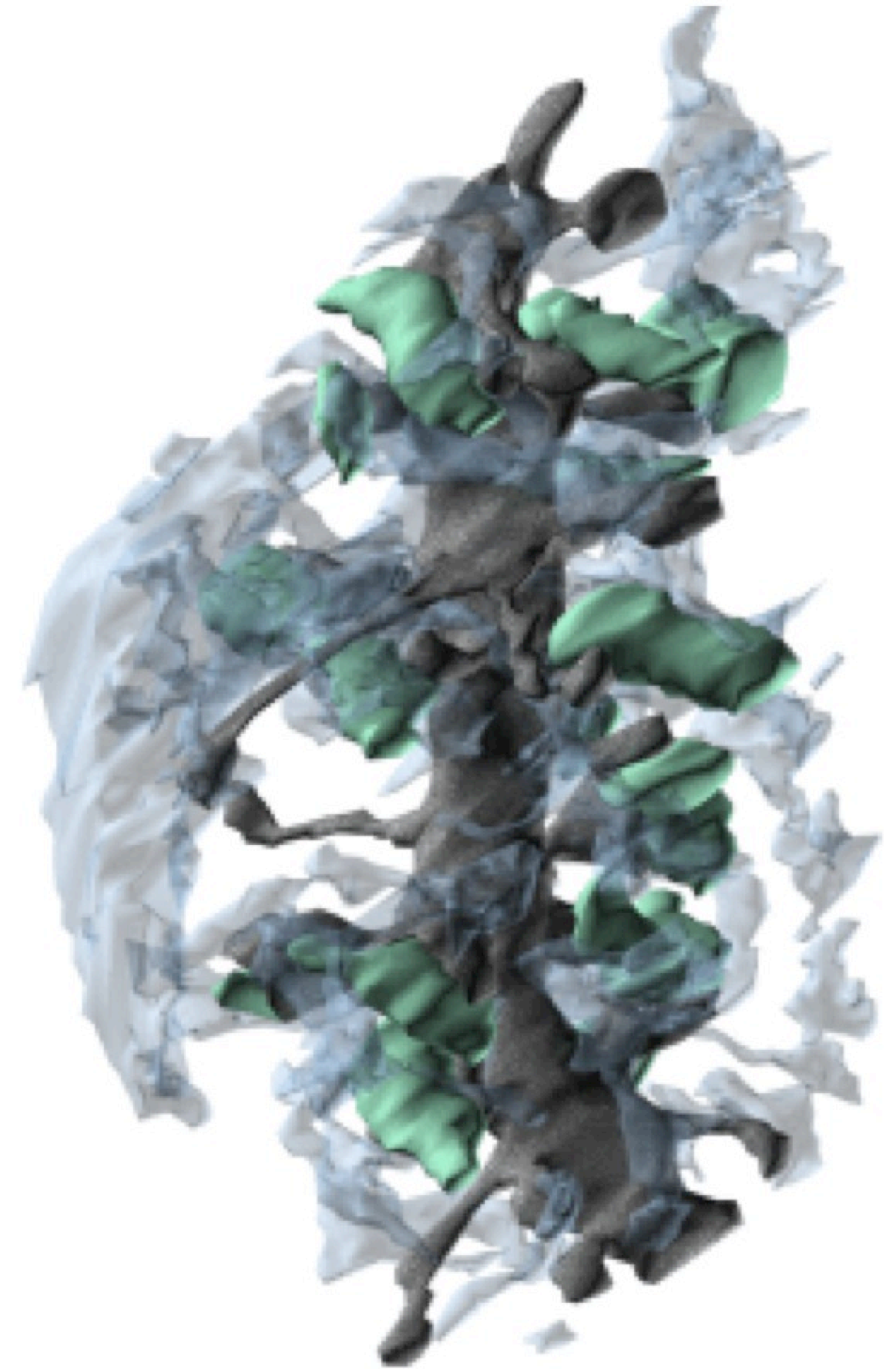
Tripartite synapse with astrocyte

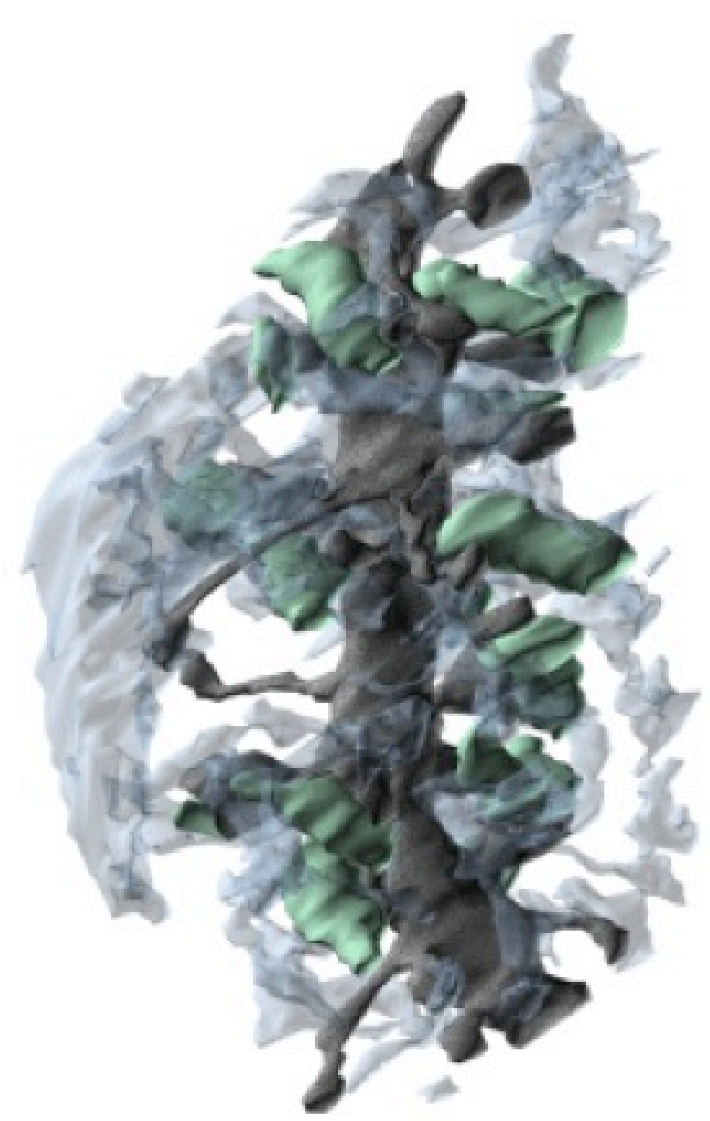




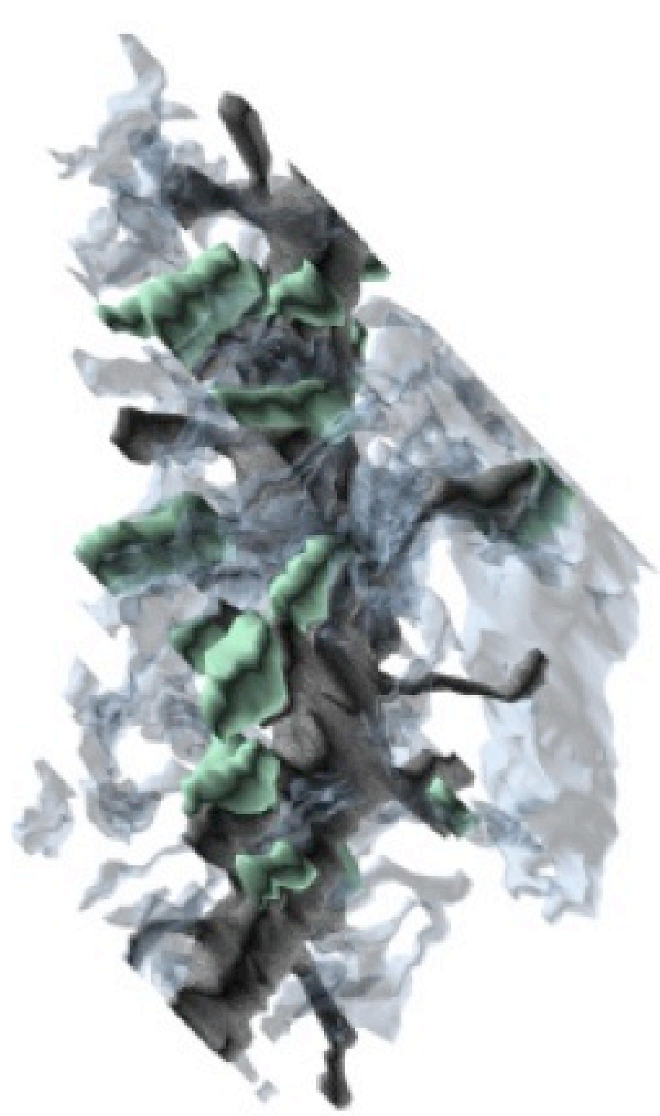
	Presynaptic Axon
	Postsynaptic Spine
	Postsynaptic Density
	Active Zone
	Astrocyte
	Coated Vesicle
	Dense Core Vesicle
	Double-Walled Vesicle
	Endoplasmic Reticulum
	Mitochondrion
	Punctum Adhaerens
	Synaptic Cleft
	Synaptic Vesicle

A dendrite with synapsing boutons and neighboring astrocyte from CA1 stratum radiatum (series K18).

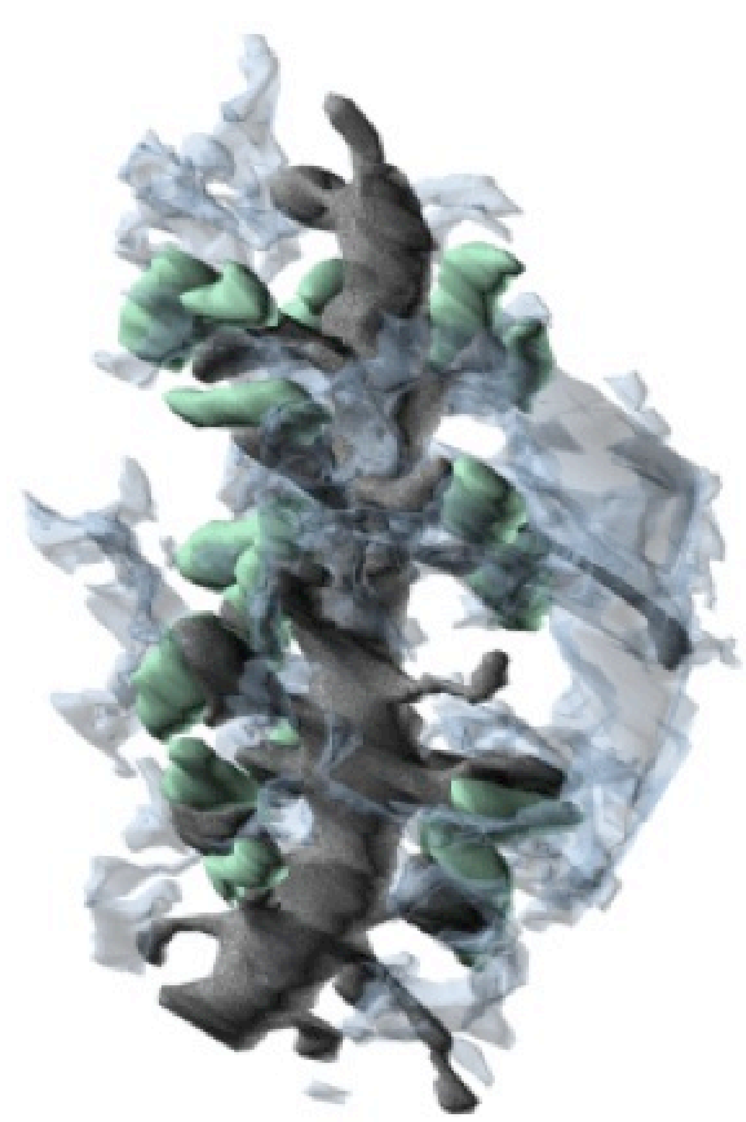




Front View



Side View



Back View

Presynaptic boutons

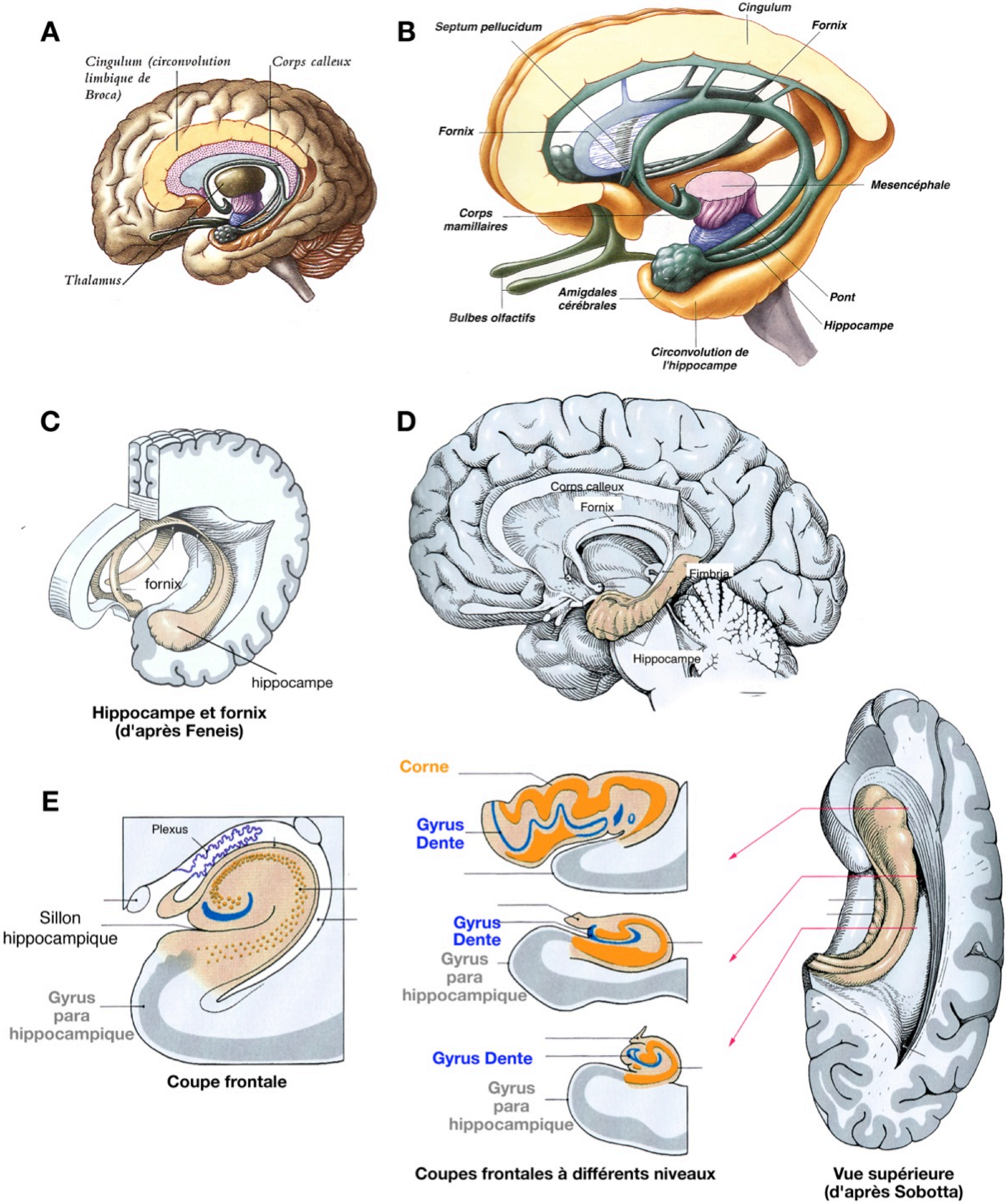
Dendrite & spines

Astrocyte

Adapted from Synapse Web, Kristen M. Harris, PI, Rachel E. Ventura, Karrin Sorra http://synapses.clm.utexas.edu/

11. Formation des synapses et plasticité synaptique in vivo

Hippocampus



Hippocampe et fornix (d'après Feneis)

Coupe frontale

Coupes frontales à différents niveaux

Vue supérieure (d'après Sobotta)

Hippocampus: A cortical structure in the medial portion of the temporal lobe; in humans, concerned with short-term declarative memory, among many other functions.

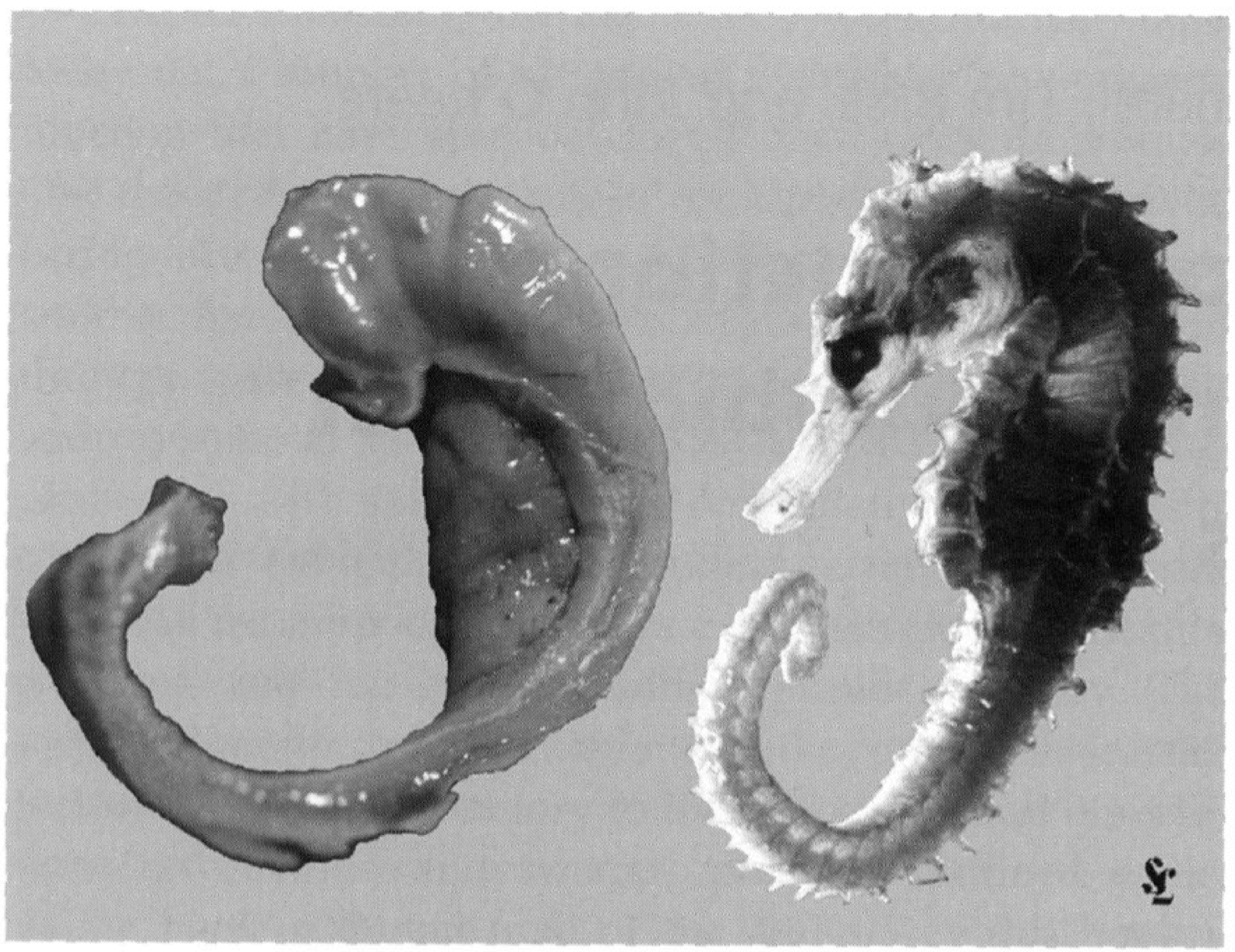
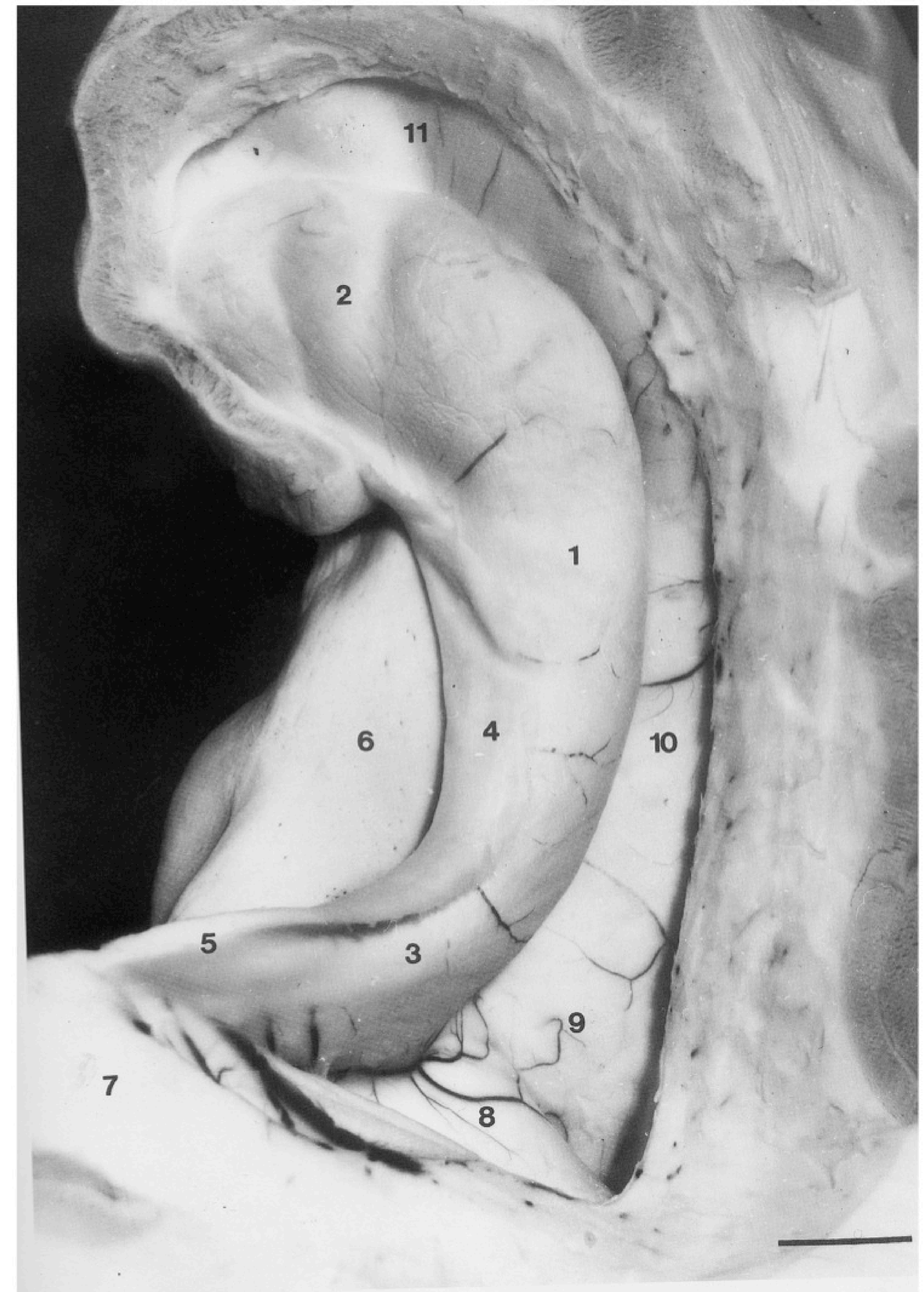
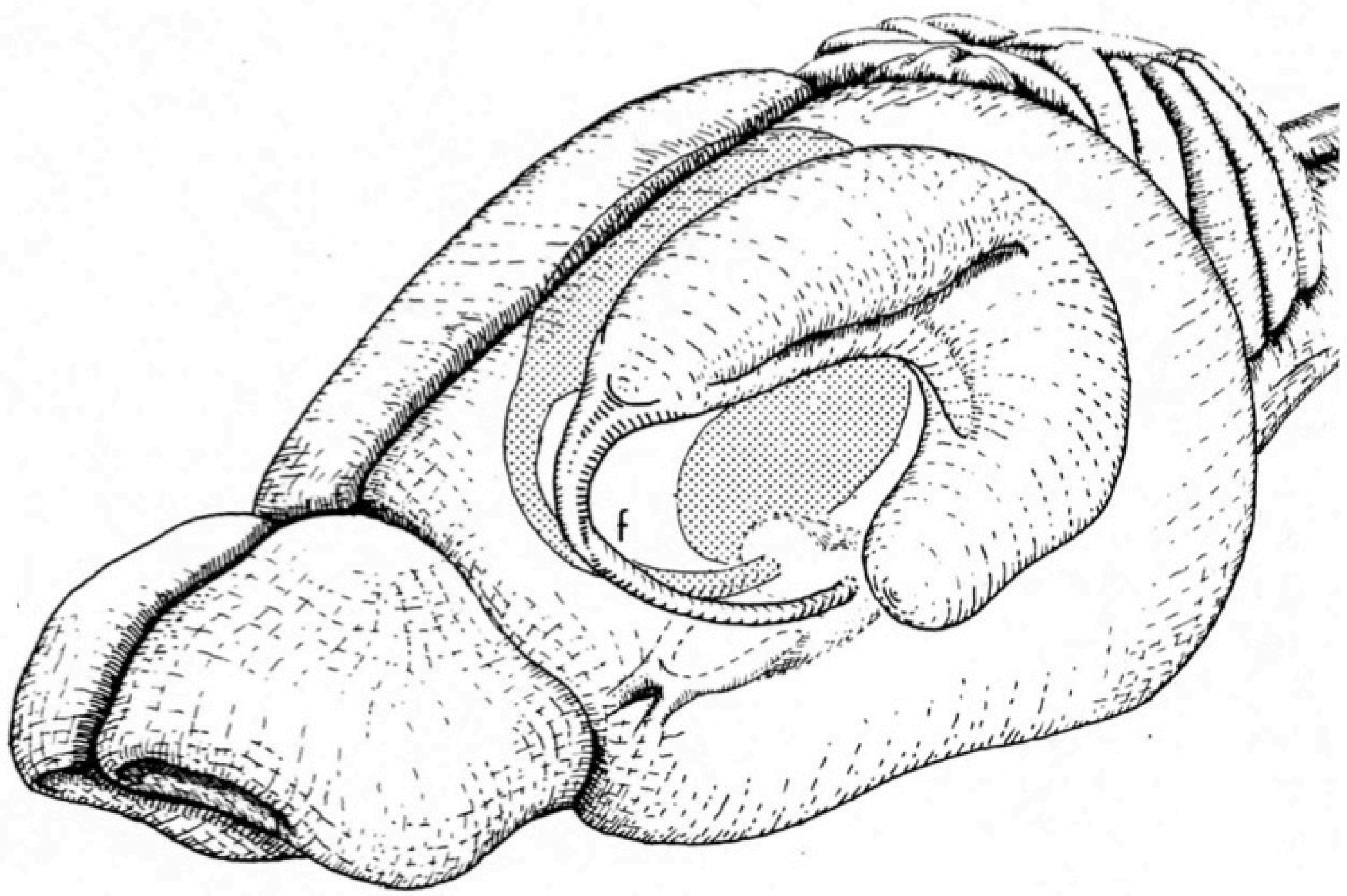
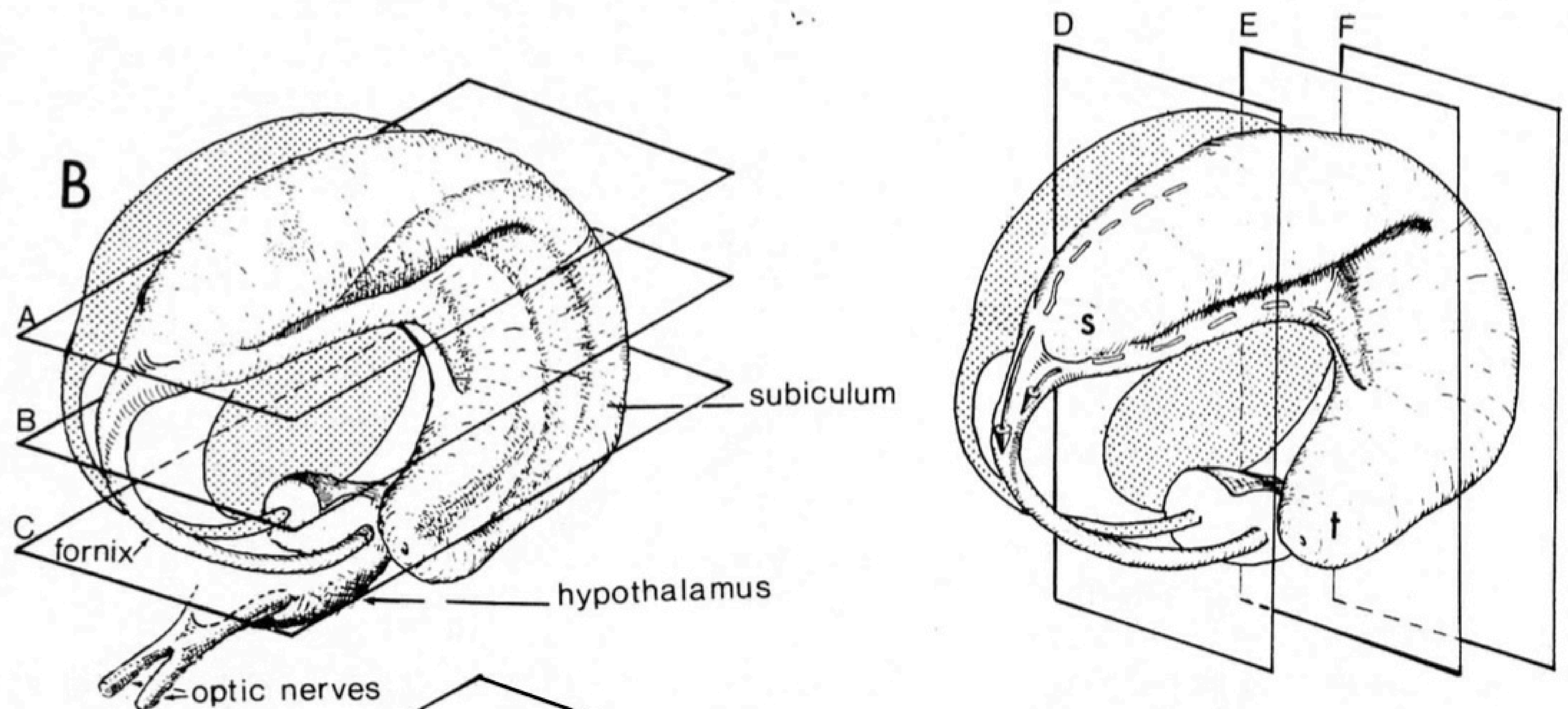


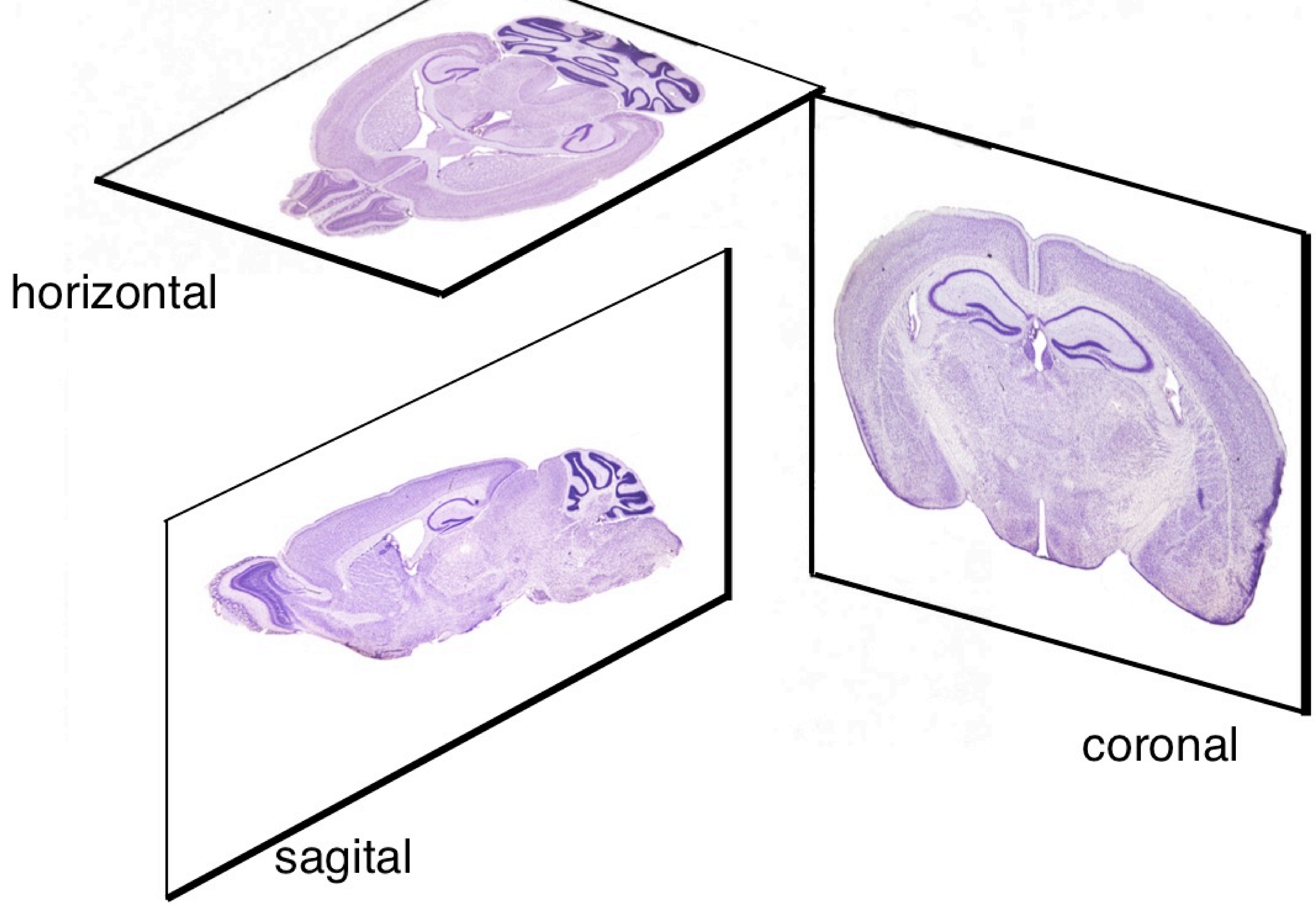
Figure 2–1. Human hippocampus dissected free (*left*) and compared to a specimen of *Hippocampus leria* (*right*). (*Source:* Courtesy of Professor Laszlo Seress, University of Pecs.)



The human hippocampus, Henri M. Duvernoy.







Hippocampal formation,
Amaral and Witter,
in The Rat Nervous System,
Edited by Paxinos, 2001

Horizontal view

Sagital view

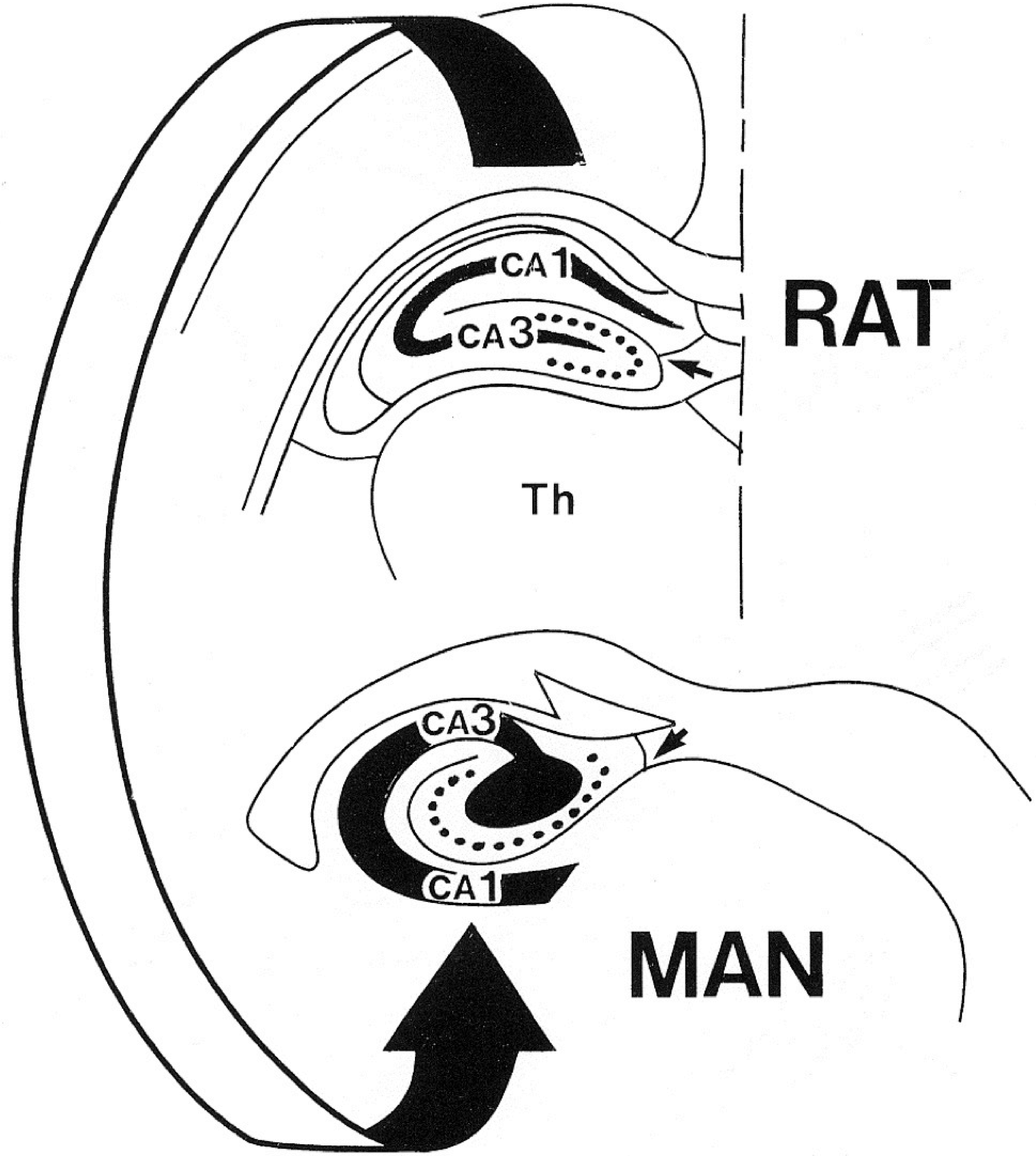


Fig. 6. Site of CA1 and CA3 in rats and humans (see p. 8). **Arrowheads** show the hippocampal sulcus. The **arrow** indicates the inversion of arrangements in the hippocampus in these two species
CA1, superior region; **CA3**, inferior region; **Th**, thalamus

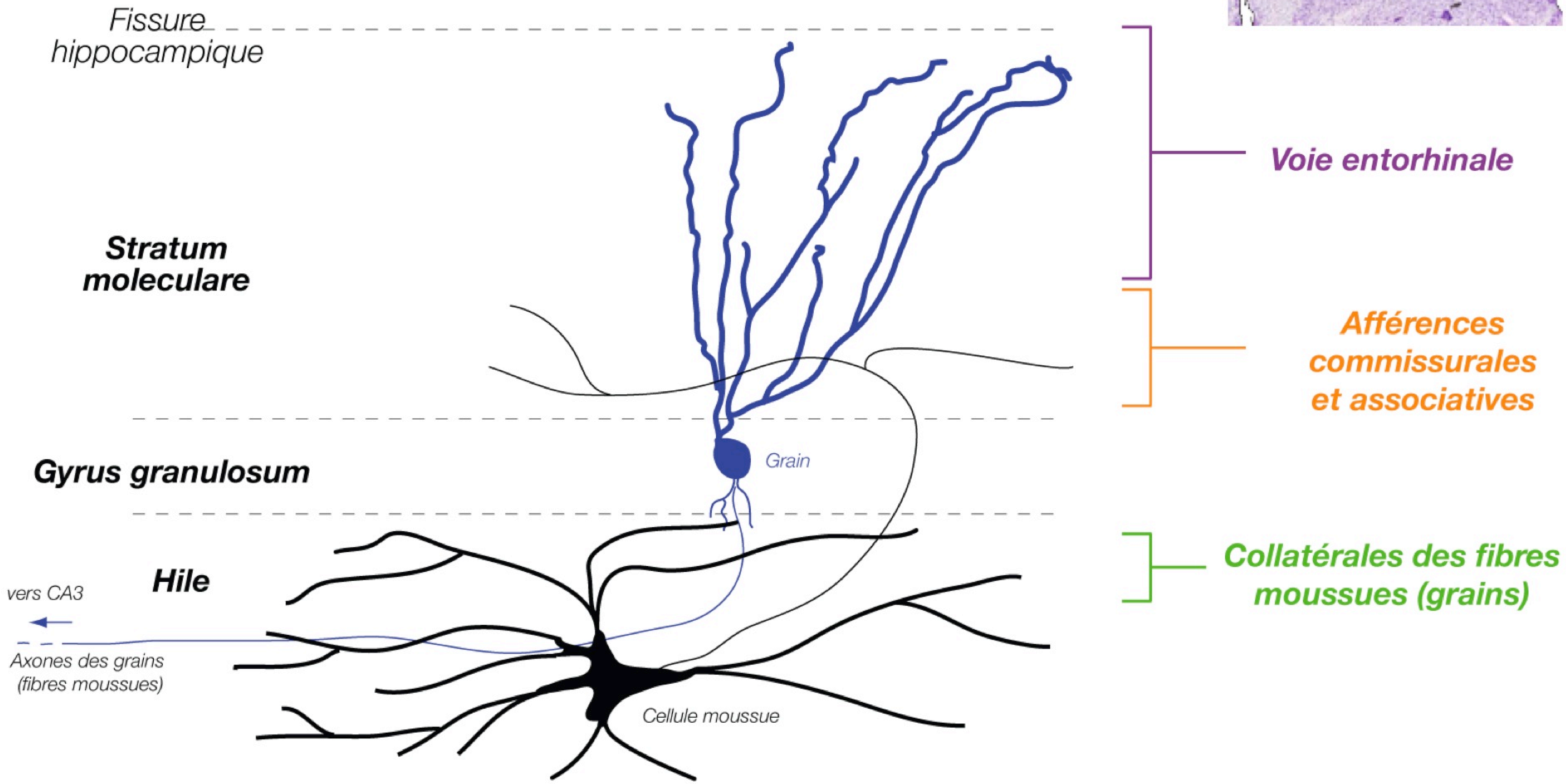
The human hippocampus, Henri M. Duvernoy.

Major excitatory connections in the rodent hippocampus : the tri-synaptic circuit.

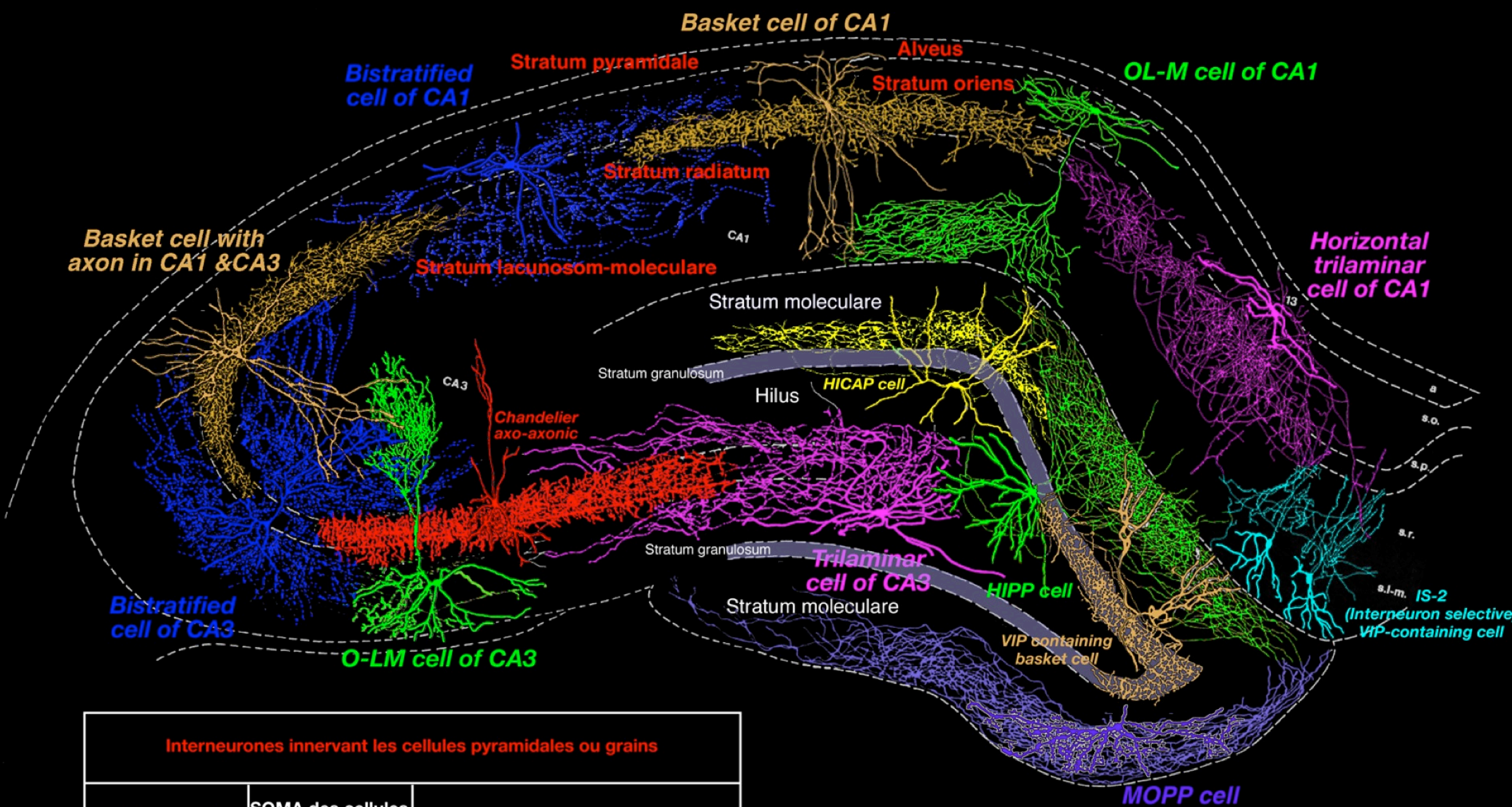
Danglot et al. (2006), Hippocampus 16: 1032-1060.

1. Perforant path : axons from the entorhinal cortex innervate granule cells.
2. The axons of the granule cells (mossy fibers) innervate pyramidal cells of CA3.
3. The axons of the pyramidal cells of CA3 (Schaffer collaterals) innervate pyramidal cells of CA1.
4. The axon of the pyramidal cells of CA1 innervate the subiculum and the entorhinal cortex.

Alveus
CA3
Axone
CA1
Collatérales récurrentes associatives
Stratum oriens
Collatérales récurrentes associatives (dans CA3)
Voie commissurale et associative
Voie commissurale et associative
Stratum pyramidale
Collatérales de schaffer (dans CA1)
Stratum lucidum uniquement dans CA3
Fibres moussues (dans CA3)
Stratum radiatum
Voie commissurale
Voie commissurale et associative
Stratum lacunosum moleculare
Voie entorhinale
Fissure hippocampique
100 um


Fissure hippocampique
Stratum moleculare
Gyrus granulosum
Hile
vers CA3
Axones des grains (fibres moussues)
Grain
Cellule moussue
Voie entorhinale
Afférences commissurales et associatives
Collatérales des fibres moussues (grains)

Classification des interneurones hippocampiques



Interneurones innervant les cellules pyramidales ou grains		
Cellule en panier	SOMA des cellules excitatrices	Parvalbumine
Chandelier	Segment initial de l'axone	
Bistratifiée Trilaminaire HICAP MOPP	Dendrites proximales (sr et so)	Calbindine
O-LM LM HIPP	Dendrites distales (slm)	Somatostatine, NPY

Interneurones innervant les interneurones		
IS-1, IS-2, IS-3	Dendrites	Calrétinine

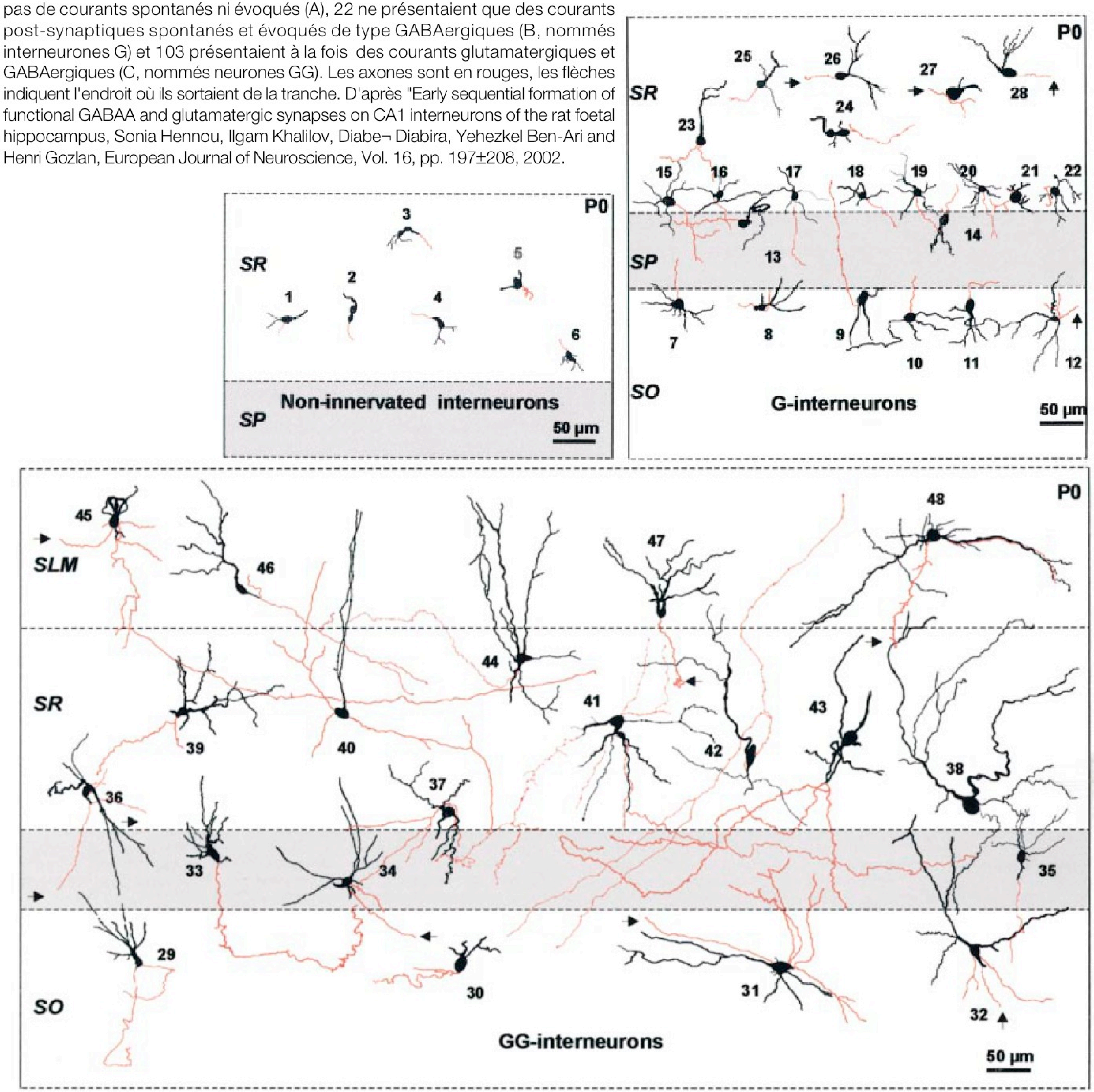
Modifié d' après Freund et al., Hippocampus,1996.

GABAergic afferences on hippocampal pyramidal cells.

Danglot et al. (2006), Hippocampus 16: 1032-1060.

Figure 52 | **Principaux ÈvËnements dans le dÈveloppement du rÈseau hippocampique chez le rat et la souris.**
Les dates indiquÈes dans les panneaux correspondent au dÈveloppement chez le rat. La correspondance entre les stades dÈveloppementaux du rat et de la souris sont indiquÈs sur l'echelle de temps.
Les donnÈes concernant la neurogenËse des cellules principales sont issues de Altman et Bayer (J. Comp. Neurol. (1990), 301: 325-342, 343-364, 365-381). Les donnÈes concernant la neurogenËse des interneurones GABAergiques sont issues de Soriano E. et al. (J. Comp. Neurol. (1989), 281: 586-602 603-611). Les donnÈes concernant l'apparition de l'immunorÈactivitÈ GAD et de l'apparition des synapses inhibitrices dans le stratum moleculare sont issues de Marty et al. 2002, Eur J Neurosci 15: 233-245; Rozenberg et al., 1989, Dev Br Res 50: 177-187; Dupuy et al., 1996, J Neurosci, 16:6919-6932). Les donnÈes concernant l'apparition des microglies sont issues de DALMAU I et al. (J. Comp Neurol (1997), 377:70ñ84) et celles des astrocytes et des microglies sont issues de Sauvageaot et al. (Curr Opin Neurobiol 12: 244-249). Les donnÈes concernant la croissance dendritique et des Èpines sont issues de Ben-Ari Y. (Tins (2001), 24: 353-360), celle du switch du chlore de Ben-Ari Y. et (Nat. rev. Neurosci. (2002), 3-728-739).

Figure 54 | **Interneurones dans la région CA1 de l'hippocampe de rat à P0.** Les interneurones ont été enregistrés électrophysiologiquement puis injectés à la biocytine, avant d'établir leur abre neuritique. Sur 131 neurones, 6 ne présentaient pas de courants spontanés ni évoqués (A), 22 ne présentaient que des courants post-synaptiques spontanés et évoqués de type GABAergiques (B, nommés interneurones G) et 103 présentaient à la fois des courants glutamatergiques et GABAergiques (C, nommés neurones GG). Les axones sont en rouges, les flèches indiquent l'endroit où ils sortaient de la tranche. D'après "Early sequential formation of functional GABAA and glutamatergic synapses on CA1 interneurons of the rat foetal hippocampus, Sonia Hennou, Ilgam Khalilov, Diabe¬ Diabira, Yehezkel Ben-Ari and Henri Gozlan, European Journal of Neuroscience, Vol. 16, pp. 197±208, 2002.



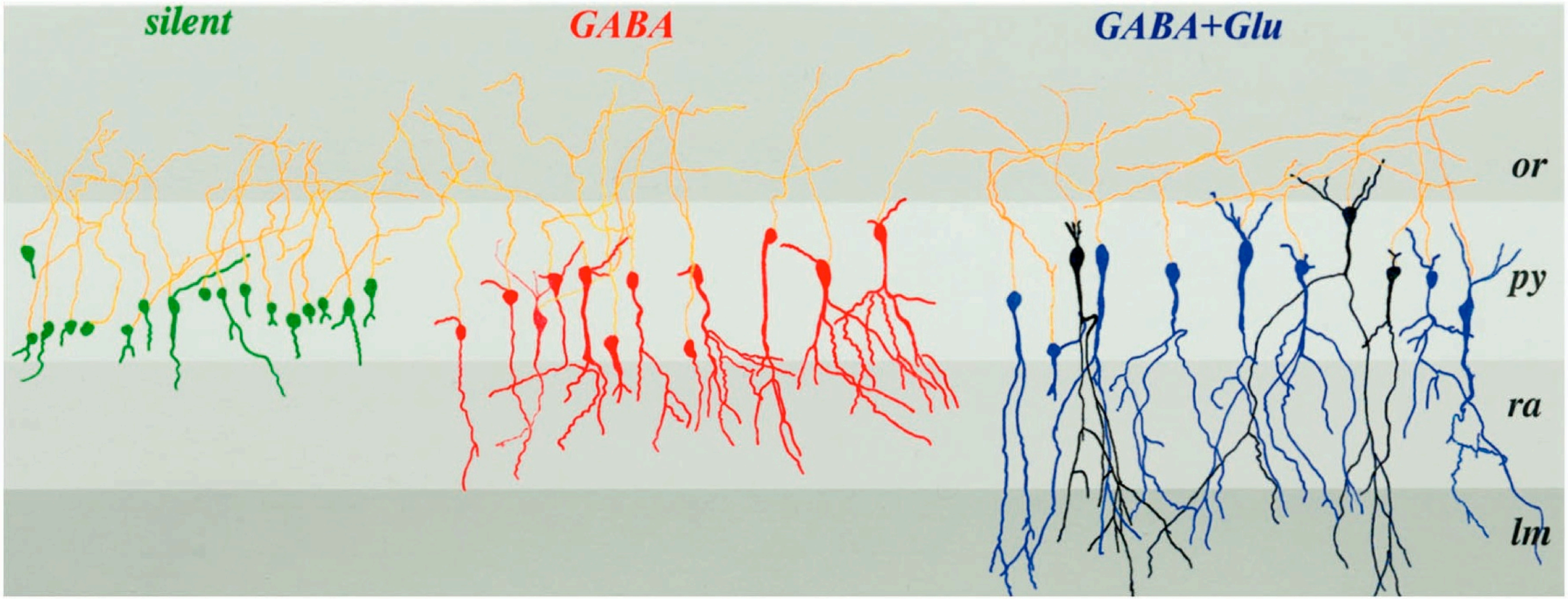


Figure 53 | **Morpholgie des cellules pyramidales dans la région CA1 de l'hippocampe de rat à P0.**
Les cellules pyramidales ont été enregistrées électrophysiologiquement puis injectées à la biocytine, avant d'établir leur arbre neuritique. Sur 60 neurones, 46 ne présentaient pas de courants spontanés ni évoqués (en vert), 8 ne présentaient que des courants post-synaptiques spontanés et évoqués de type GABAergiques (en rouge) et 5 présentaient à la fois des courants glutamatergiques et GABAergiques (en bleu : GABA +NMDA; en noir: GABA+NMDA+AMPA).
Les auteurs indiquent que les neurones recevant uniquement des courants GABA, ne diffèrent de ceux recevant des courants glutamatergiques que par la présence d'un dendrite apical dans le stratum lacunosum moleculare.
D'après "The Establishment of GABAergic and Glutamatergic Synapses on CA1 Pyramidal Neurons is Sequential and Correlates with the Development

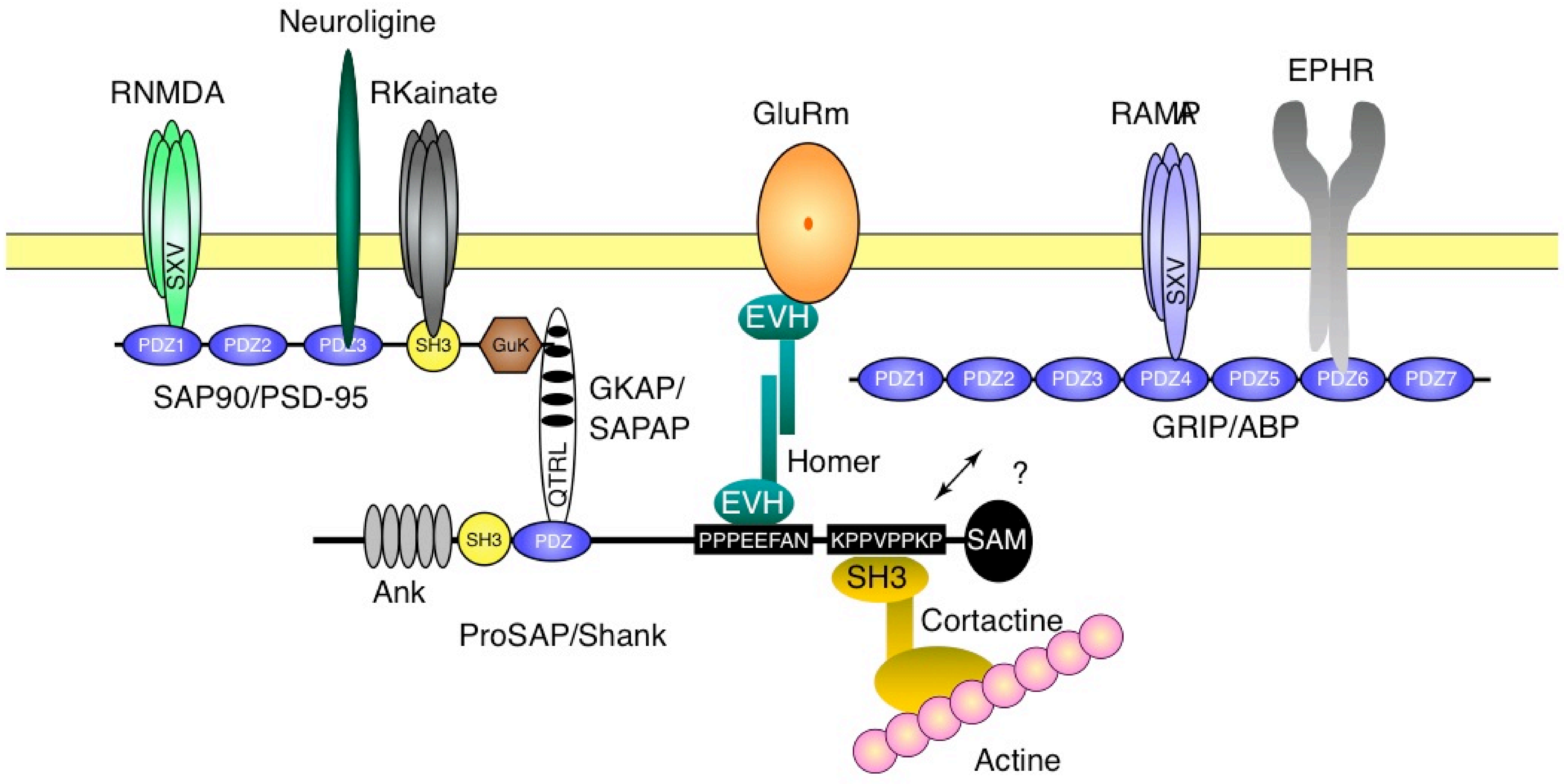


Figure 20 | **Interactions protéine-protéine entre les protéines synaptiques de la densité post-synaptique .**
Le regroupement et la localisation postsynaptique des récepteurs au glutamate sont médiés par différentes protéines-adaptateur. Le membre le mieux caractérisé est PSD95 de la famille des MAGUK. Le domaine PDZ de PSD95 interagit avec la portion C-terminale du RNMDA, et avec la molécule d'adhésion neuroligine. Les récepteurs kaïnate interagissent avec le domaine SH3 de PSD 95. Le domaine GUK de PSD 95 fournit un lien avec le cytosquelette post-synaptique via GKAP. A son tour, GKAP lie domaine C-terminal de Shank. Sjhank interagit avec le domaine EVH de Homer, qui est une protéine de liaison des récepteurs métabotropiques au glutamate (RGlum), et avec le domaine SH3 de la cortactine, une protéine liant l'actine. Un composant majeur de la PSD est la protéine GRIP/ABP qui contient sept domaines PDZ et interagit à la fois avec les RAMPA et les récepteurs aux éphrines (EPHR).
D'après Garner et al. (2000), Trends in Cell Biology, 10: 274-280.

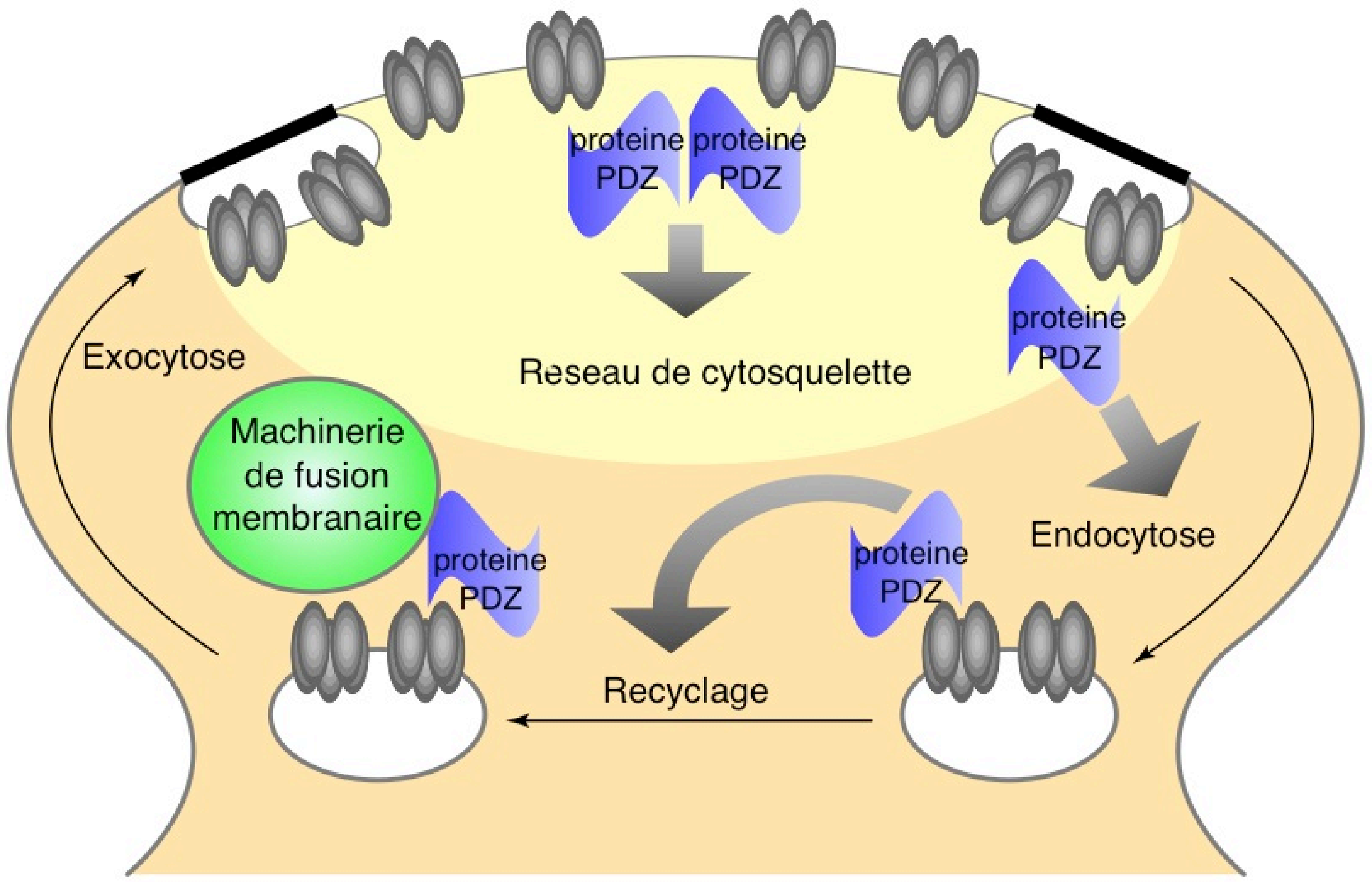


Figure 21 | **Mécanismes impliqués dans le recyclage des récepteurs à la membrane post-synaptique.**
Les protéines PDZ fournissent un cytosquelette pour la localisation, le regroupement et la maintenance des récepteurs au glutamate dans la densité post-synaptique . Des données convergentes suggèrent que les protéines à domaines PDZ participent aussi au trafic et au recyclage des récepteurs entre la membrane plasmique et les pools endosomaux.
D'après Garner et al. (2000), Trends in Cell Biology, 10: 274-280.

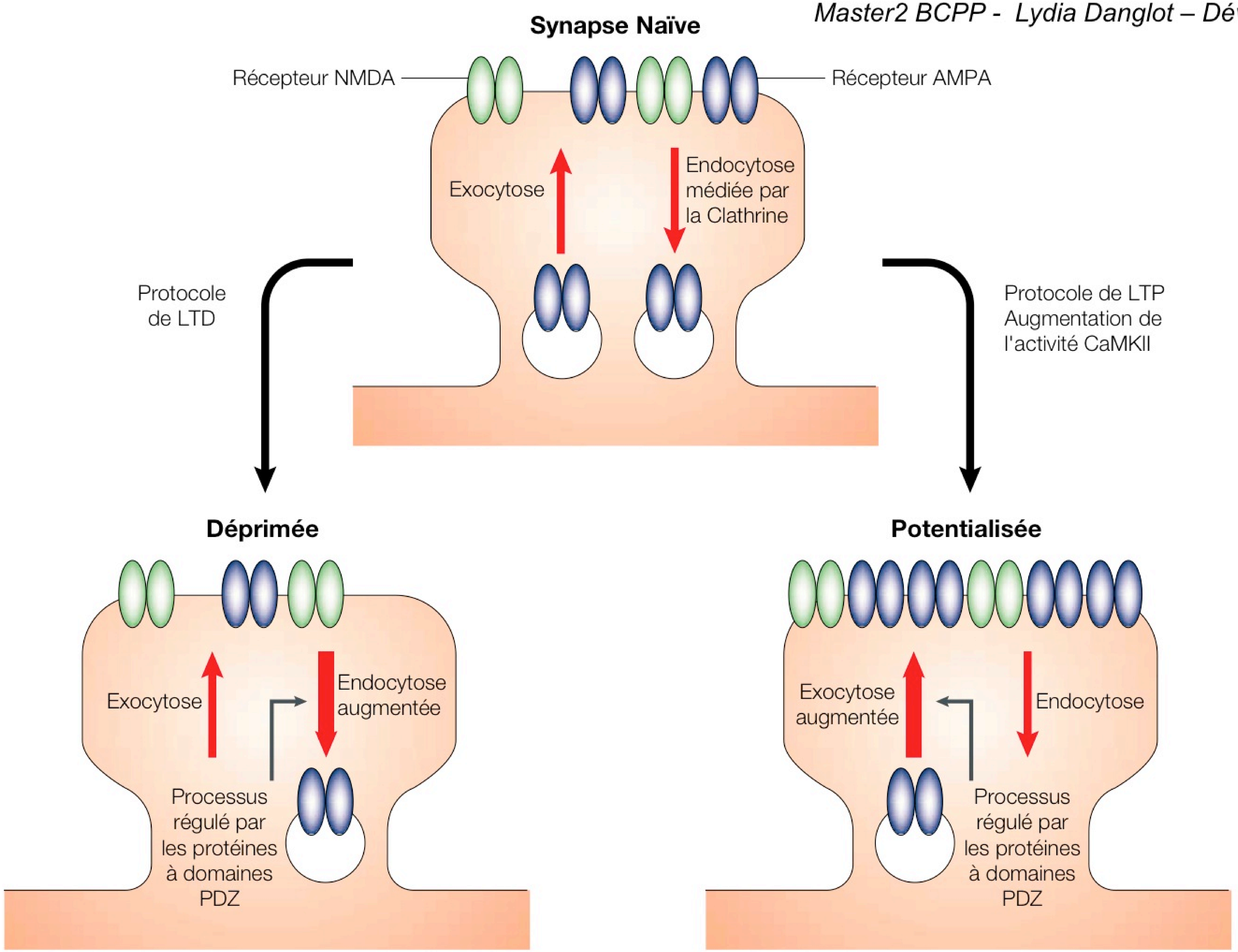
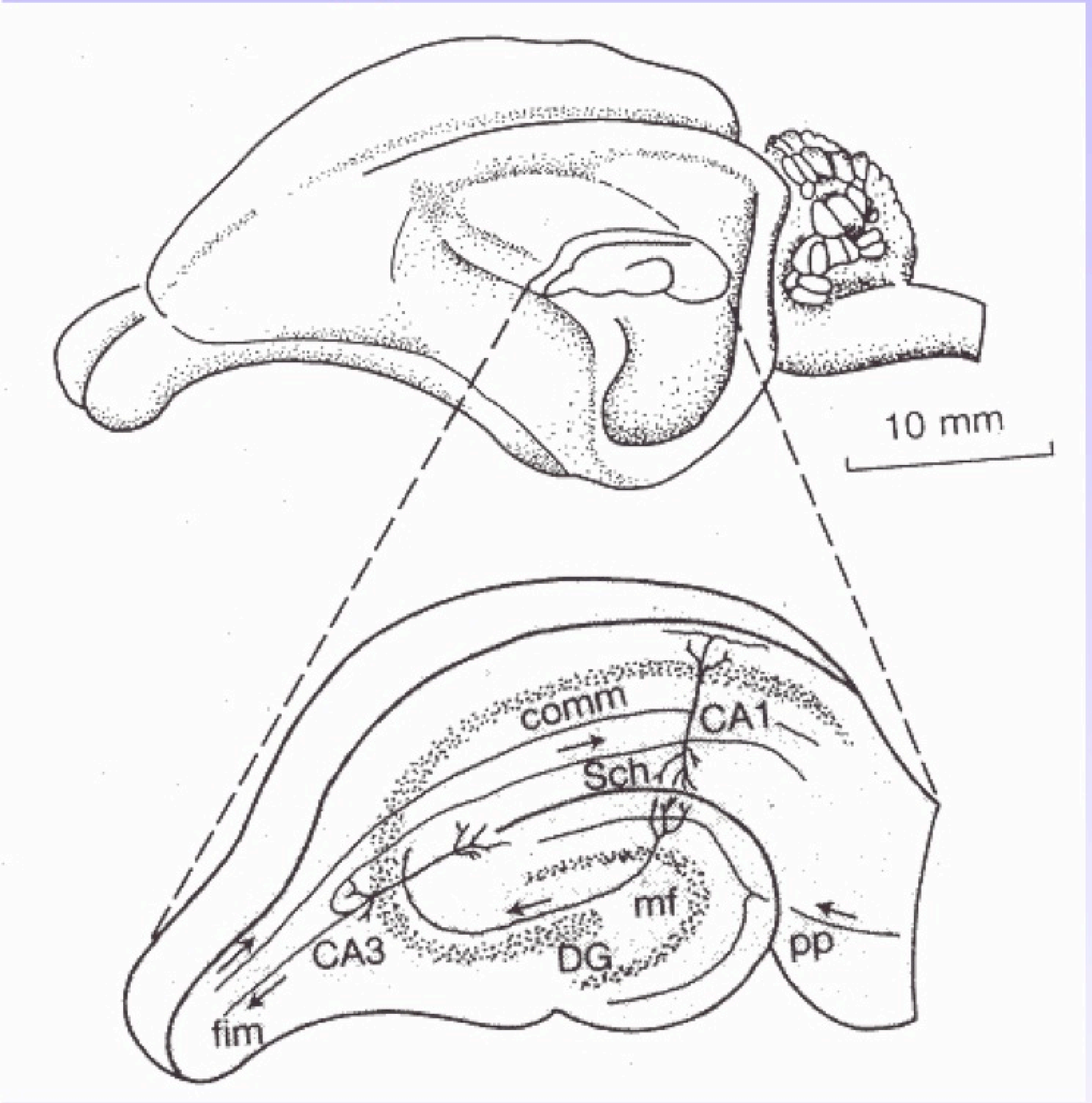


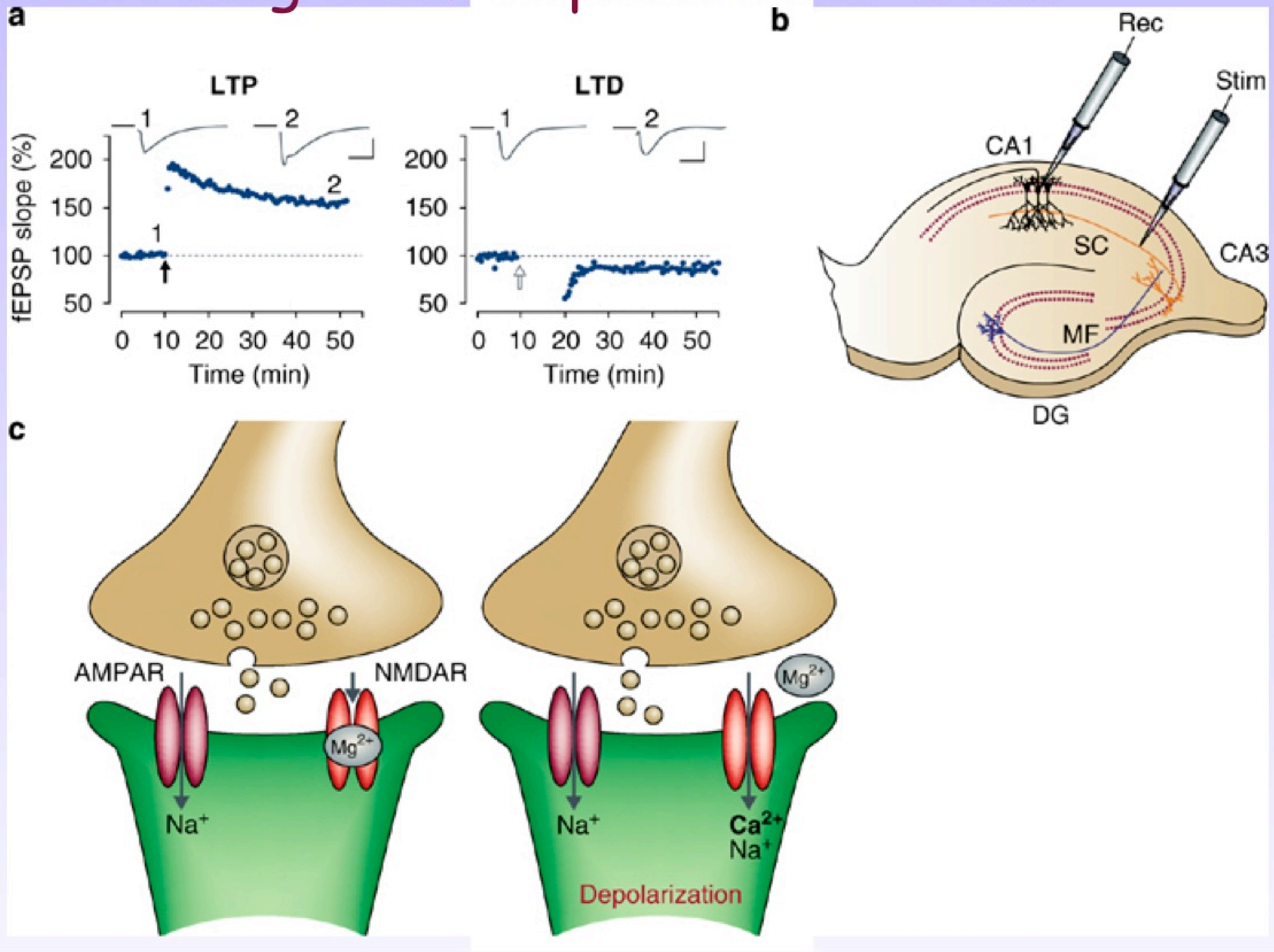
Figure 16 | **Régulation dynamique de la structure post-synaptique.**
Ce diagramme montre les régulations dynamiques des récepteurs au glutamate à la membrane post-synaptique. La modulation de l'efficacité synaptique semble être due à un mécanisme régulé de l'expression en surface des RAMPA. Les protocoles qui induisent la LTP (Long Term Potentiaton) semblent augmenter le niveau de RAMPA. Inversement, les protocoles de LTD (Long Term Depression) résultent en une baisse de récepteurs AMPA synaptiques. La régulation de la densité des RAMPA au niveau de la membrane plasmique s'effectue par l'intermédiaire de plusieurs étapes au cours du trafic du récepteur: depuis son insertion et son endocytose, jusqu'à sa stabilisation. De récentes études ont montré que chaque processus est régulé par des interactions avec des protéines à domaines PDZ.
D'après Robert H. Scannevin and Richard L.Huganir (Nat Rev Neurosci, 2000, 1: 133-141).

Synaptic Plasticity: a Cellular Model of Memory?

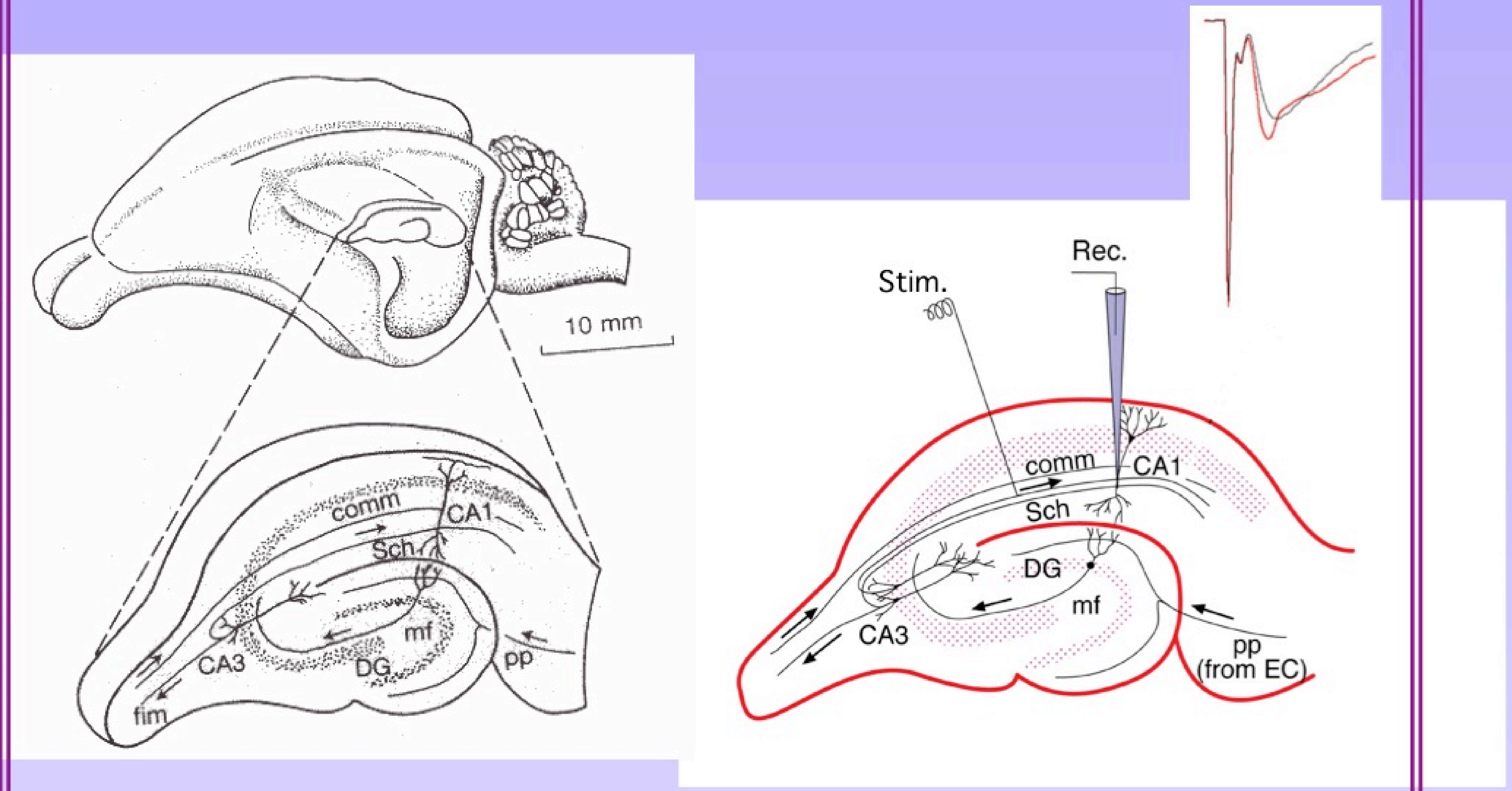
- Memory formation might be due to a persistent increase in synaptic strength, called long-term potentiation or LTP.

- The opposite mechanism of the LTP: long-term depression or LTD.

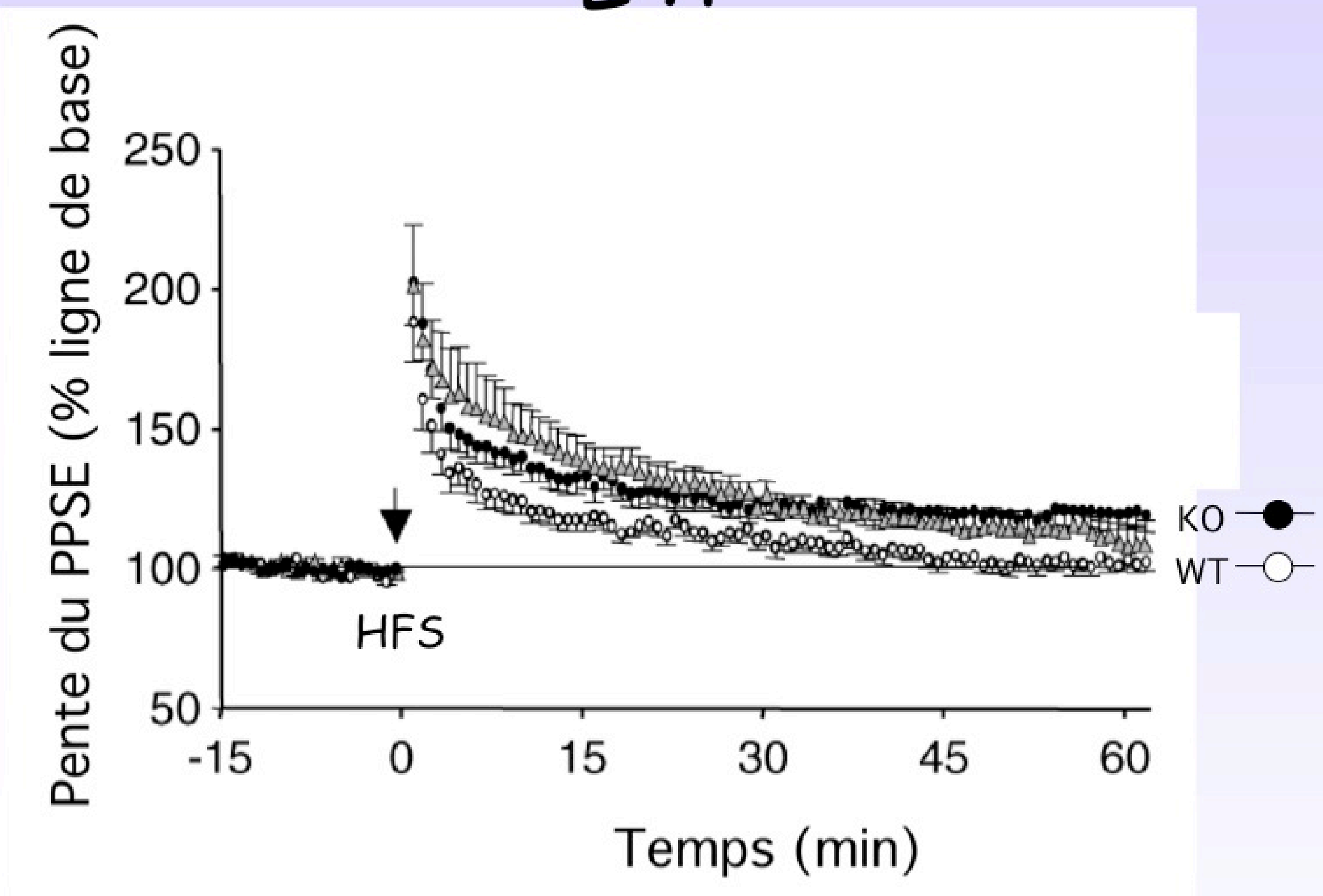




Long-Term Potentiation: LTP



LTP

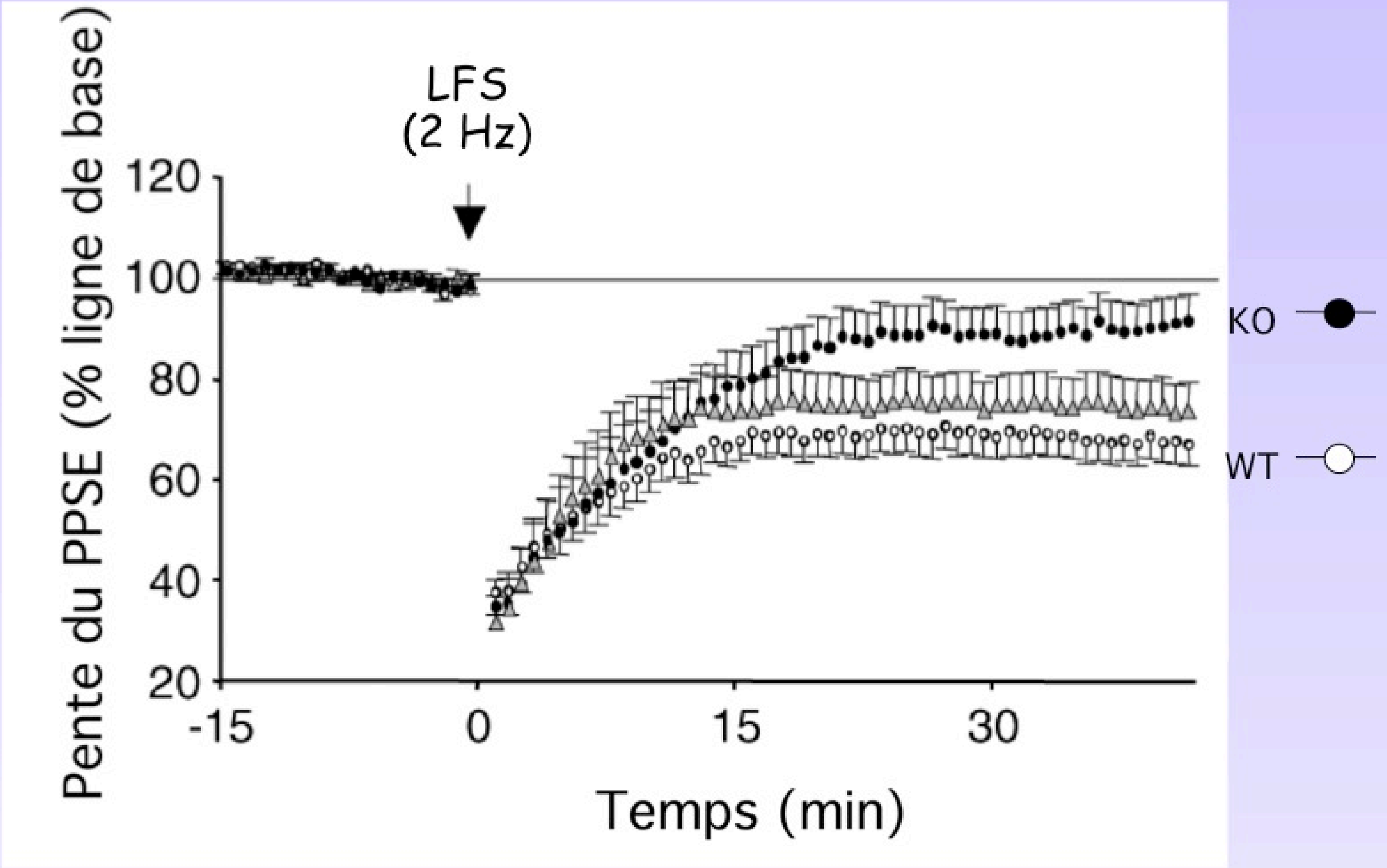


➡ Enhanced LTP in DAT-/- mice

(Morice *et al.* 2007)

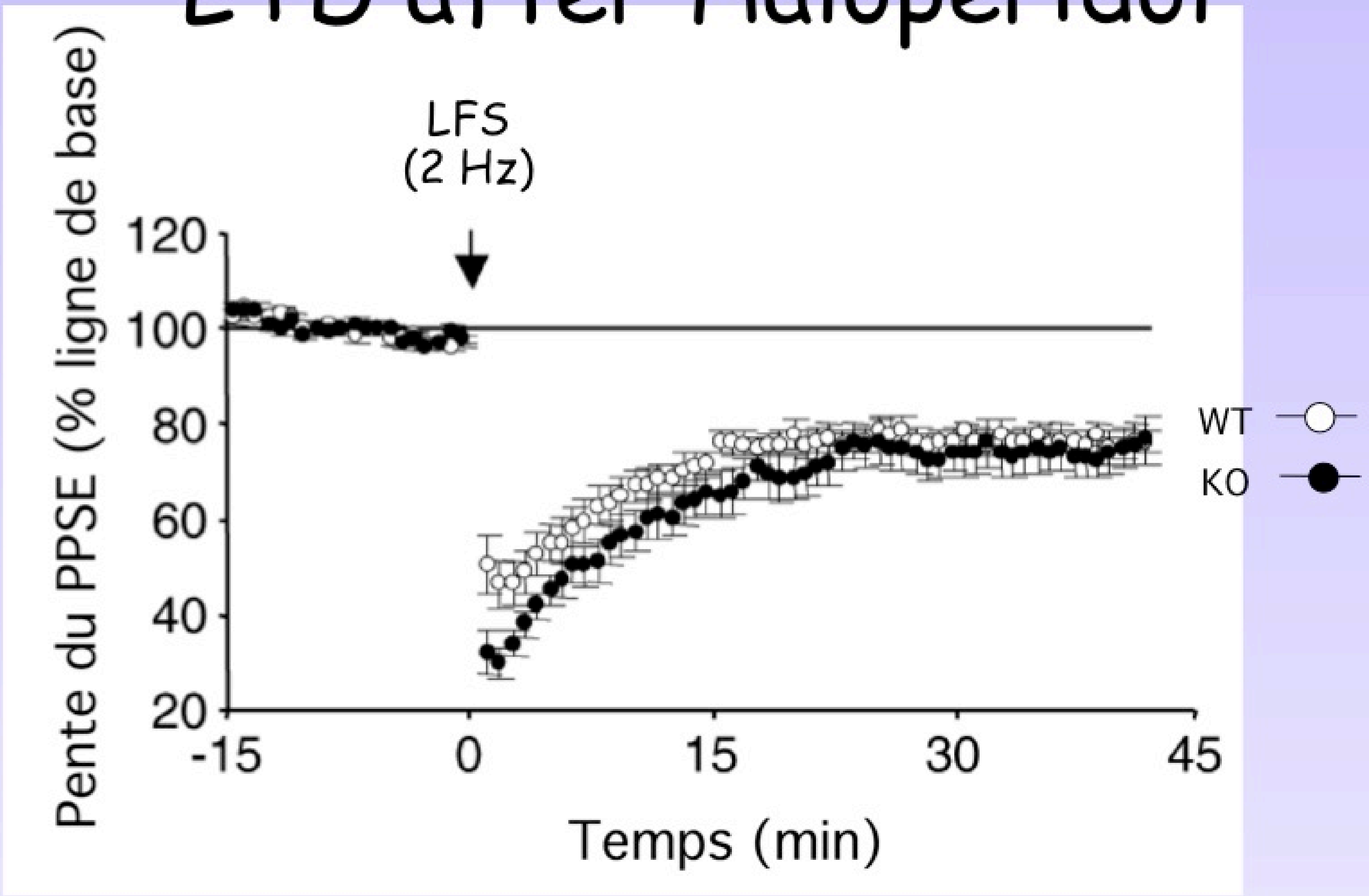
Long-Term Depression: LTD

LTD



➜ LTD Deficit in DAT-/- mice

LTD after Haloperidol

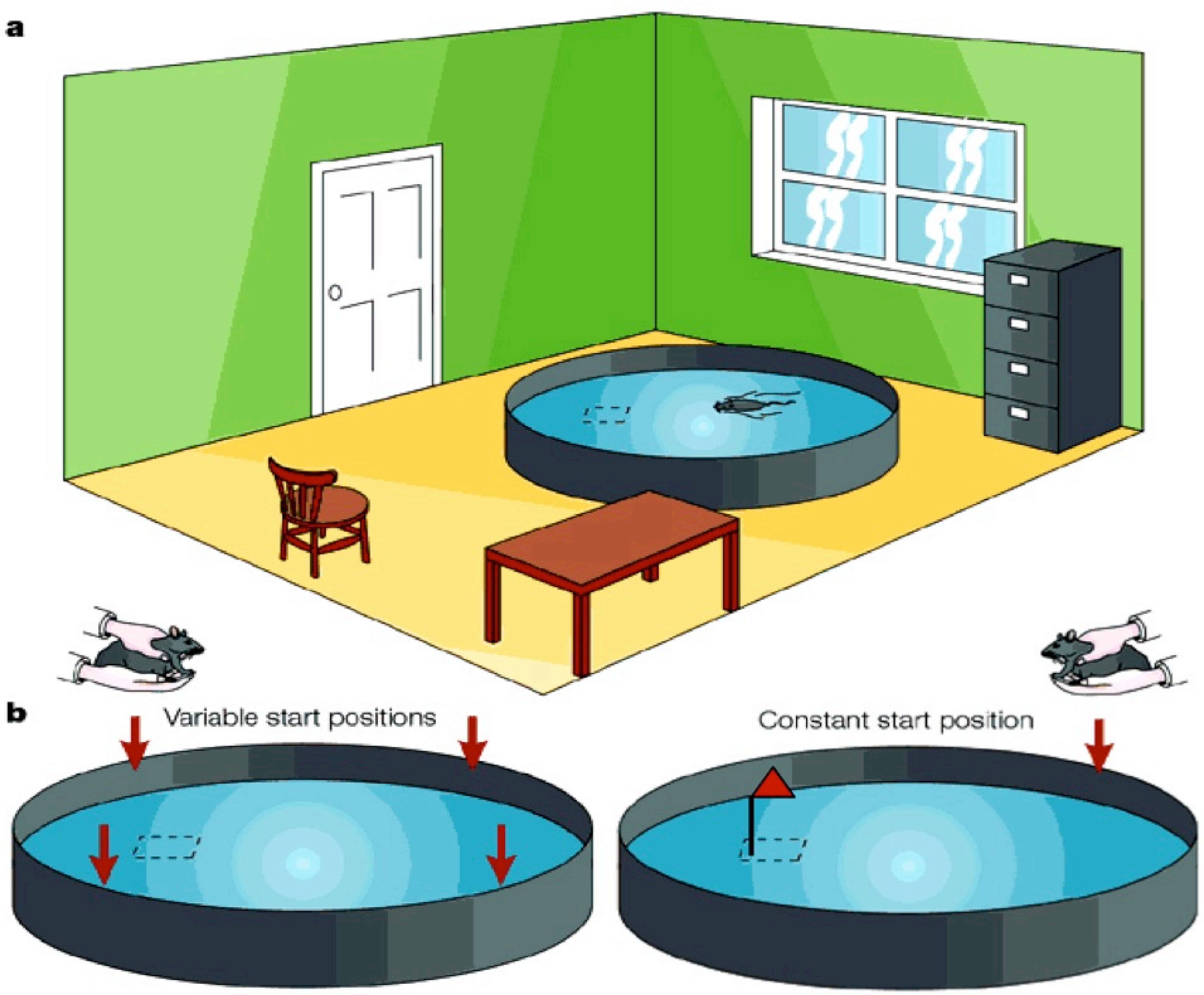


➜ Haloperidol fully restores LTD

Haloperidol possesses a strong activity against delusions and hallucinations, most likely due to an effective dopaminergic receptor blockage in the mesocortex and the limbic system of the brain.

(Morice *et al.* 2007)

Piscine de Morris (Morris Water Maze)



SPATIAL VERSION
Platform: fixed position

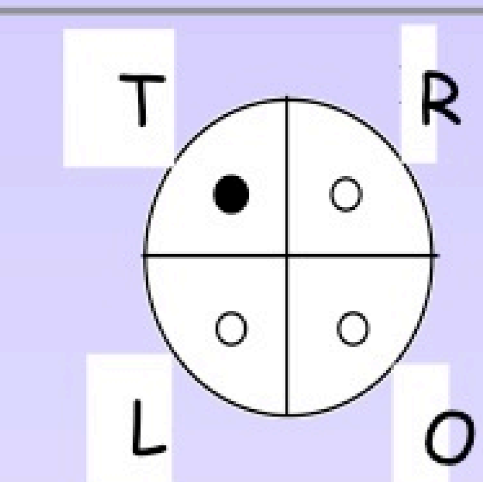
CUED VERSION
Platform: variable position

ACQUISITION TRIALS

Duration of test: 90 sec
Variables:

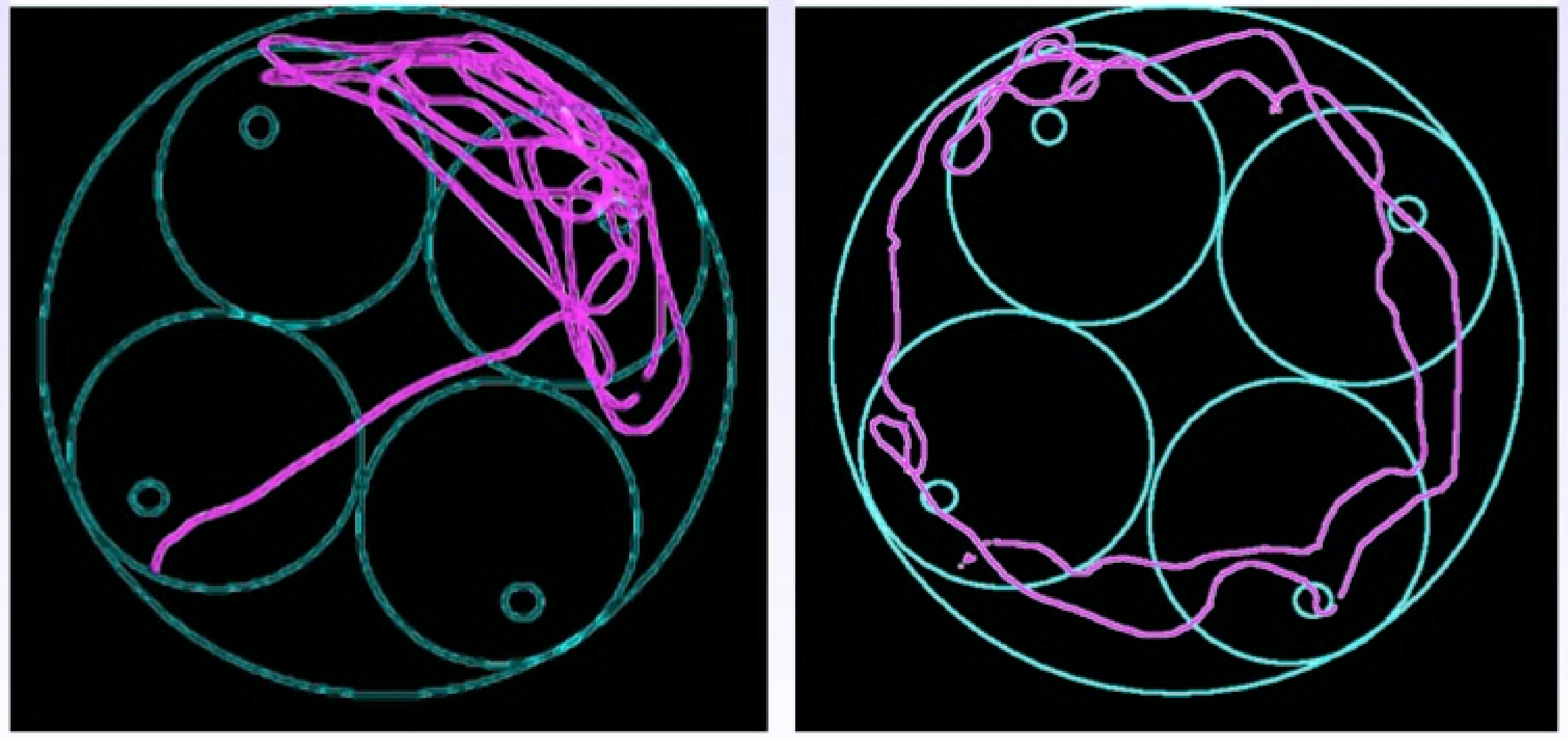
- **Inactivity** (s)
- **Swim speed** (cm/min)
- **Latency** (s)
- **Distance** travelled (cm)
- **Successful trials** (proportion)

PROBE TRIAL



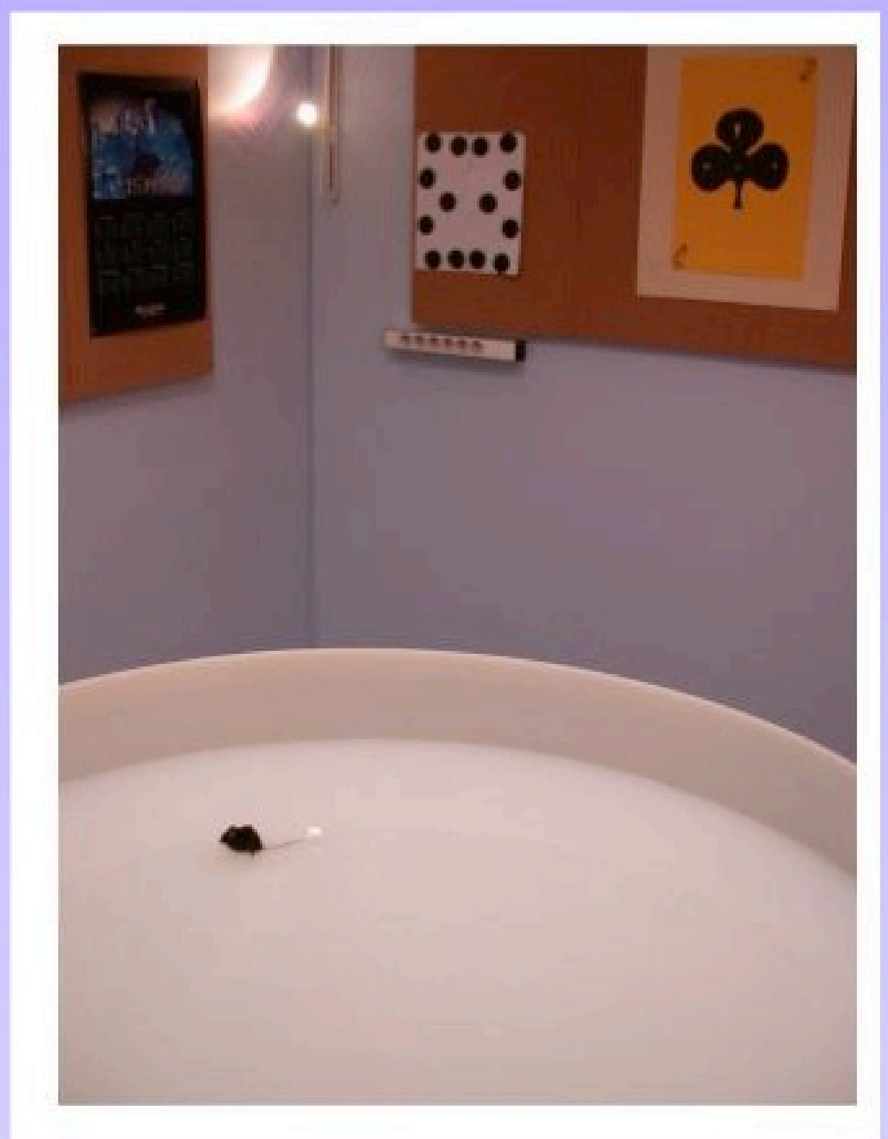
Duration of test: 60 sec
Variables:

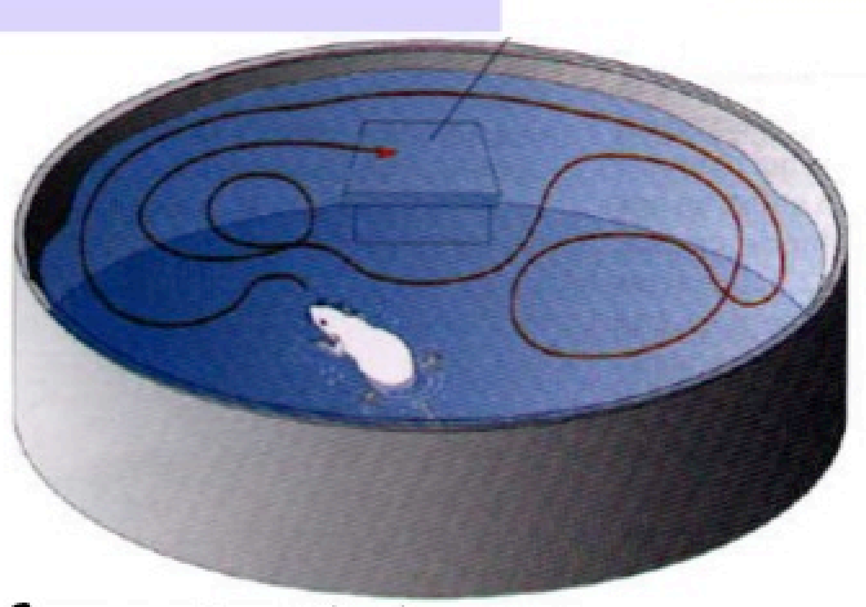
- **Distance travelled (%)** per quadrant
- **Number of annulus crossings**



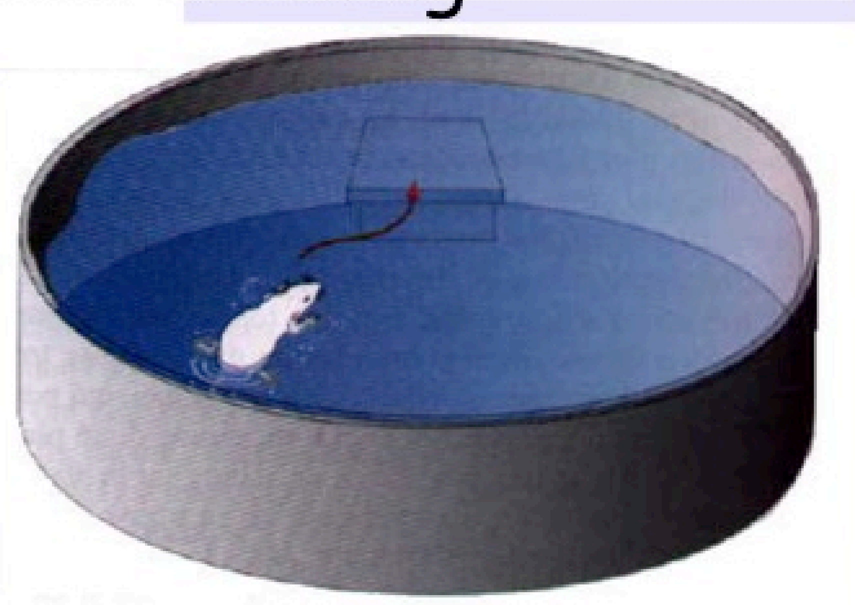
Piscine de Morris (Morris Water Maze)

Spatial Learning and Memory





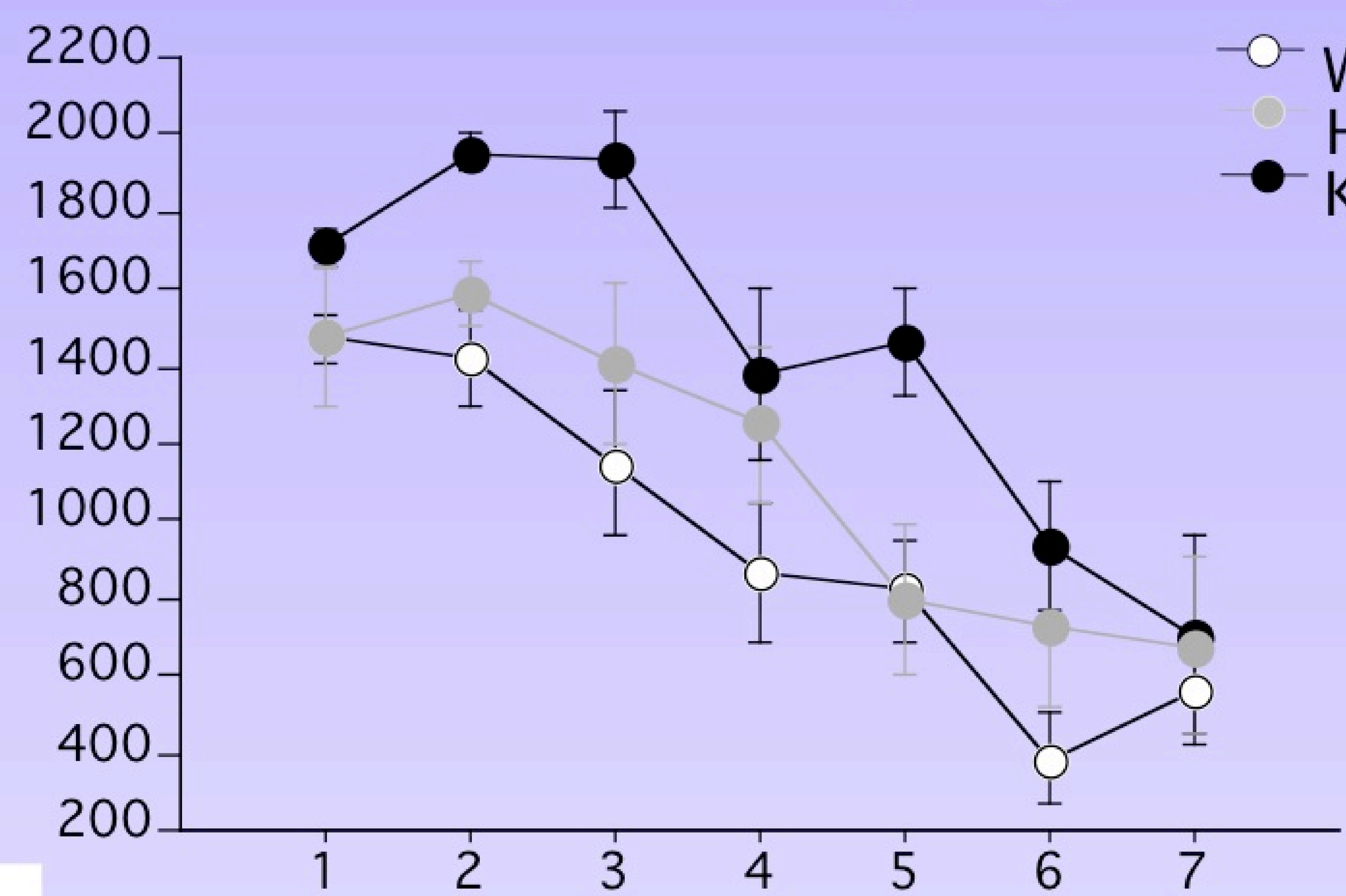
Before training



After training

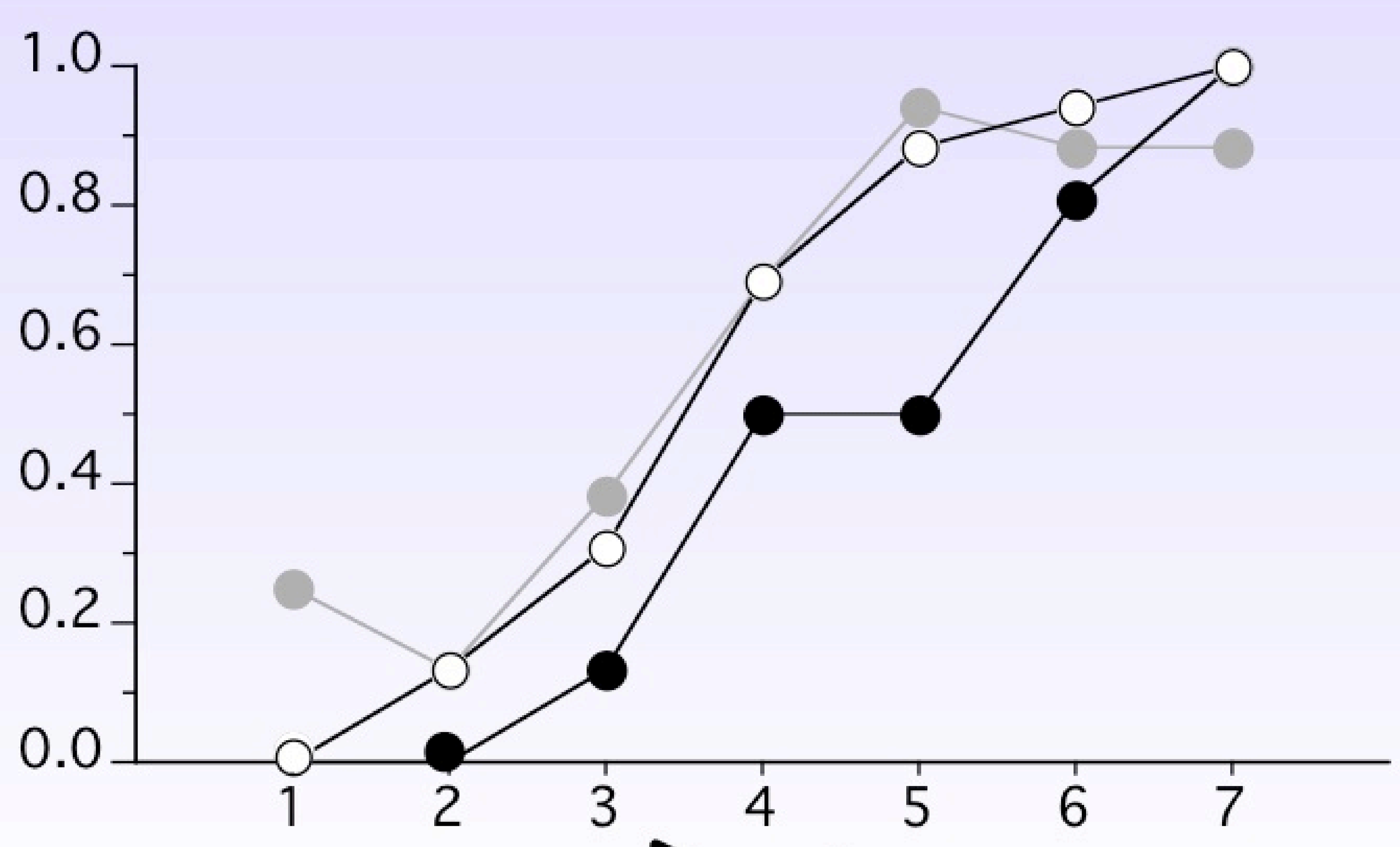
Acquisition trials

Distance (cm)



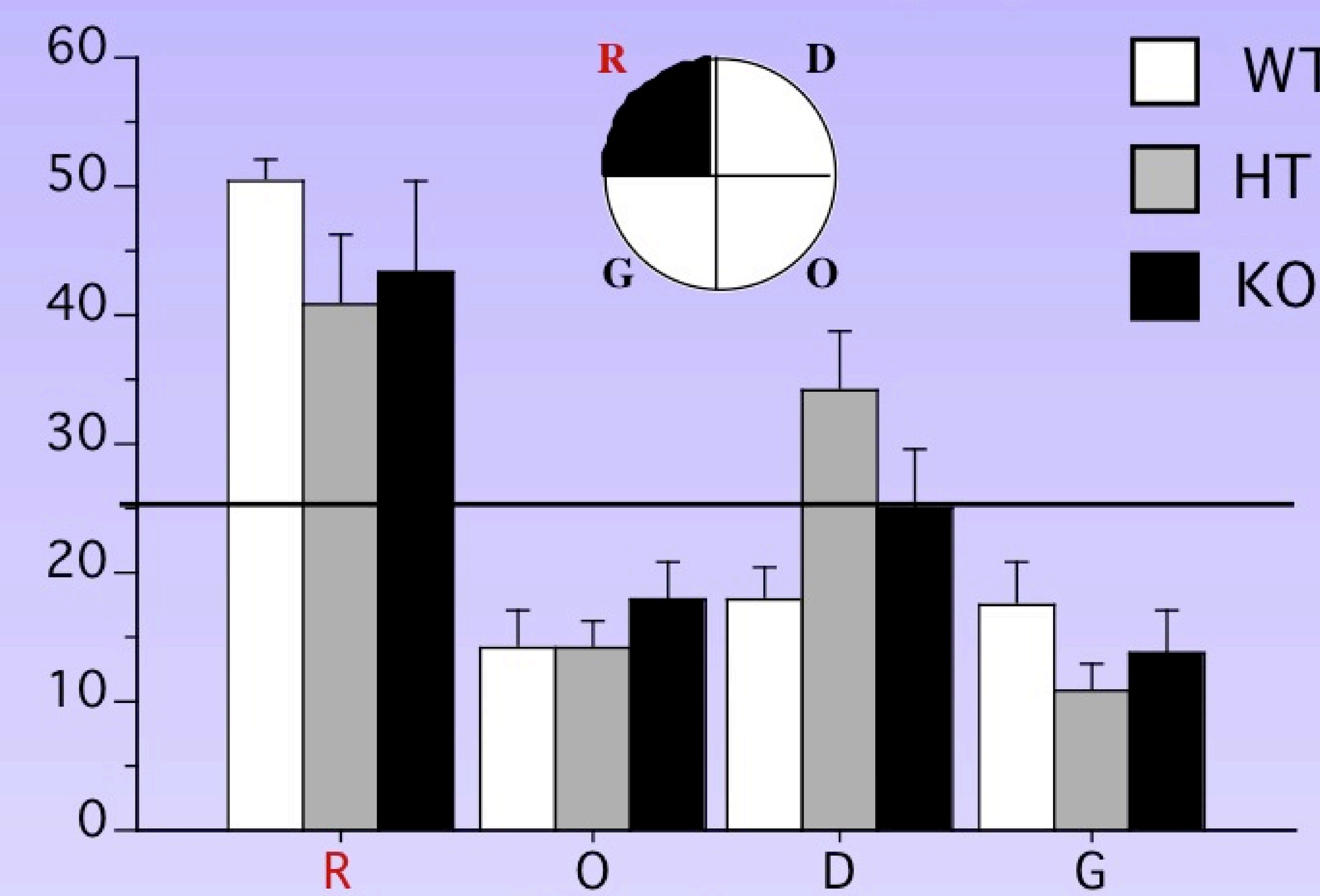
Successfull trials

(proportion)

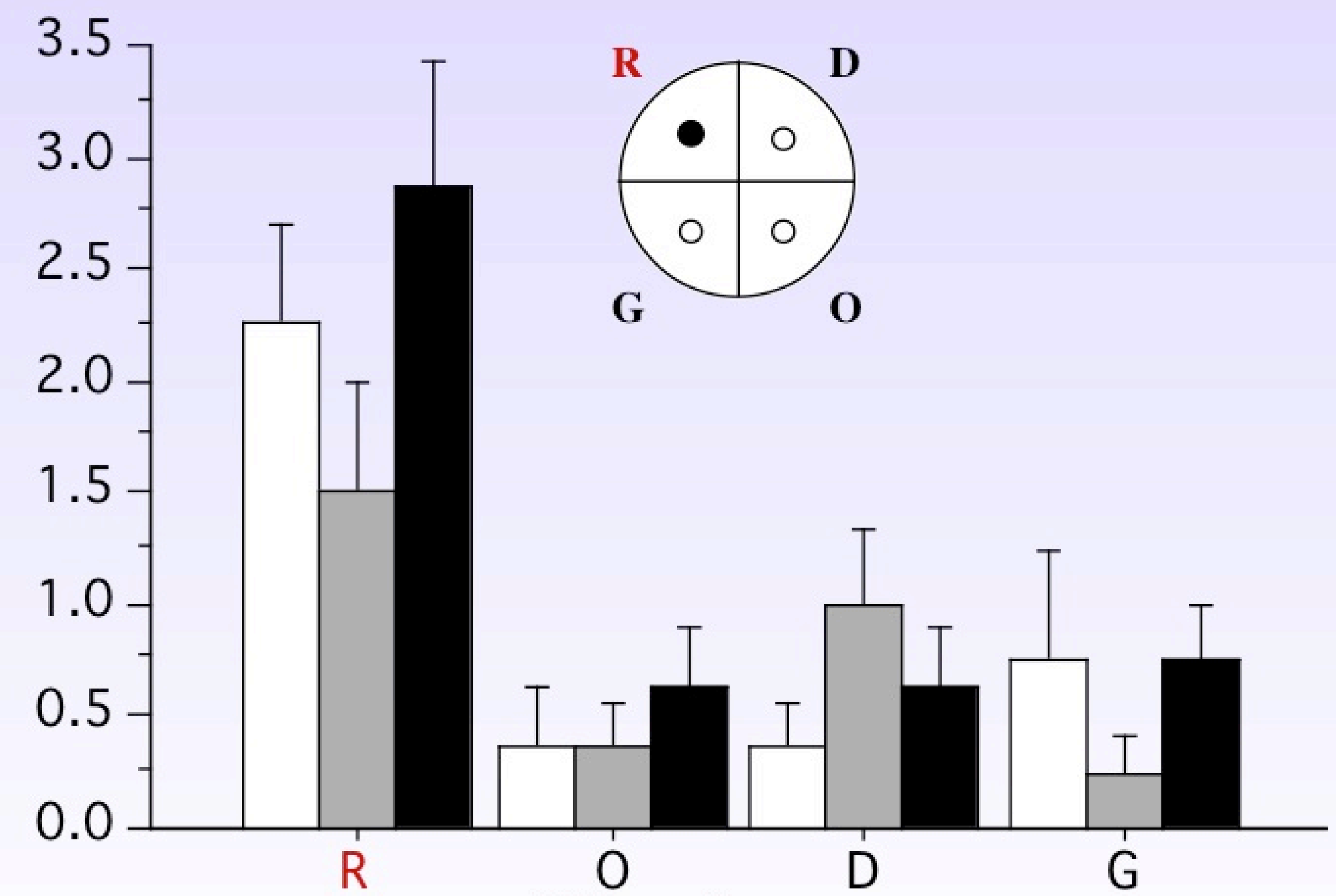


Probe trial

Distance (%)



Number of annulus crossings



(Morice *et al.* 2007)

Formation et développement de la synapse

1. **Le neurone: unité fonctionnelle polarisée**
2. **La croissance neuritique**
3. **Transport sélectif ou rétention**
4. **Le recyclage des vésicules synaptiques**
5. **Le cycle des vésicules synaptiques**
   - Voie lente: endocytose médiée par la clathrine
   - Voie courte: kiss and run
   - Les différents « pool » vésiculaires
6. **Comment mesurer l'exocytose ?**
   - Capacitance
   - Ampérométrie
   - GFP pH sensible: la Phluorin
   - Utilisation des SNARE inversée
7. **Comment mesurer le recyclage ?**
   - Utilisation des anti-synaptotagmine
   - Sondes fluorescentes de type FM
8. **Formation de la Synapse dans les neurones en culture**
9. **Structure des épines dendritiques**
10. **Formation de la synapse plasticité synaptique in vivo**

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