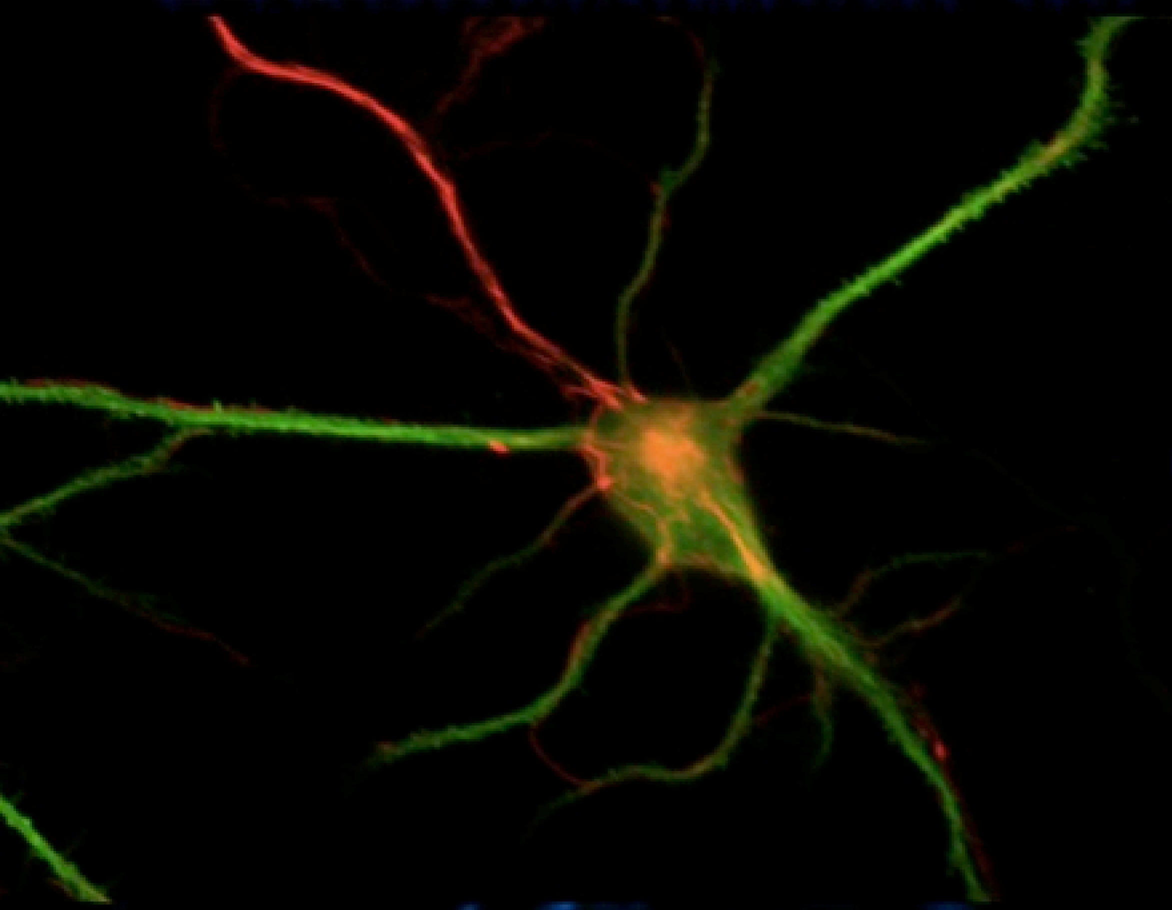
Etablissement de la polarité neuronale…



Synapses
& Synaptogenèse
Master 2 - 2013

… et trafic membranaire

Lydia Danglot

INSTITUT JACQUES MONOD

Trafic membranaire et morphogenèse neuronale et épithéliale - IJM - Paris

Lydia Danglot web page

Life Science & Imaging

Novembre 2, 2009

- Thème de recherche
- Publications
- Enseignement
- Liens favoris
- CONTACT

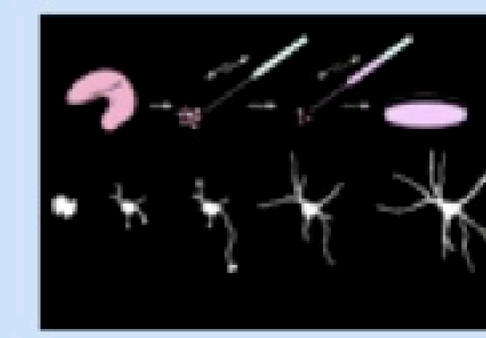
French English

Enseignement

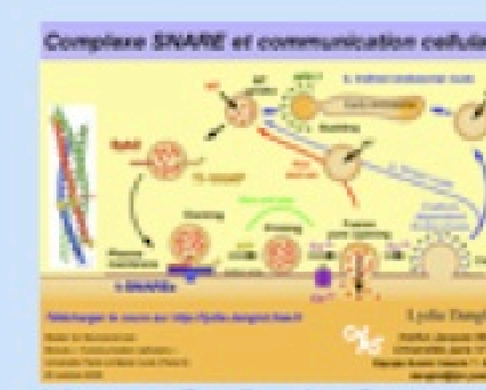
Cours

- 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.***

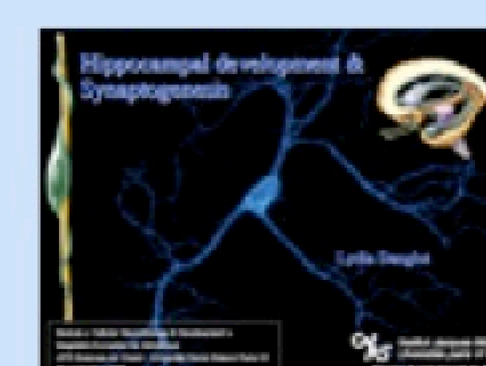
MANUEL de cours



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

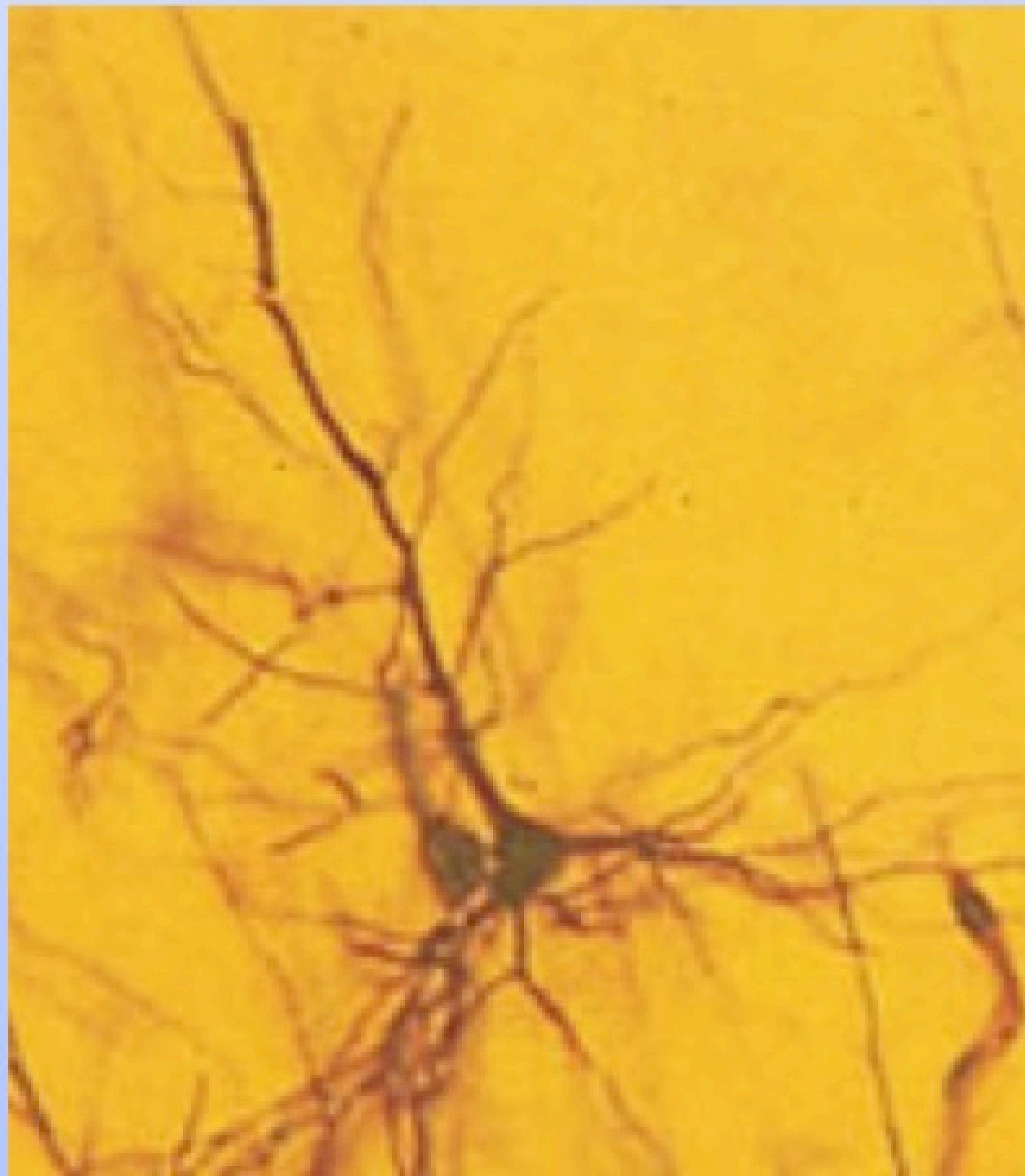
Plan

1. Le neurone: unité fonctionnelle polarisée
2. D'où proviennent les neurones
3. Polarité neuronale: les domaines
4. Rôle du trafic membranaire dans la neuritogenèse
5. Polarité et cytosquelette
6. La métaphore (imparfaite) épithéliale
7. Formation de la Synapse

1. Le Neuron: *unité fonctionnelle polarisée*

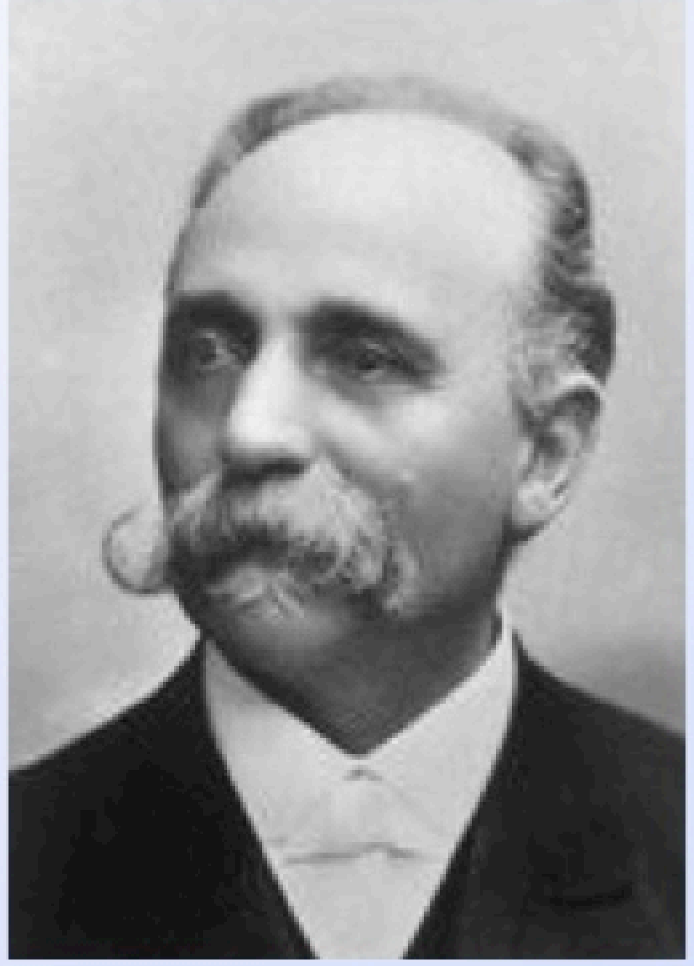
- 1800's: les techniques de microscopies permettent l'examen de tissus nerveux
  Refinement of the microscope allows scientists to examine brain tissue

- 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...



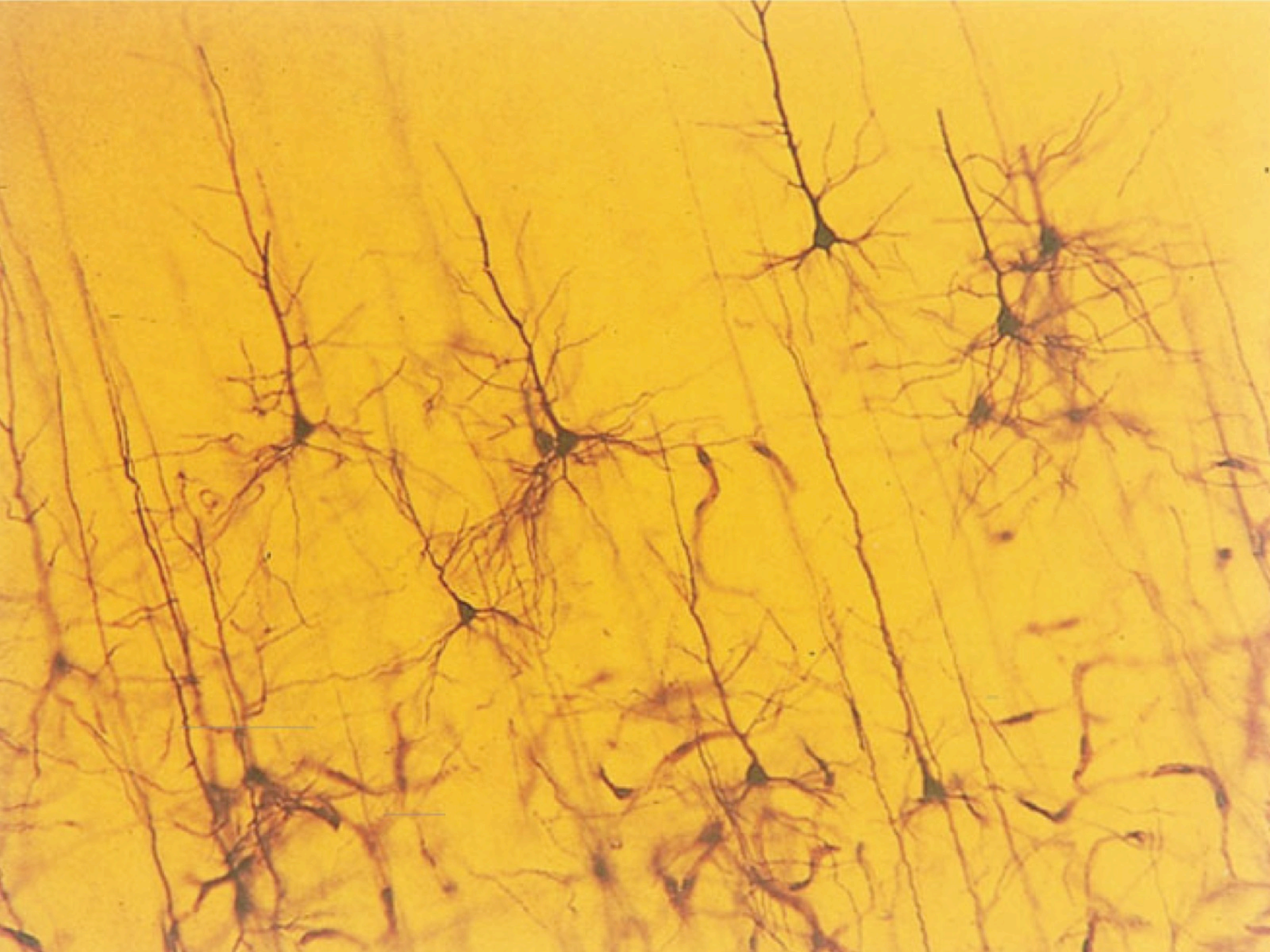
- Théorie réticulaire (C. Golgi): Il existe une continuité cytoplasmique entre les neurones (tunnel).

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

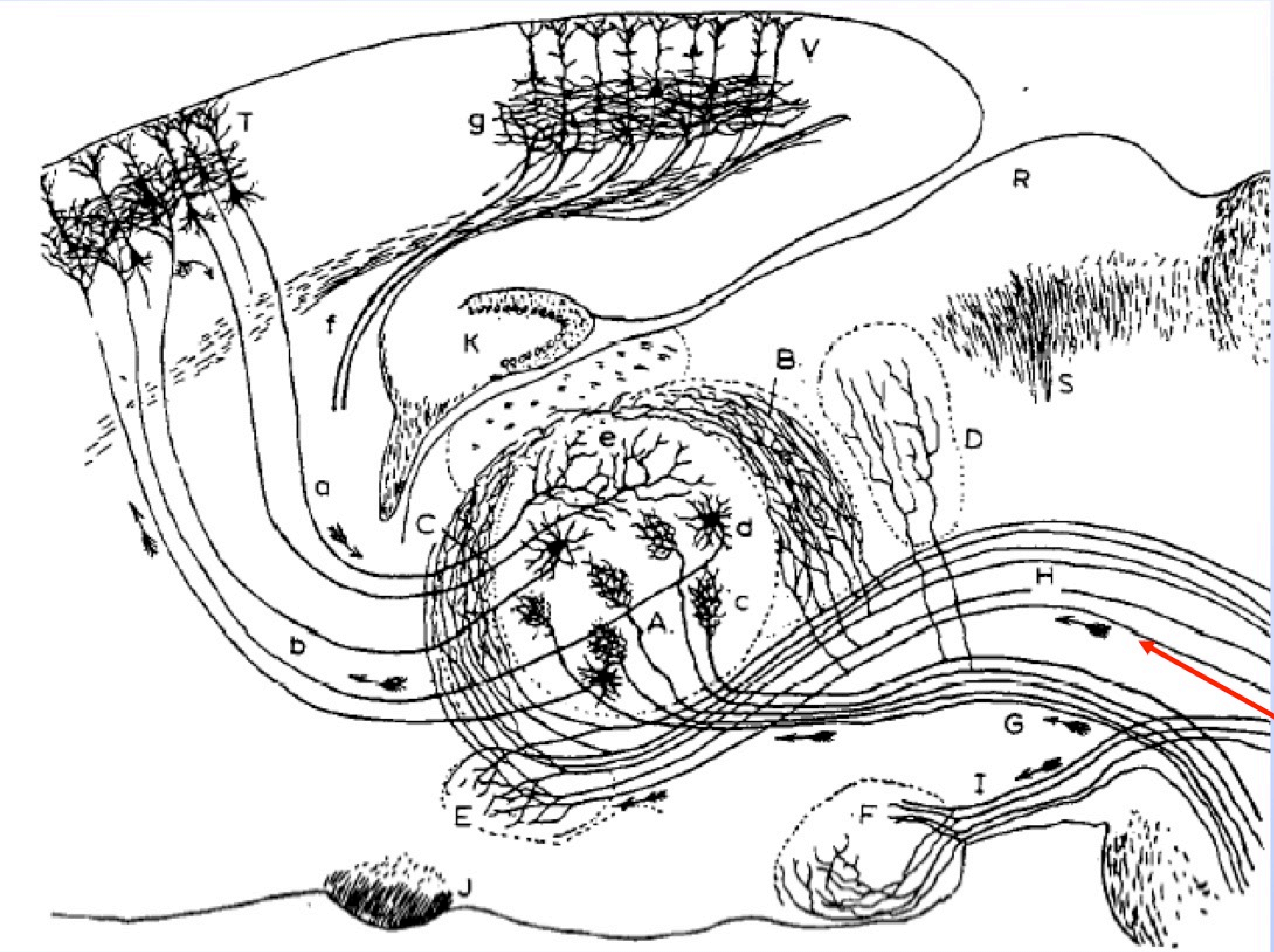
Aujourd'hui, les scientifiques donnent raison à Cajal

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

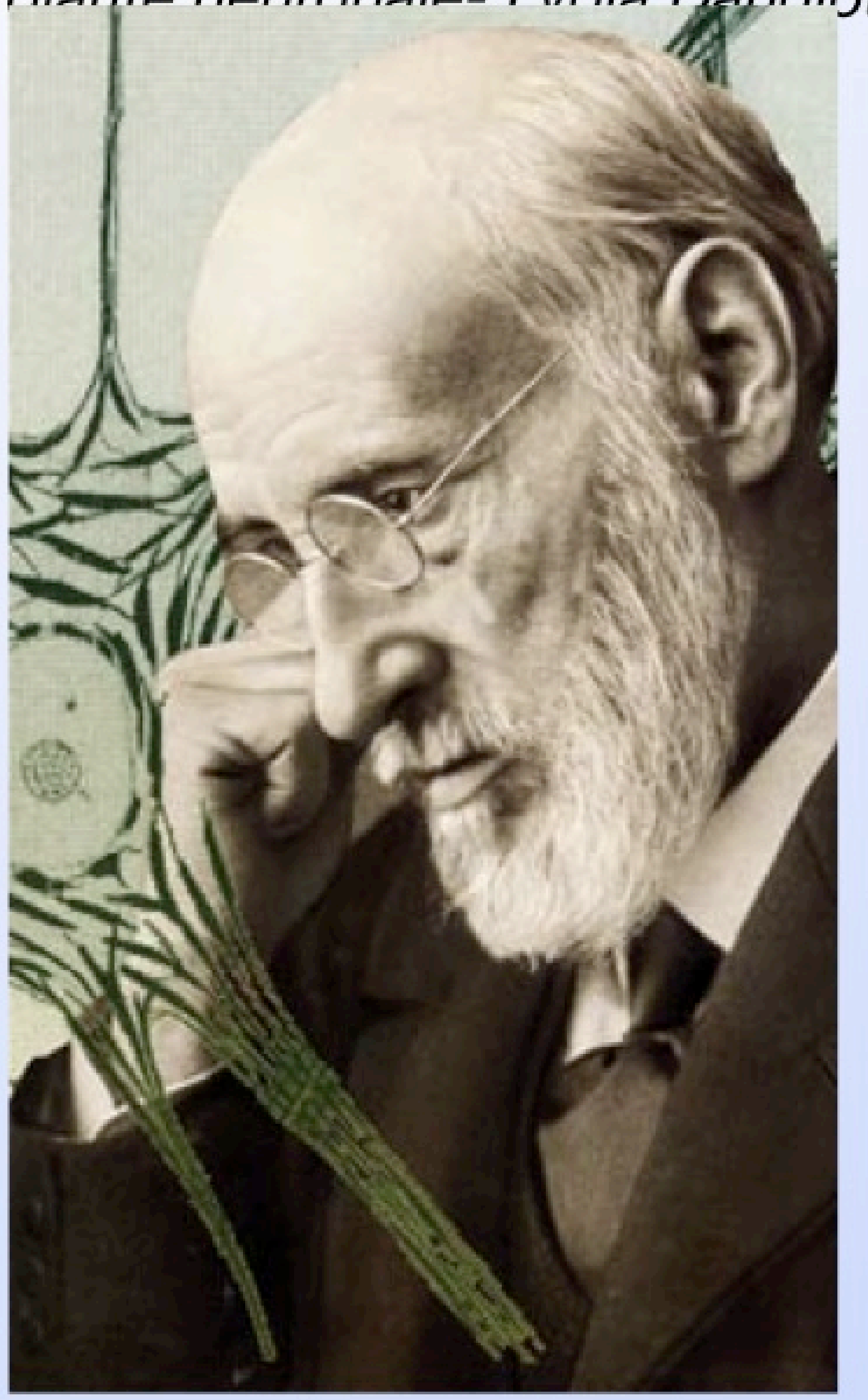
(Les deux ont reçu le prix Nobel en 1906)



Les neurones sont des cellules polarisées



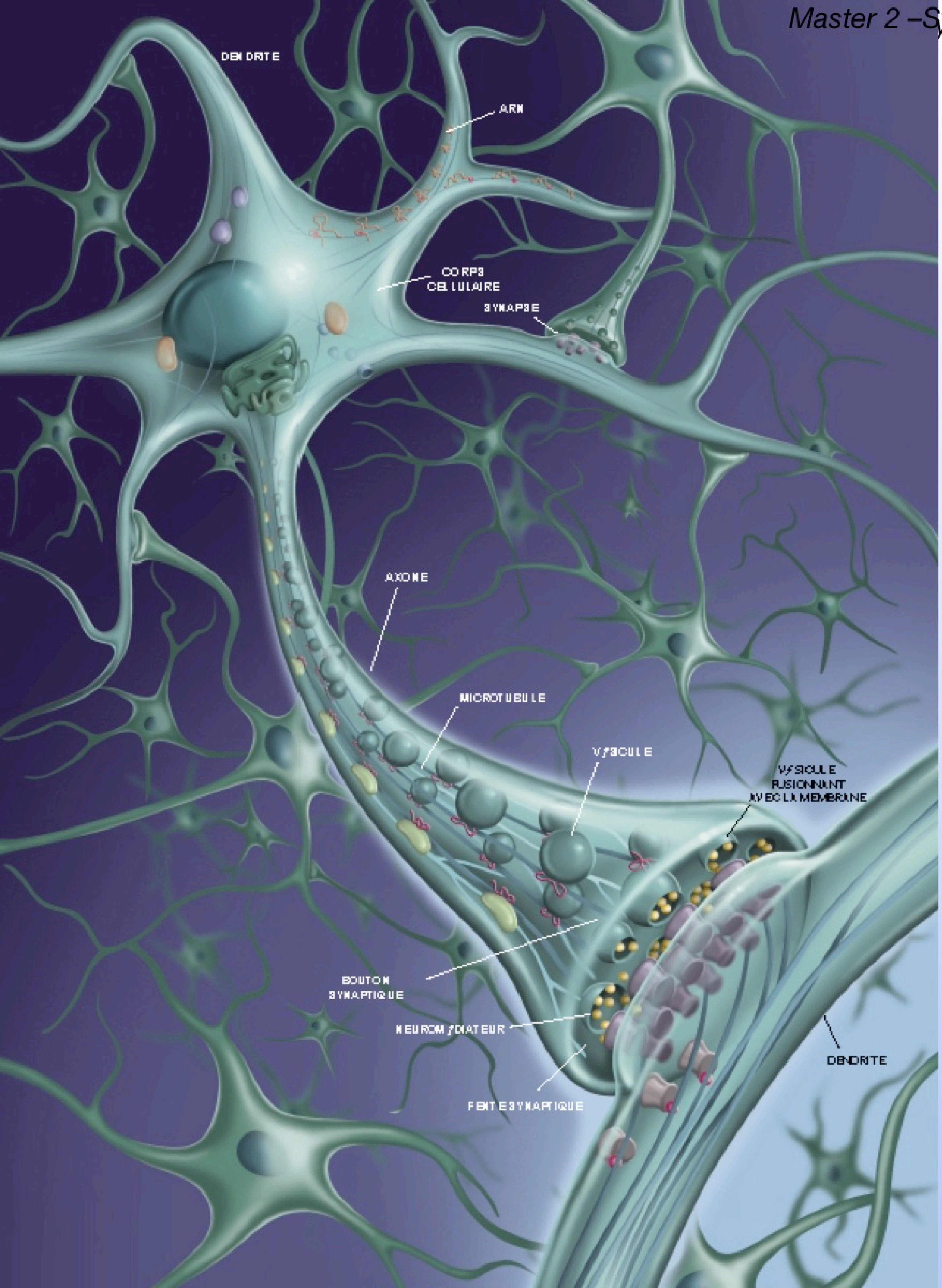
Direction de l'influx nerveux



Santiago Ramón y Cajal

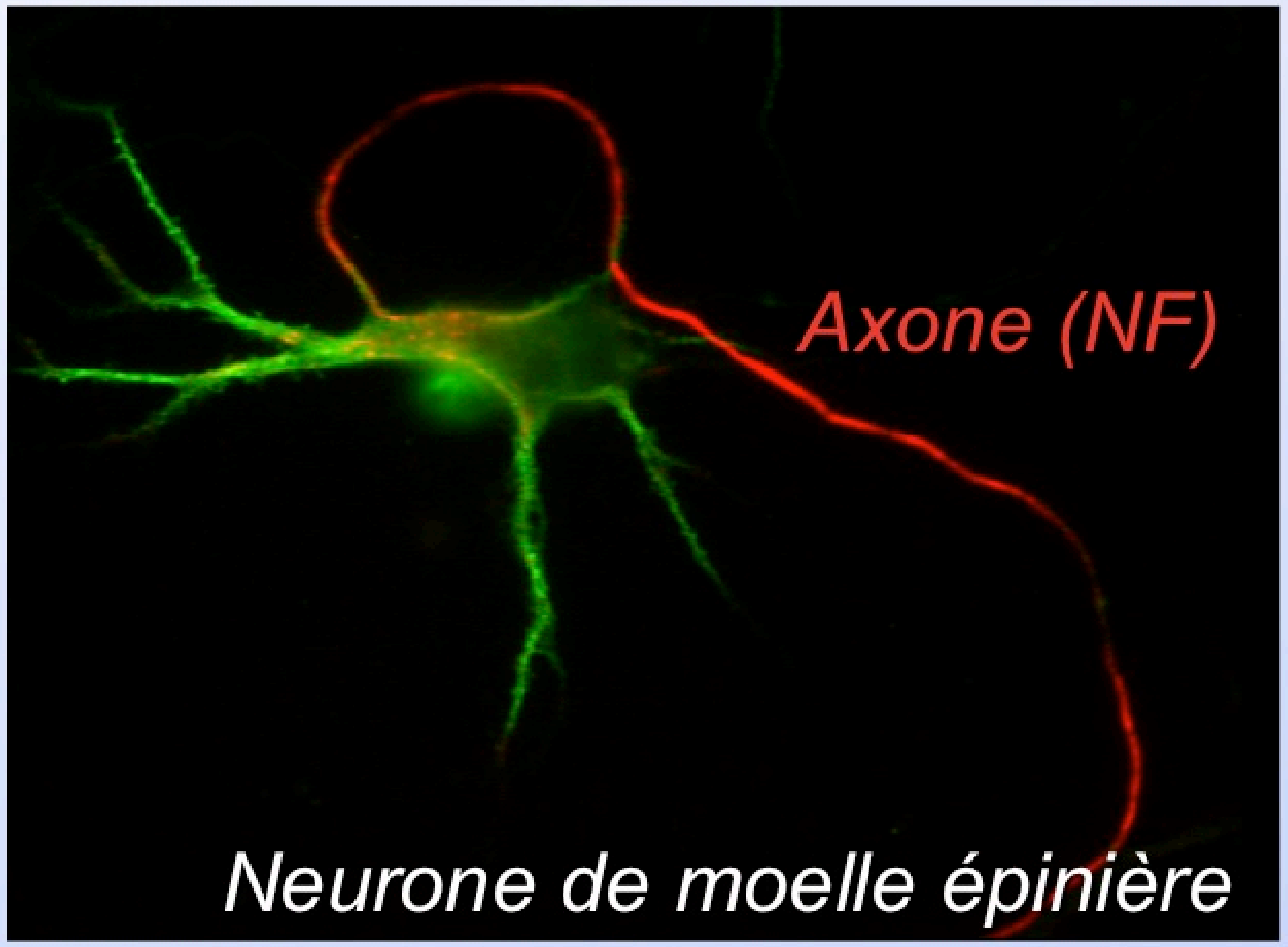
(1852-1934)

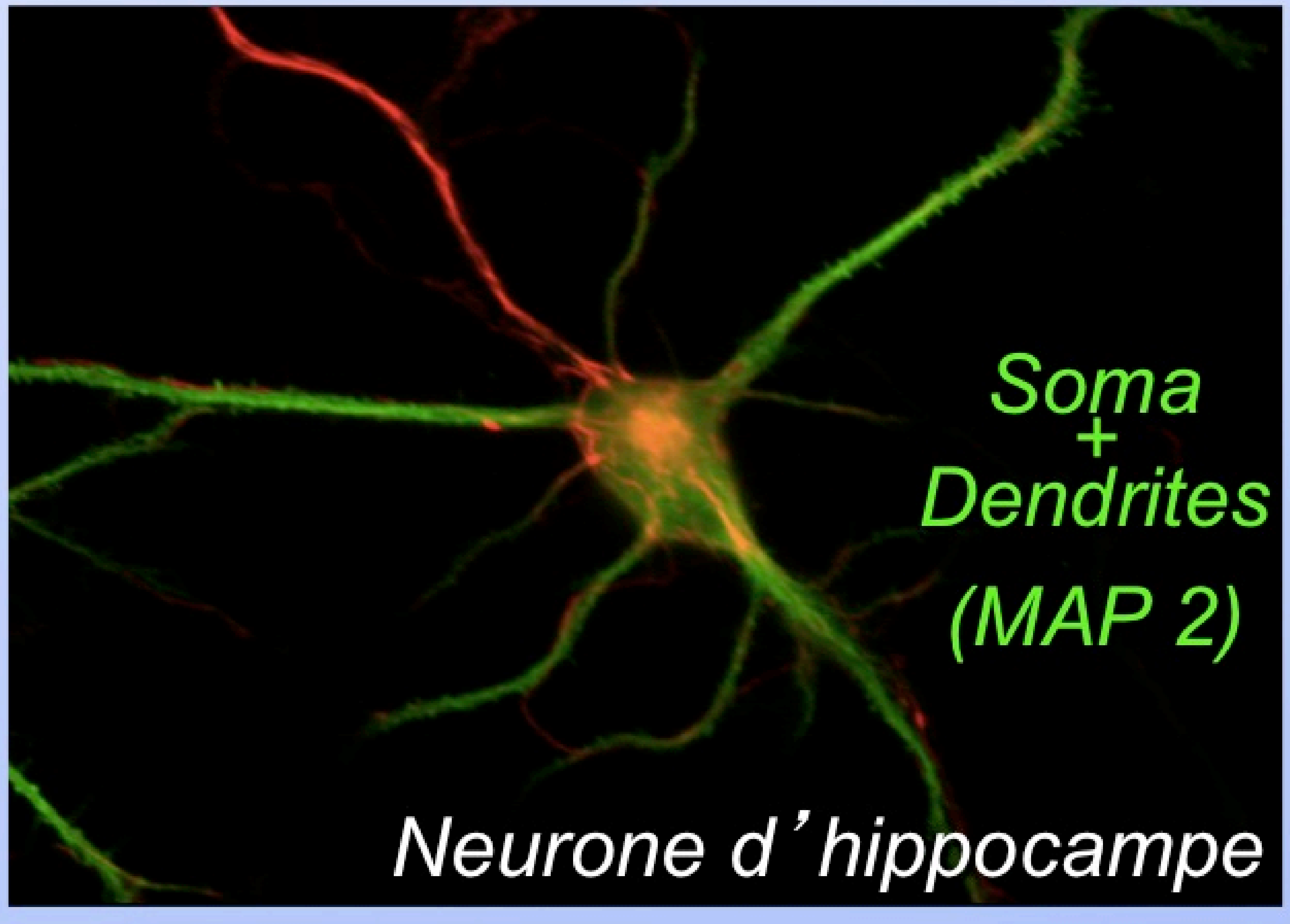
ynapse & synaptogenèse –Polarité neuronale- Lydia Danglot

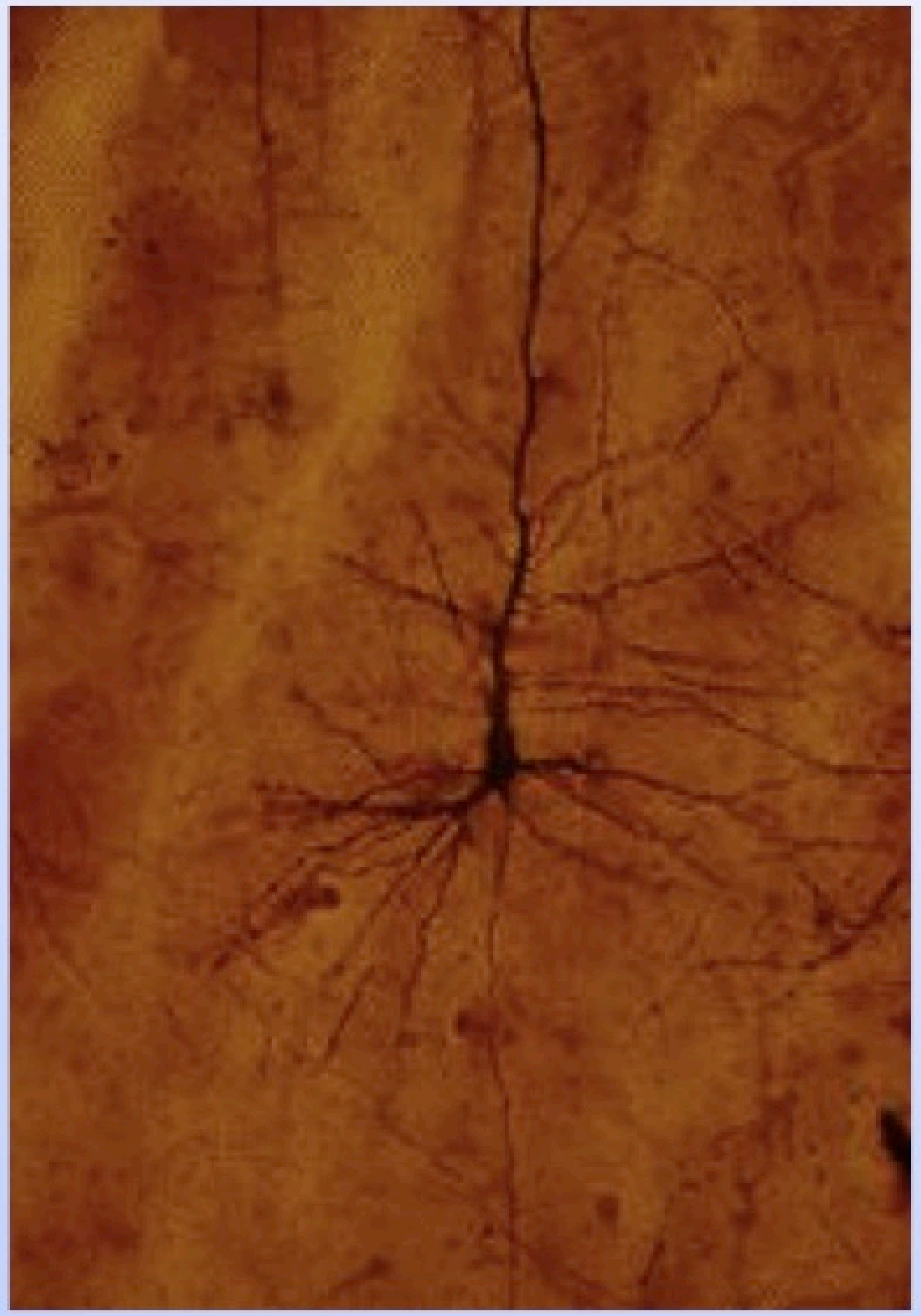


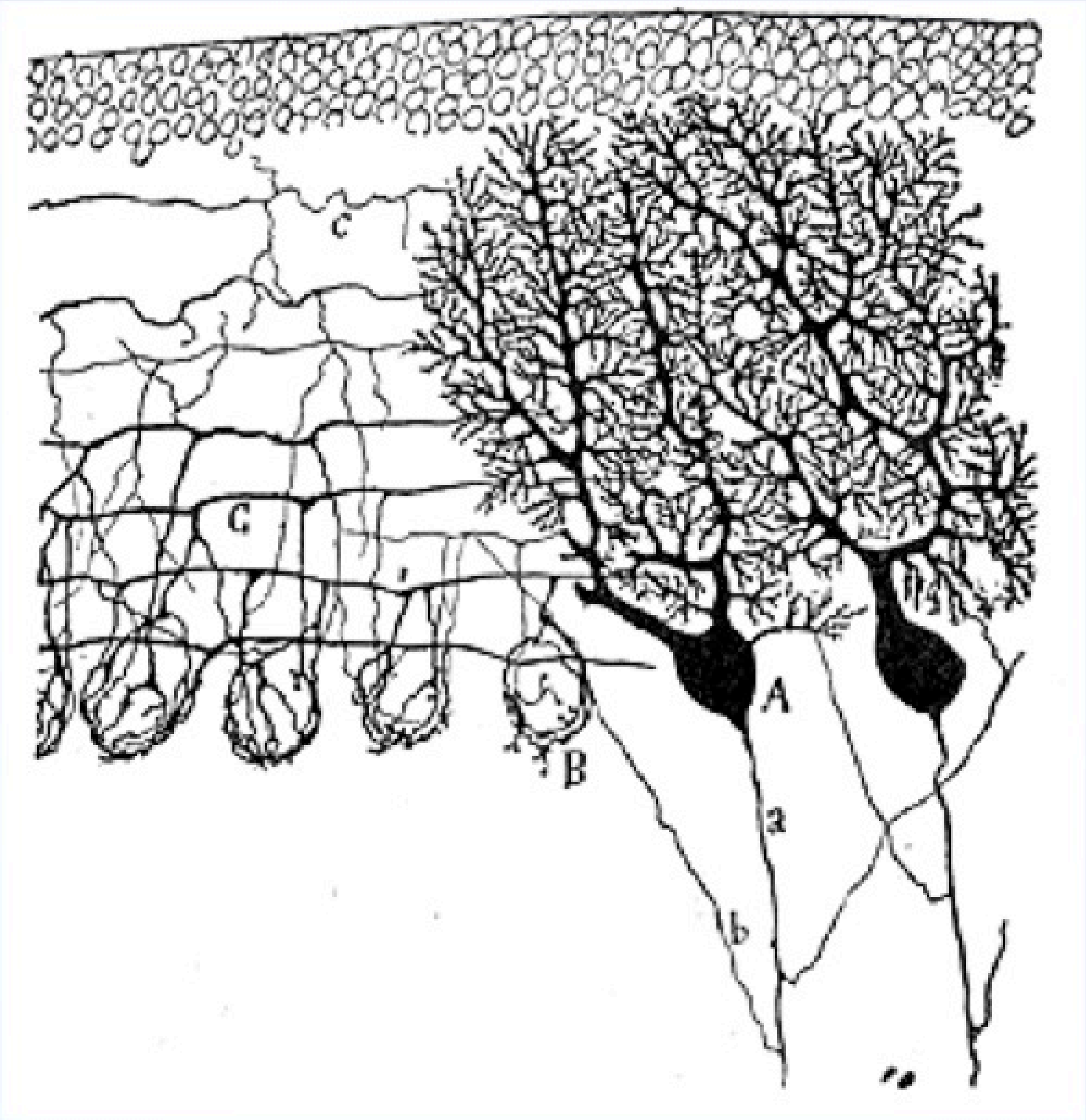
2 compartiments:

- *Axonal*
- *Somatodendritique*





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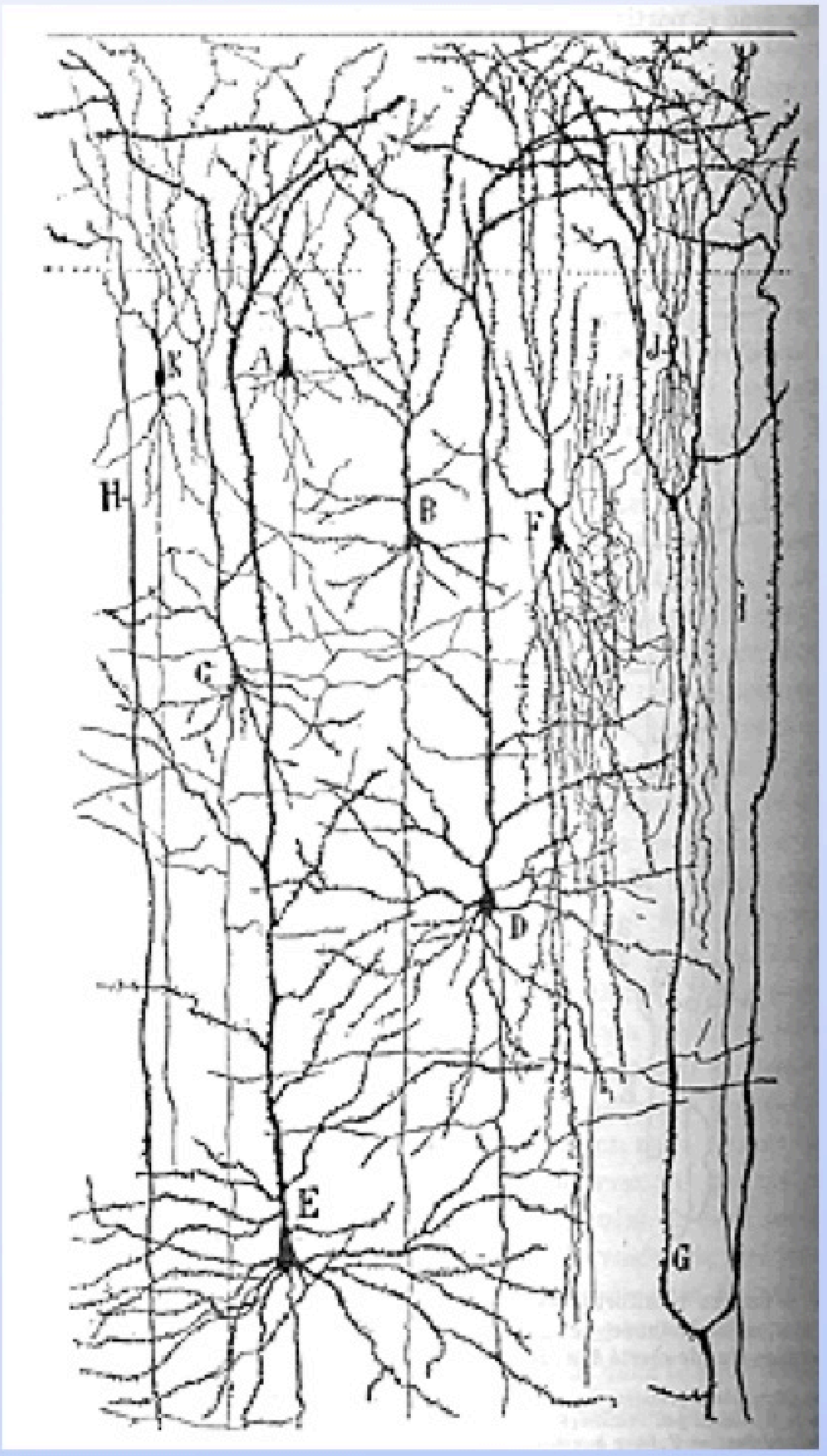
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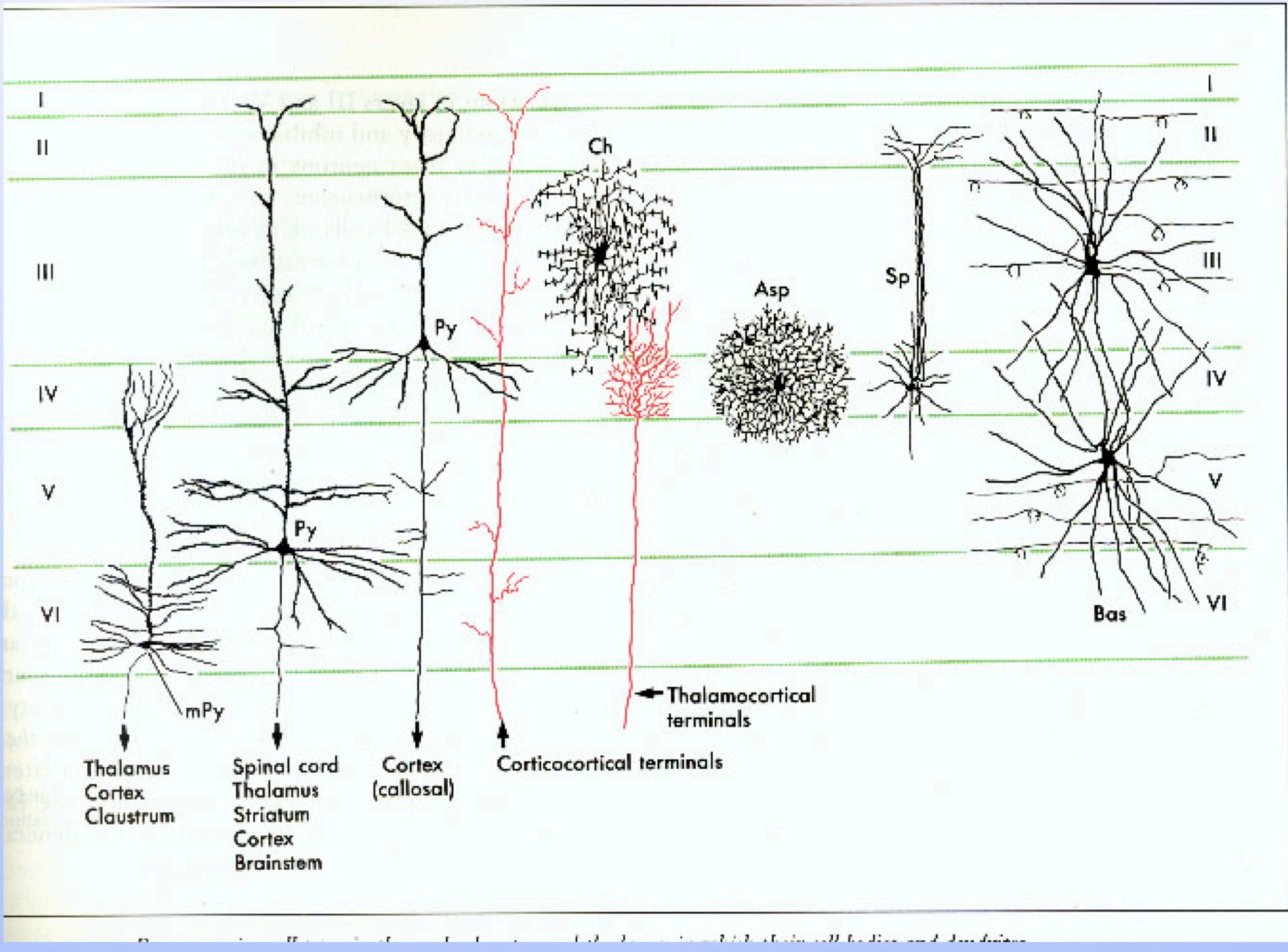
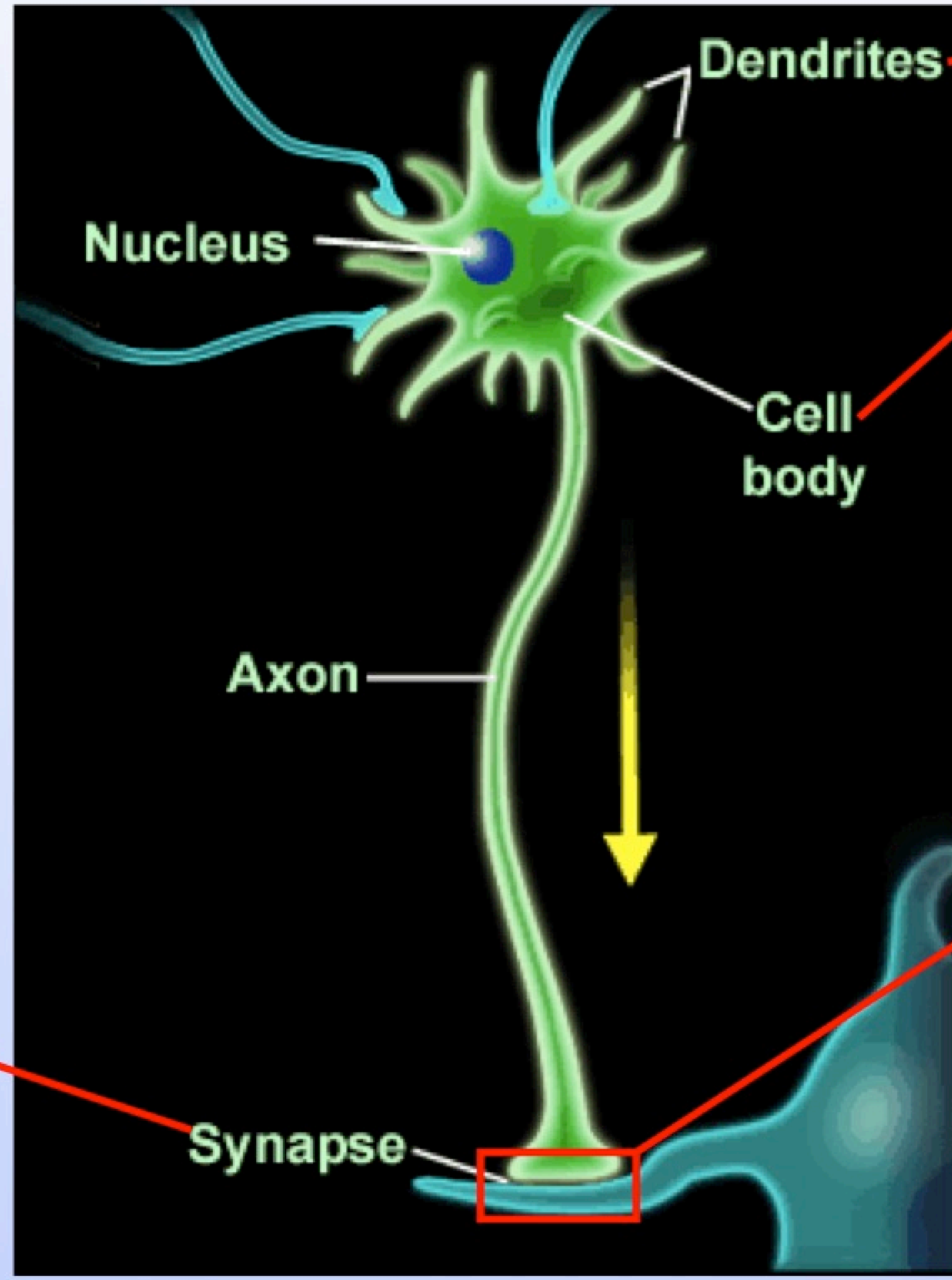
Cerebral Cortex



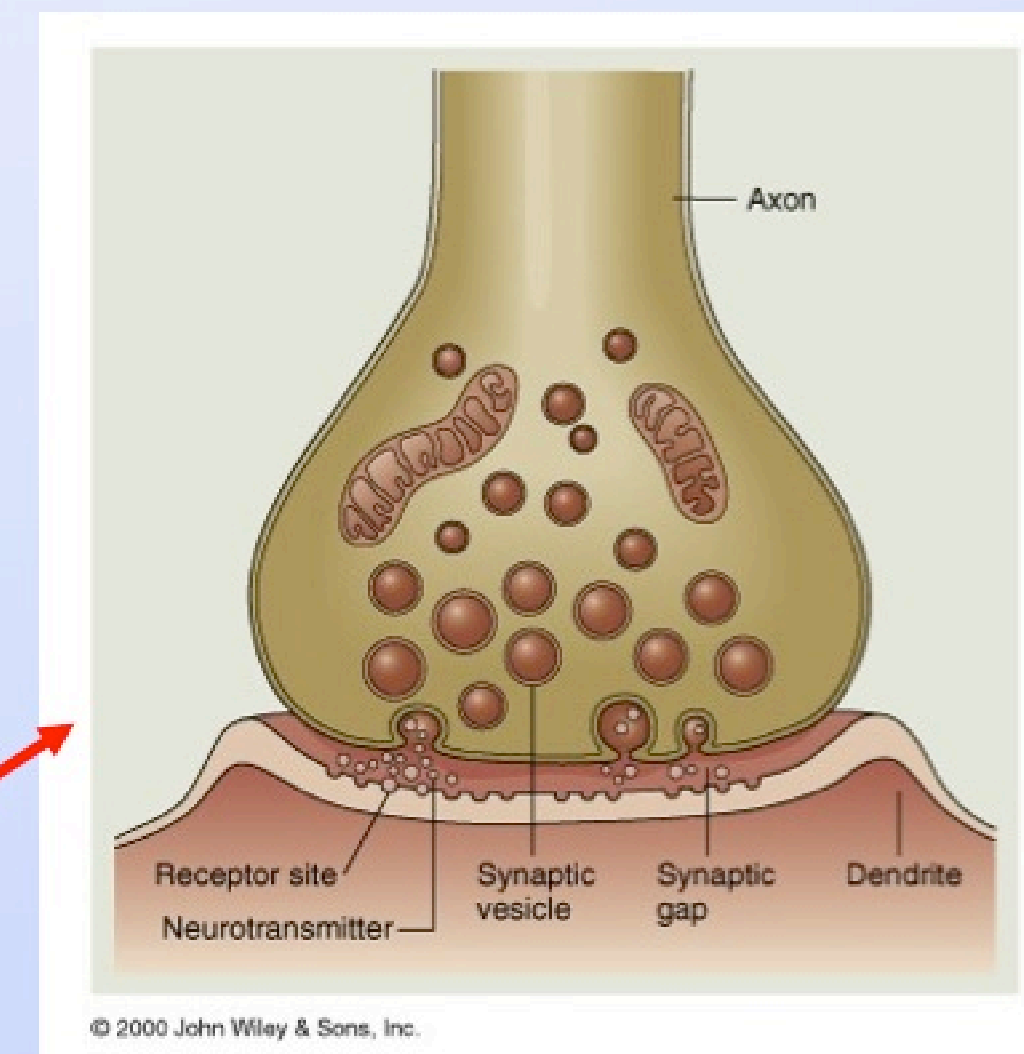
Domaines du neurones

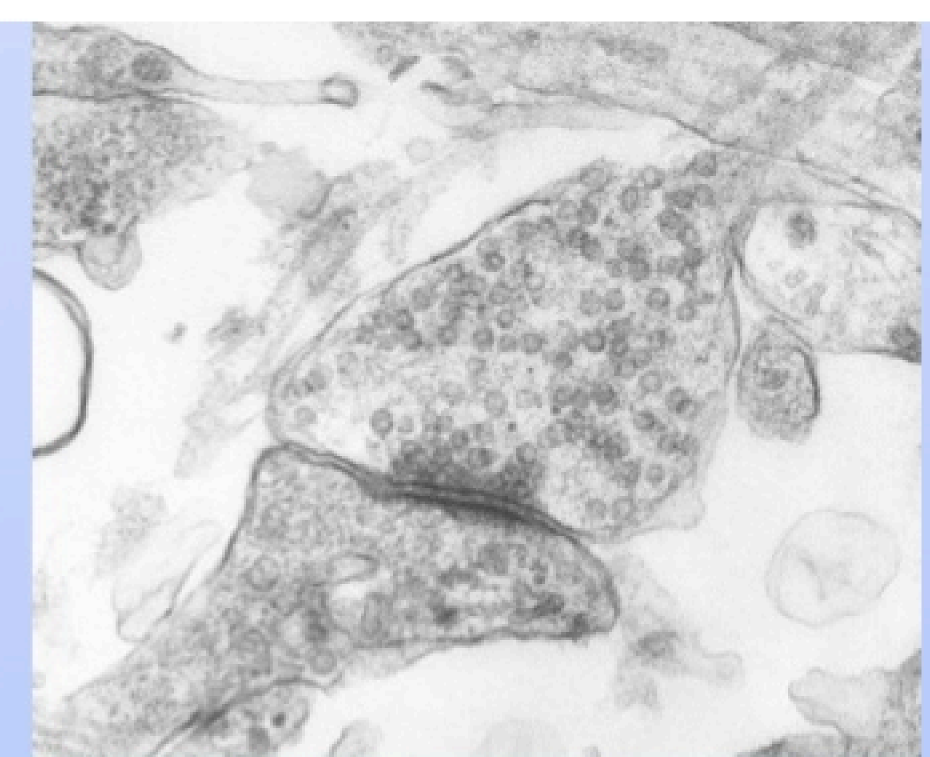
1 mm^3 de substance grise du cortex peut contenir 5 milliards de synapses.

Pôle récepteur



Pôle émetteur





La question centrale :

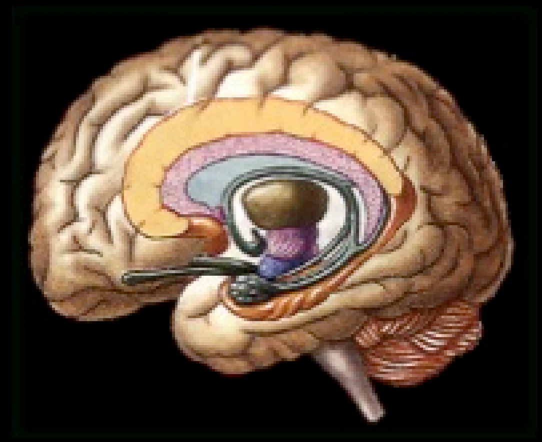
Polarisation neuronale: développement de l'axone et des dendrites.

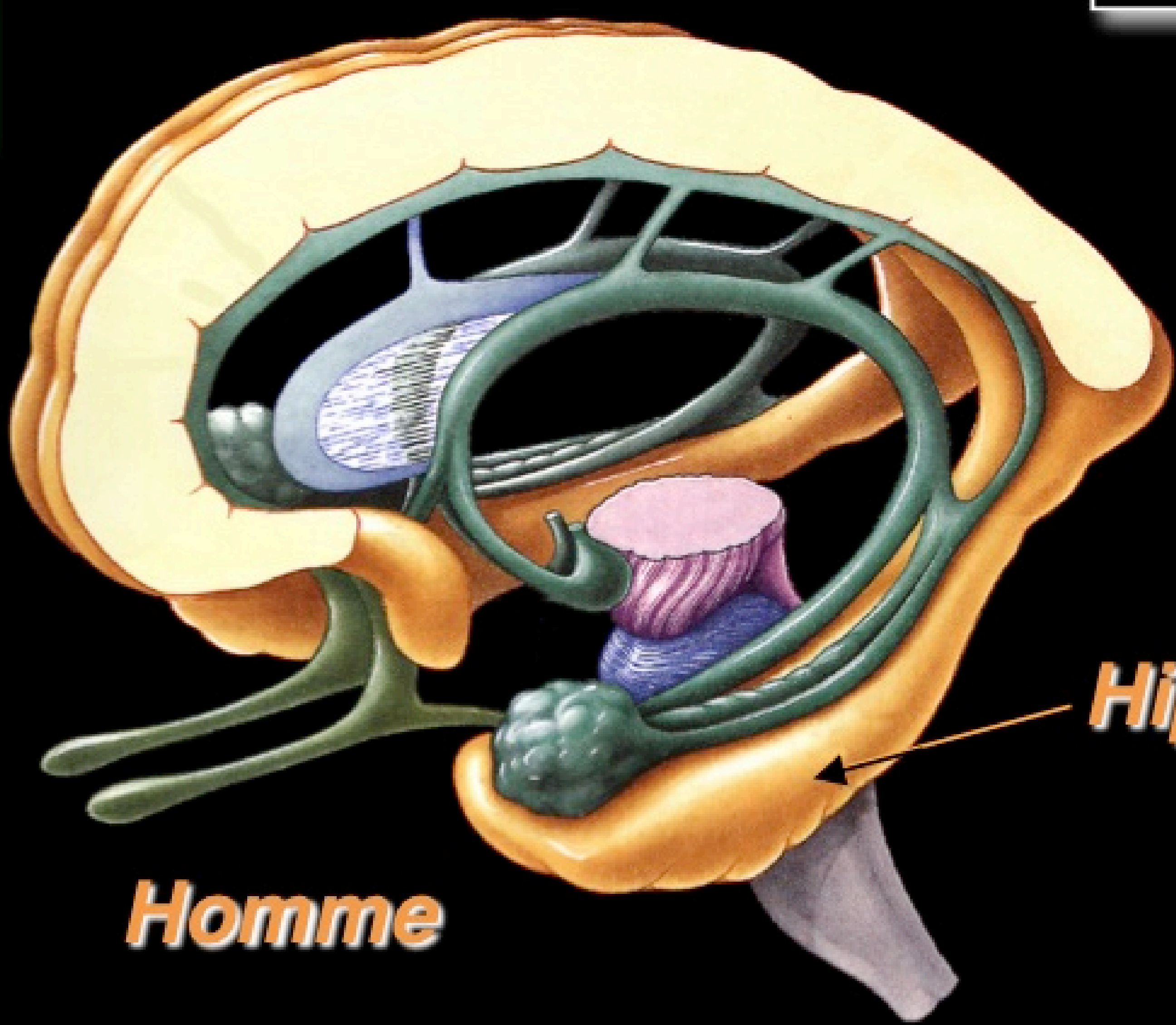
Molecular markers for axon or dendrite

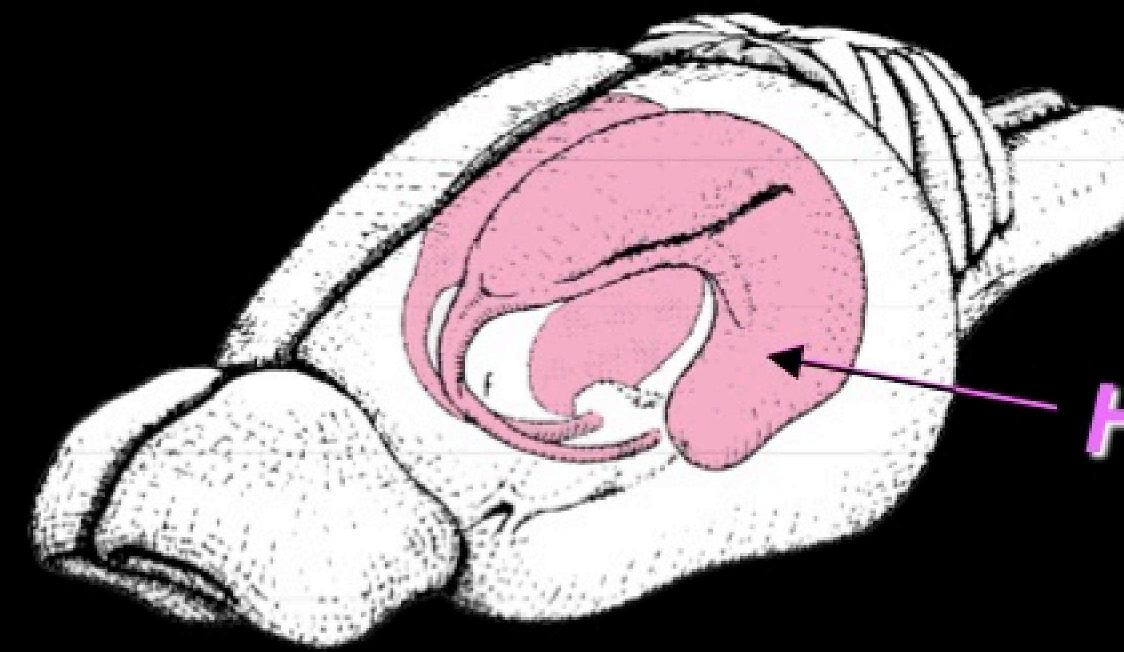
Axon: Tau1, GAP43, synapsin, synaptotagmin …

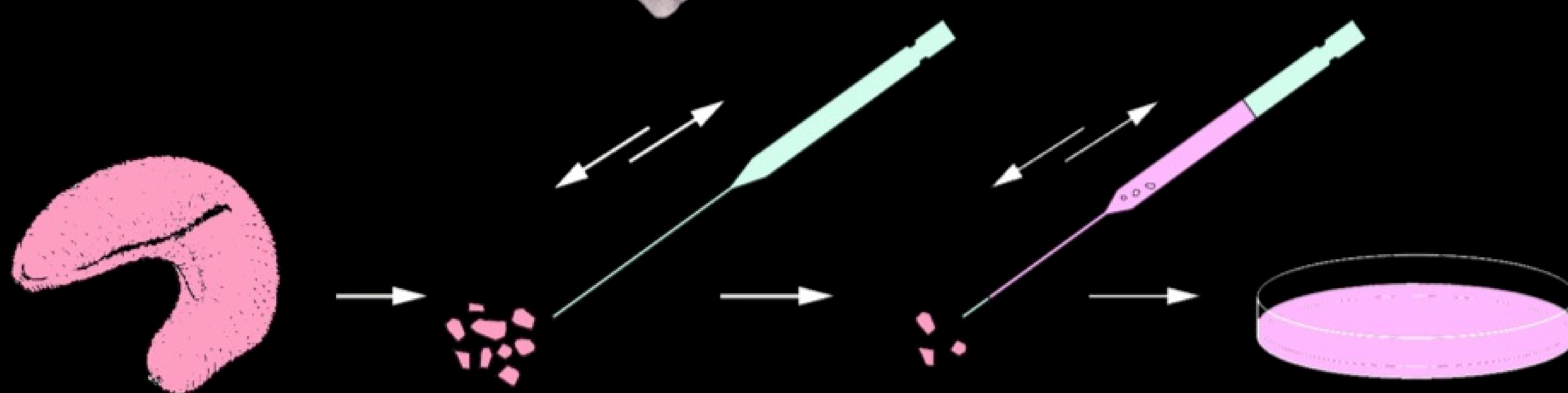
Dendrite: MAP2, Glycine receptor, GABAa receptor …

Neurones d'hippocampe en culture



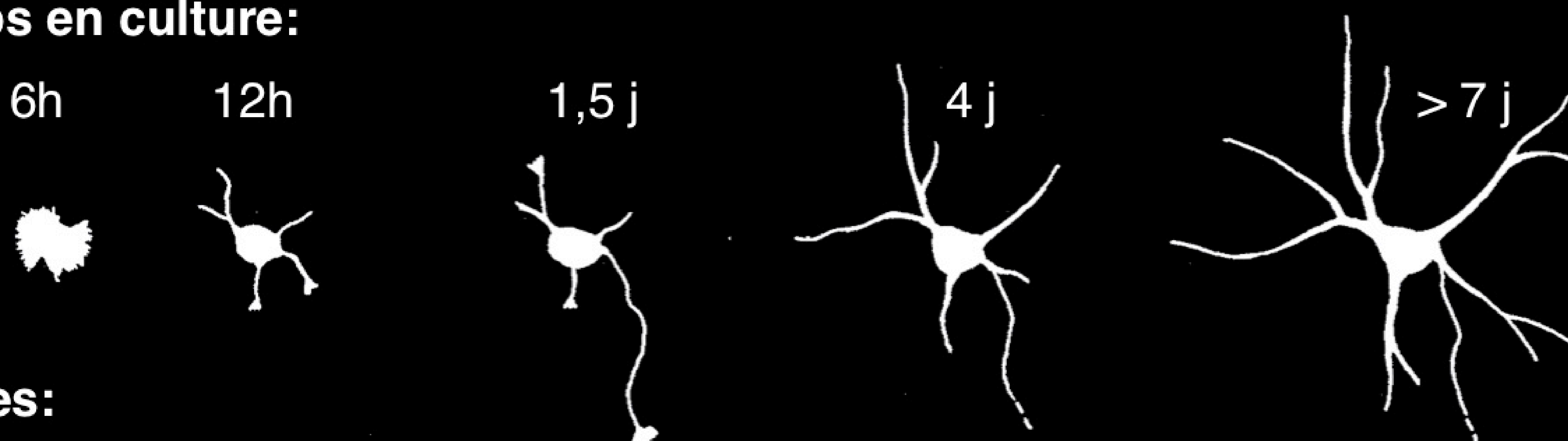






Temps en culture:

6h 12h 1,5 j 4 j > 7 j

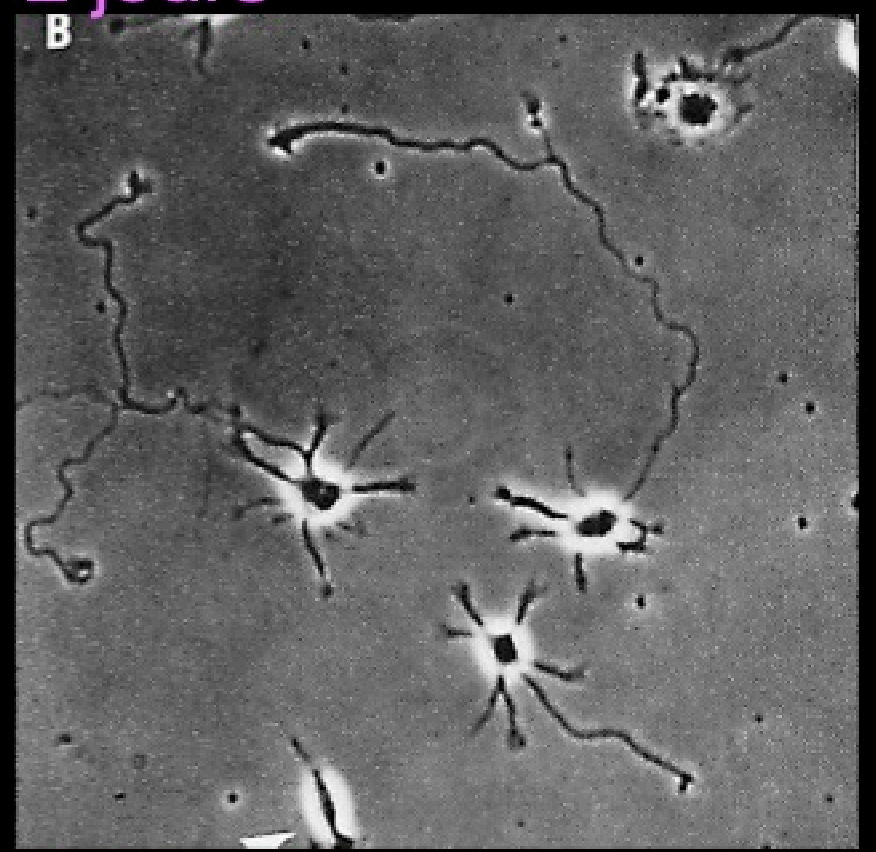


Stades:

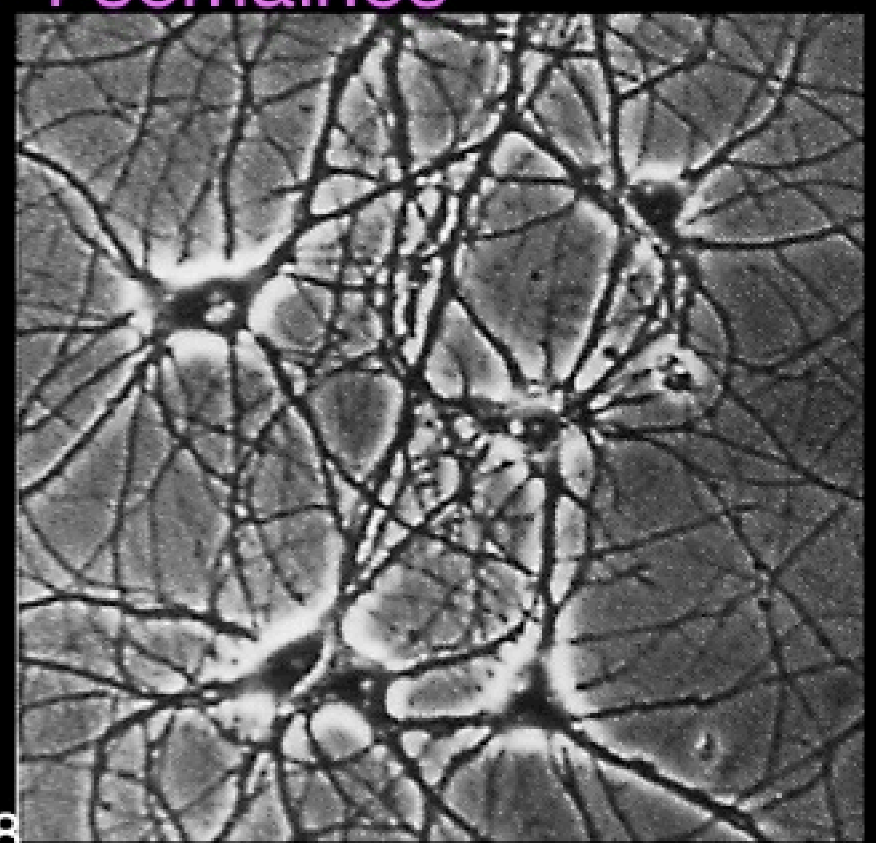
1 Lamellipodes
2 Neurites mineurs
3 Croissance axonale
4 Croissance dendritique
5 Maturation

D'après Dotti et al. 1988

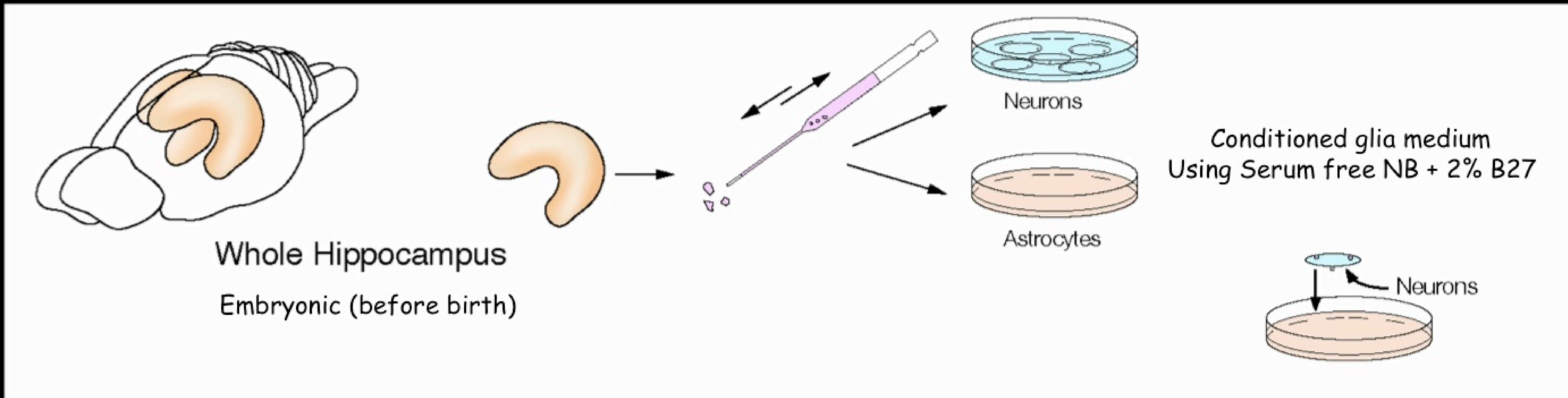
2 jours

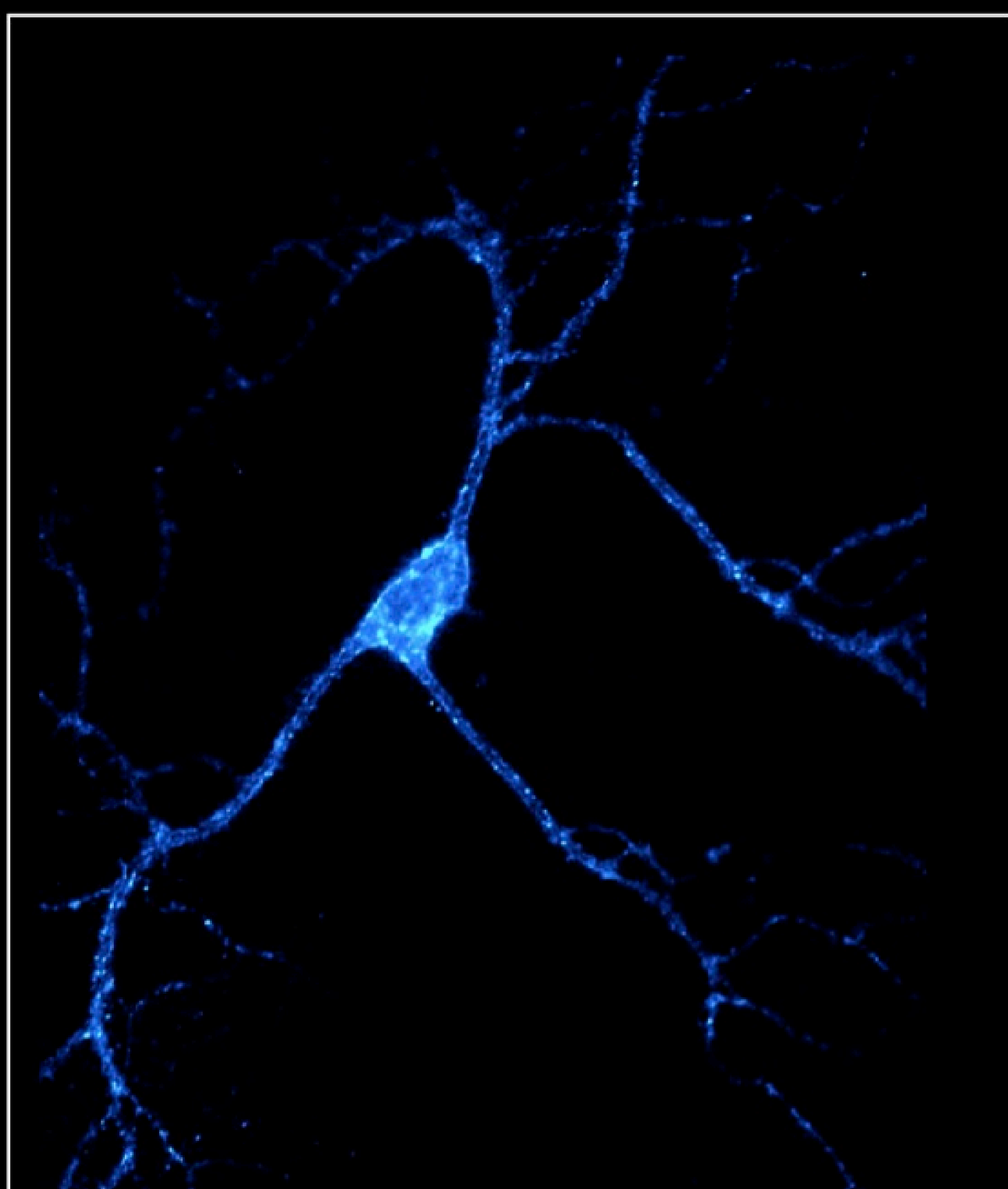


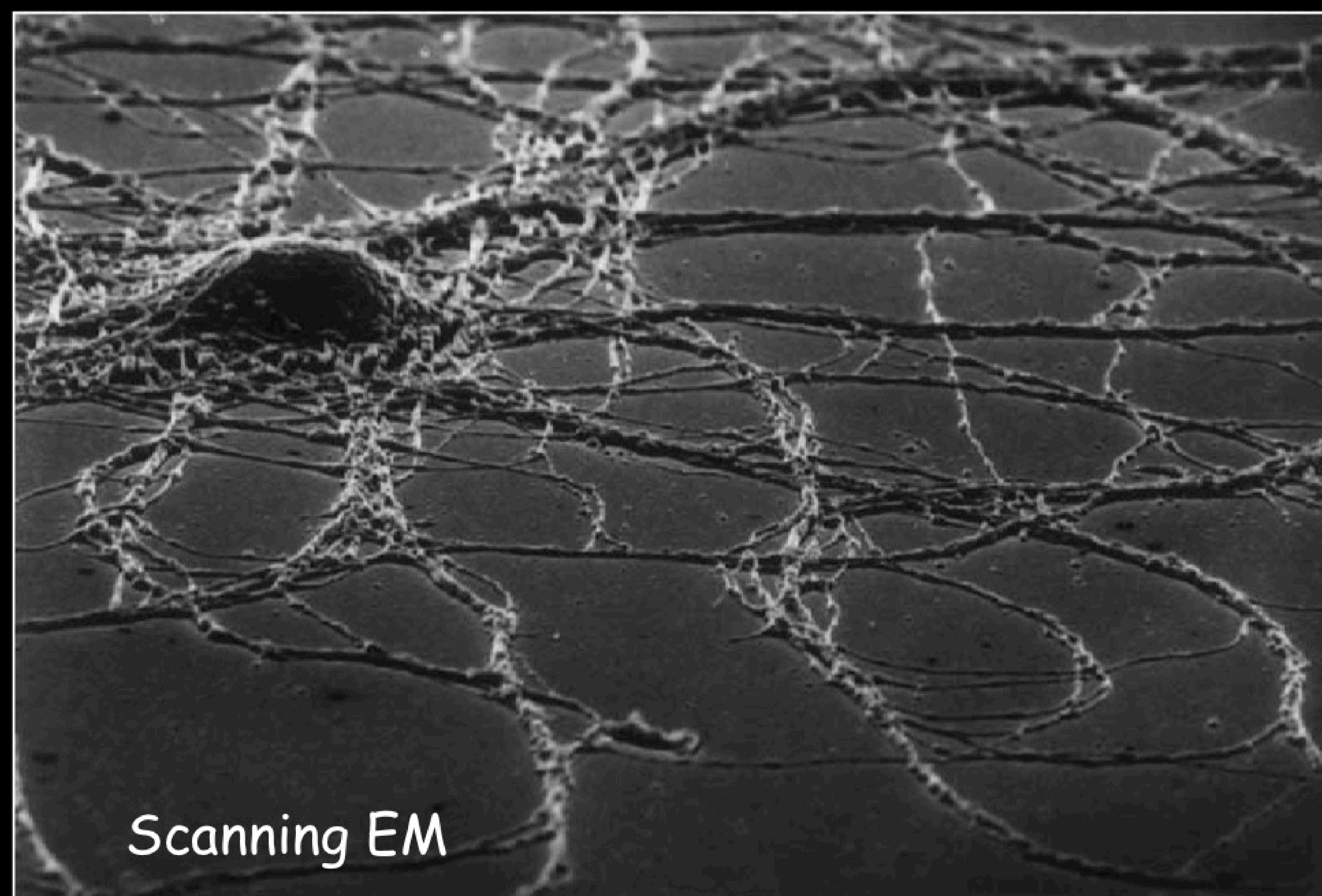
4 semaines



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





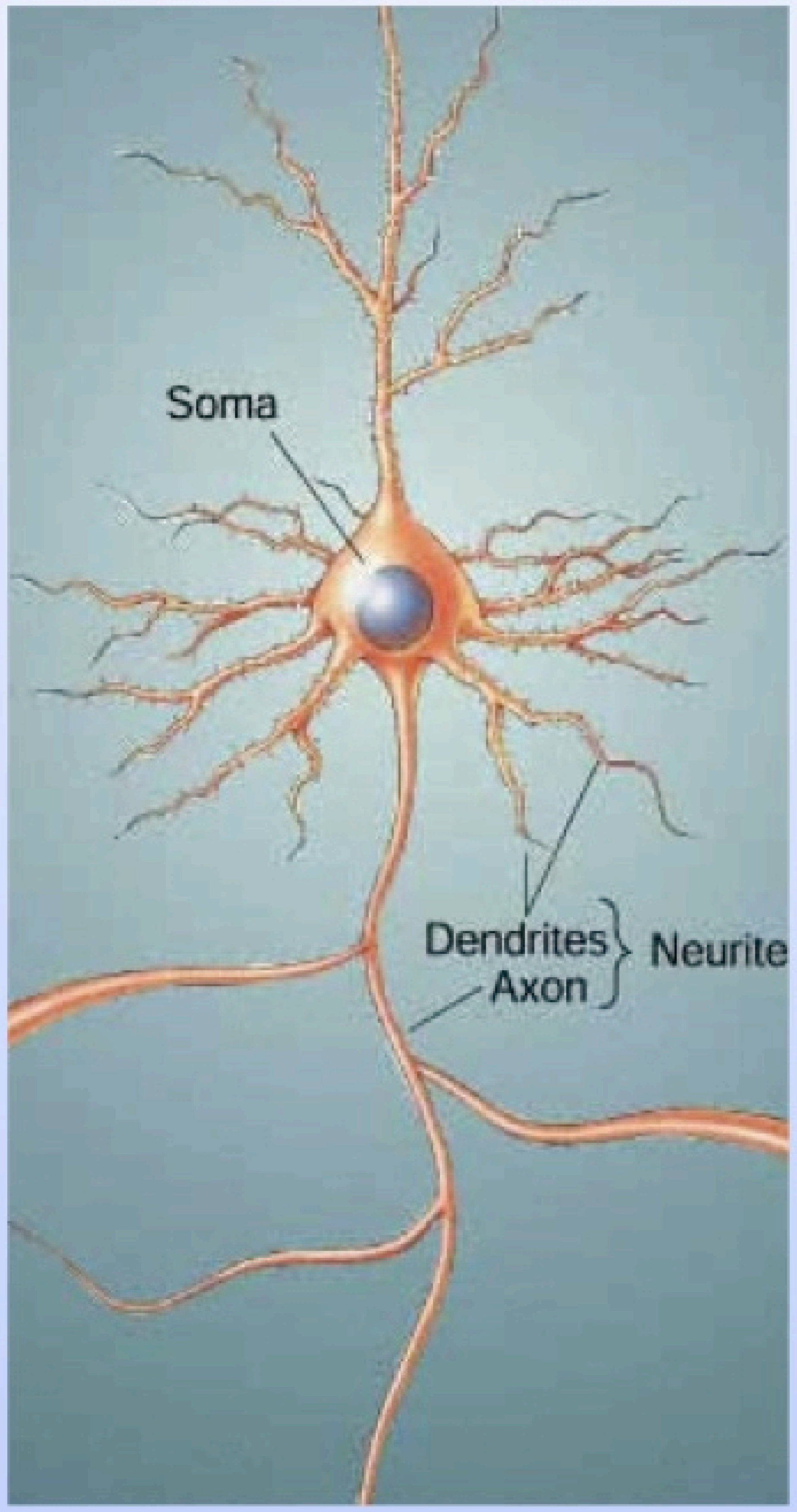


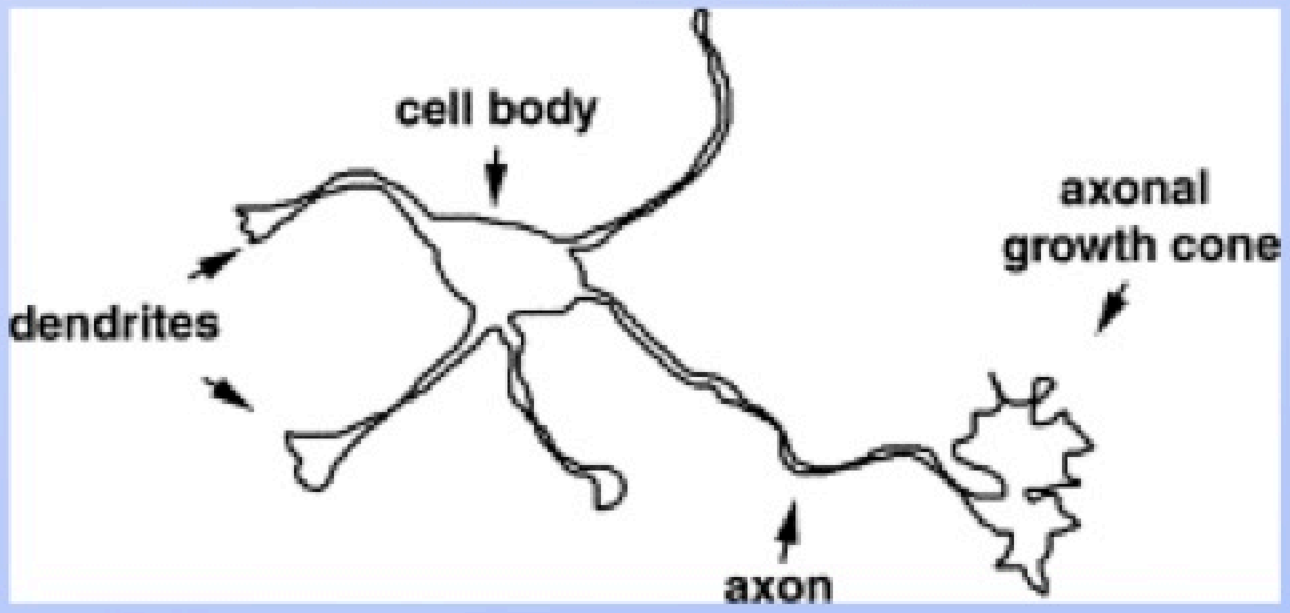
Danglot et al (2003), Mol Cell Neurosci. 23:264-278.

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

3. D'où proviennent les neurones ?

- Neurones et glie : neuroectoderm (progenitor stem cells)
- Neuroblastes -> neurones
- Astroblastes -> astrocytes





3. D'où proviennent les neurones ?

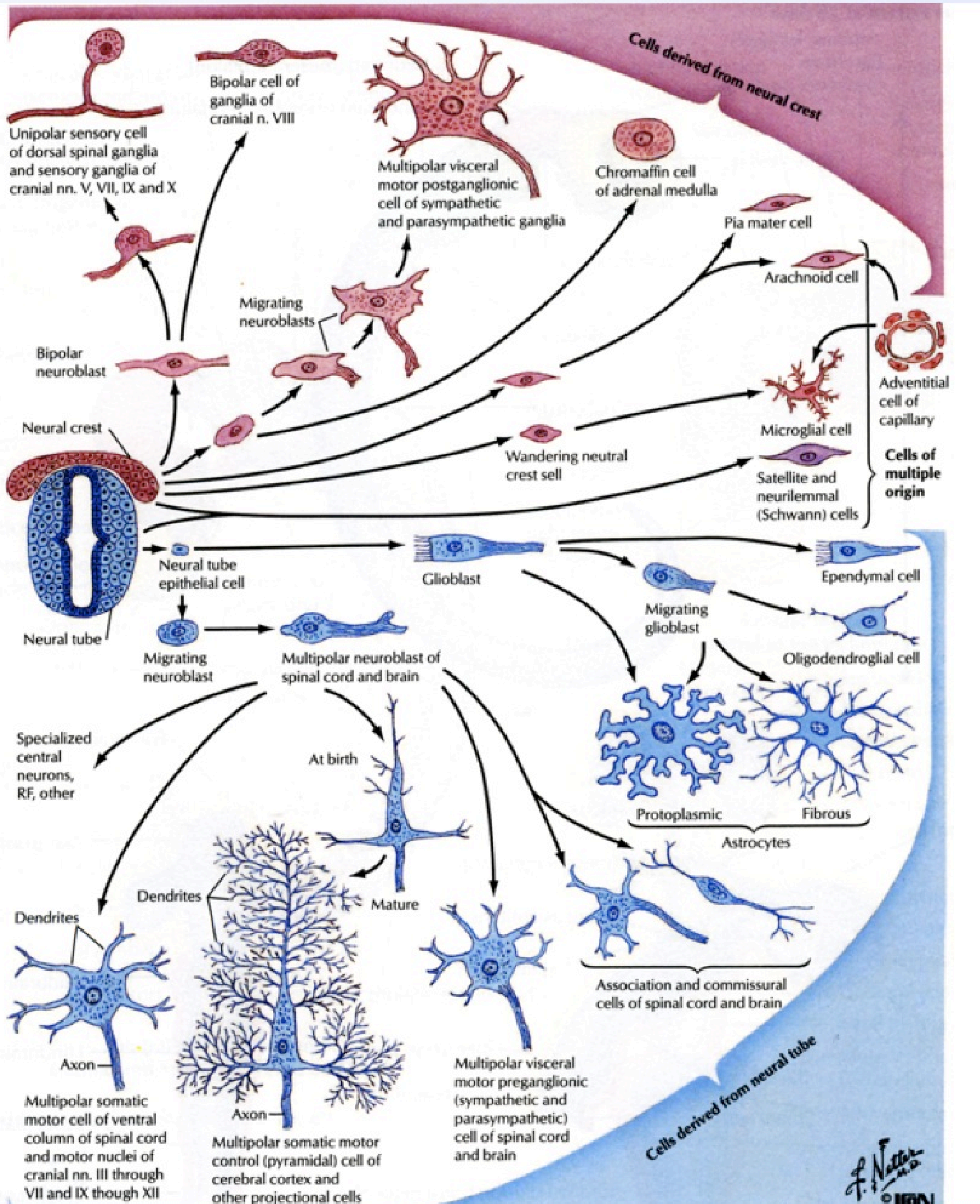
Derived cells from neural tube and neural crest

Neural crest:

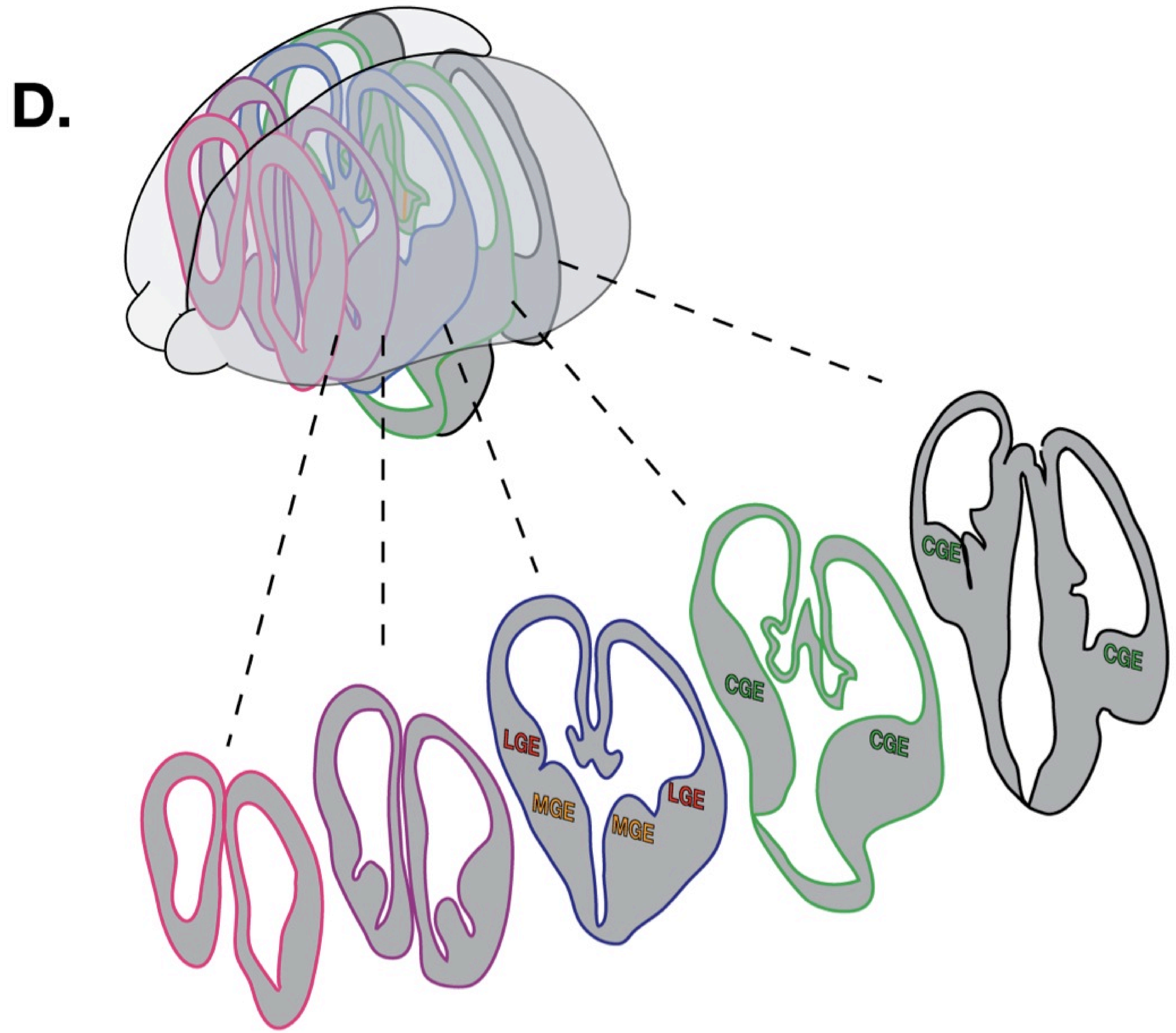
- Ganglionar cells (spinal & ANS)
- Chromaffin cells
- Glial cells:
  - Schwann cells
  - Microglia

Neural tube:

- Neurons (brain & spinal cord)
- Ependymal cells
- Glial cells:
  - Oligodendrocytes
  - astrocytes



Migration of neurons during development



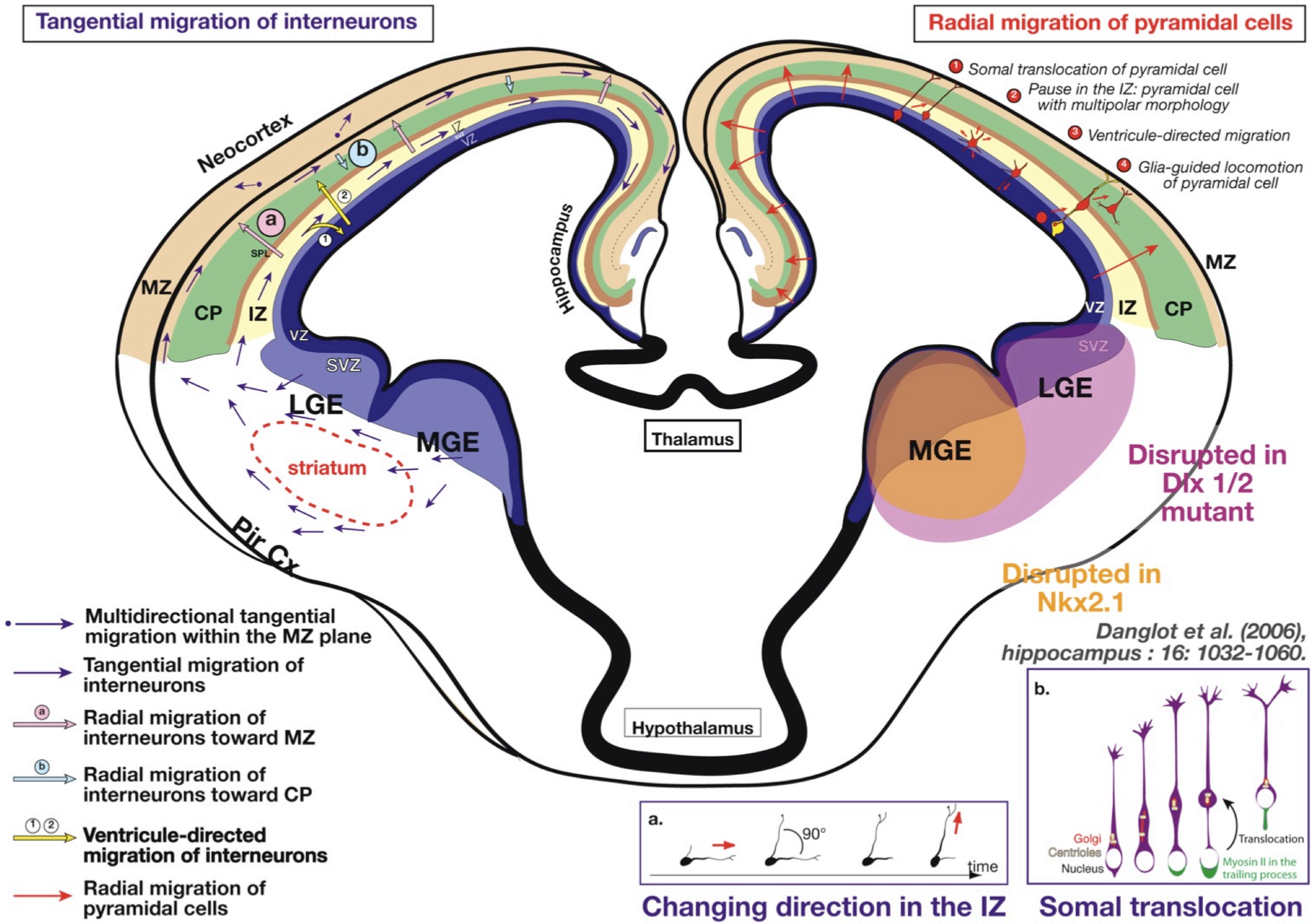
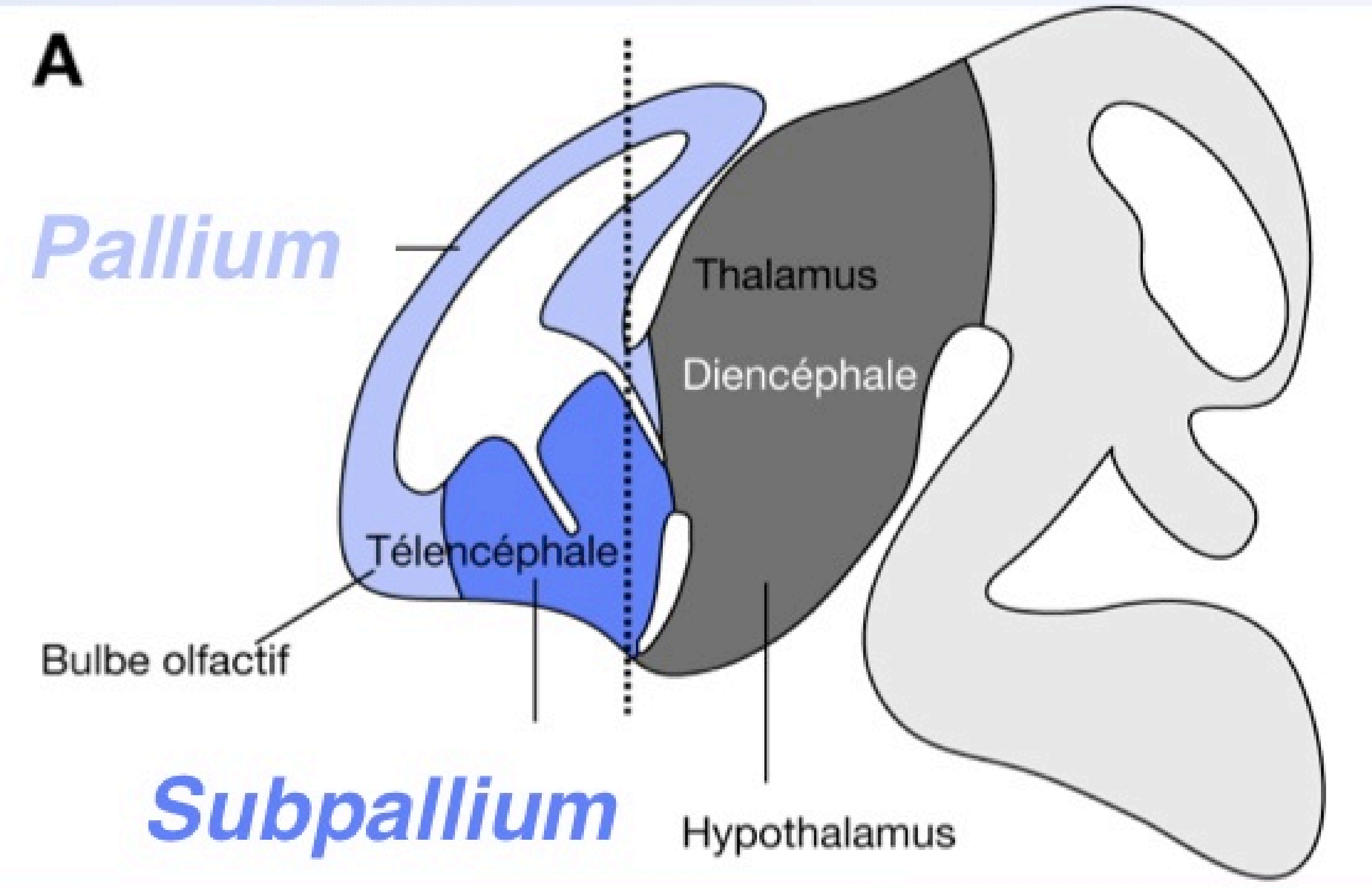
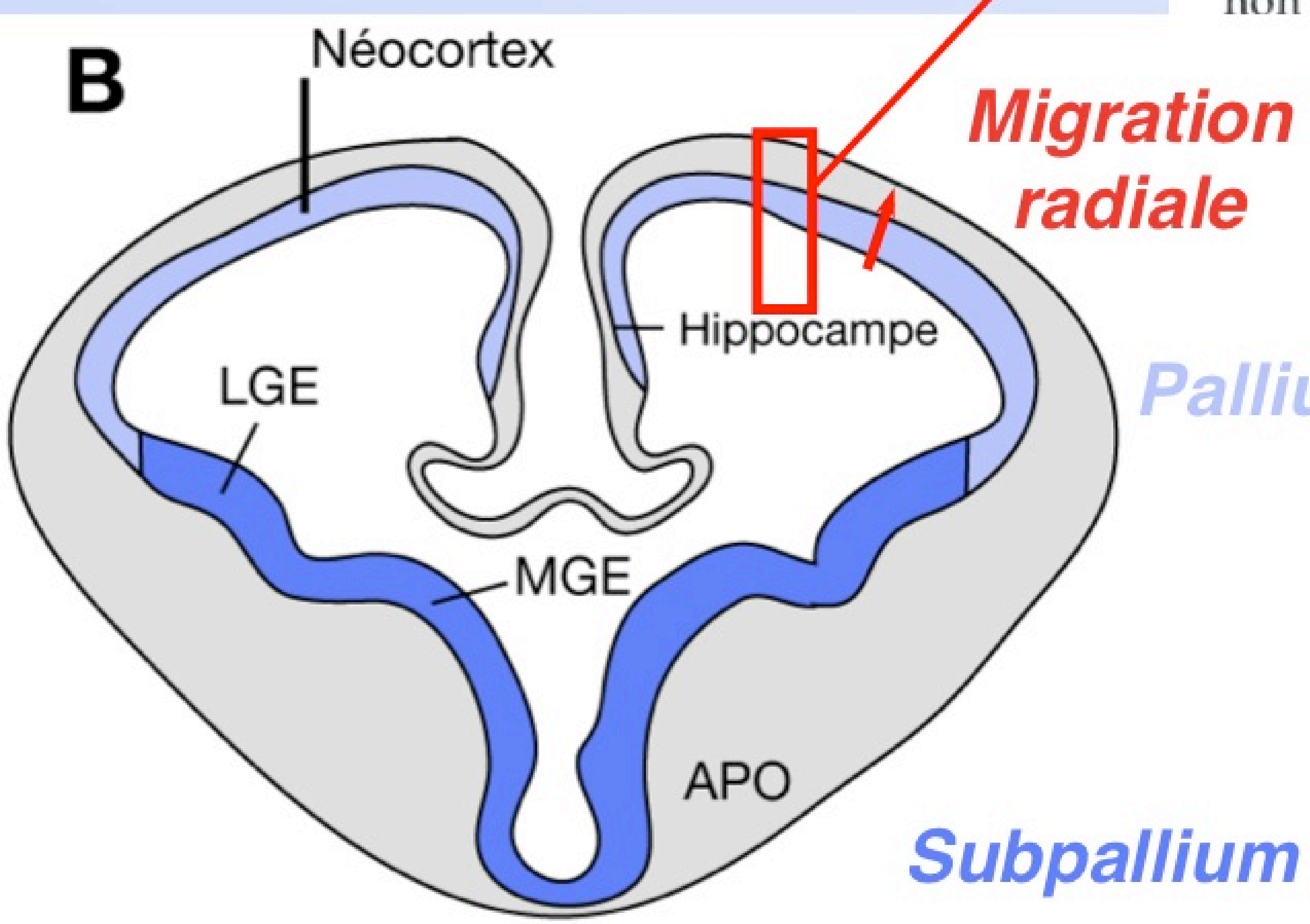
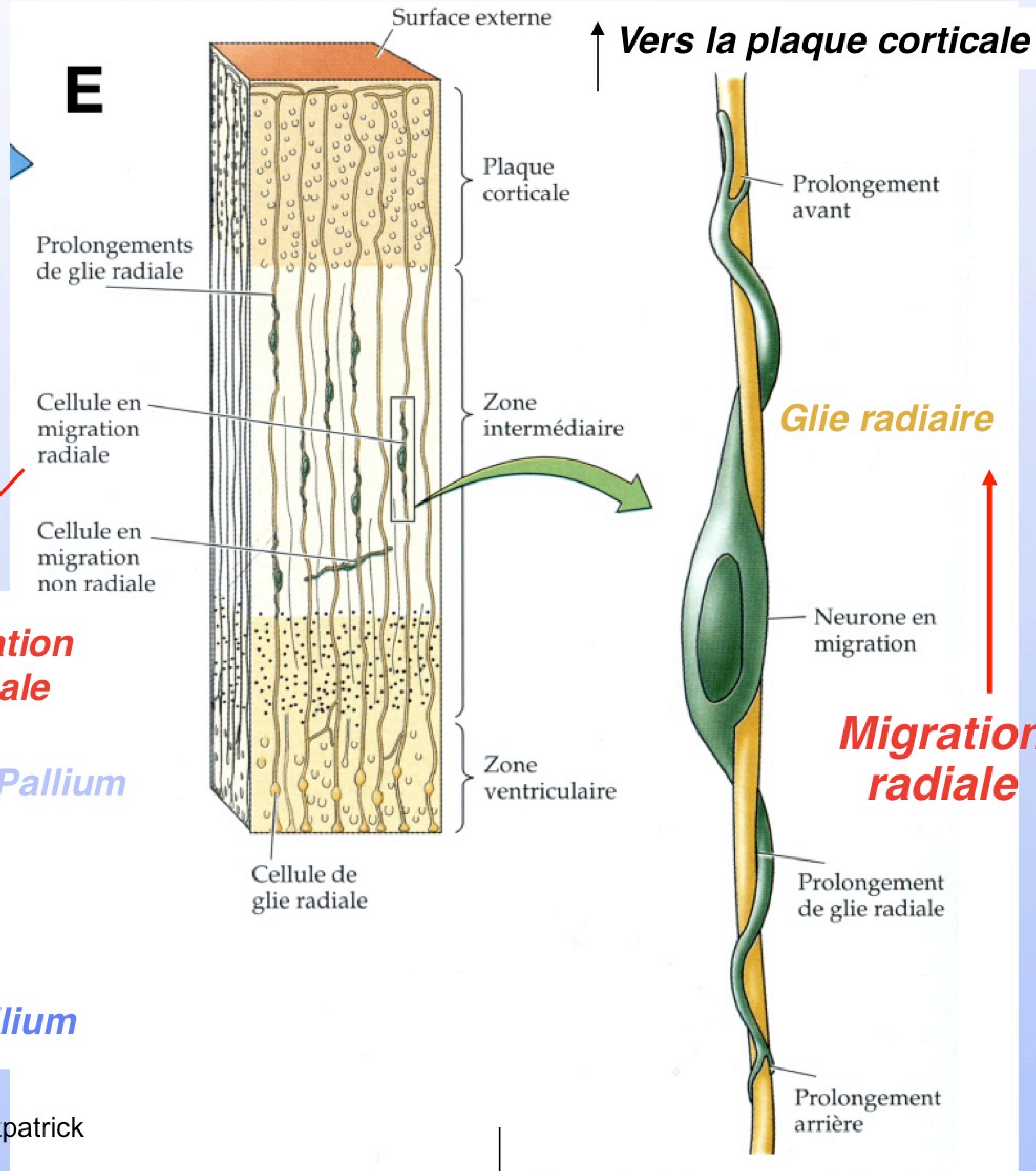


Figure 5 : Modes of migration of interneurons from the subpallial telencephalon toward the cortical and hippocampal anlagen.

Télencéphale







Adapted from « Neurosciences »:
De Dale Purves, George J. Augustine, David Fitzpatrick
Traduit par Jean-Marie Coquery
De Boeck Université 3ème édition, 2005

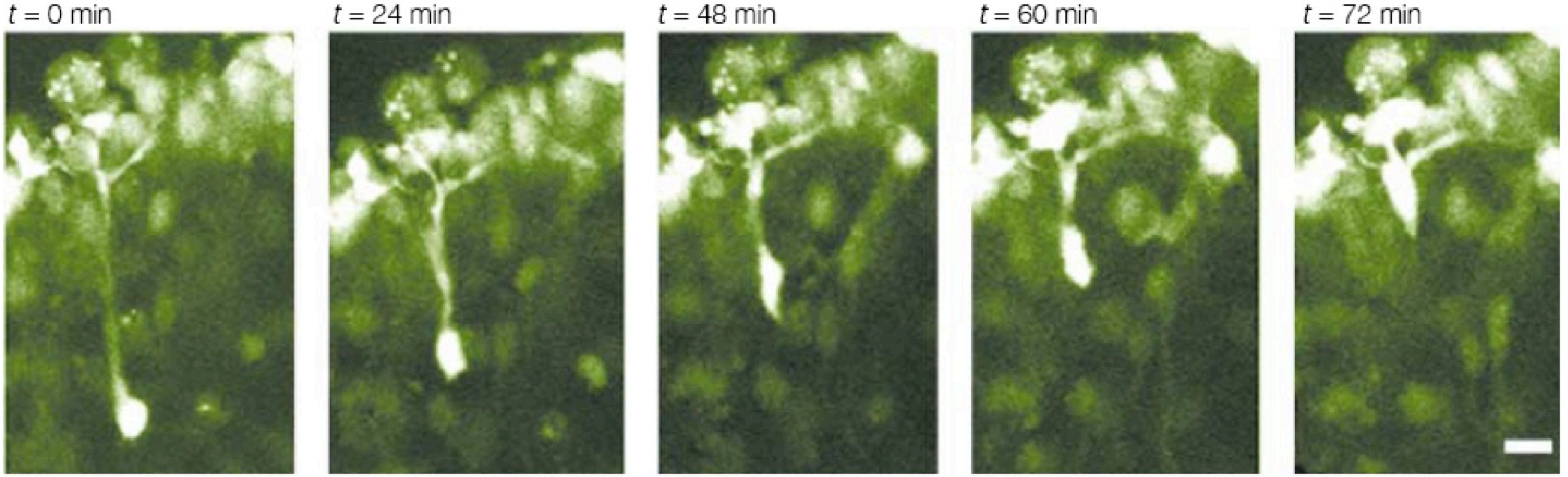
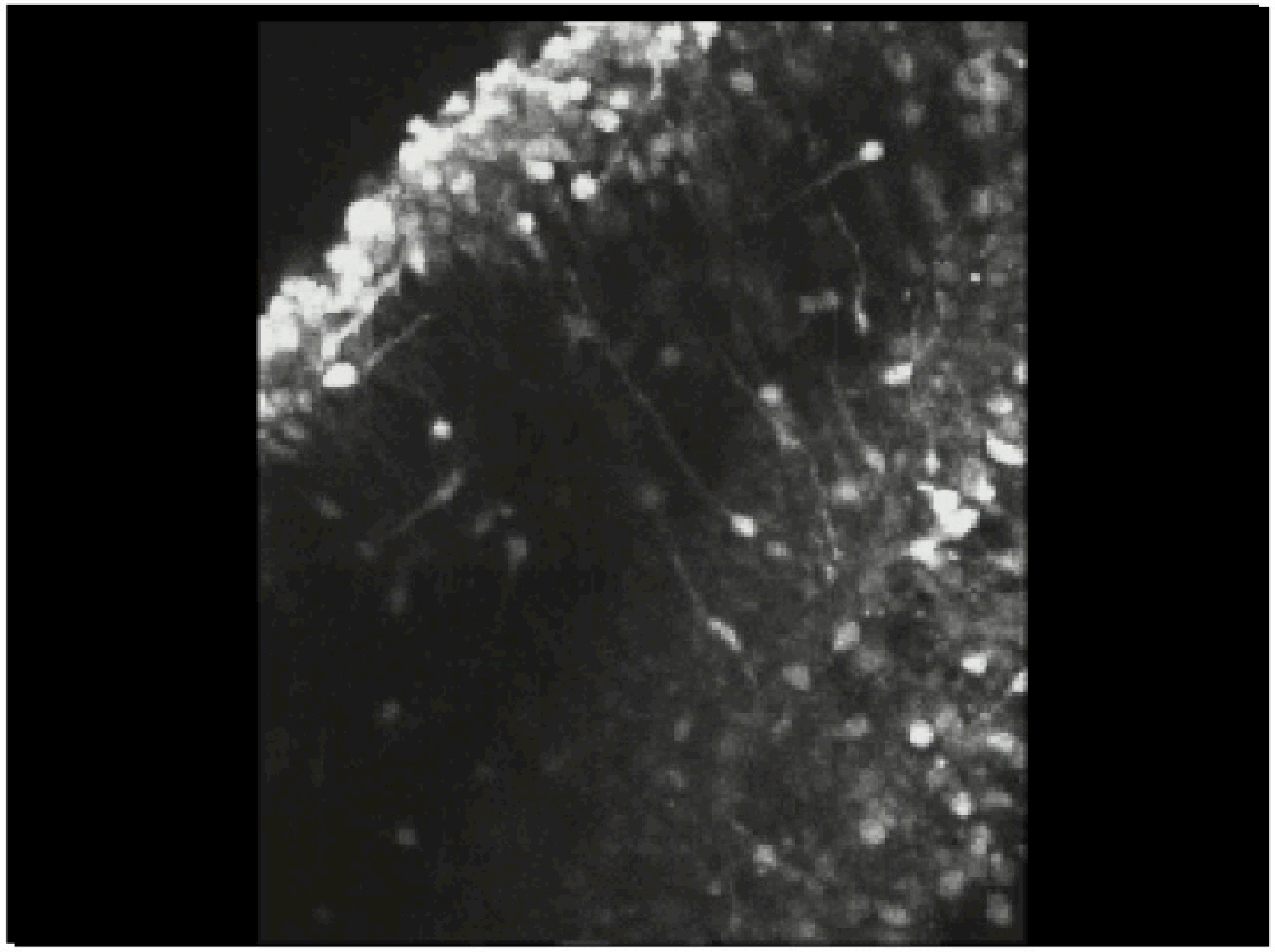


Figure 3 | **Somal translocation.** Time-lapse images of a cell showing somal translocation in a mouse cortical slice that was labelled with Oregon Green BAPTA-1 488 AM. Images were acquired every minute and each frame shows a single optical section. Scale bar, 10 µm. See Supplementary Movie from REF. 31 © 2001 Macmillan Magazines Ltd.



Nadarajah & Parnavelas
Nat Rev Neur (2002)vol.3:423.

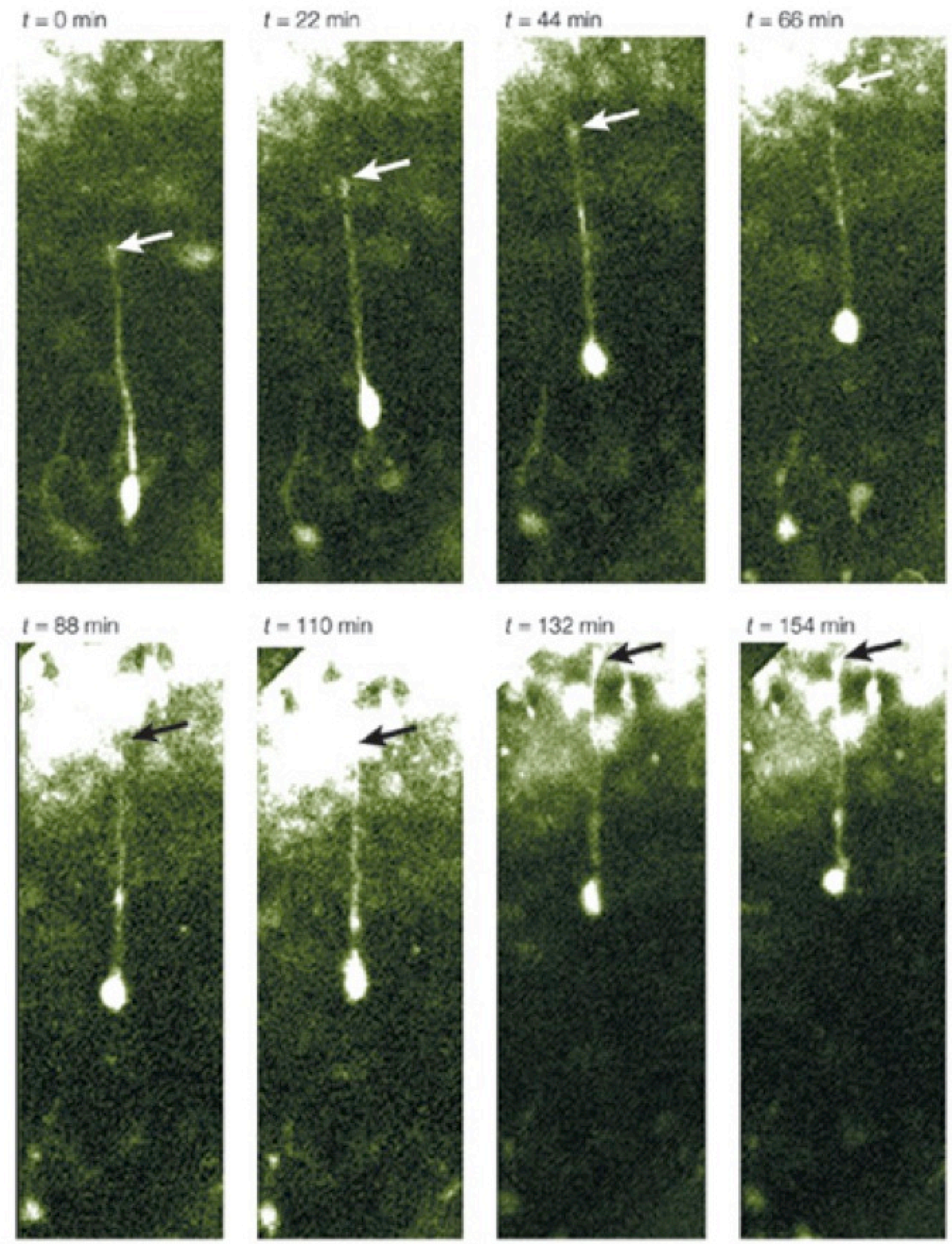
Somal translocation

E16 - 300min

Nadarajah, Nature Neurosci. 4, 143–150 (2001).

Glia-guided locomotion

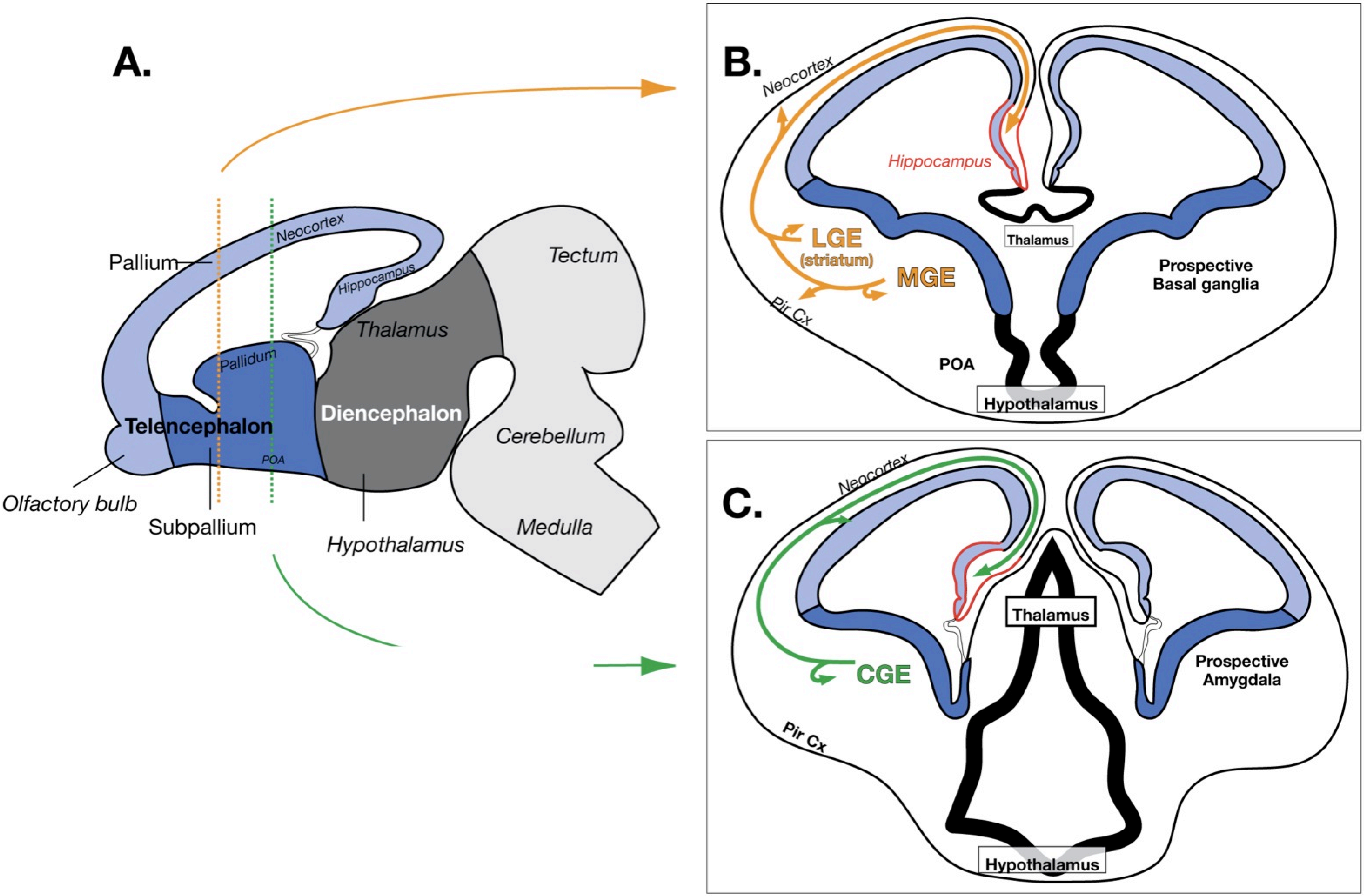
Nadarajah, Nature Neurosci. 4, 143–150 (2001).



Nadarajah & Parnavelas
Nat Rev Neur (2002)vol.3:423.

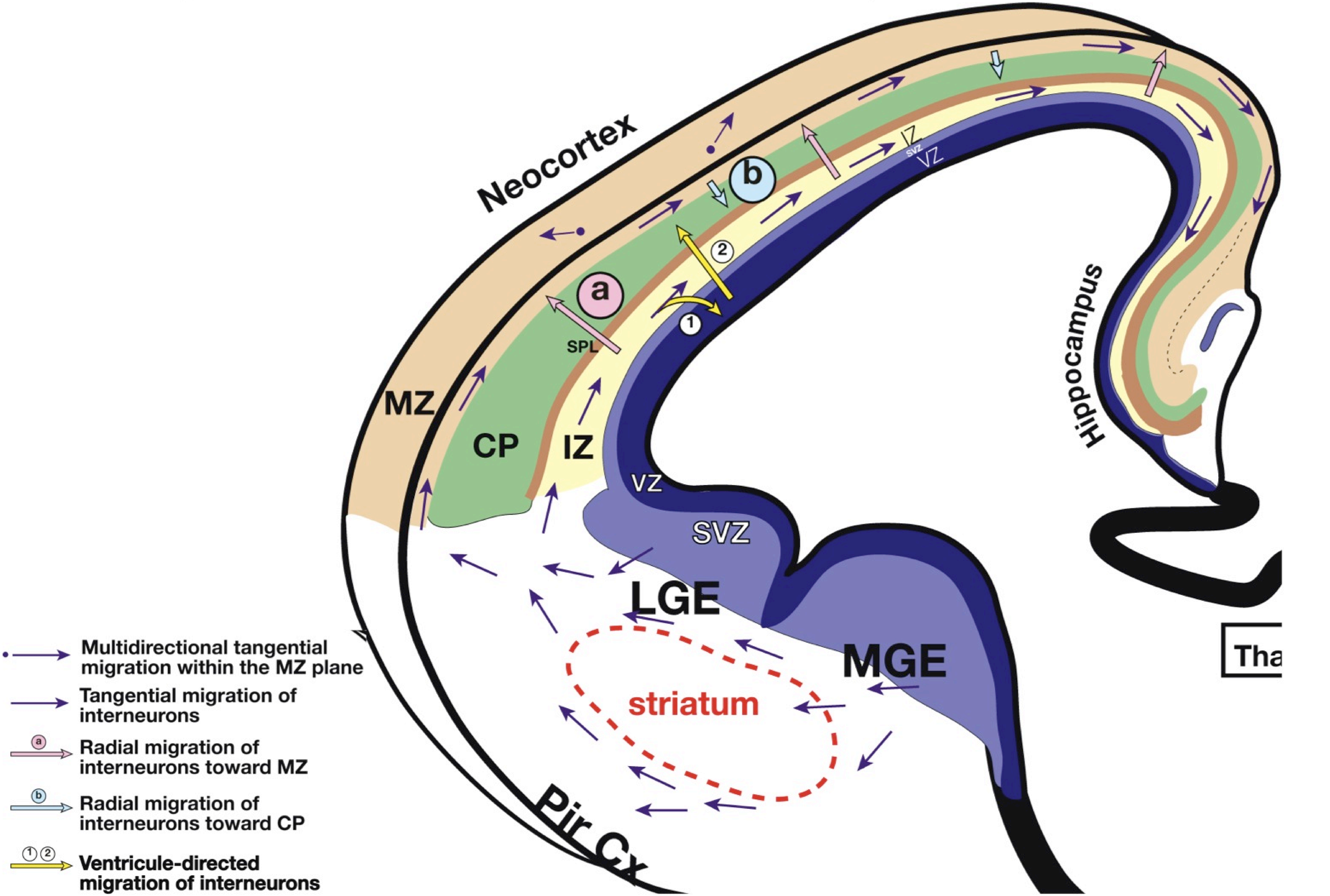
Nature Reviews | Neuroscience

Migration of inhibitory interneurons



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

Tangential migration of interneurons

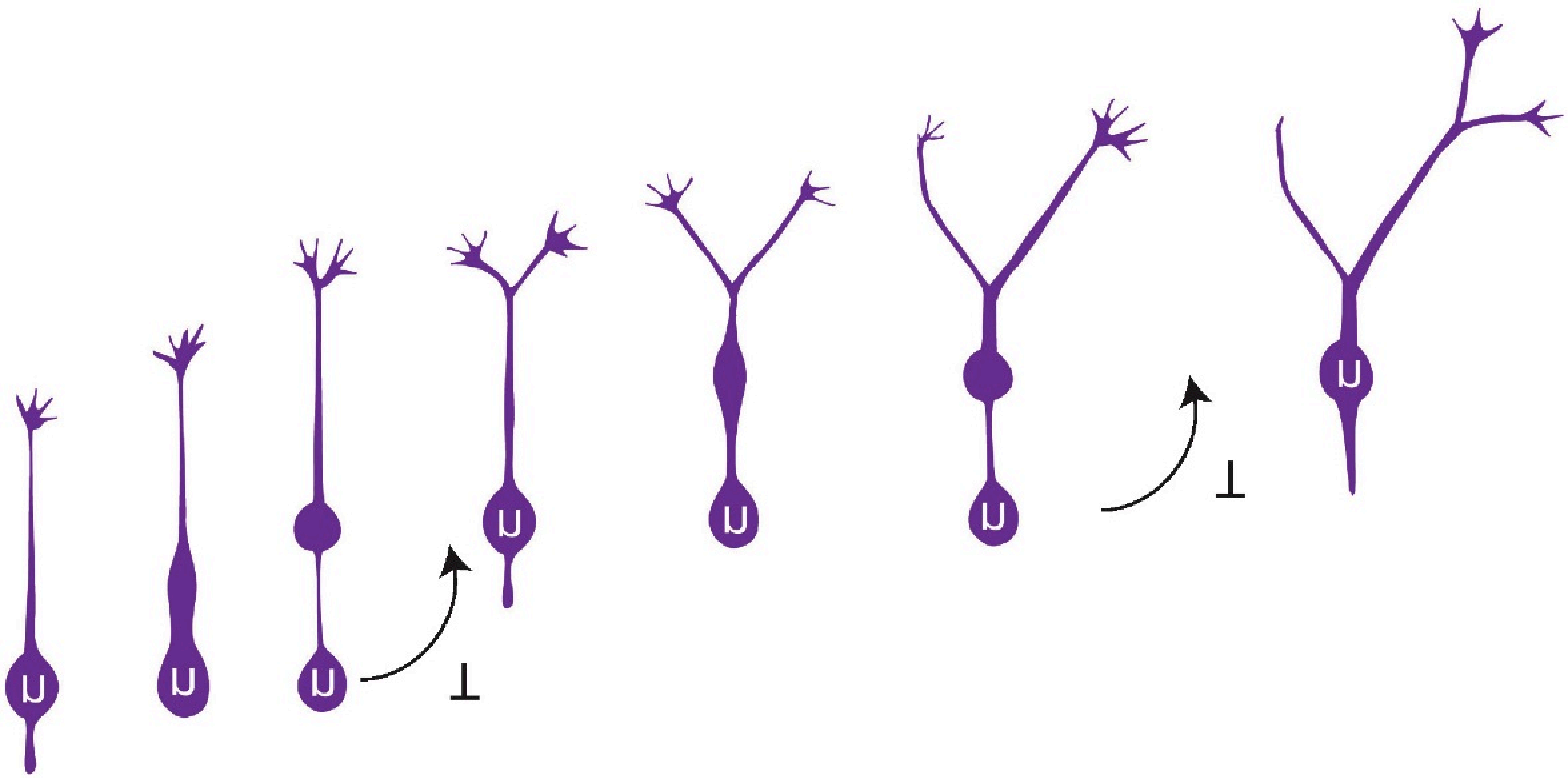


European Journal of Neuroscience, Vol. 23, pp. 894–900, 2006 doi:10.1111/j.1460-9568.2006.04630.x

REVIEW ARTICLE

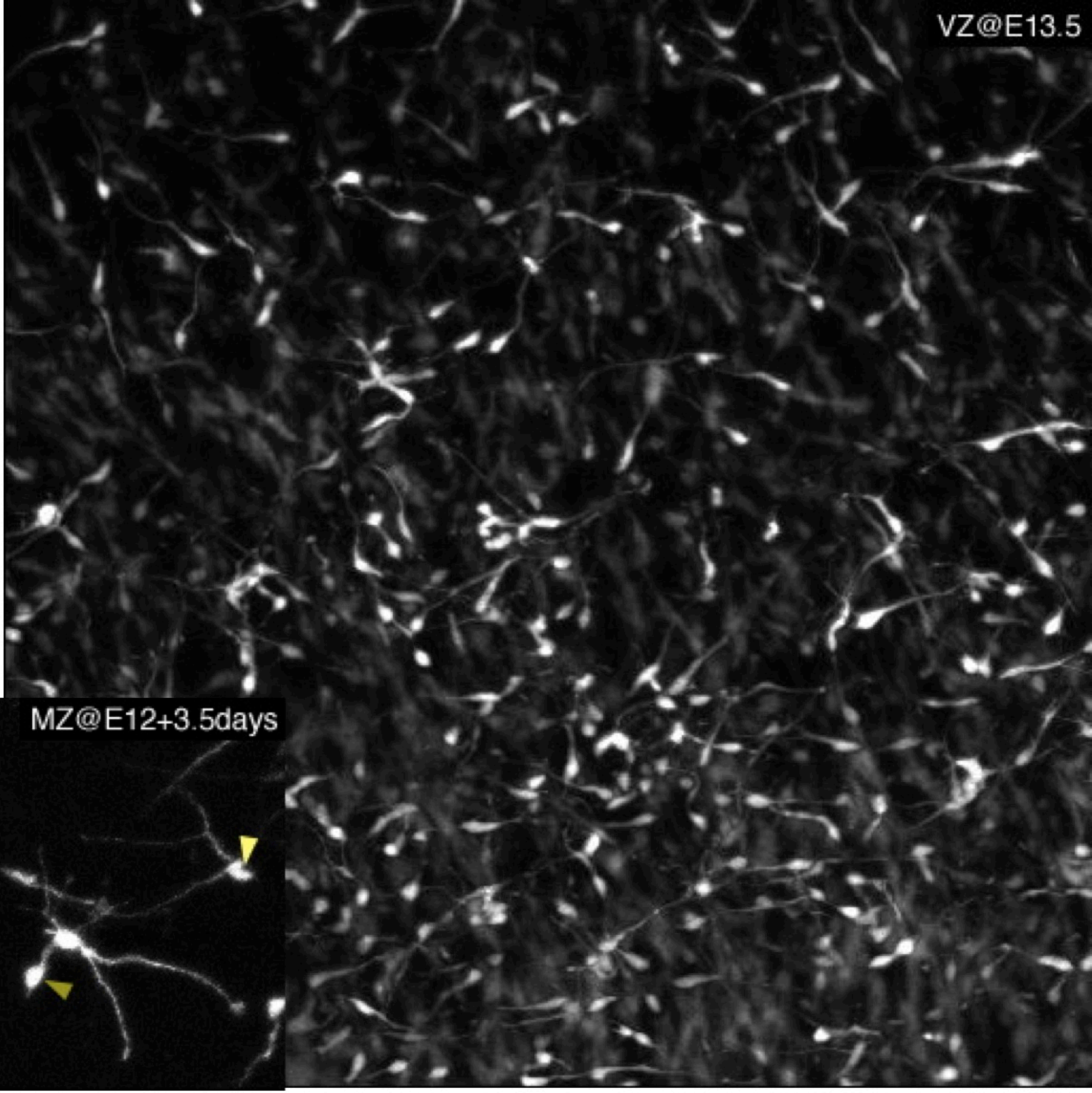
Cell and molecular mechanisms involved in the migration of cortical interneurons

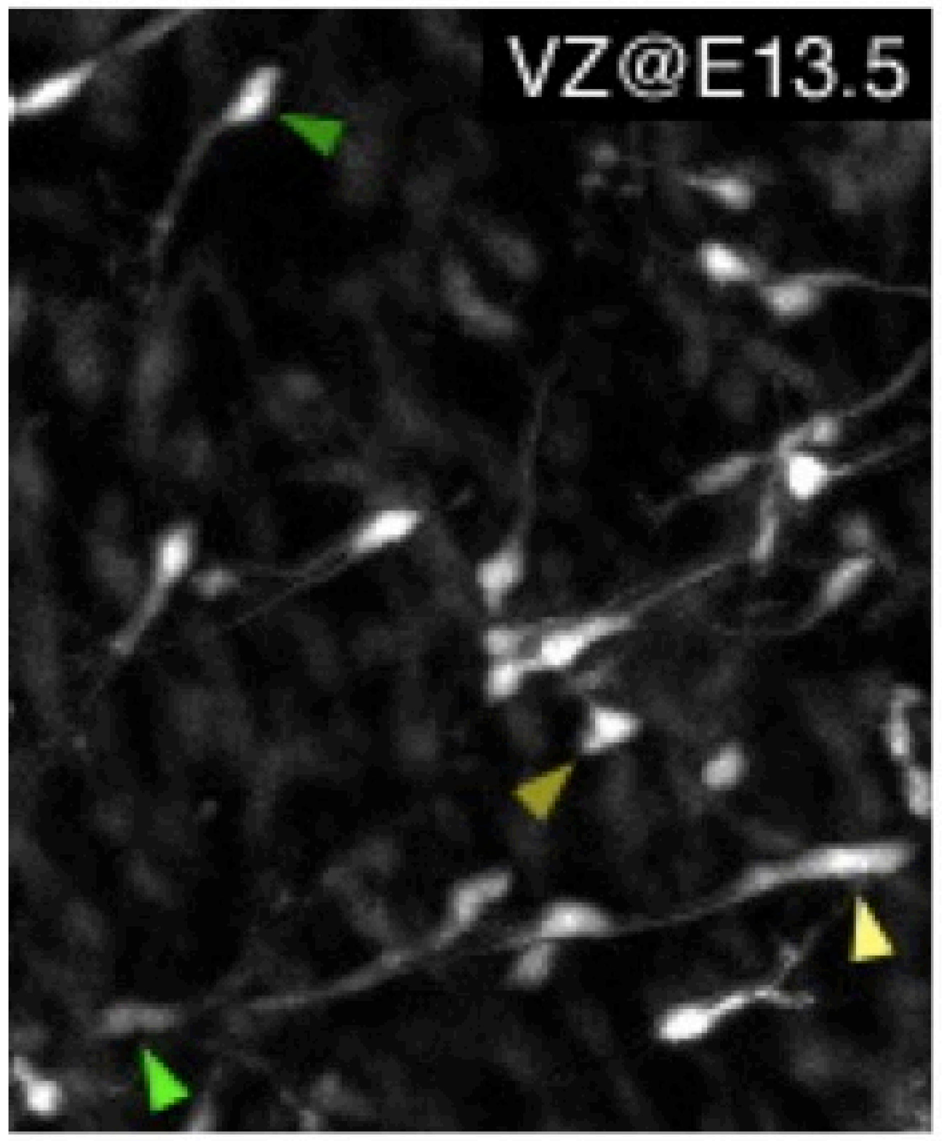
Christine Métin,[1,2] Jean-Pierre Baudoin,[1,2] Sonja Rakić[3] and John G. Parnavelas[3]

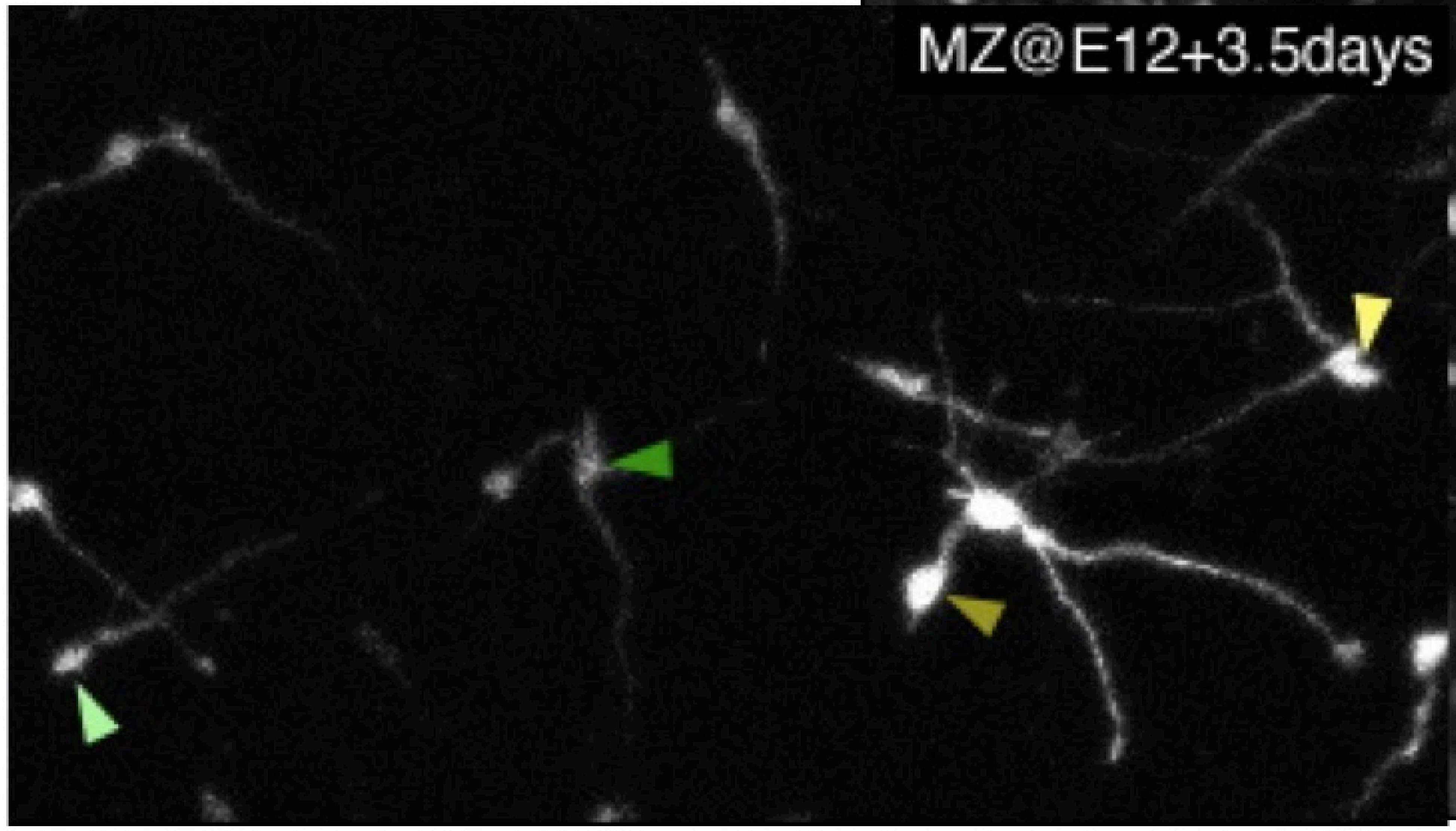


Multidirectional and multizonal tangential migration of GABAergic interneurons in the developing cerebral cortex

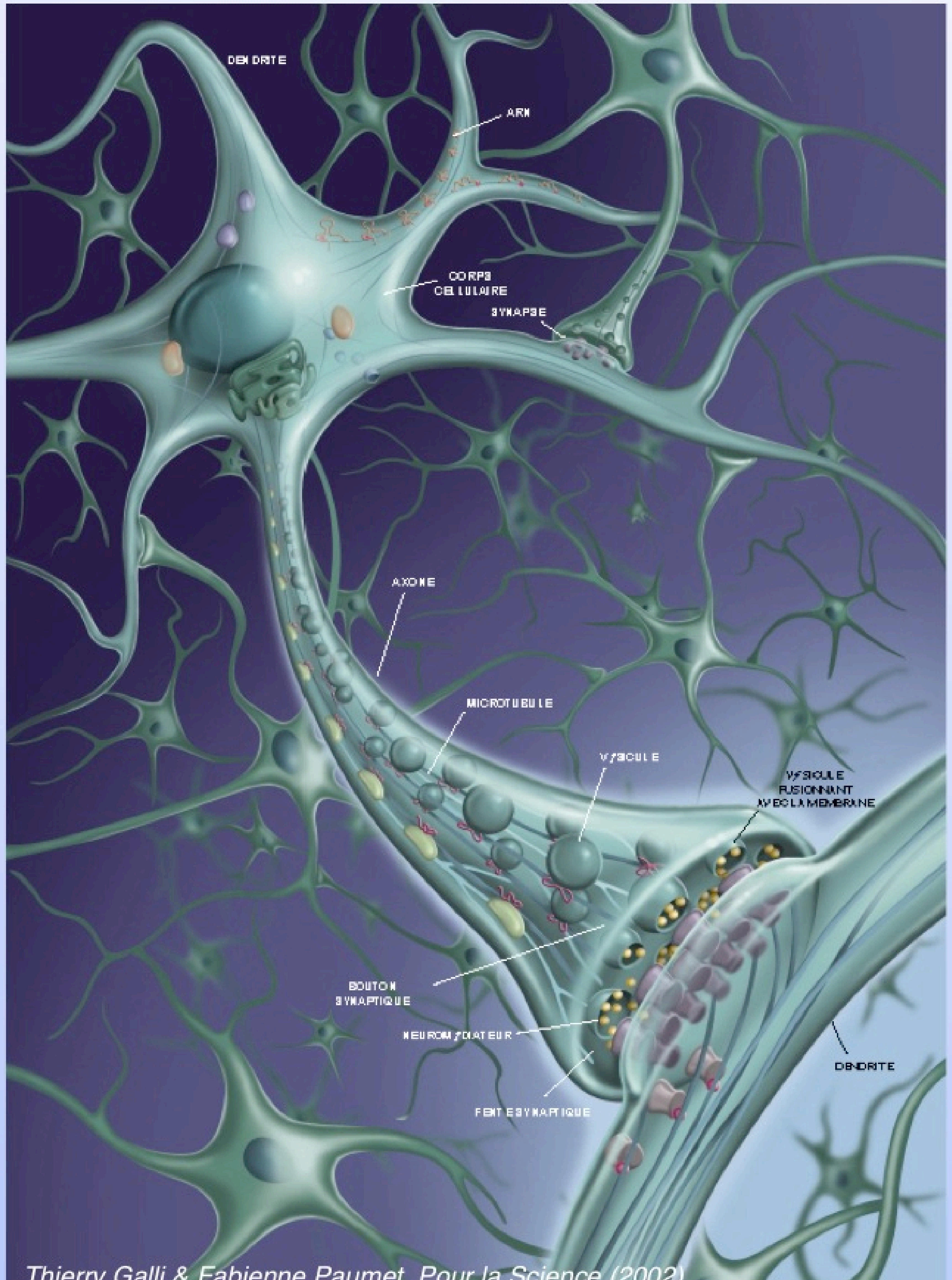
Development 133, 2167-2176 (2006)



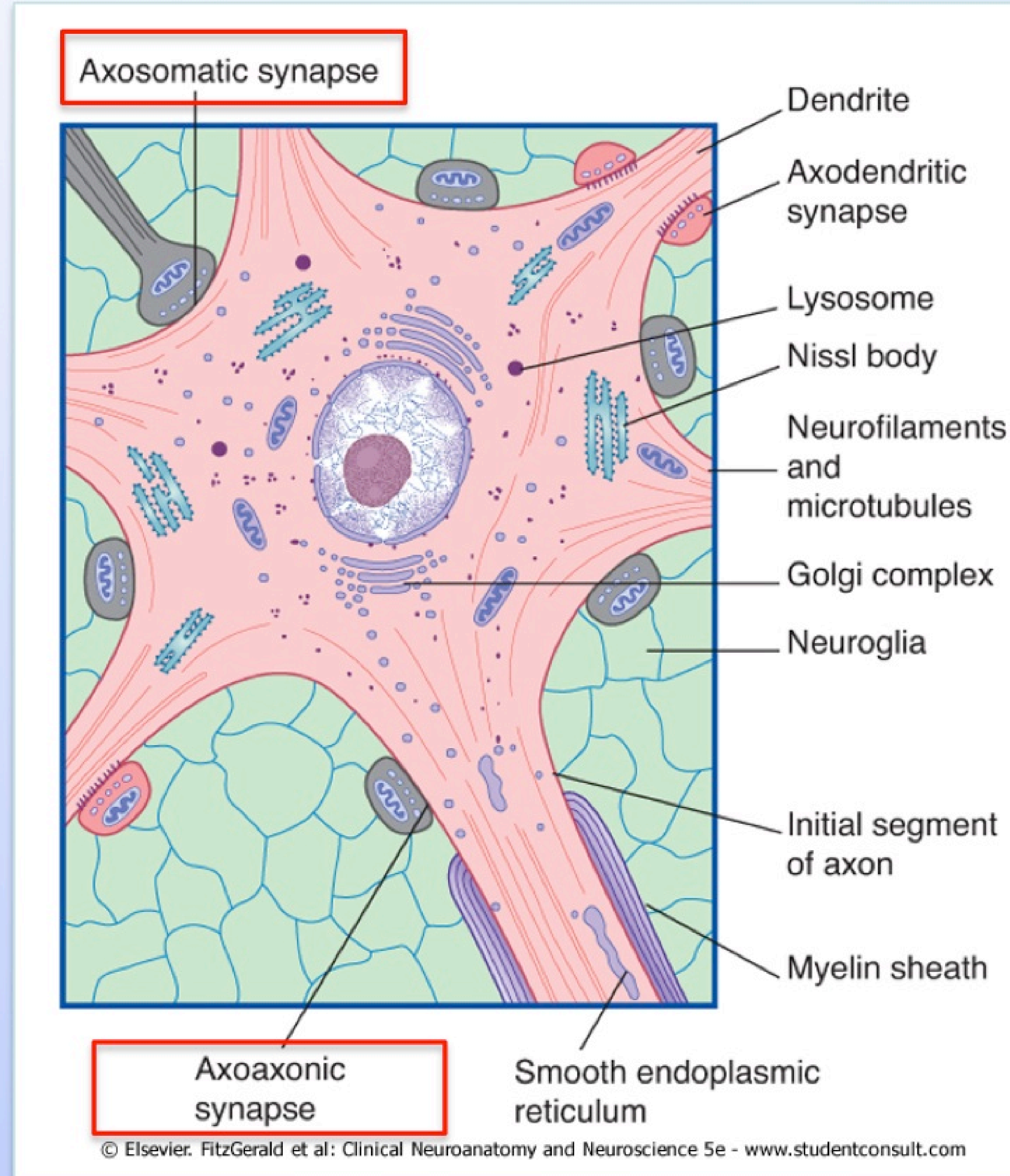




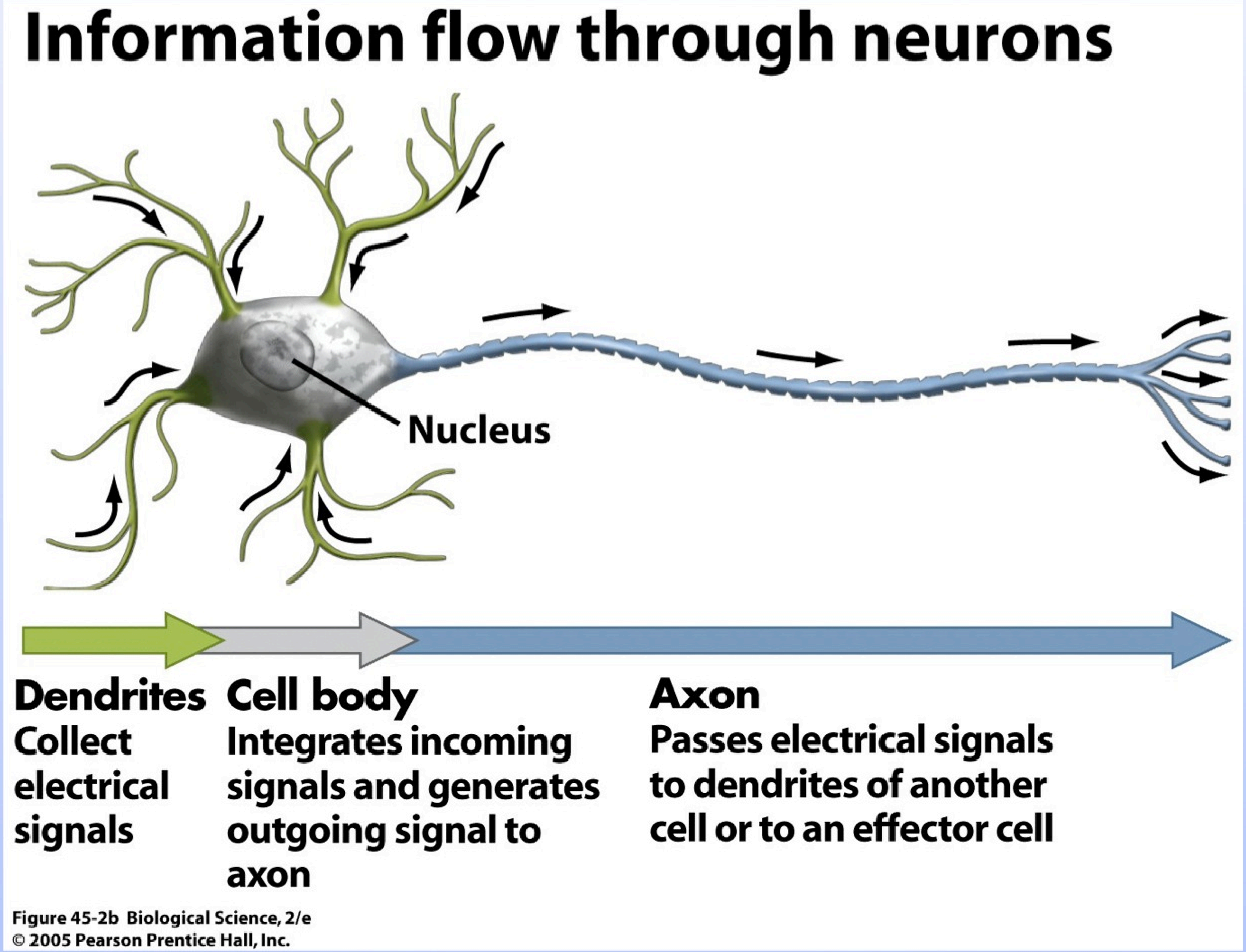
4. Polarité neuronale : les domaines



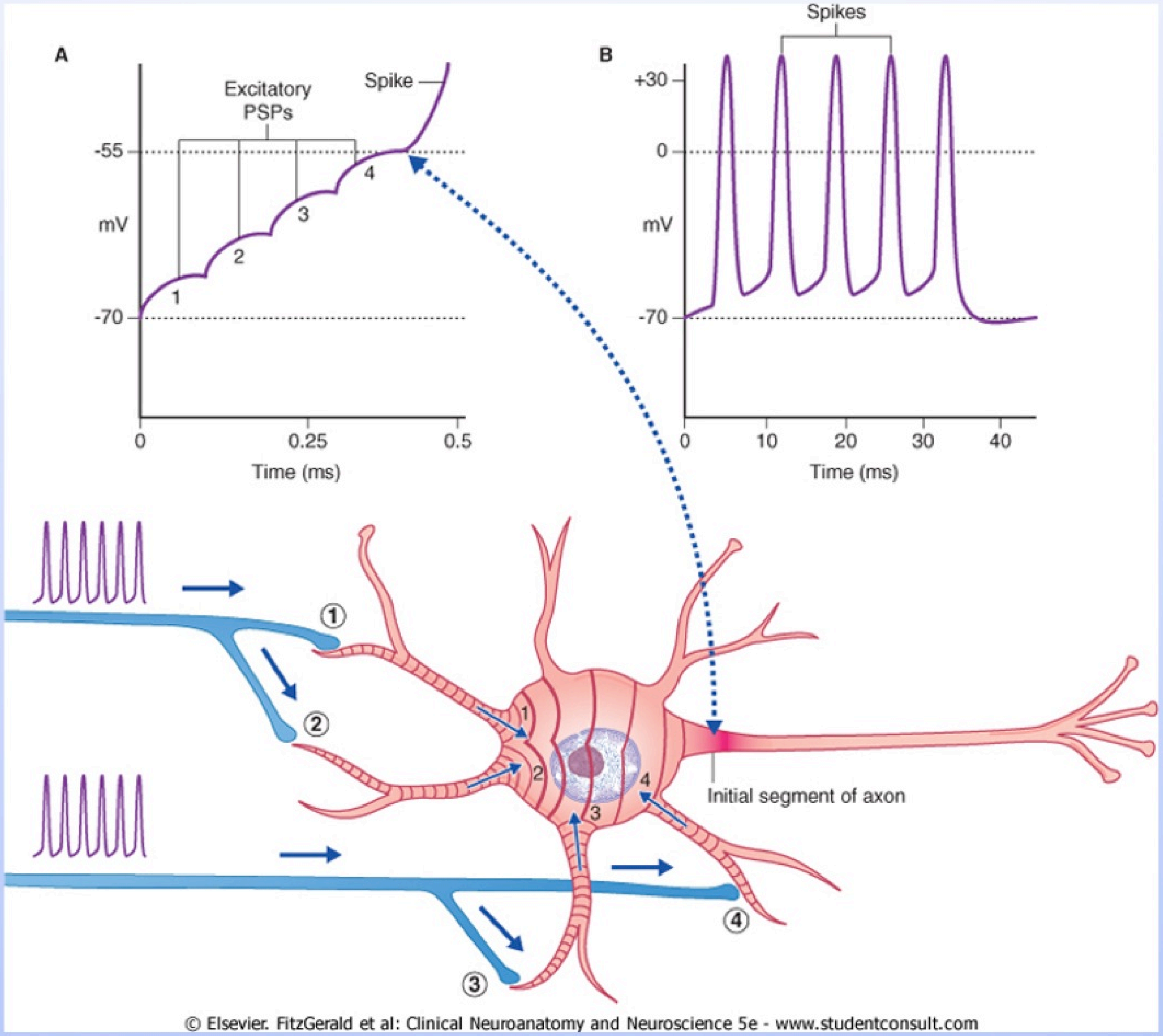
Thierry Galli & Fabienne Paumet, Pour la Science (2002)



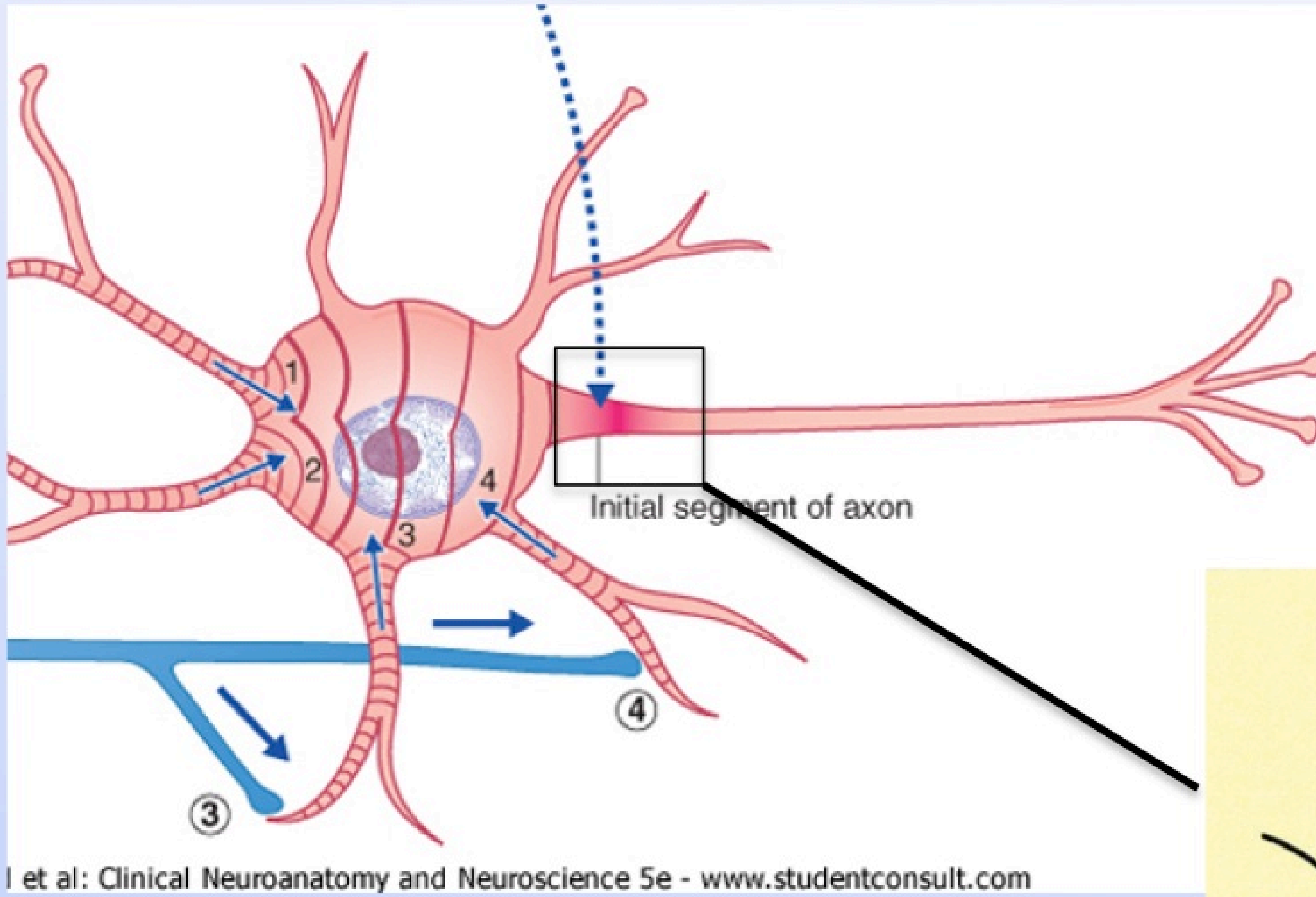
La signalisation neuronale nécessite la polarité



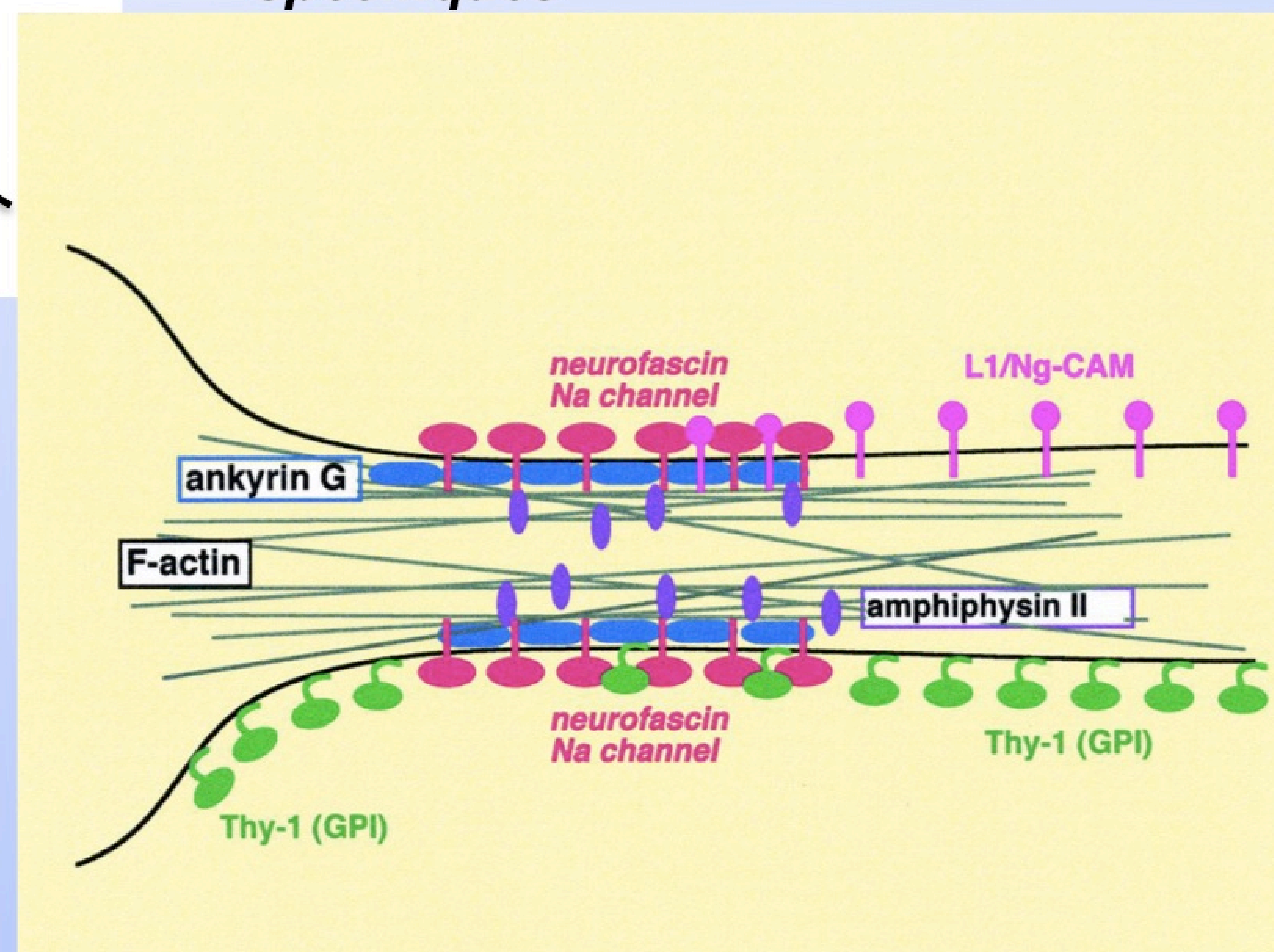
La signalisation neuronale nécessite la polarité



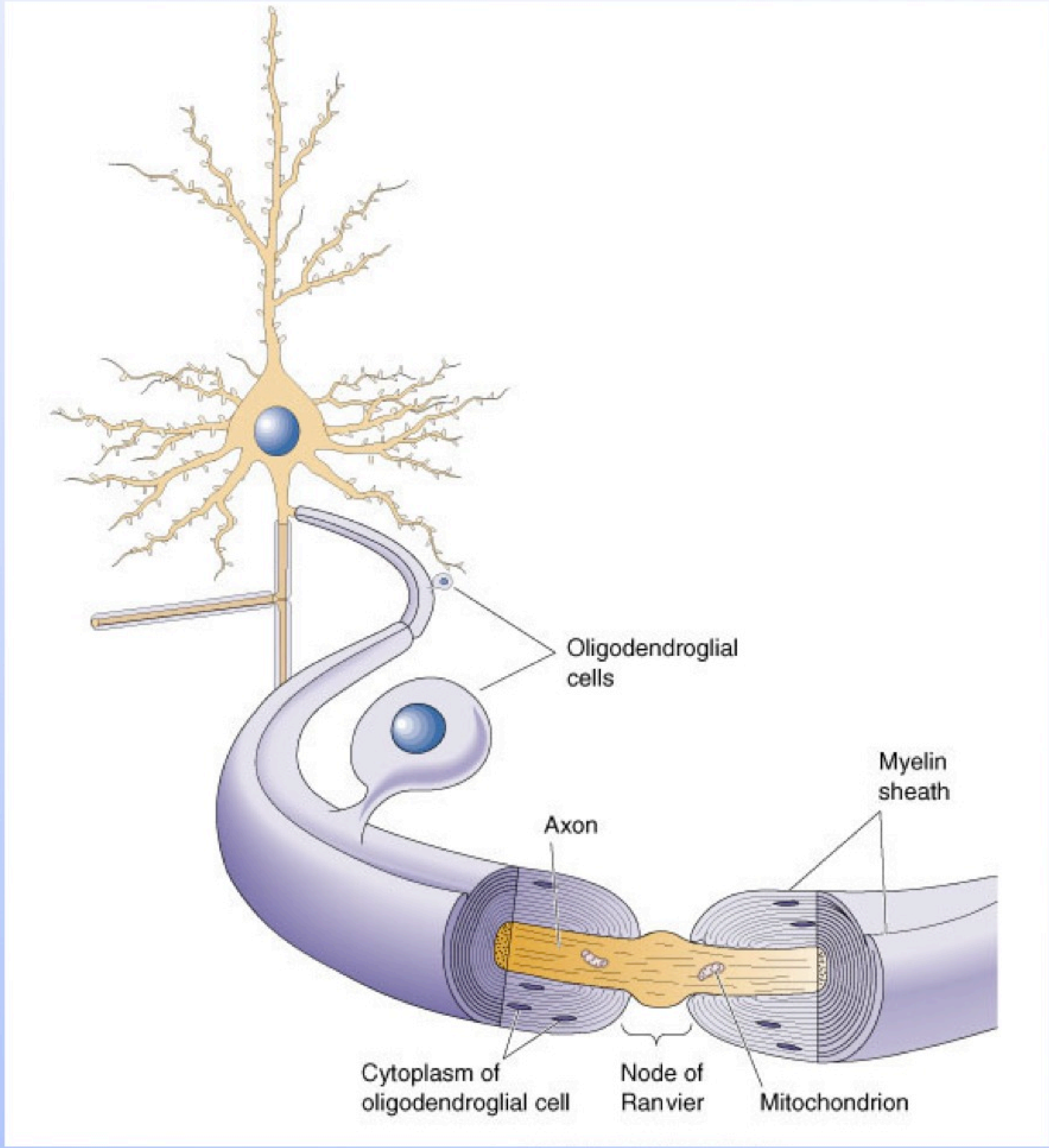
4. Les domaines: segment initial de l'axone

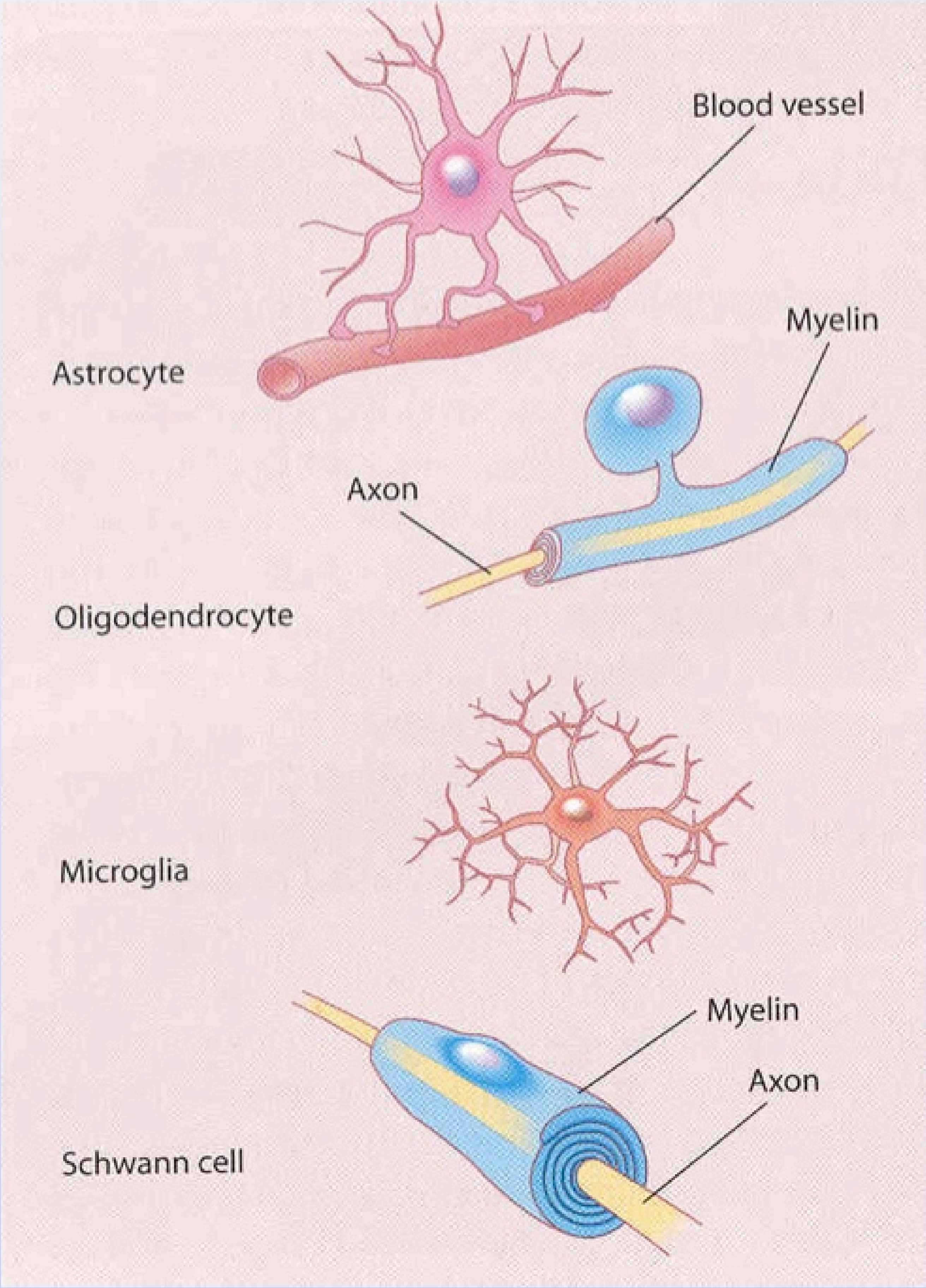


Expression et ciblage de protéines spécifiques

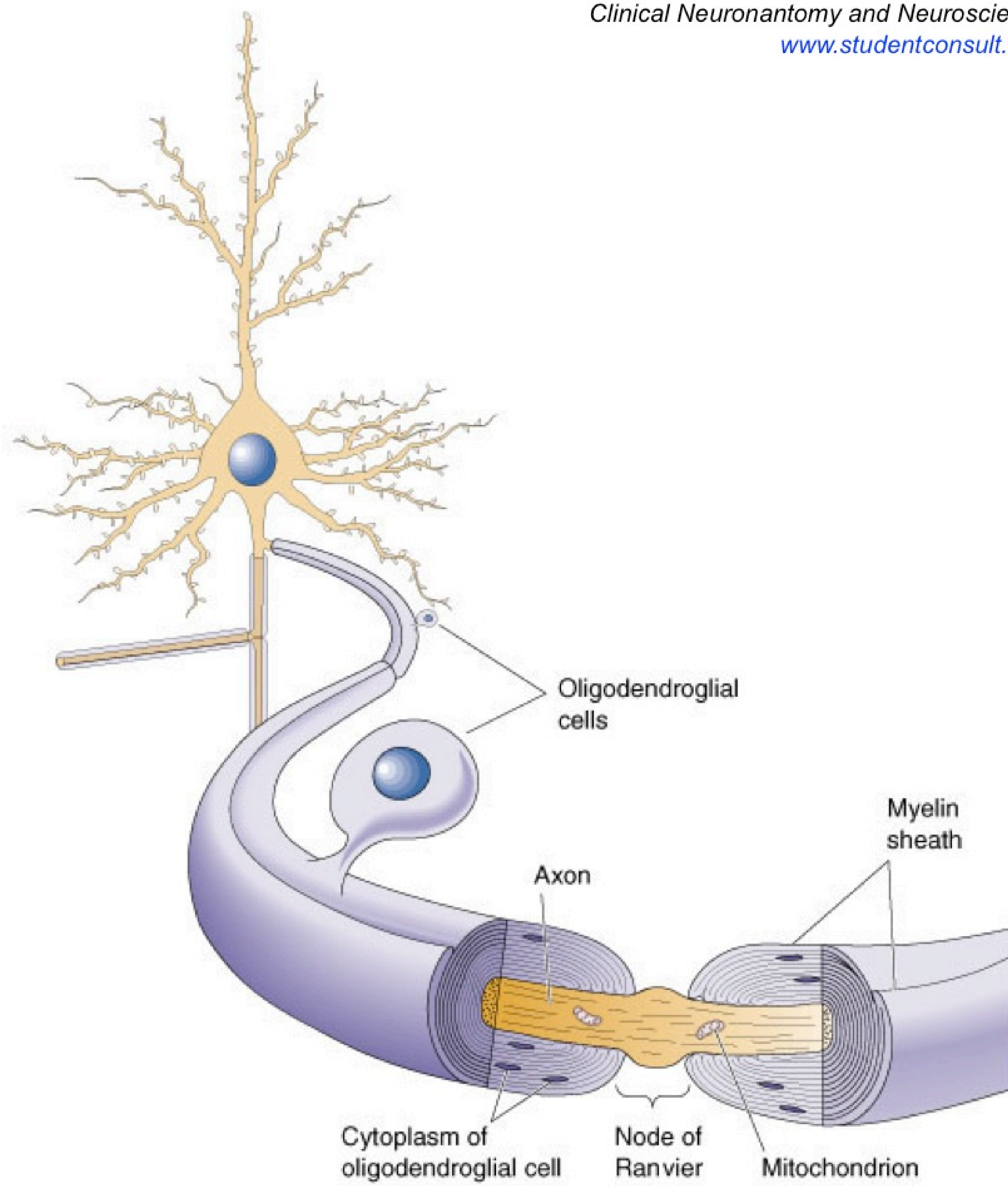


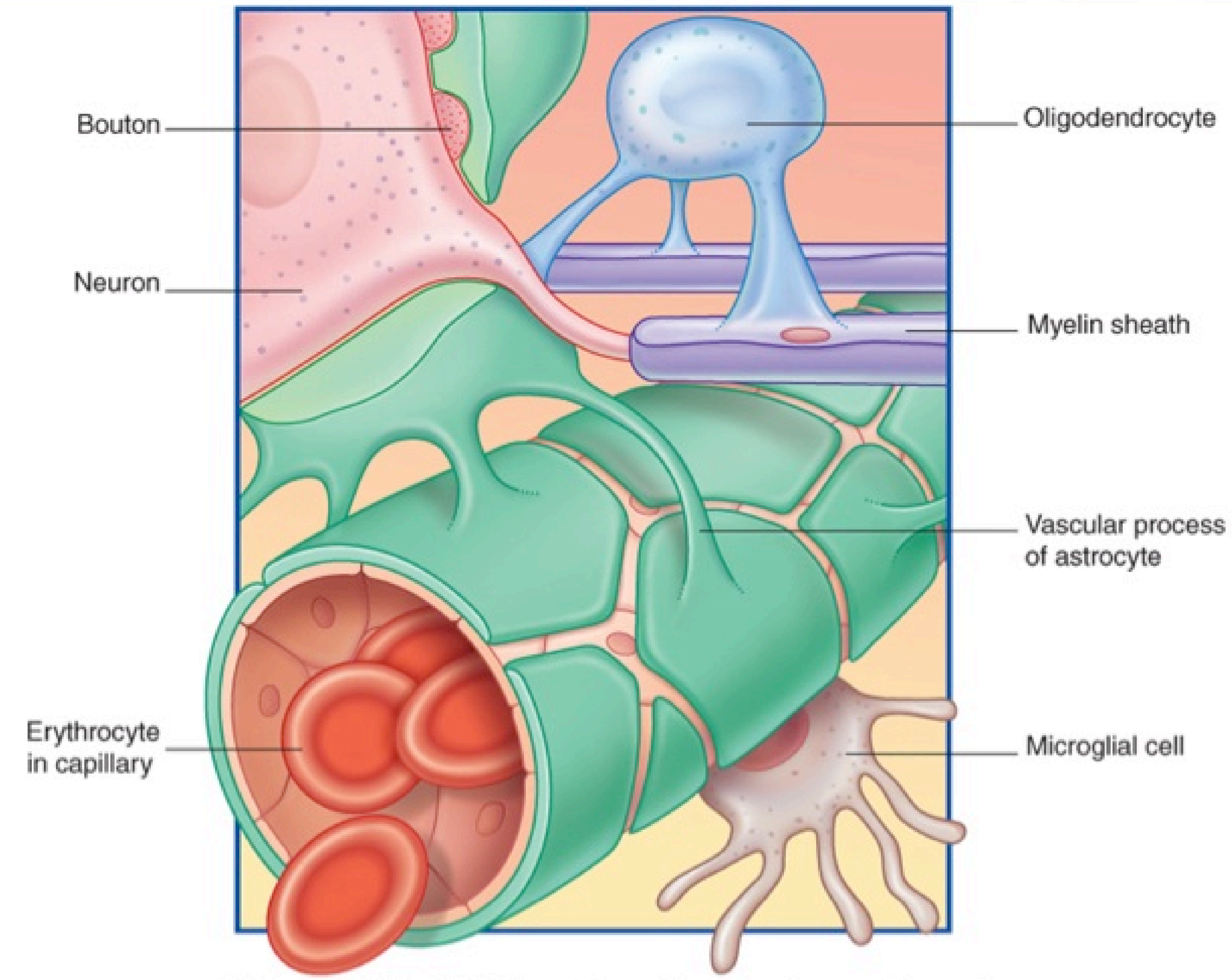
Oligodendrocytes and the formation of myelin

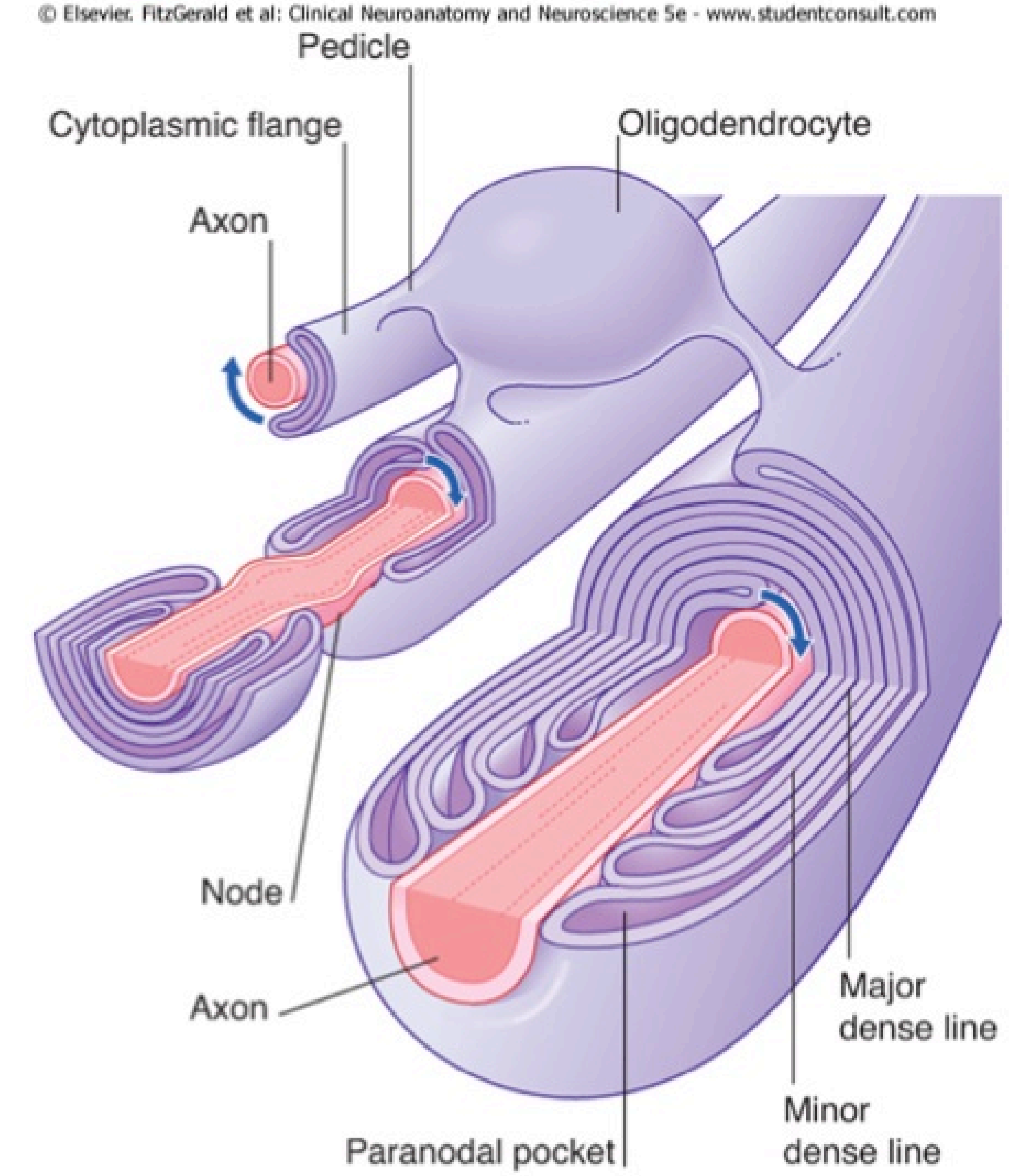




Oligodendrocytes and the formation of myelin







PYRAMIDAL NEURON (HIPPOCAMPAL)

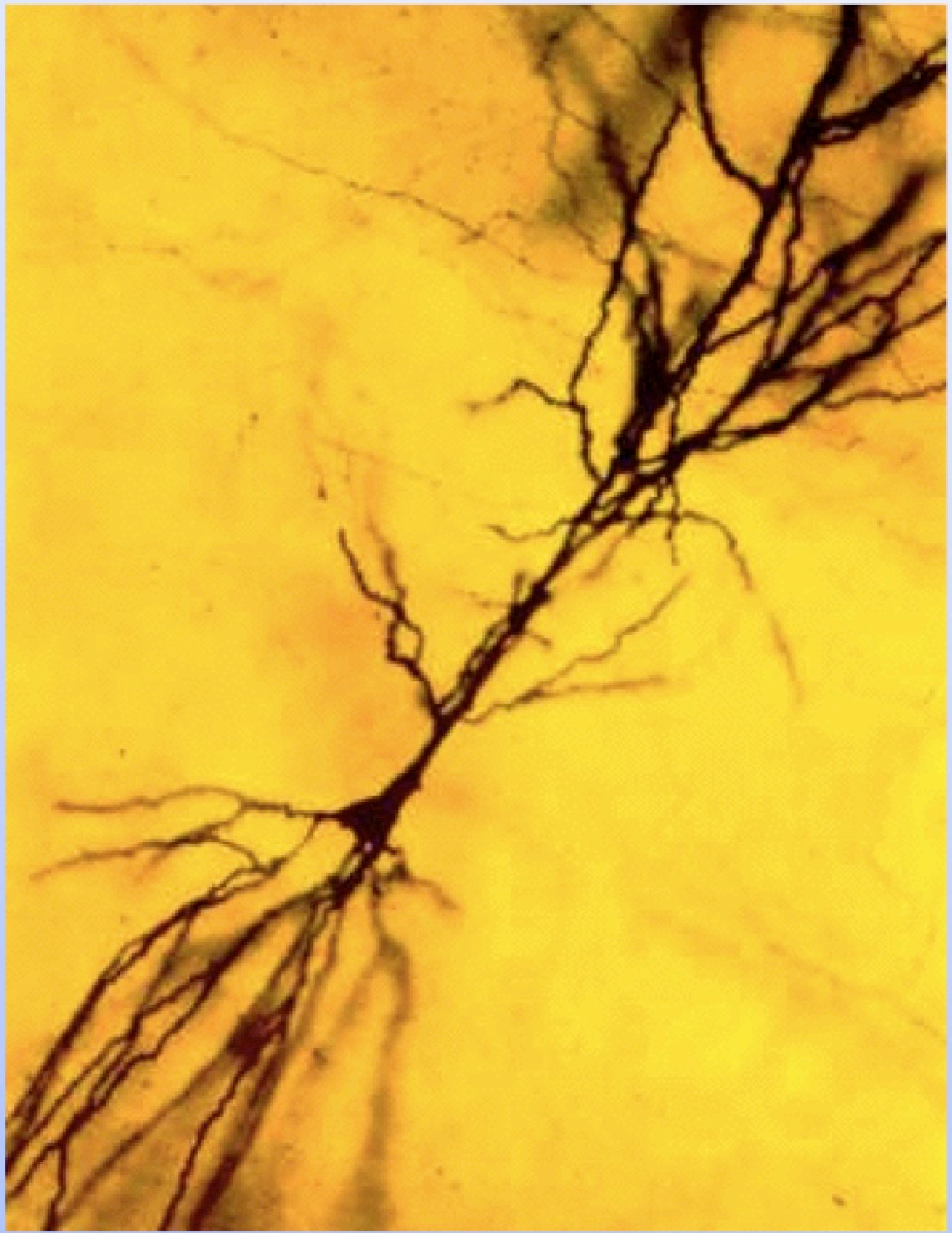


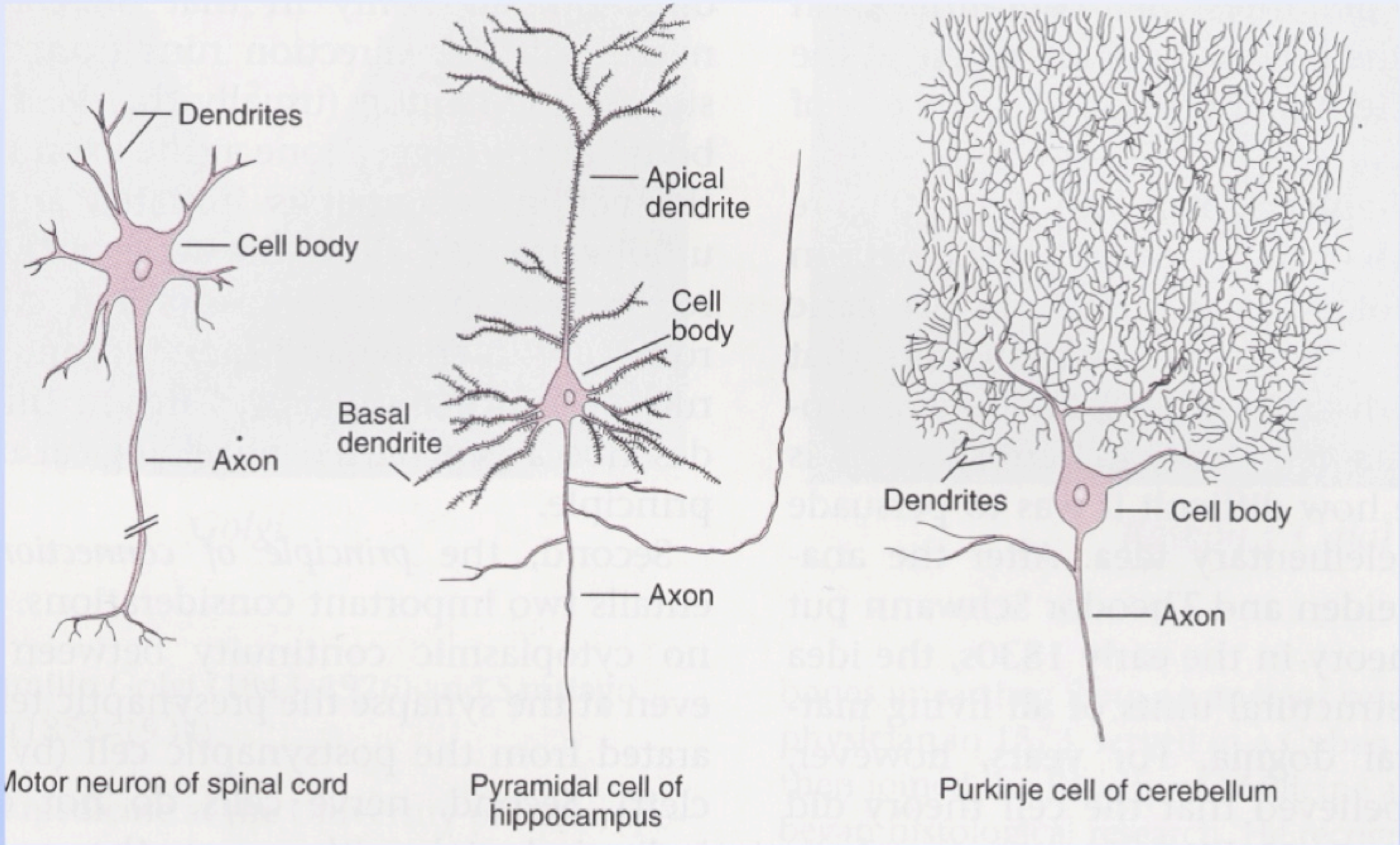
Photo from http://synapses.clm.utexas.edu/lab/lab.stm

GOLGI STAIN: ***potassium dichromate + silver nitrate=silver chromate*** precipitates within the matrix of the cytoplasm

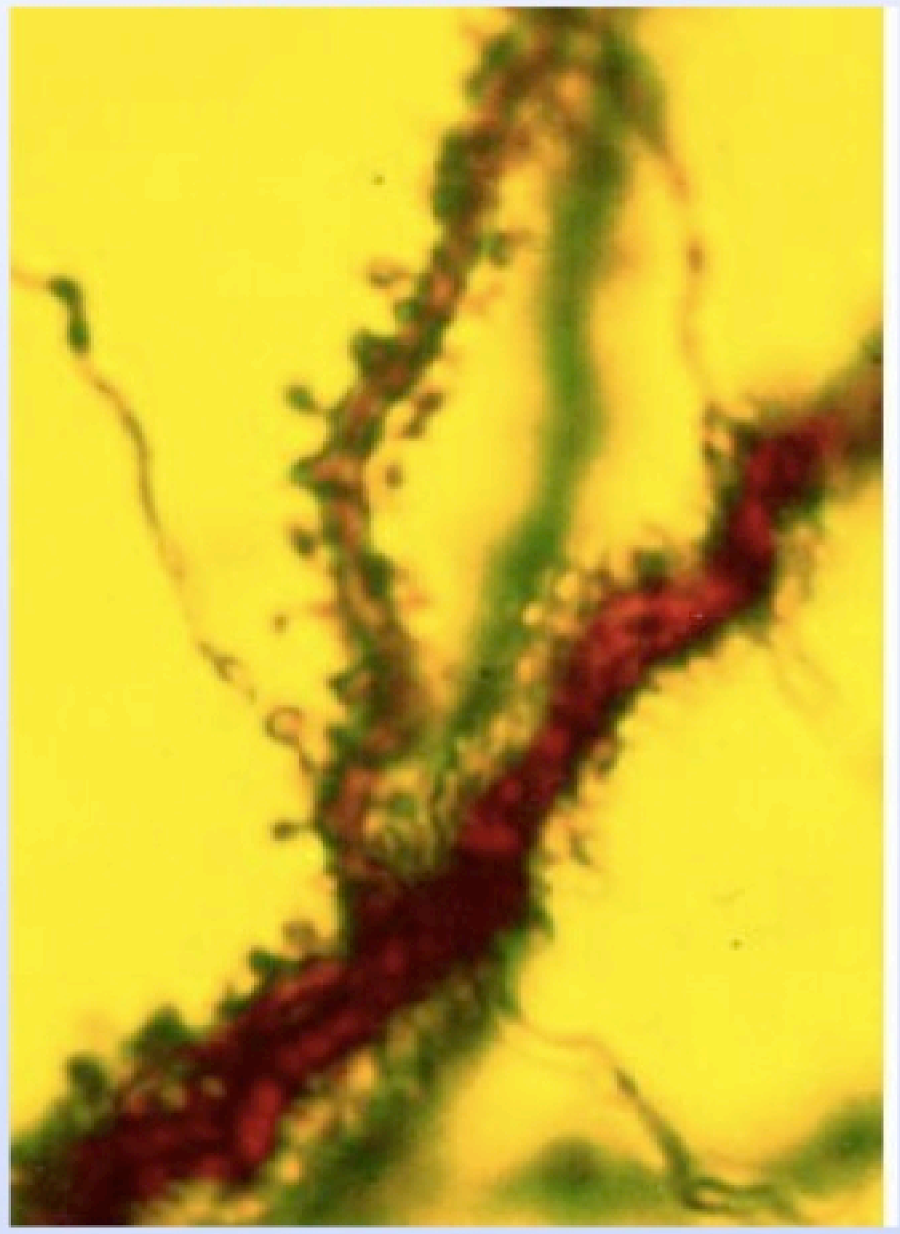
SERENDIPITOUSLY RANDOM!!

USED BY RAMON Y CAJAL TO FORMULATE THE NEURON DOCTRINE

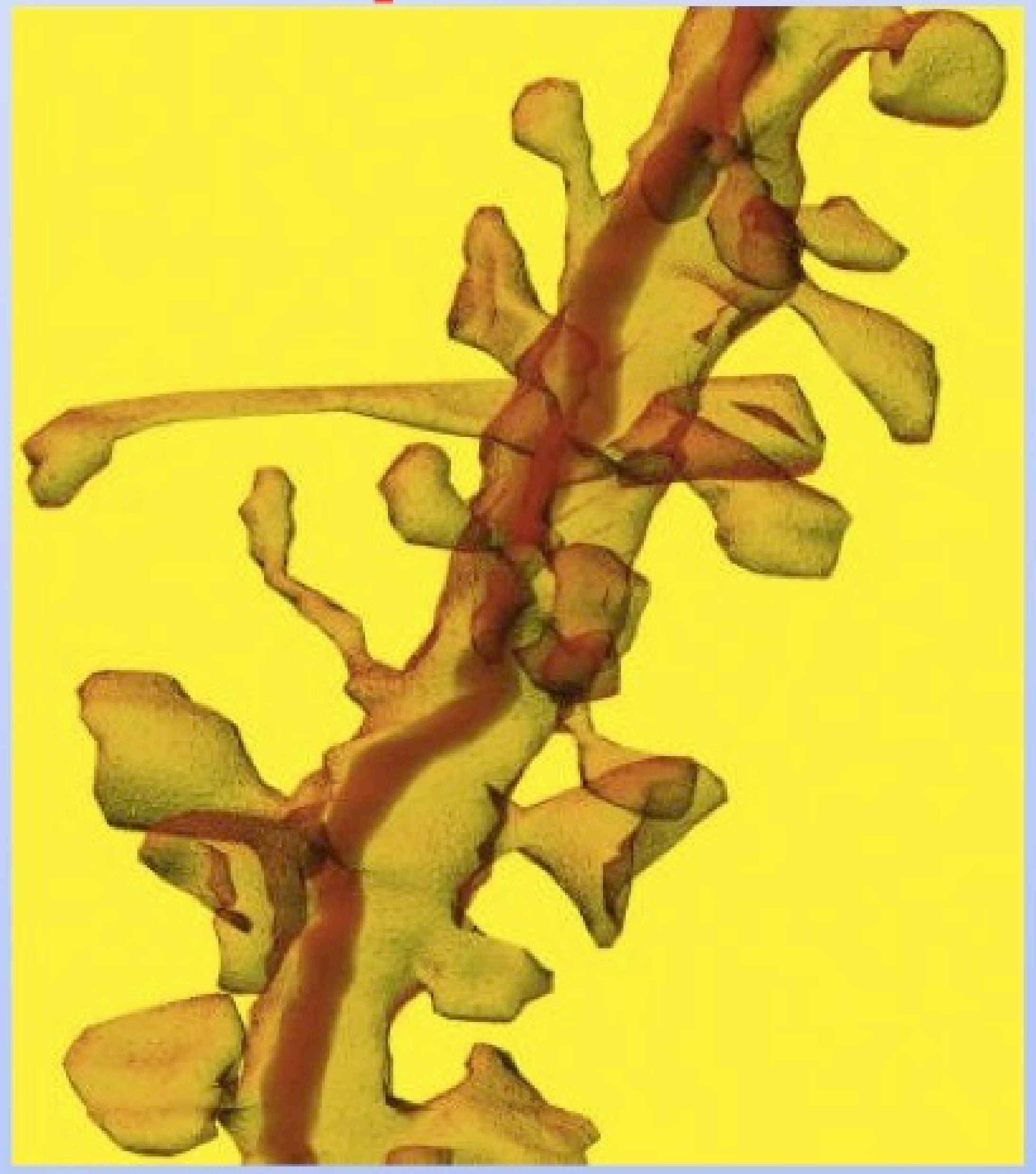
Different forms of dendrites



dendrite

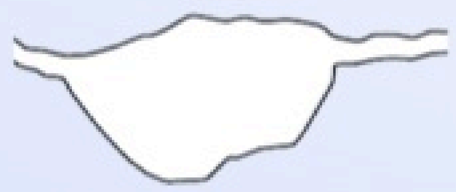
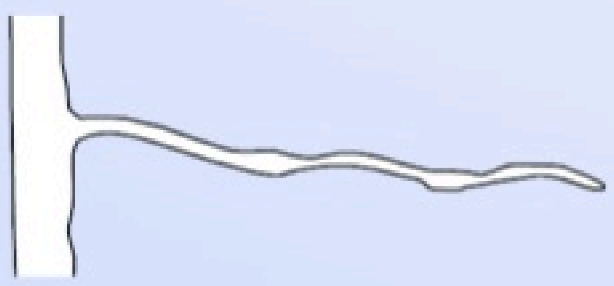
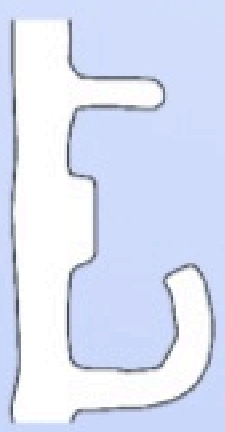
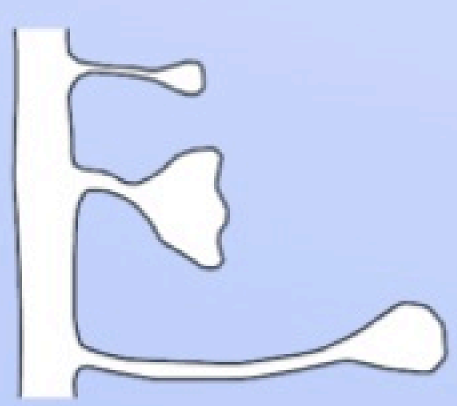
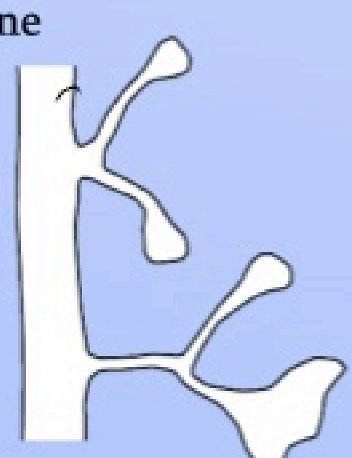


spine

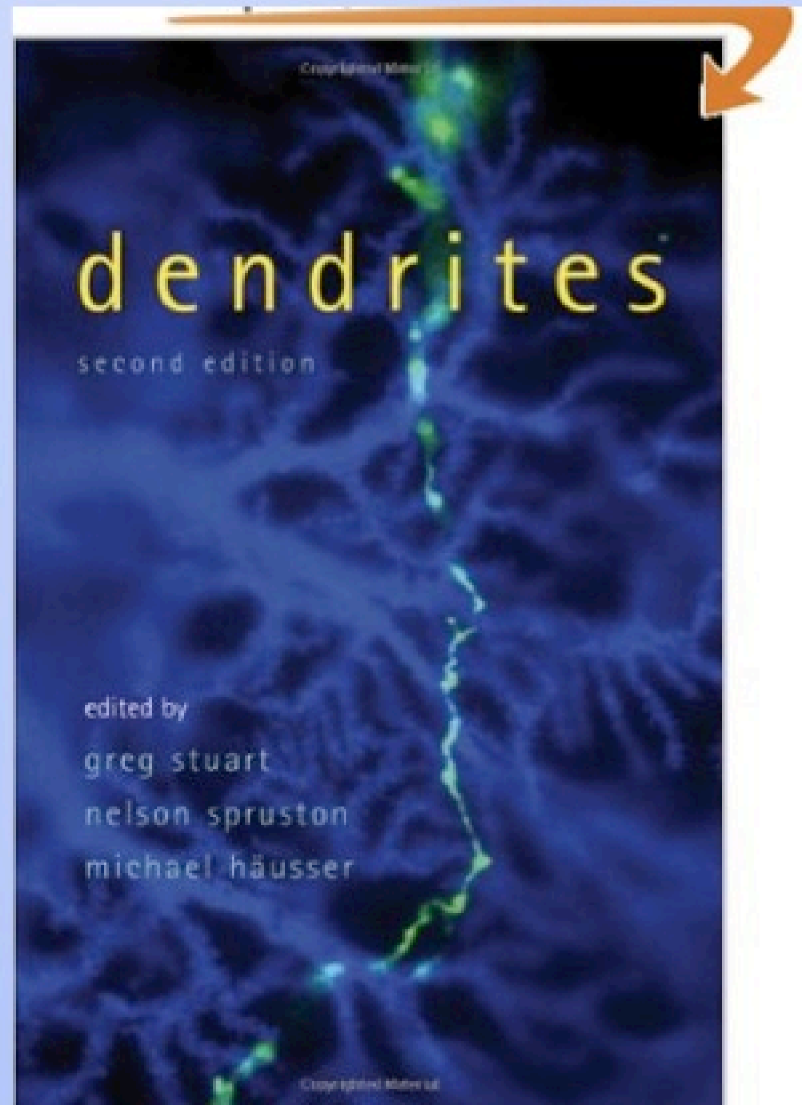


3D reconstruction from Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

Different forms of spines

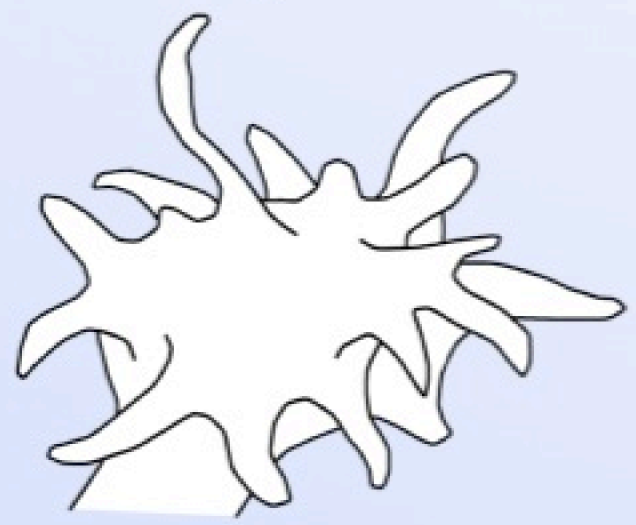
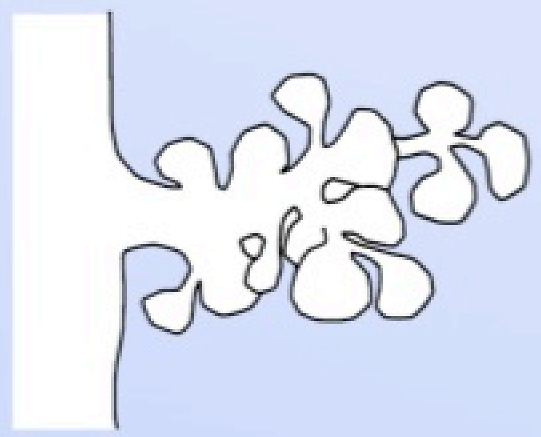
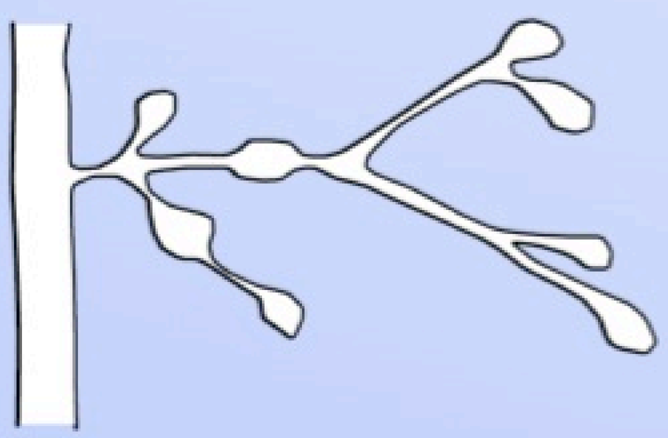
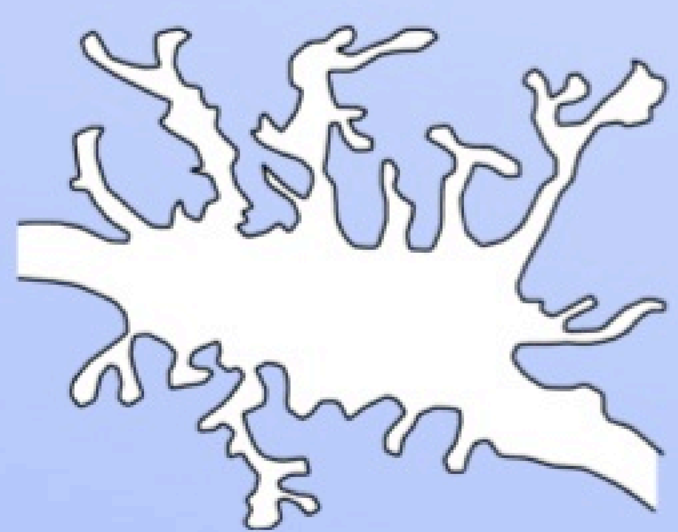
Pattern	Characteristics	Examples
Varicosity 	An enlargment in a thinner dendrite associated with synaptic contacts	Retinal amacrine cells
Filopodium 	A long, thin protrusion with a dense actin matrix and few internal organelles	Normally only seen during development
Simple Spine Sessile 	Synaptic protrusions 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/

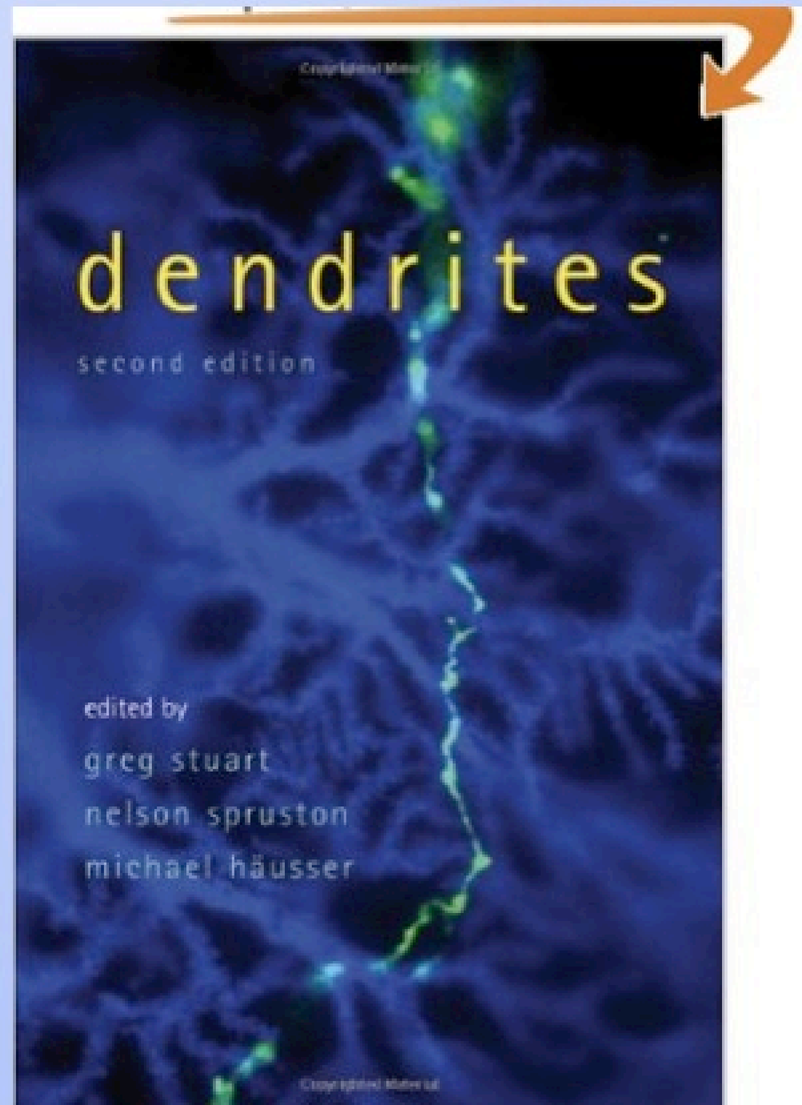


Adapted from Fiala JC, Harris KM (1999) Dendrite Structure. In: G Stuart, N Spruston, M Häusser, (eds.) Dendrites. Oxford University Press: Oxford UK.

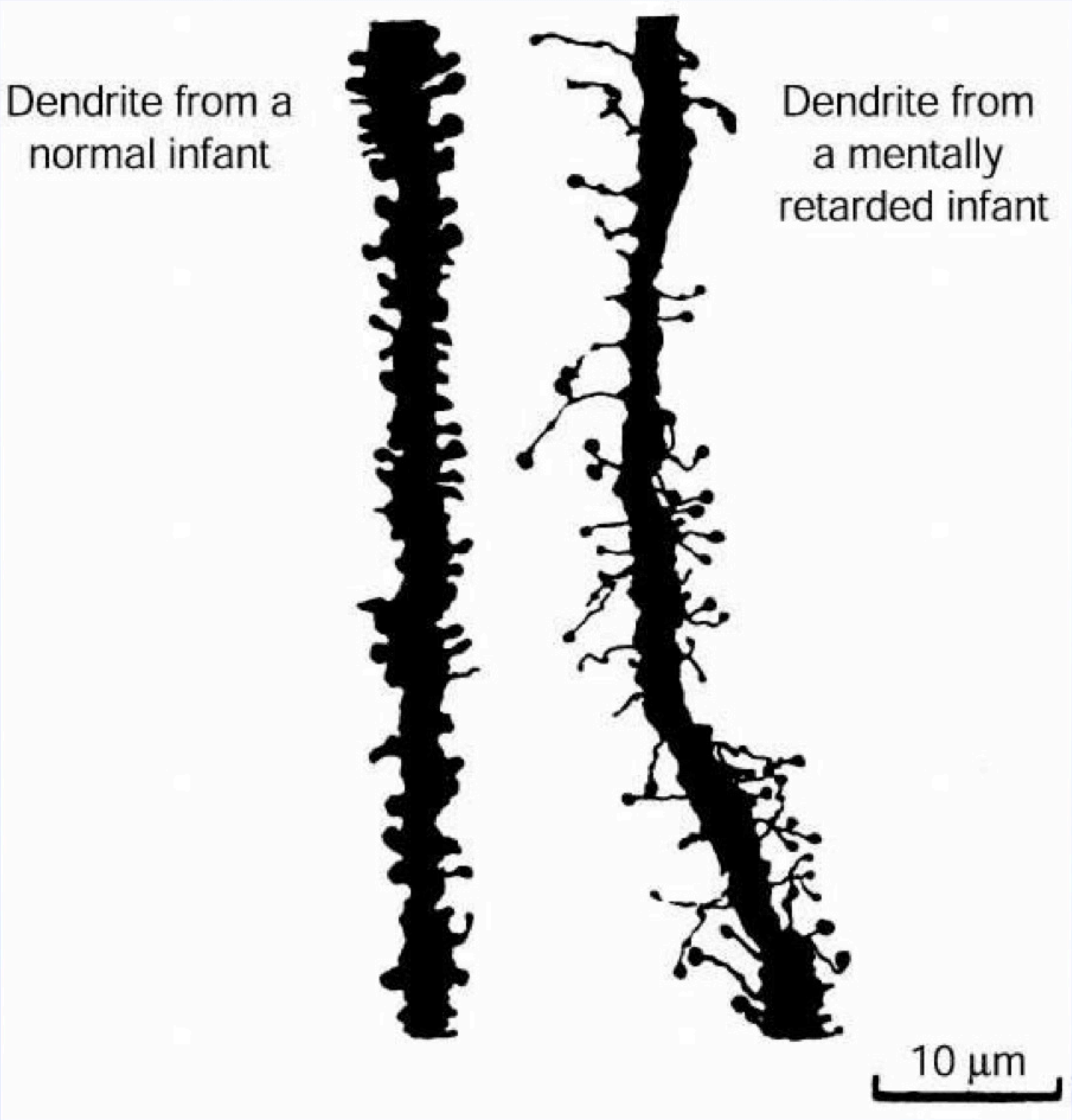
Different forms of spines

	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

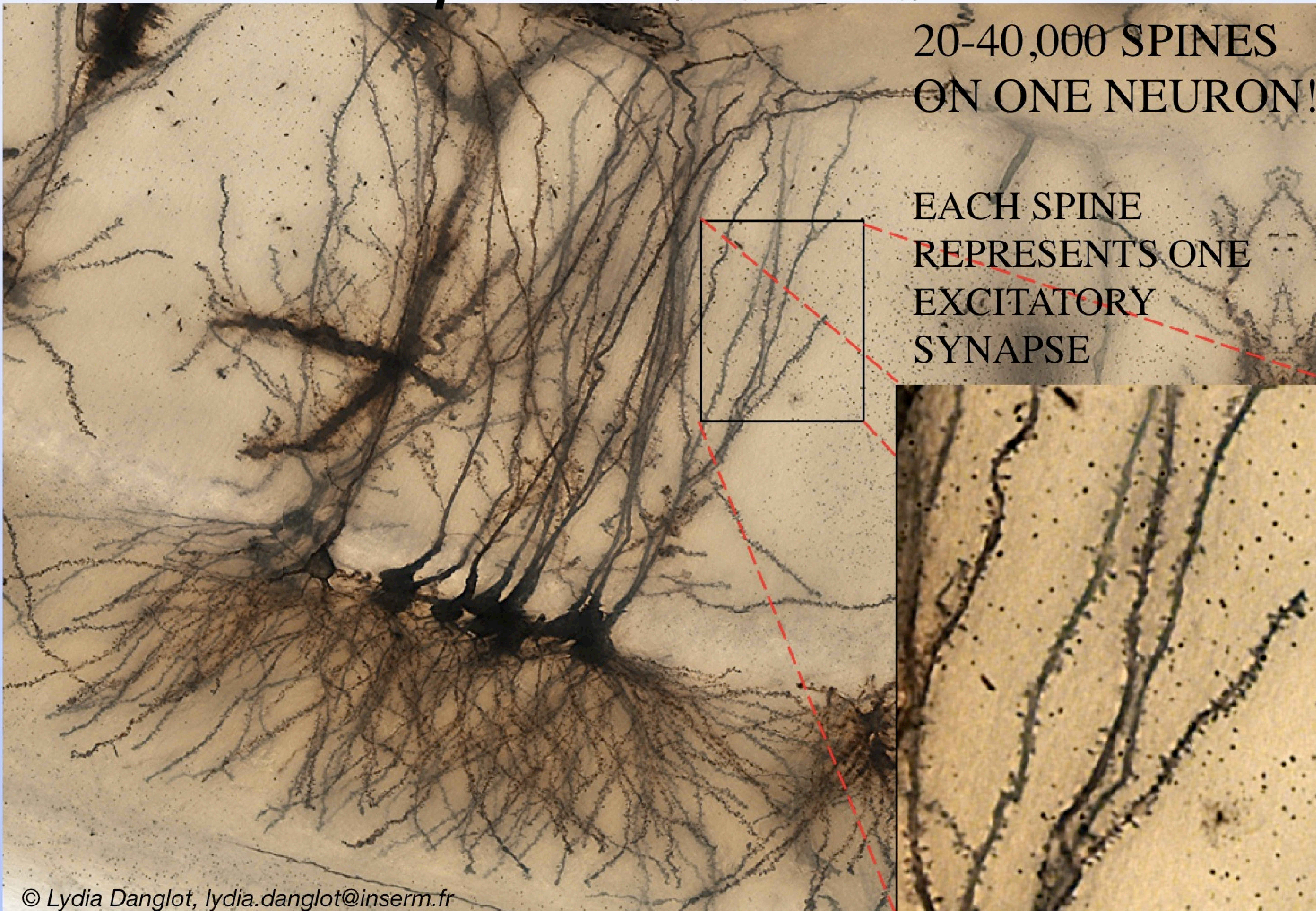
3D reconstruction from Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/



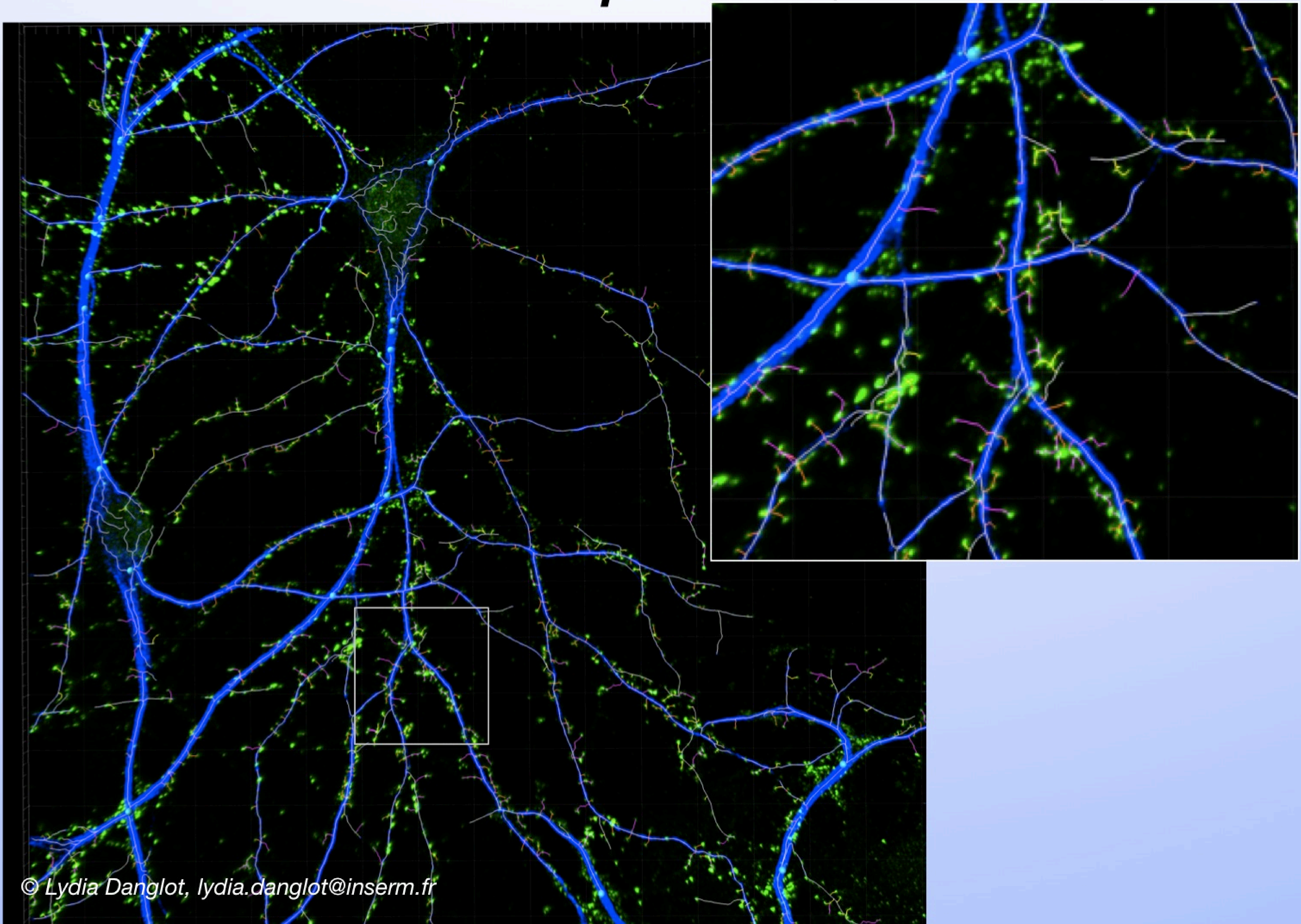
Fiala JC, Harris KM (1999) Dendrite Structure. In: G Stuart, N Spruston, M Häusser, (eds.) Dendrites. Oxford University Press: Oxford UK.


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

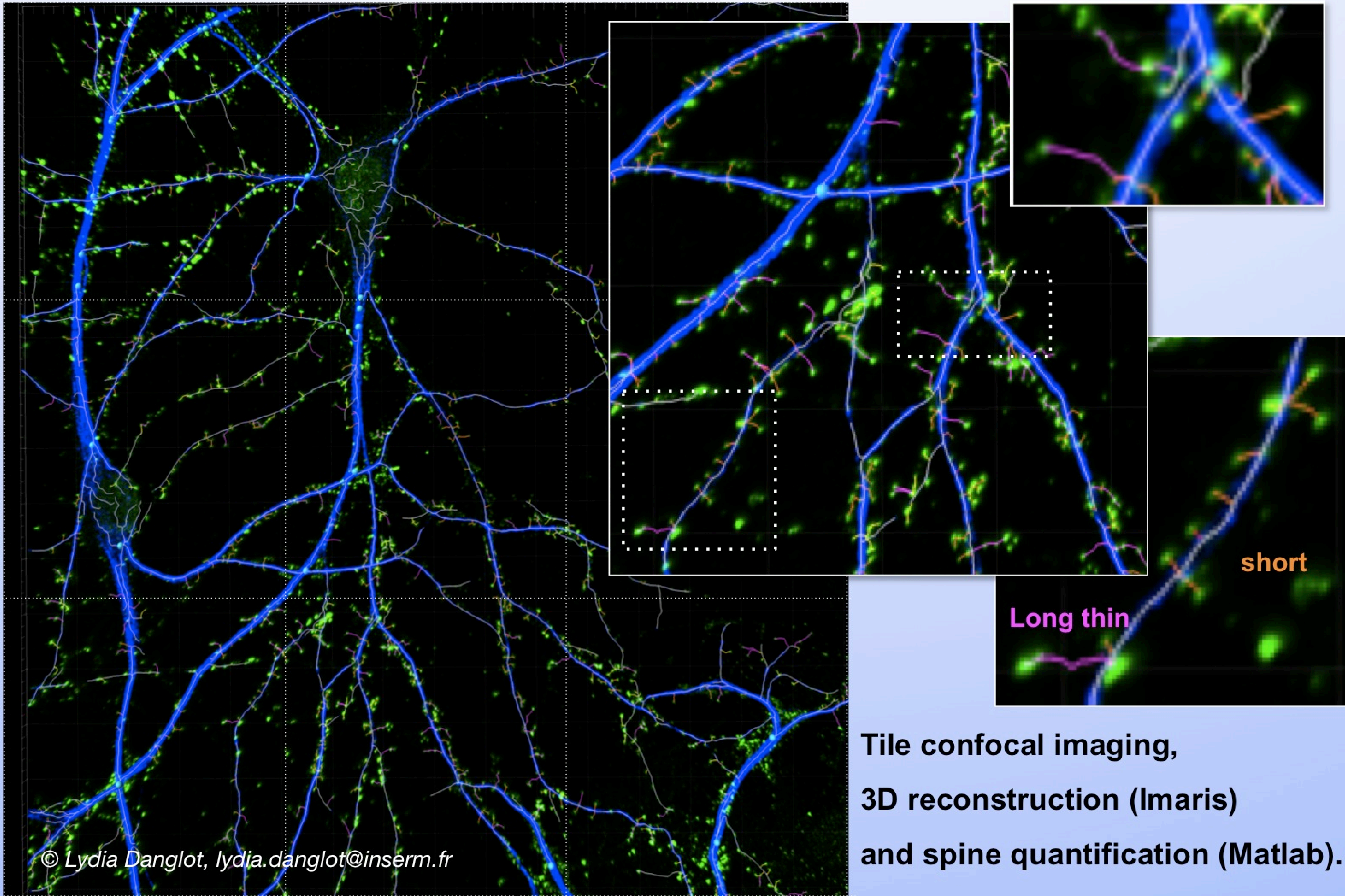
Dendritic Spines *(golgi staining)*



Dendritic Spines *(fluorescence)*



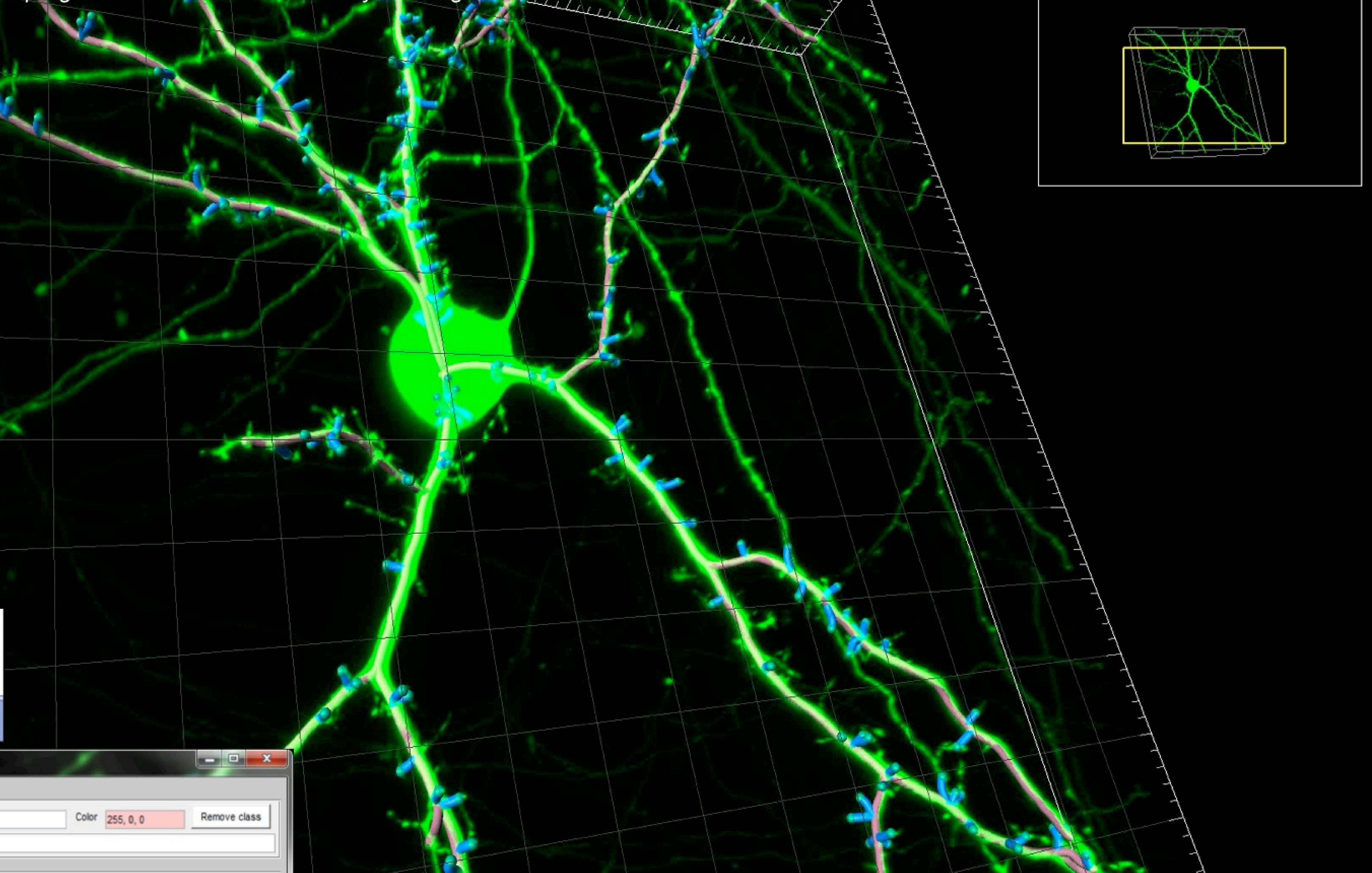
Spine morphology?

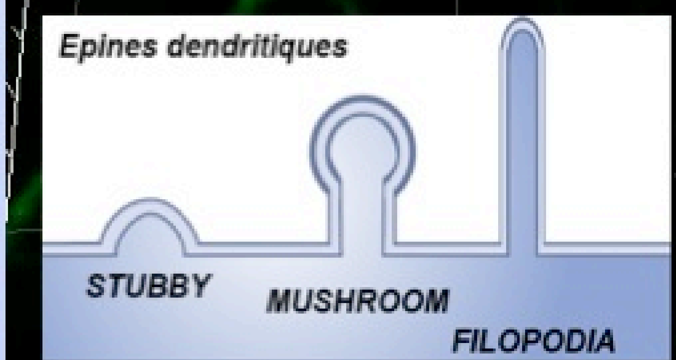


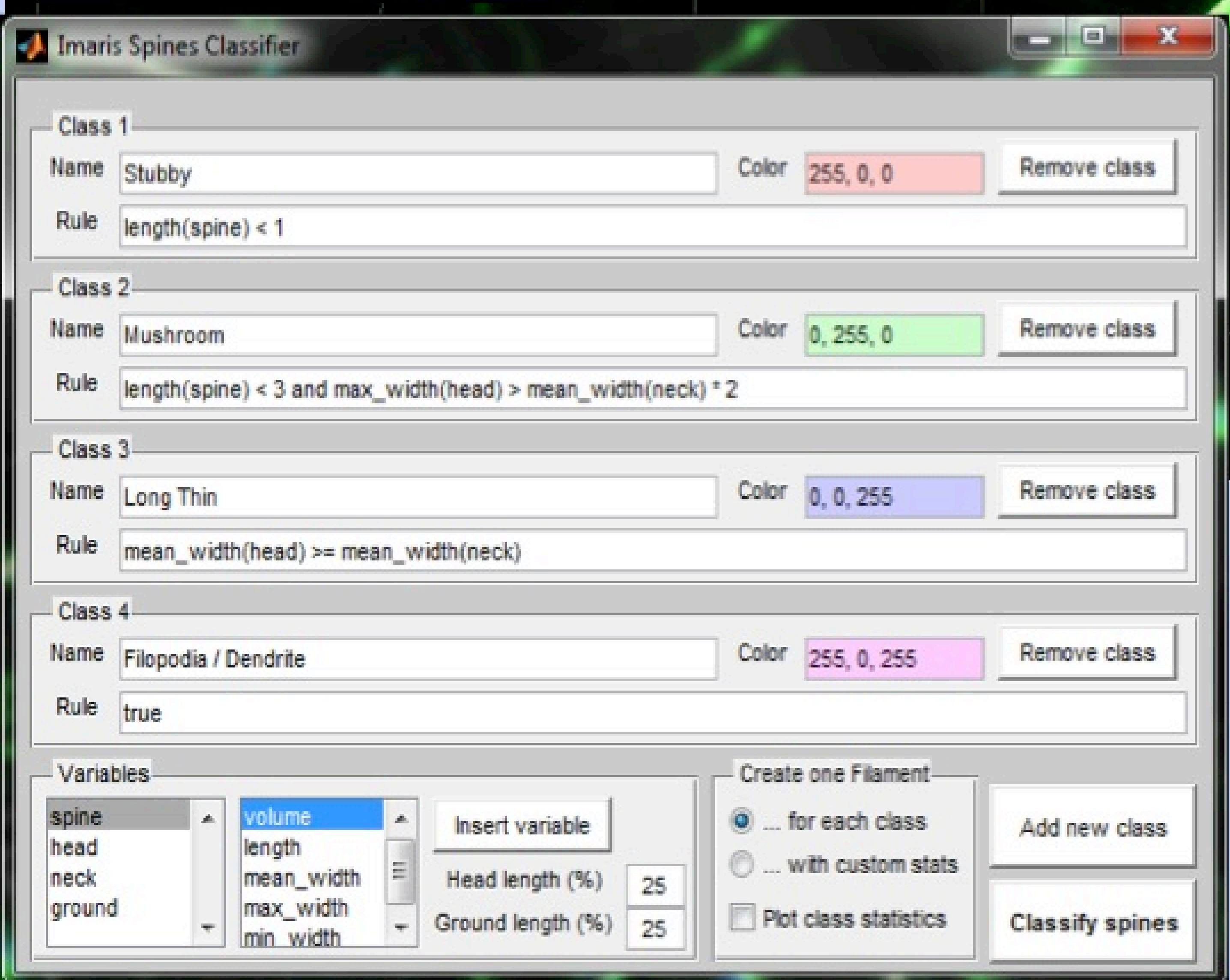
Tile confocal imaging,

3D reconstruction (Imaris)

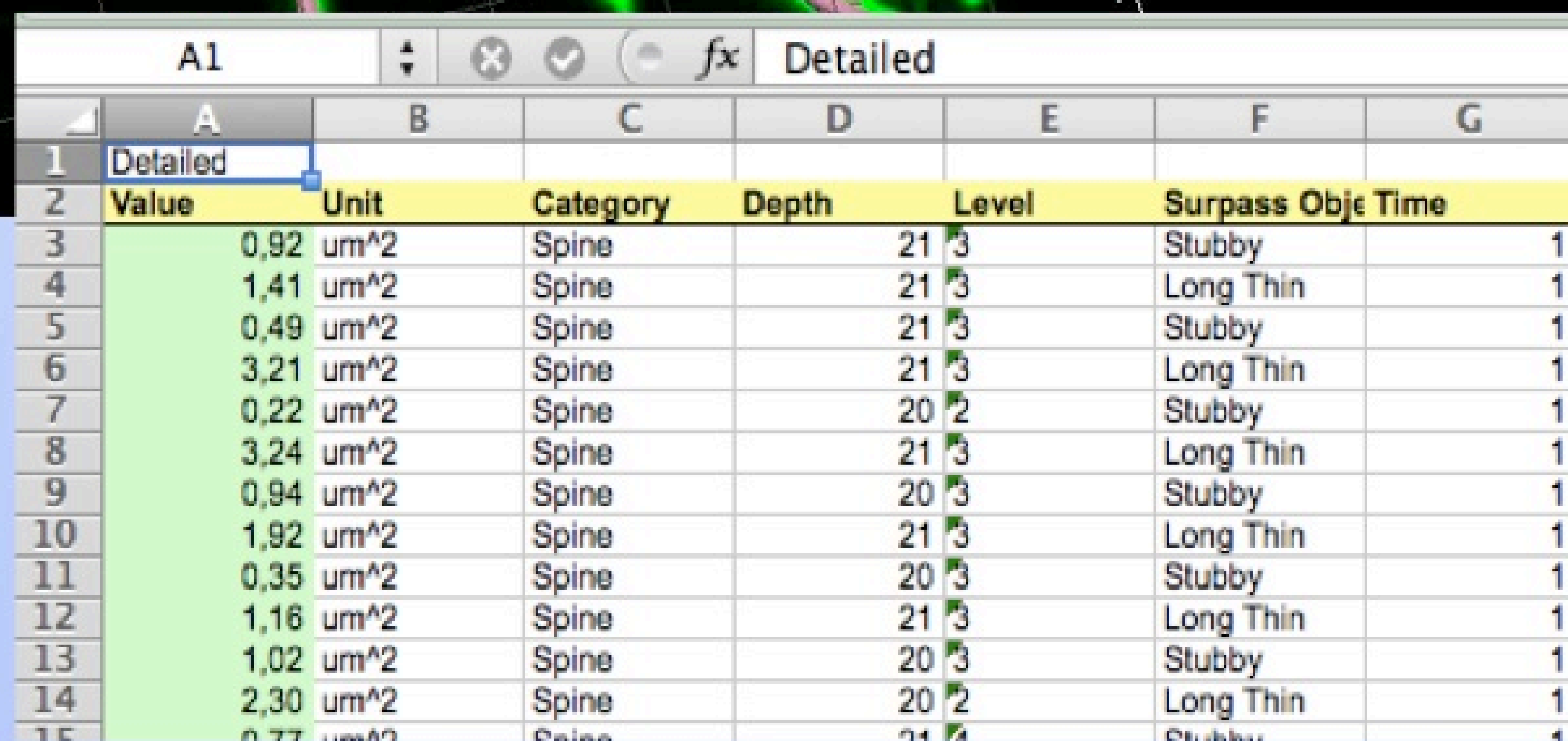
and spine quantification (Matlab).







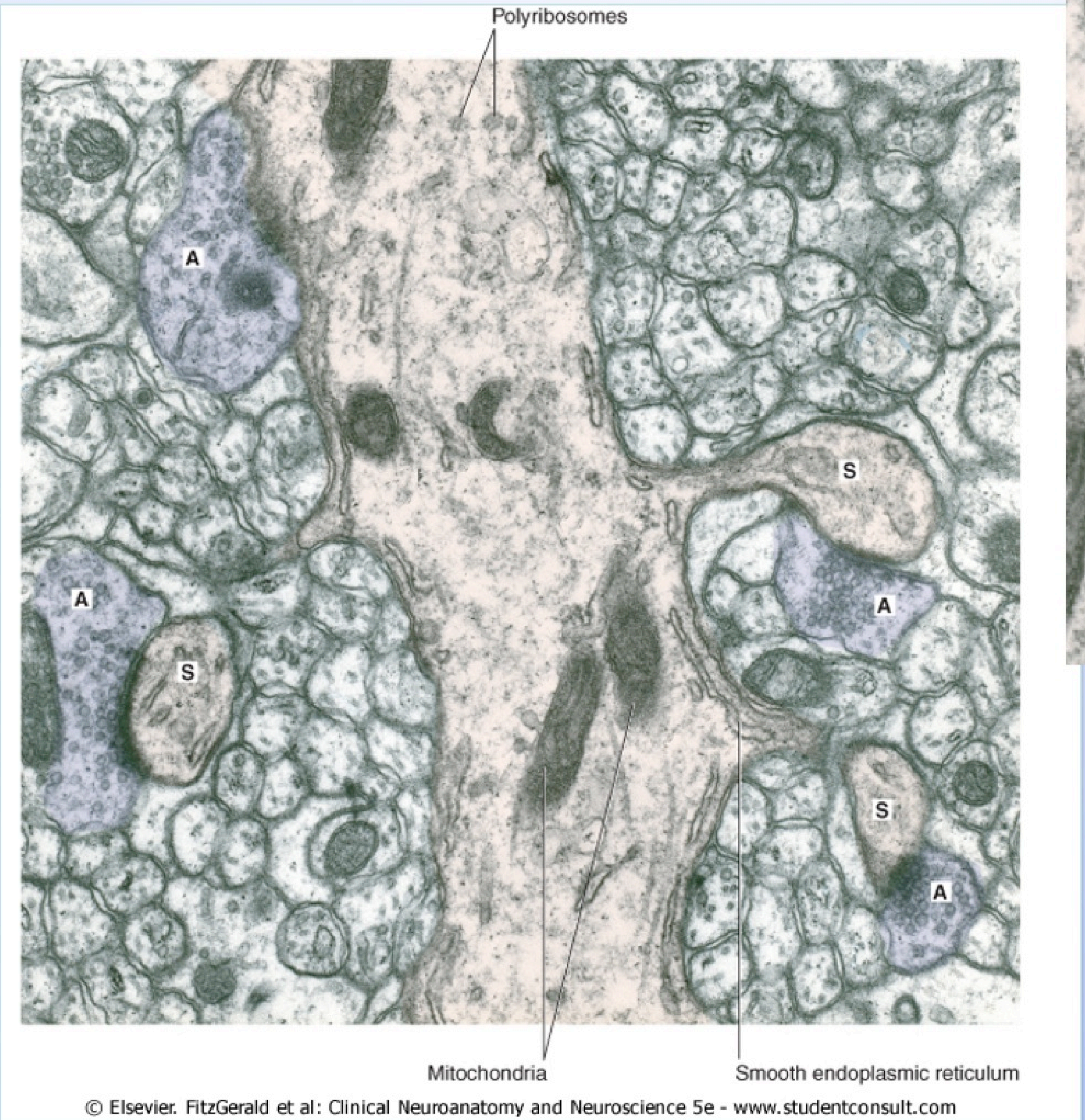
Excel file



A1 | Detailed

Value	Unit	Category	Depth	Level	Surpass Object	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

Dendritic Spines (electron microscopy)

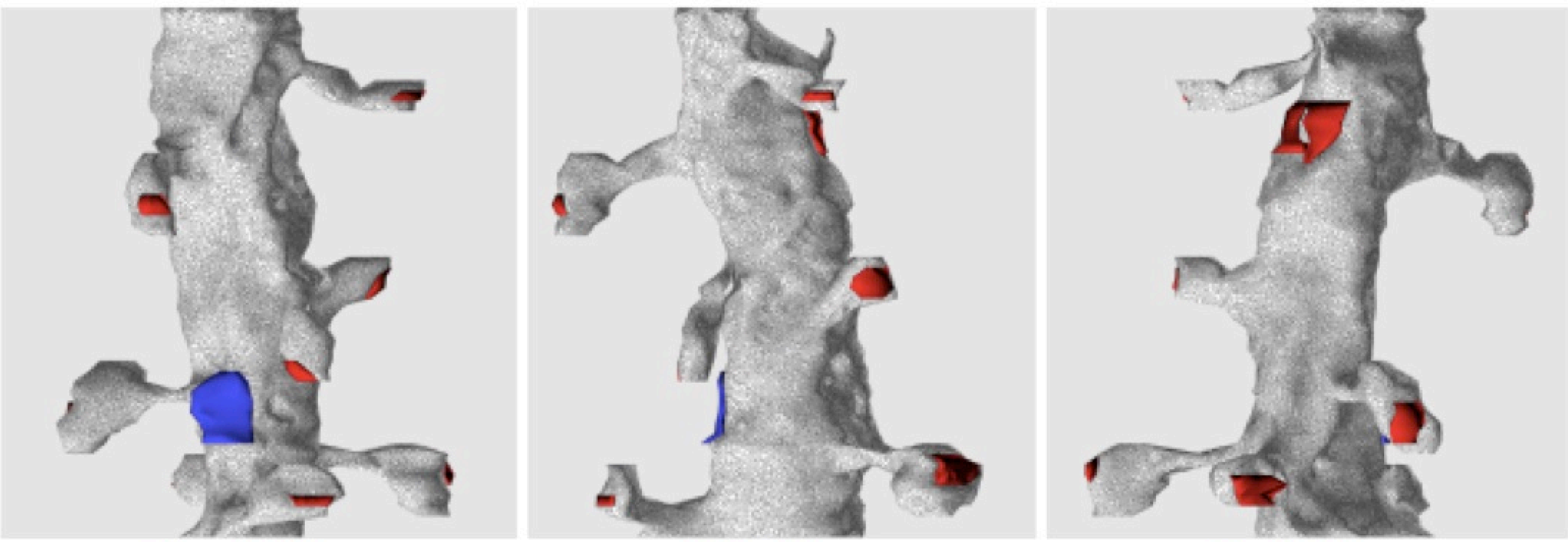


Different forms of spines

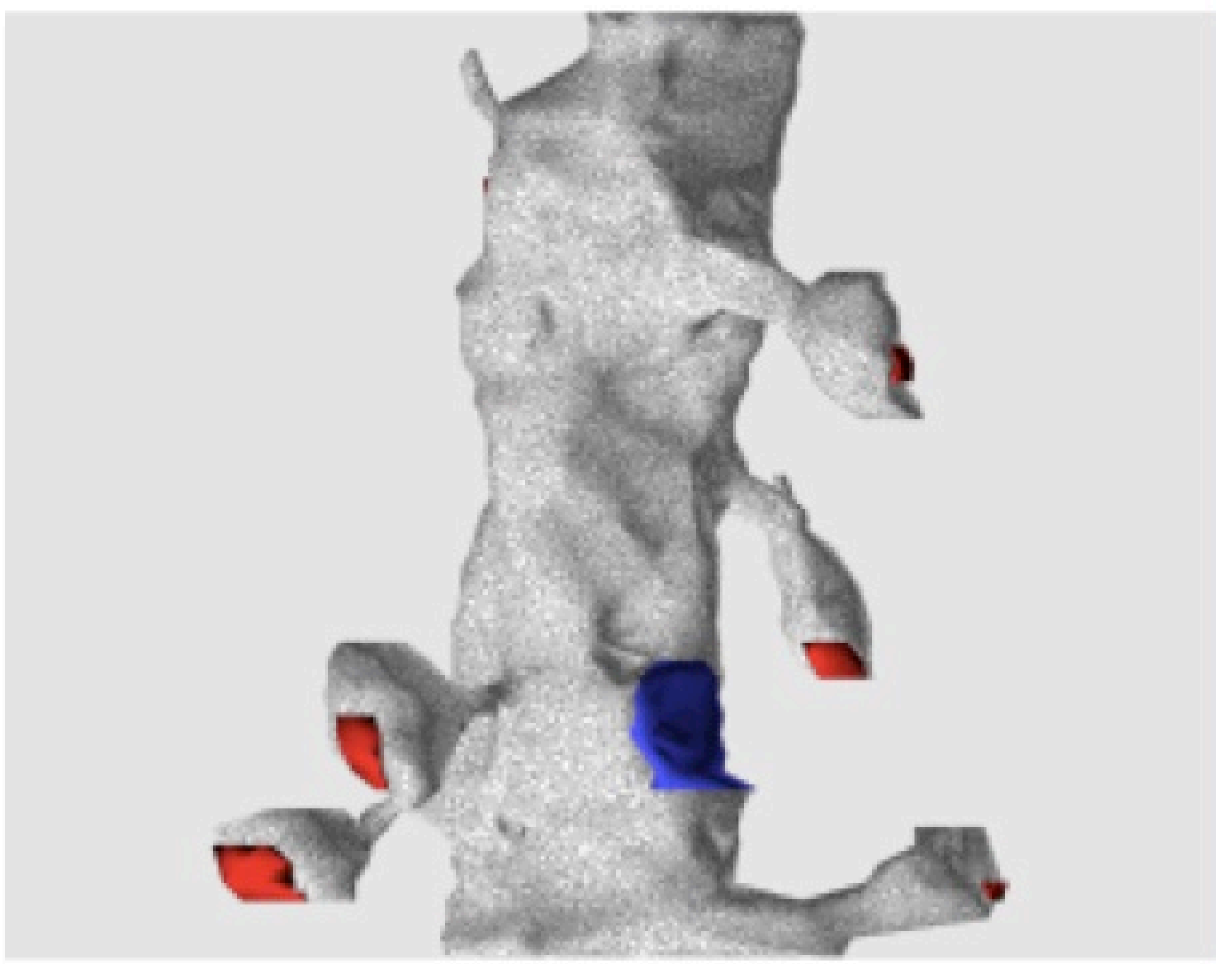
SynapseWeb

About **Anatomy** **Links** **Publications**

A spiny dendrite from CA1 stratum radiatum (series K24).



Front View **Side View** **Back View**



1.0 µm

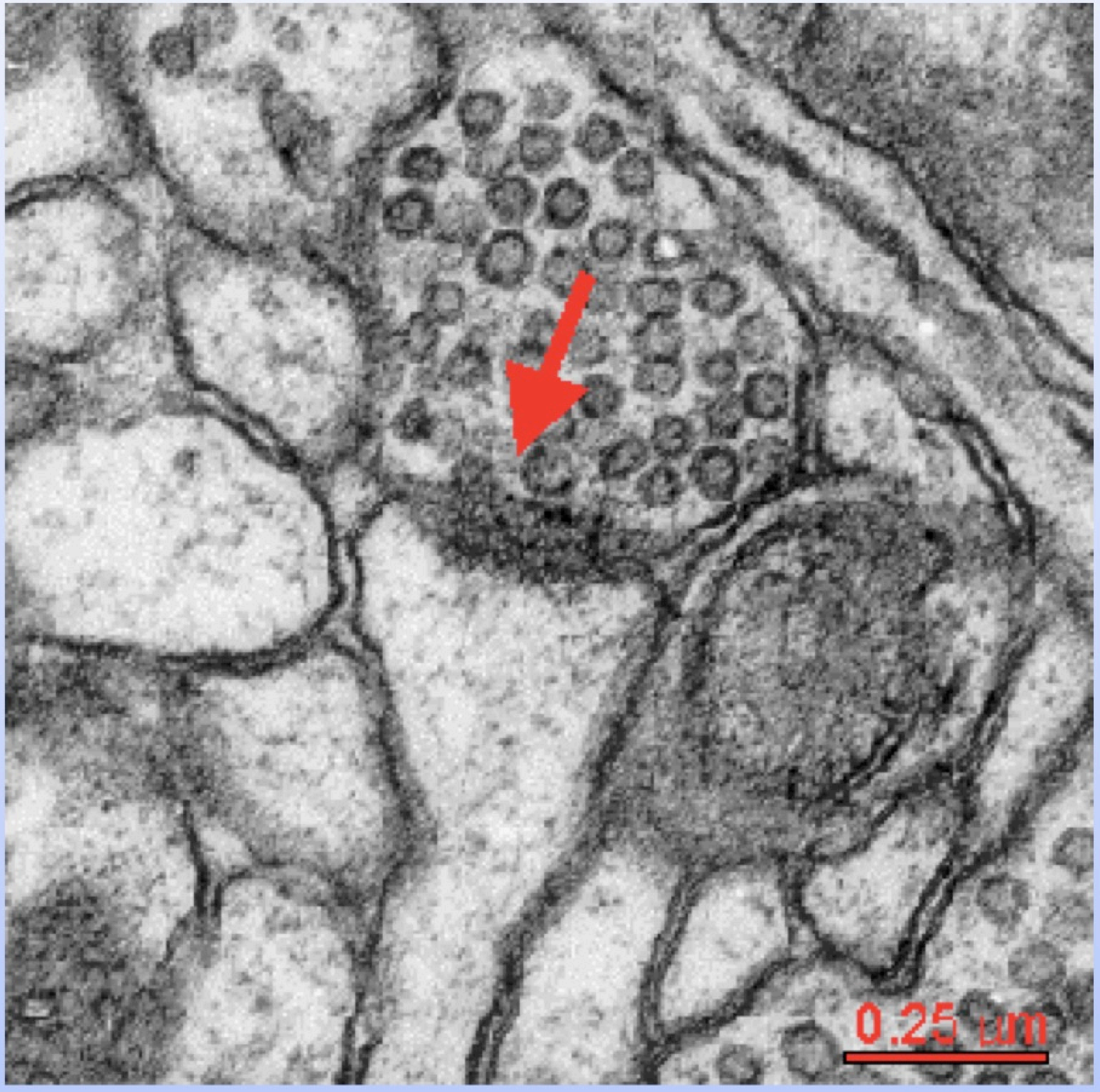
Excitatory synapses are shown in red. Inhibitory synapses in blue.

Excitatory synapse

Inhibitory synapse

3D View this dendrite as a flip book of serial section images (2.62Mb Javascript). View a three-dimensional reconstruction of this dendrite as a rotating GIF (126Kb), as a VRML 1.0 object* (450Kb). You may also download (4.65Mb) the data which appears in the flipbook (46 sections) as an IGL Trace** series of Windows Bitmap images.

Asymmetric synapse (GRAY'S TYPE I) excitatory

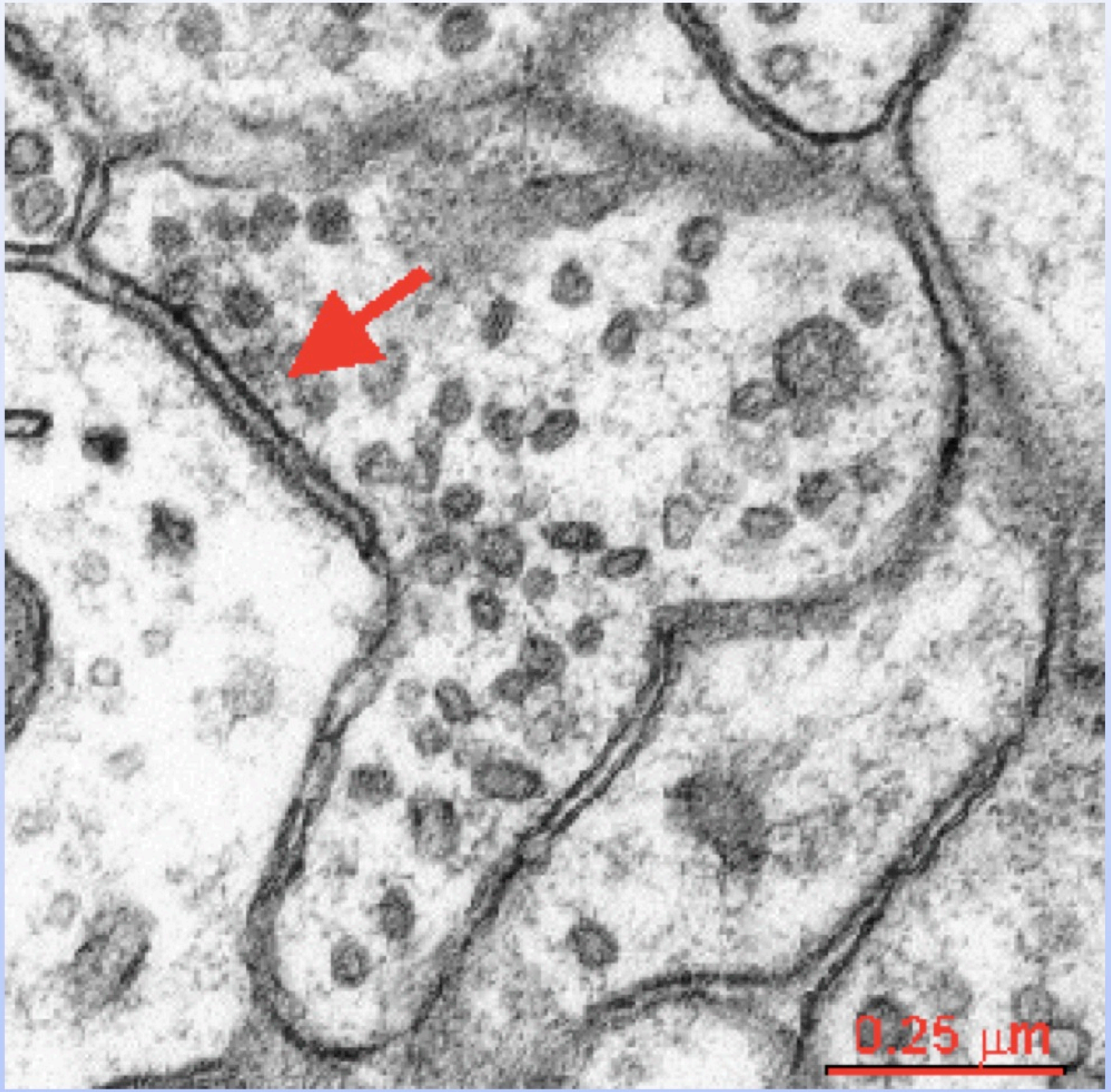


from **Karin Sorra,** Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

WHAT TYPE OF SYNAPSE?

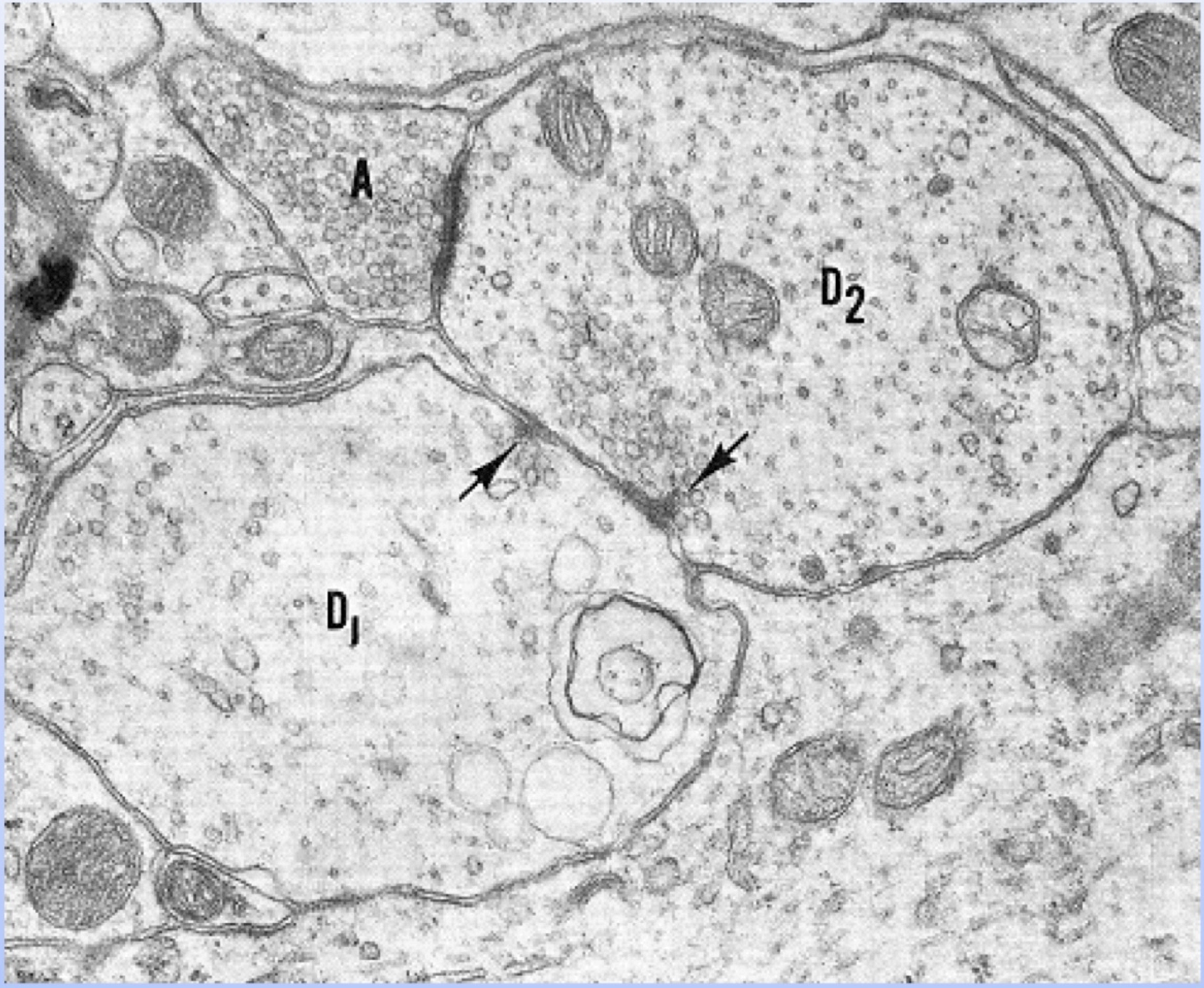
*from **Karin Sorra,** Synapse Web, Kristen M. Harris, PI*
http://synapses.clm.utexas.edu/

SYMMETRIC (GRAY'S II) : INHIBITORY

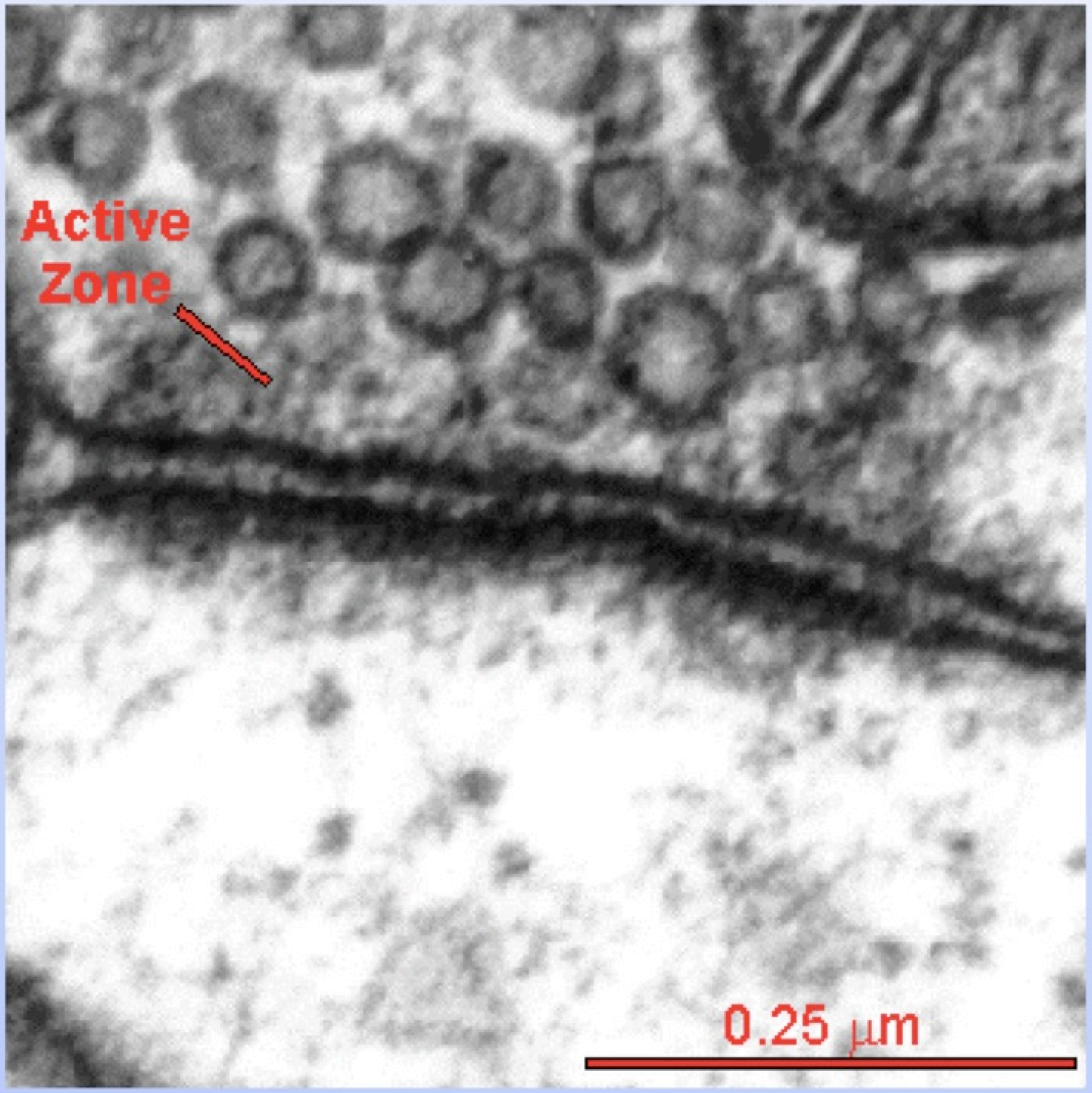


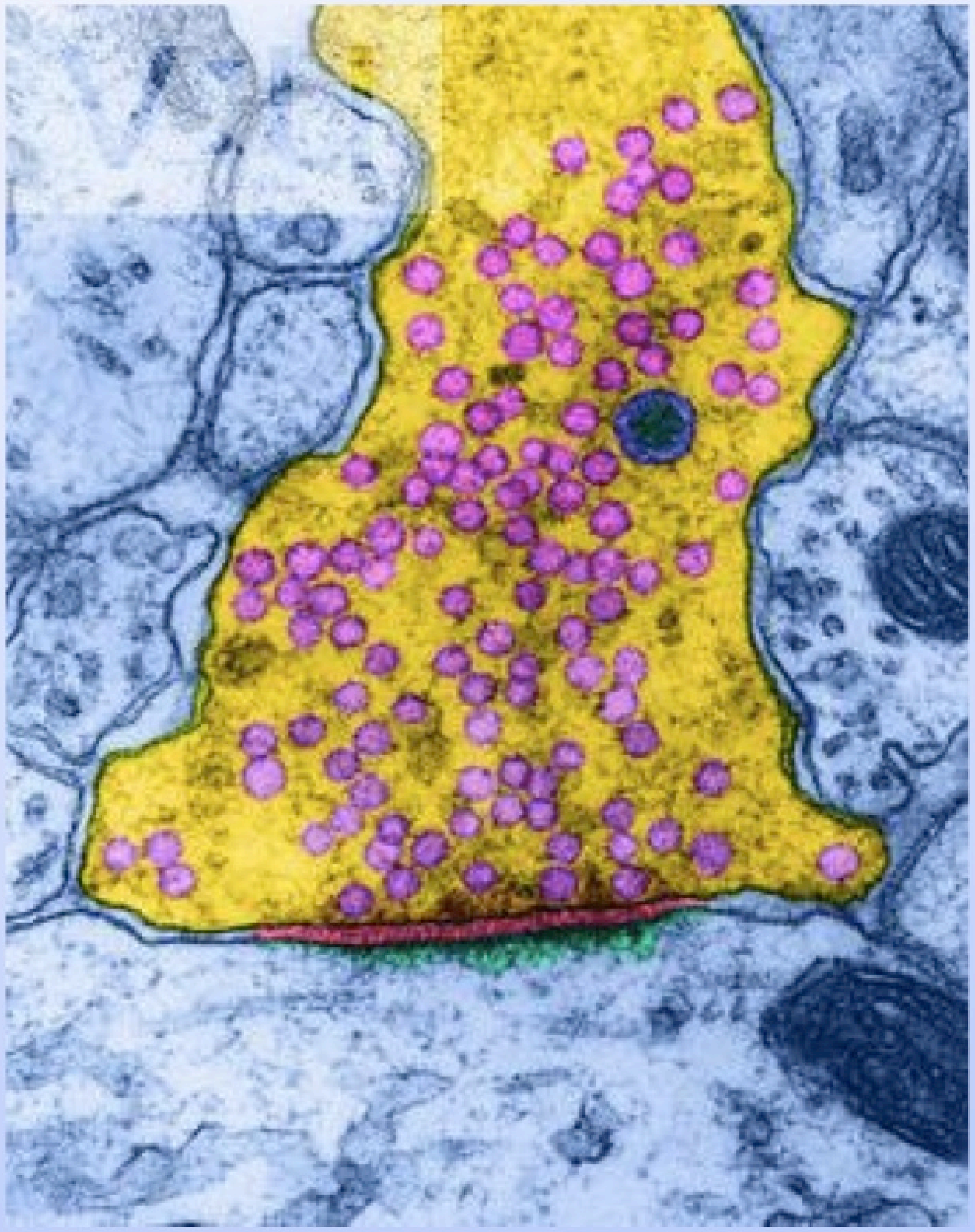
from **Karin Sorra,** Synapse Web, Kristen M. Harris, PI
http://synapses.clm.utexas.edu/

DENDRO-DENDRITIC SYNAPSE

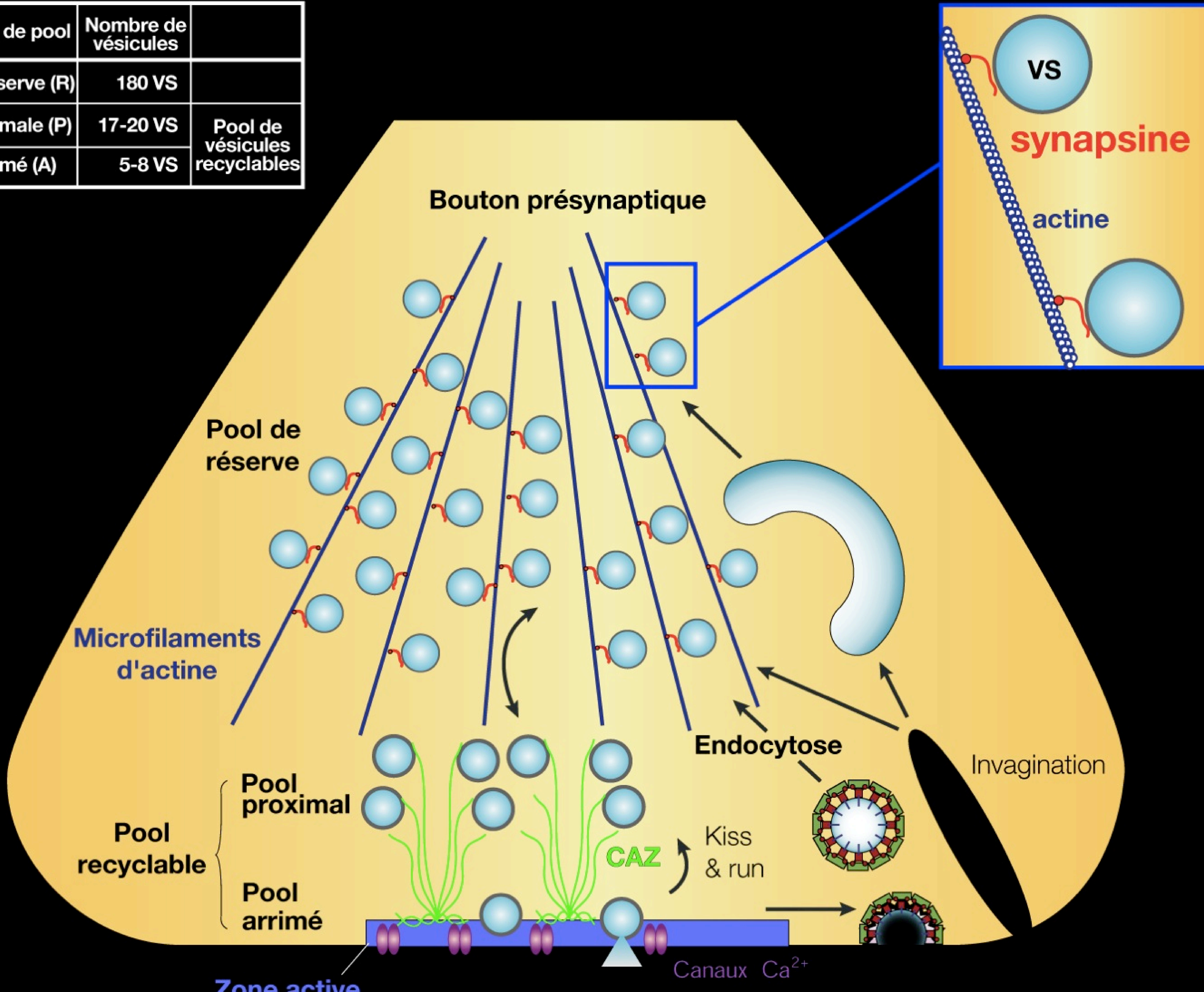


Presynaptic Active Zone



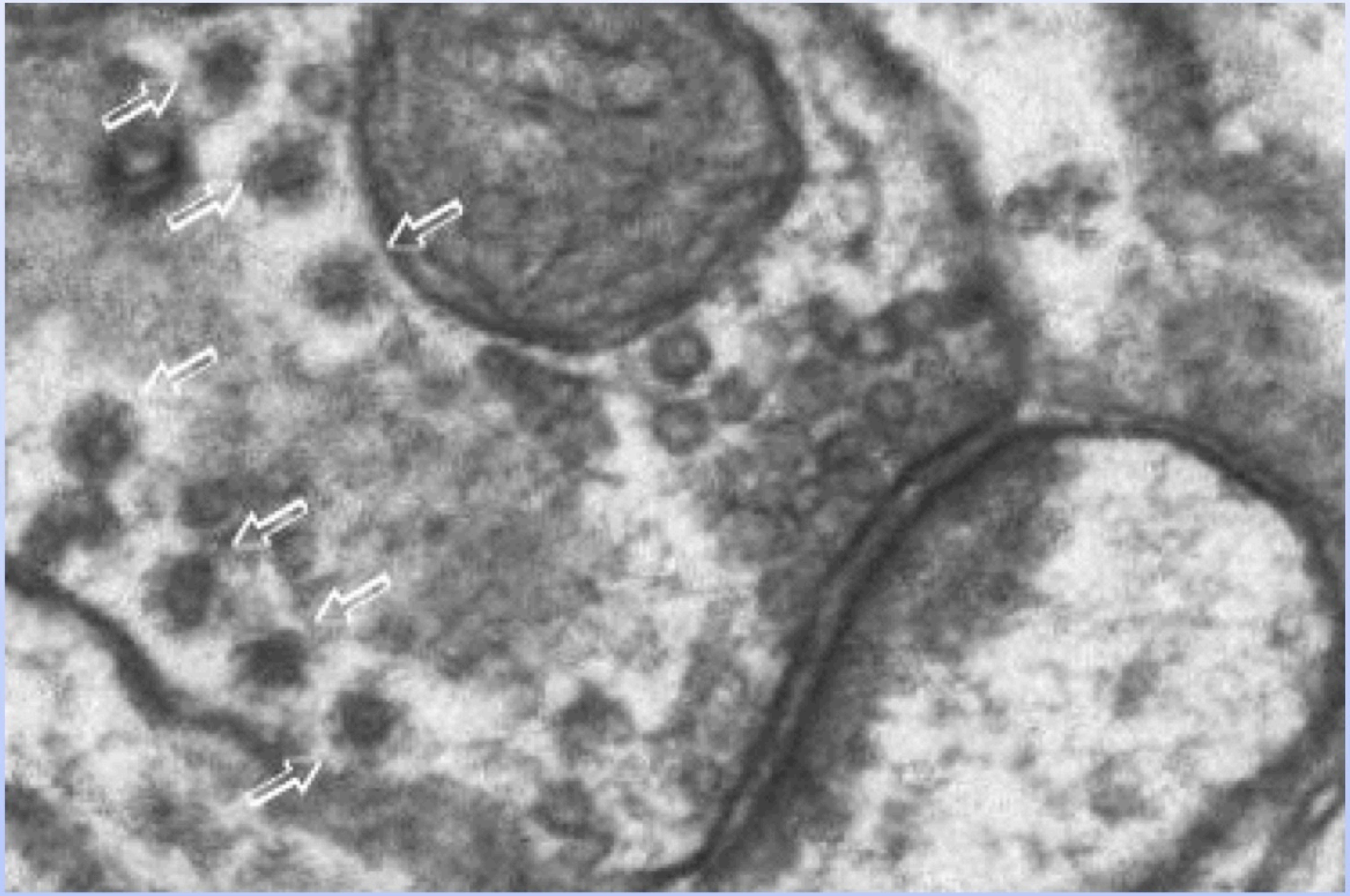


Type de pool	Nombre de vésicules	
de réserve (R)	180 VS	
proximale (P)	17-20 VS	Pool de vésicules recyclables
arrimé (A)	5-8 VS	

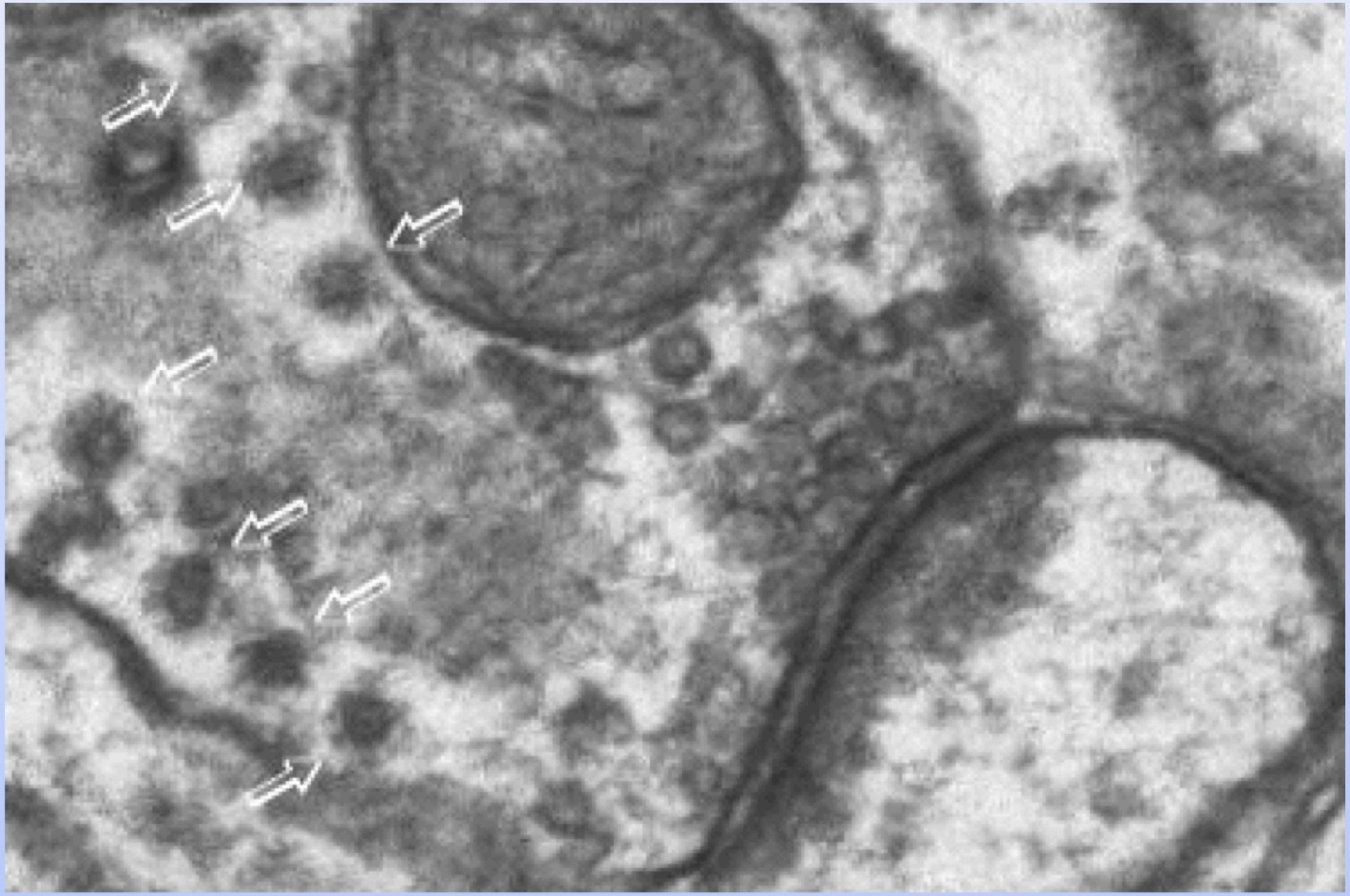


Modifié d'après Current Opinion in Neurobiology 2000, 10:321–327 et Natrure Review Molecular Cell Biology, 2003, 4:127-139.

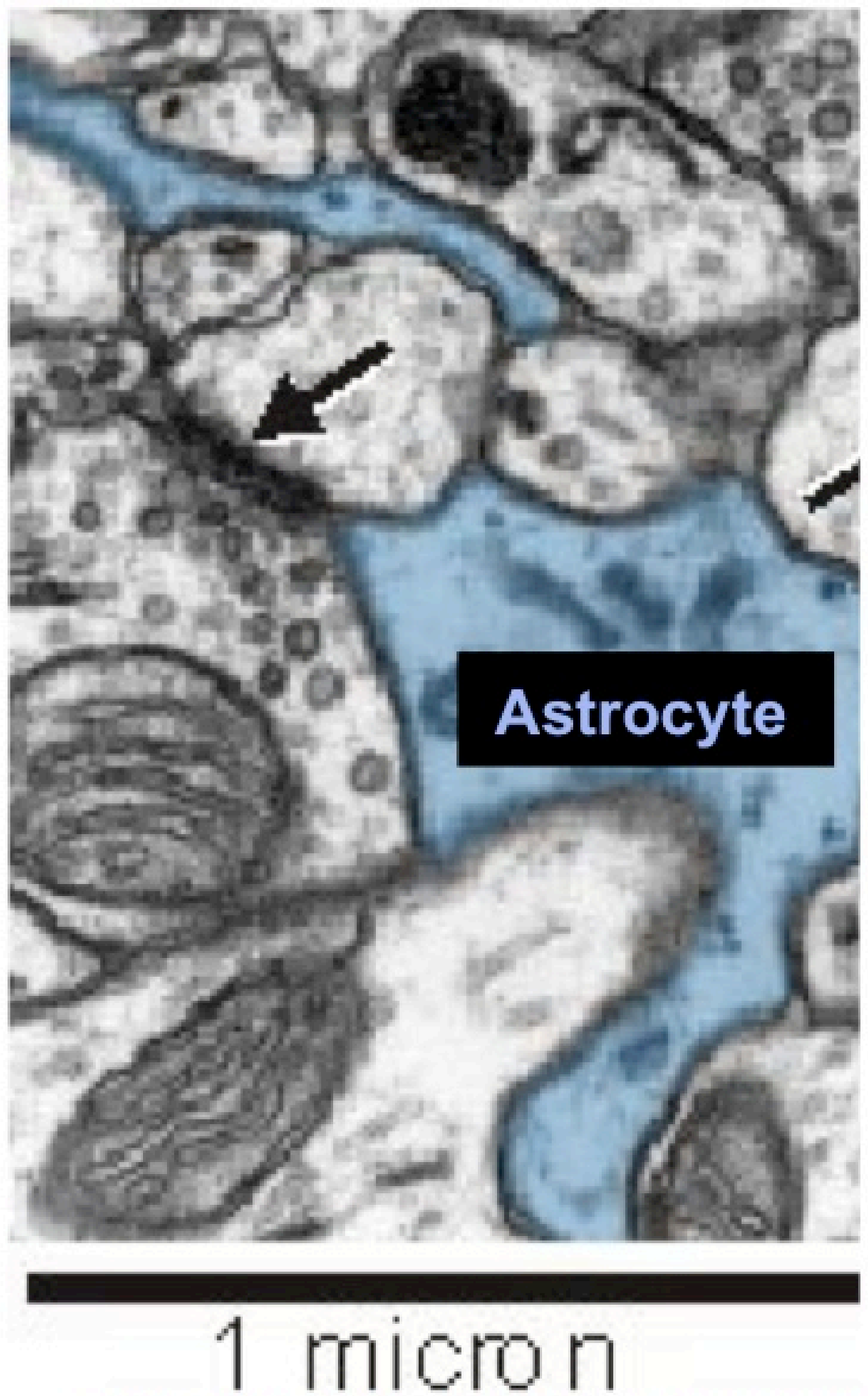
?

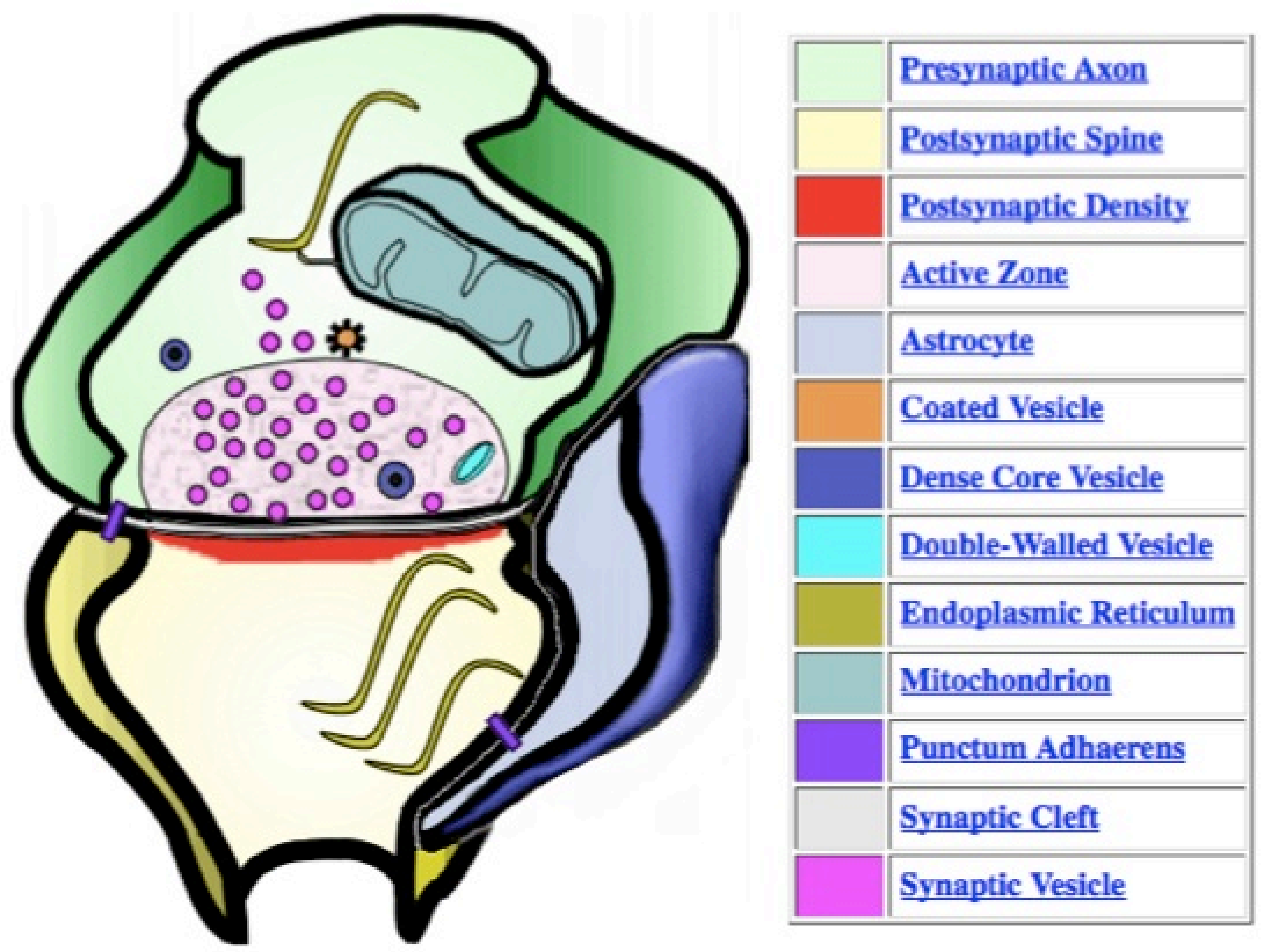


COATED VESICLES (CLATHRIN) MEMBRANE RECYCLING AT THE TERMINAL

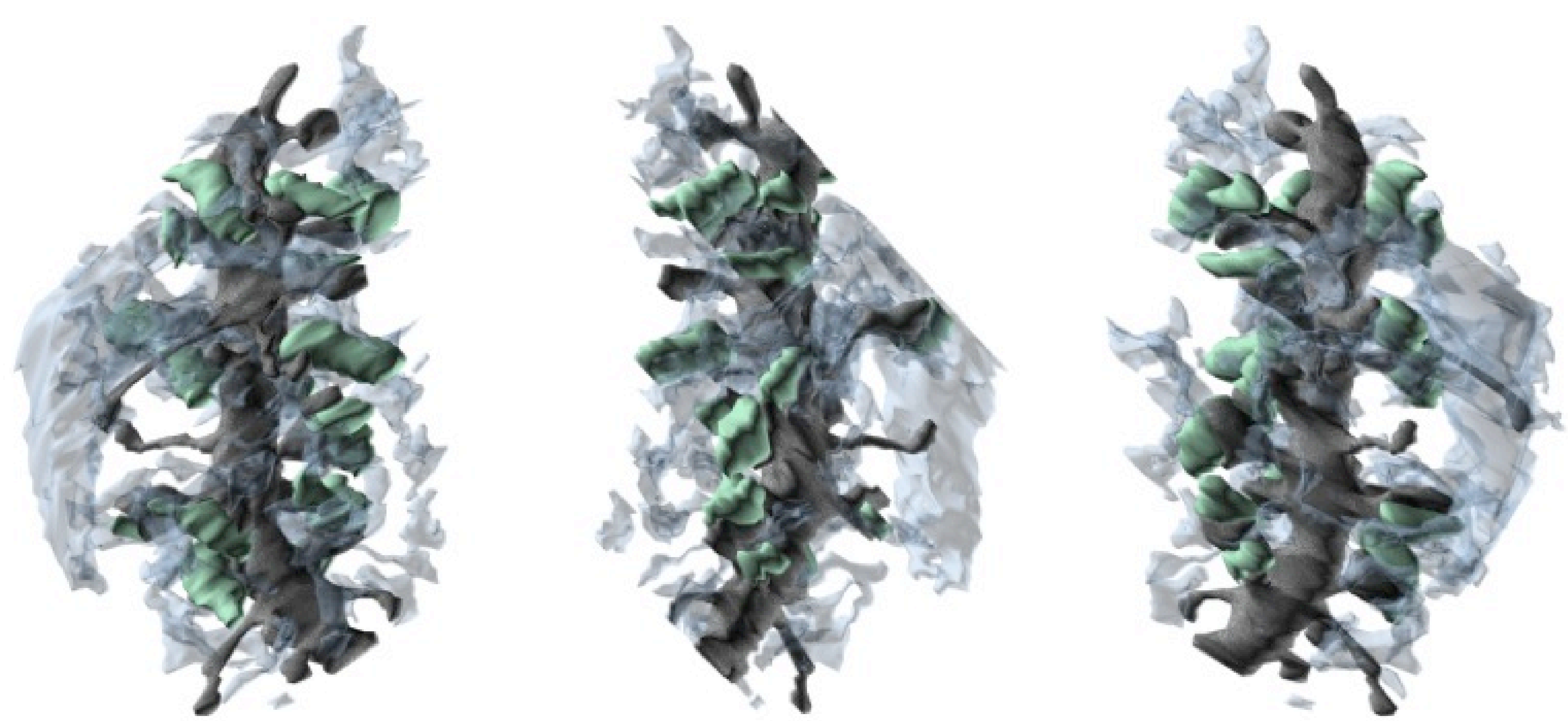


Tripartite synapse with astrocyte

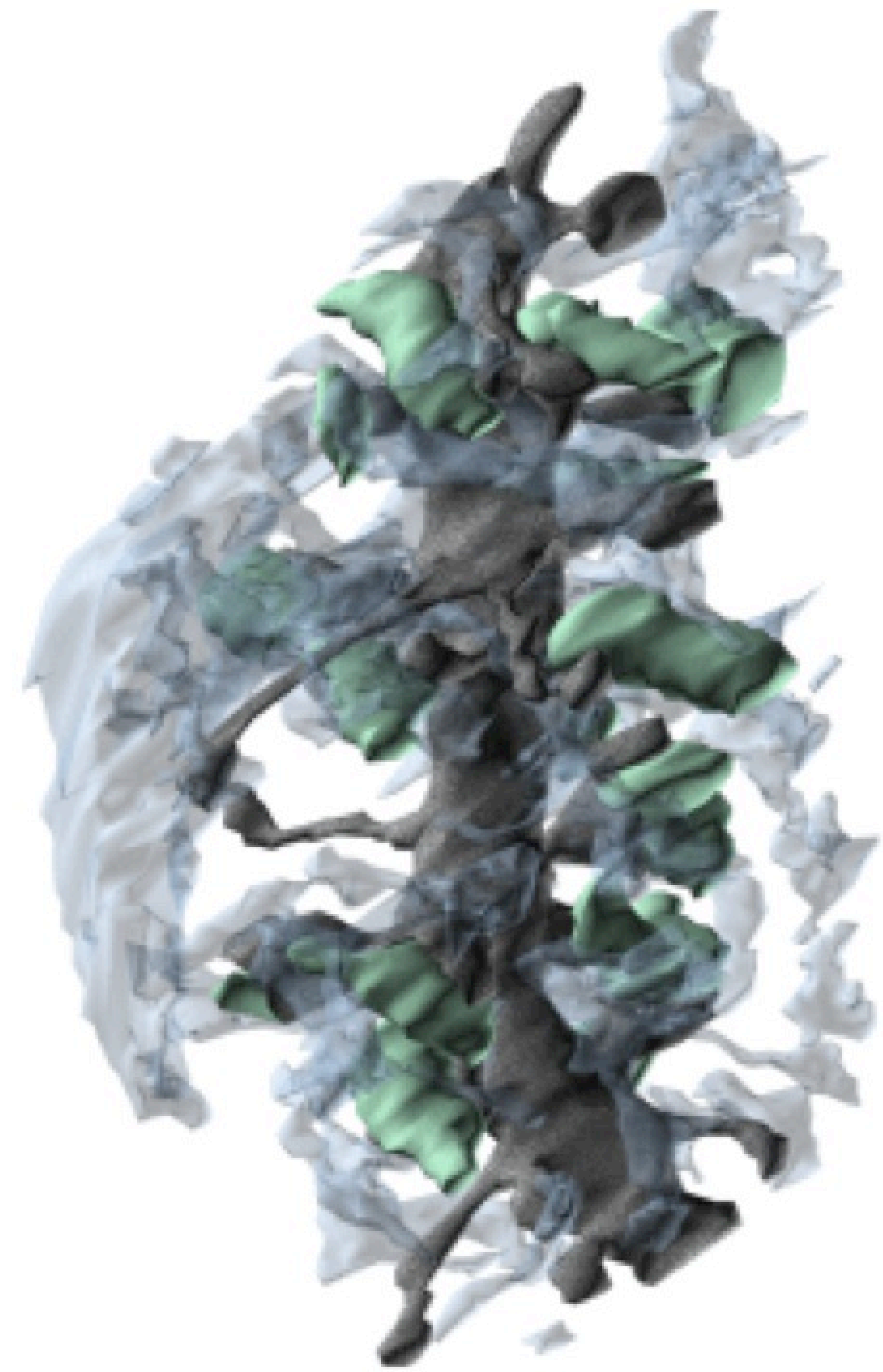




A dendrite with synapsing boutons and neighboring astrocyte from CA1 stratum radiatum (series K18).



Front View **Side View** **Back View**

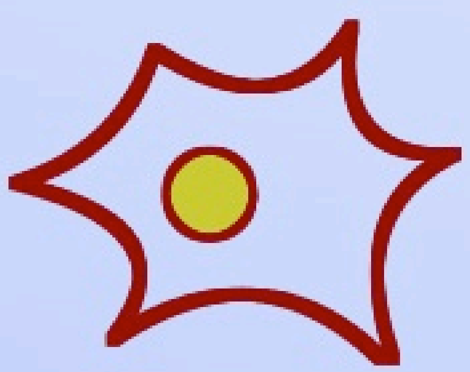


Adapted from Synapse Web, Kristen M. Harris, PI, ***Rachel E. Ventura, Karrin Sorra*** *http://synapses.clm.utexas.edu/*

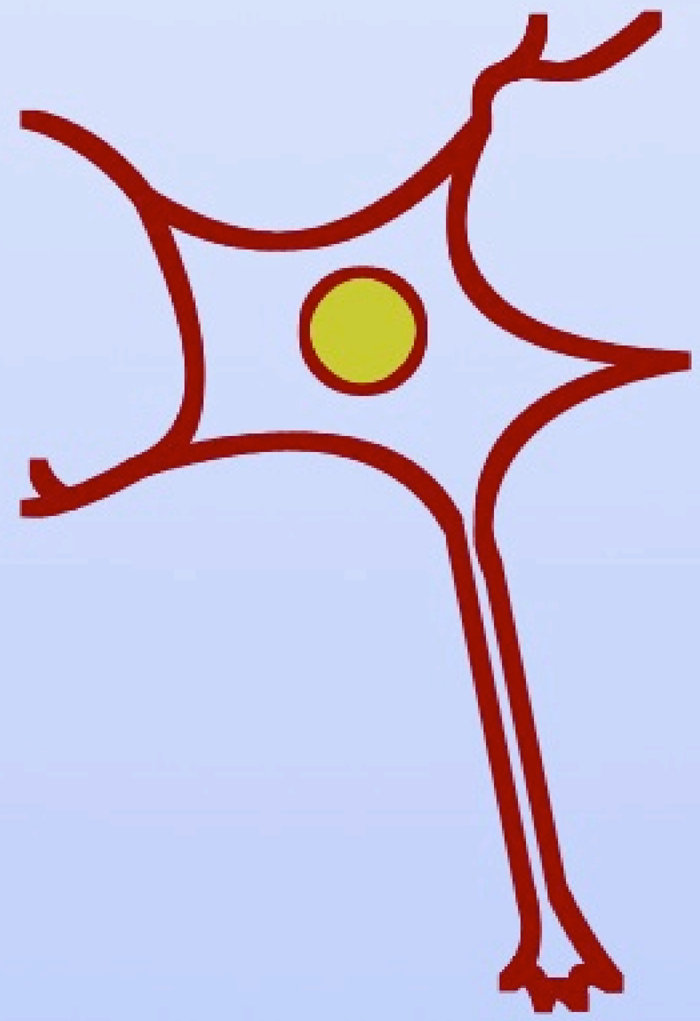
4. Role of membrane trafficking in neuritogenesis

I. Croissance membranaire
II. Ciblage polarisé des molécules

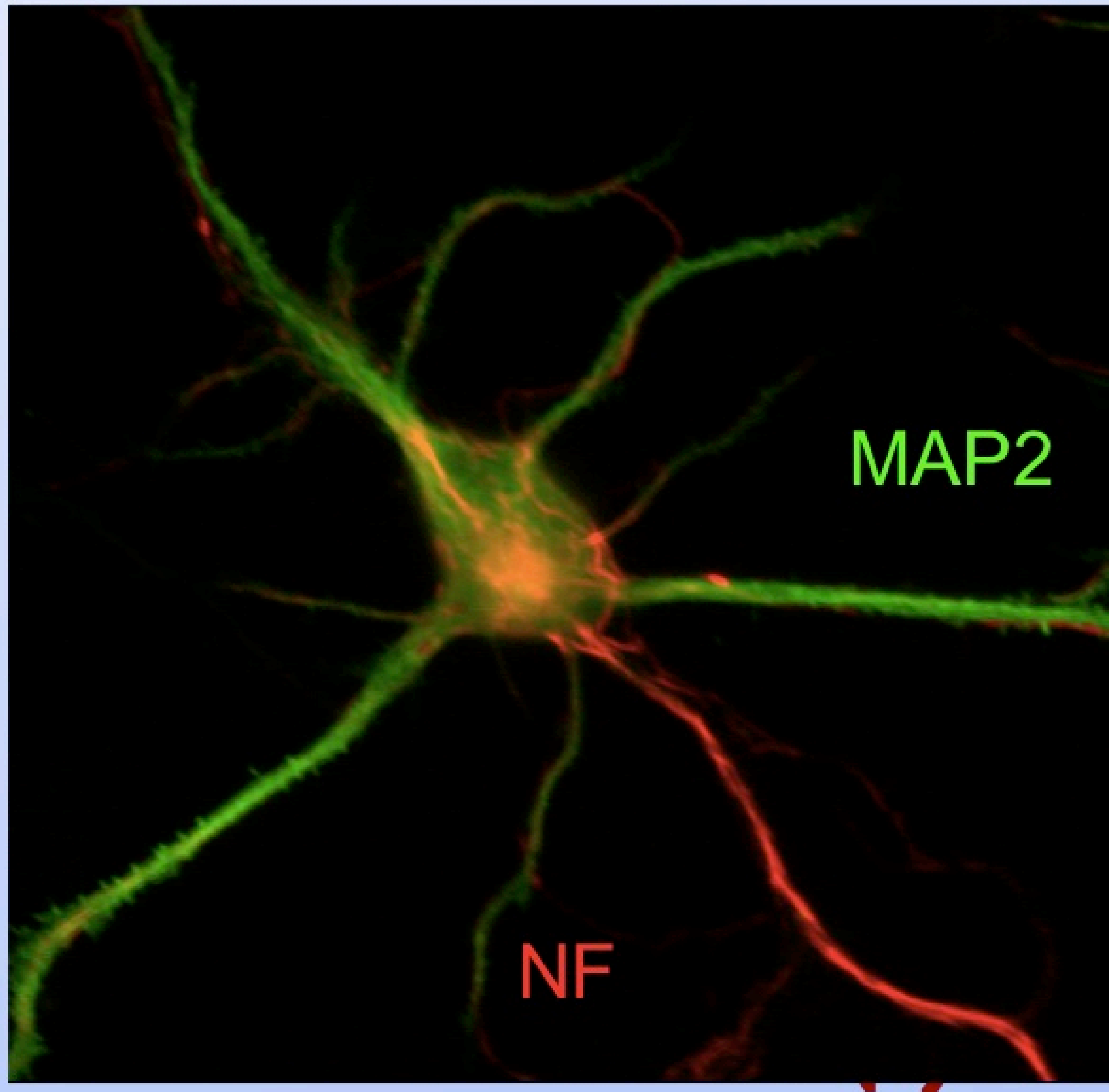
Précurseur



Neurone immature



Neurone mature



5. Role of membrane trafficking in neuritogenesis

Movie from http://www.silvermanlab.orgl

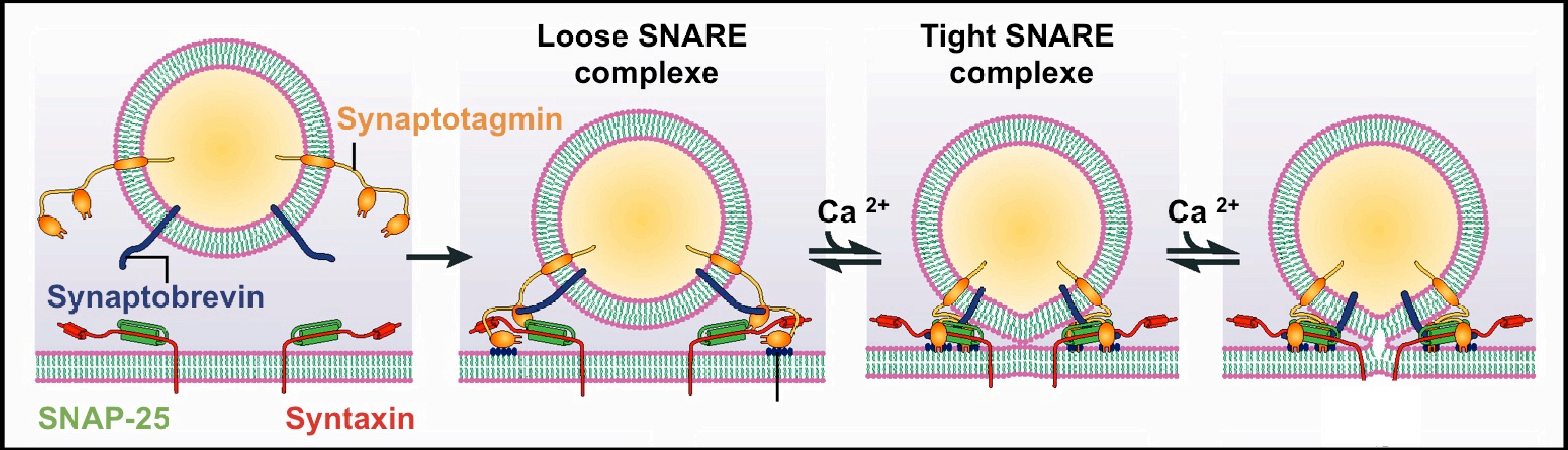
I. Croissance membranaire

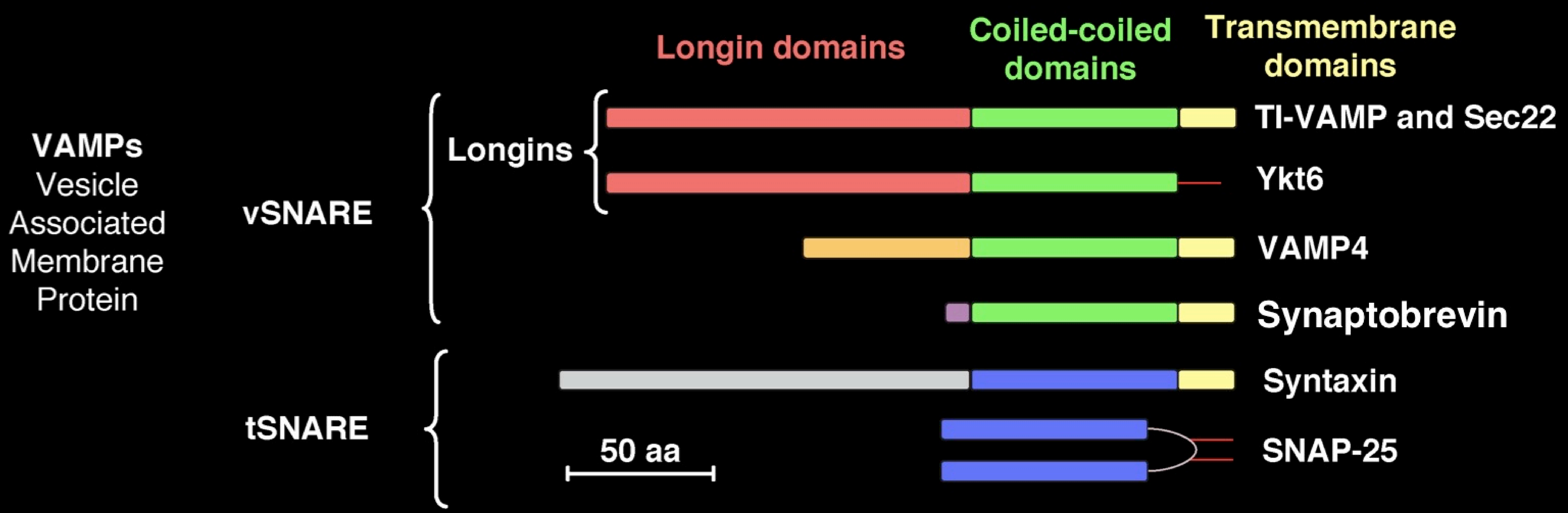
Phase contrast time-lapse recording of a neuron forming an axon. The video shows a rapid playback of a 16 hour recording with one image taken every 10 minutes.

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

Quels sont les mécanismes permettant l’expansion membranaire ?

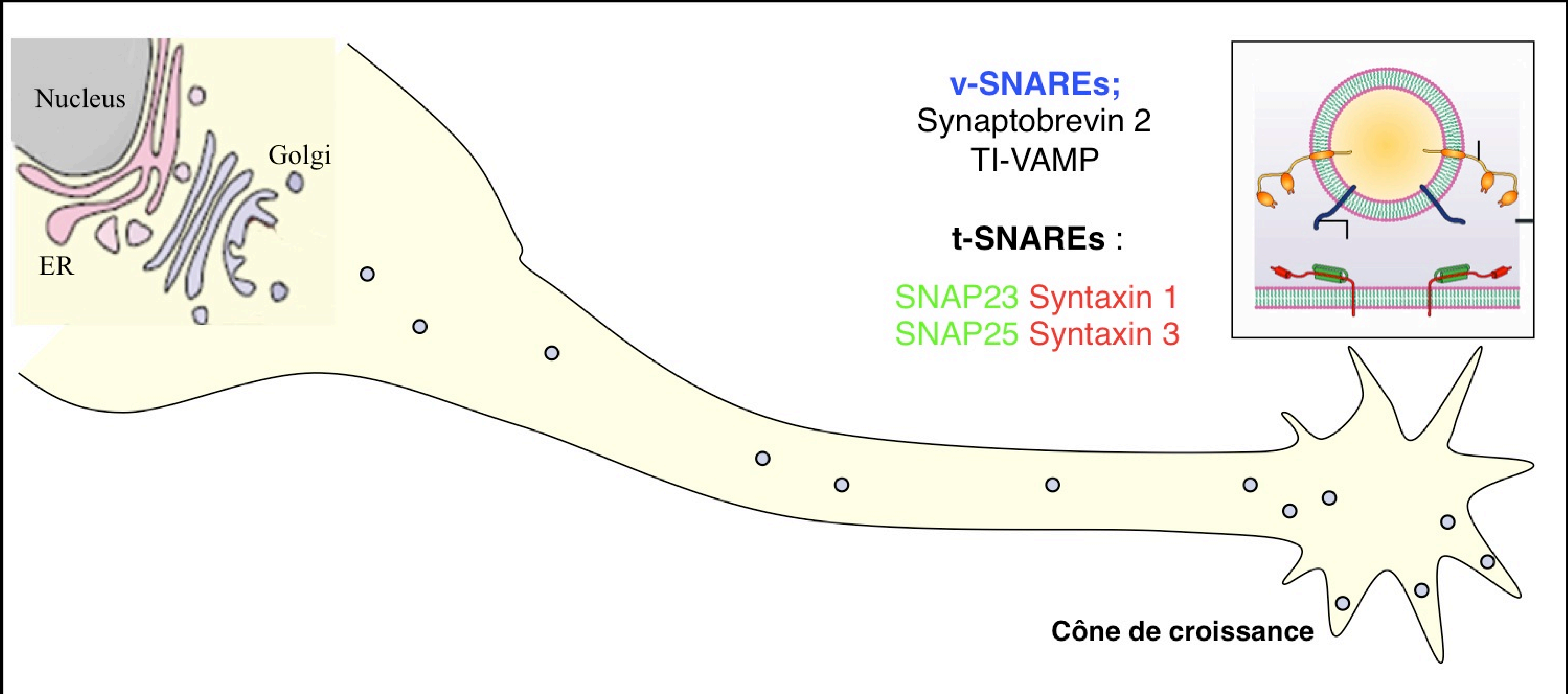
Le complexe SNARE





Adapted from Rossi et al. (2004) TRENDS in Biochemical Sciences Vol.29 No.12:682-688.

SNAREs in neurite outgrowth



SNAREs à la synapses

v-SNAREs: Synaptobrevin 2

t-SNAREs : Syntaxin 1, SNAP25

Perte de Syb2 ou SNAP25:
Perte de sécrétion évoquée
Devpt cerveau normal, croissance neur. normale

SNAREs au cône de croissance

v-SNAREs: Synaptobrevin 2, TI-VAMP

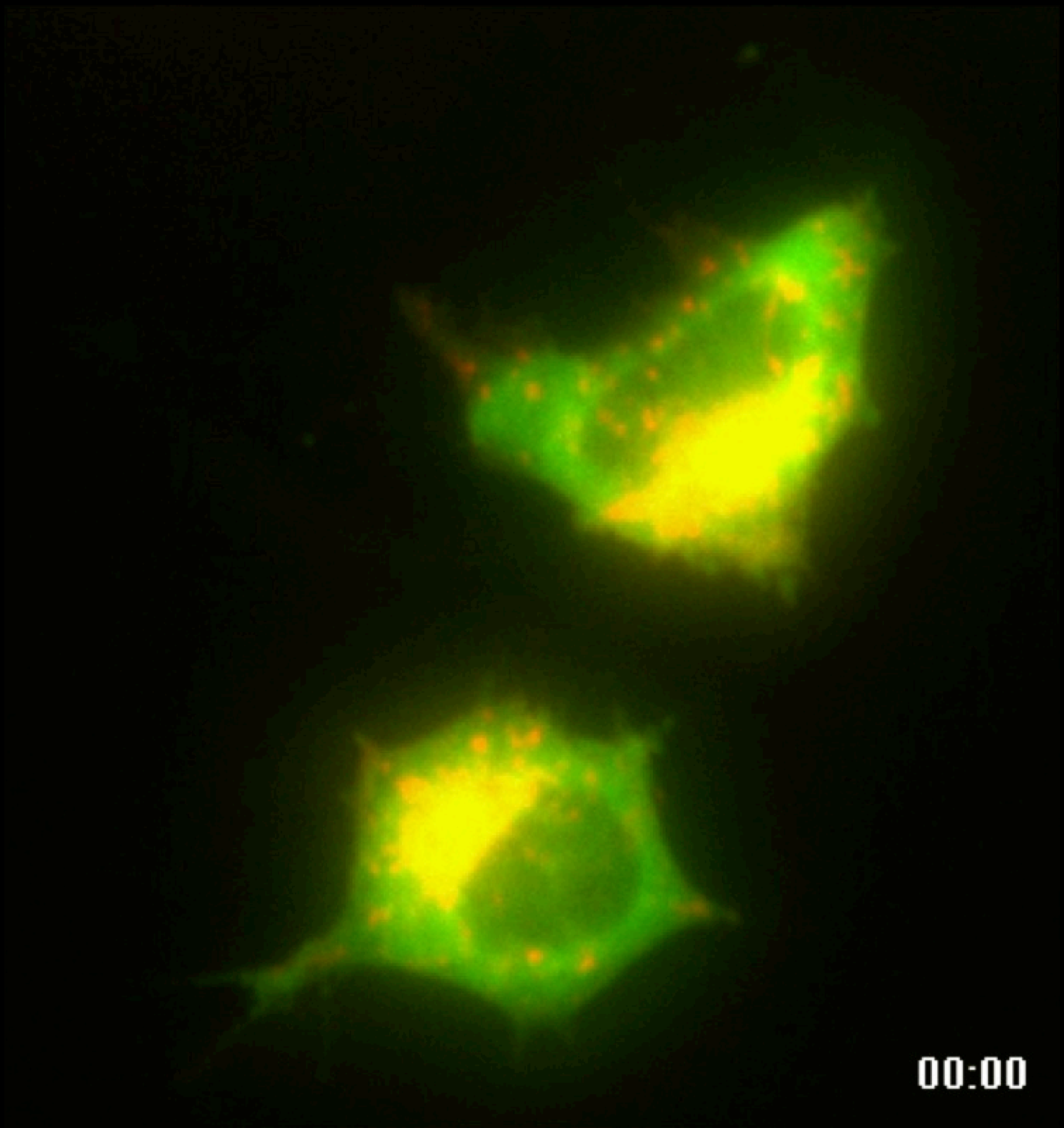
t-SNAREs : Syntaxin 1,3, SNAP23,25

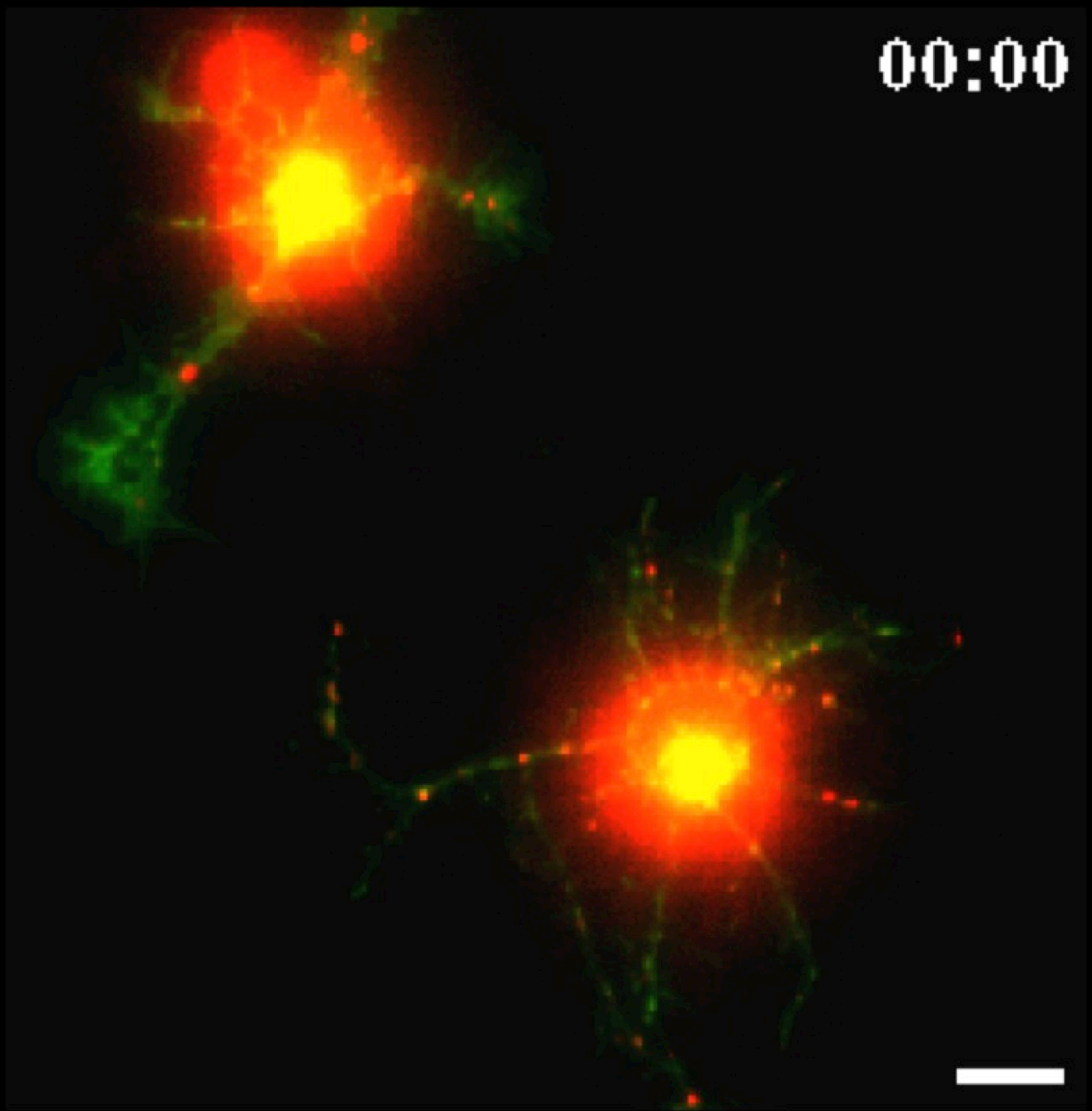
Croissance neuritique:
Résistante à la TeNT qui clive Syb2
Nécessite TI-VAMP et stx3

TI-VAMP et la neuritogenèse

PC12 cells

Hippocampal neurons

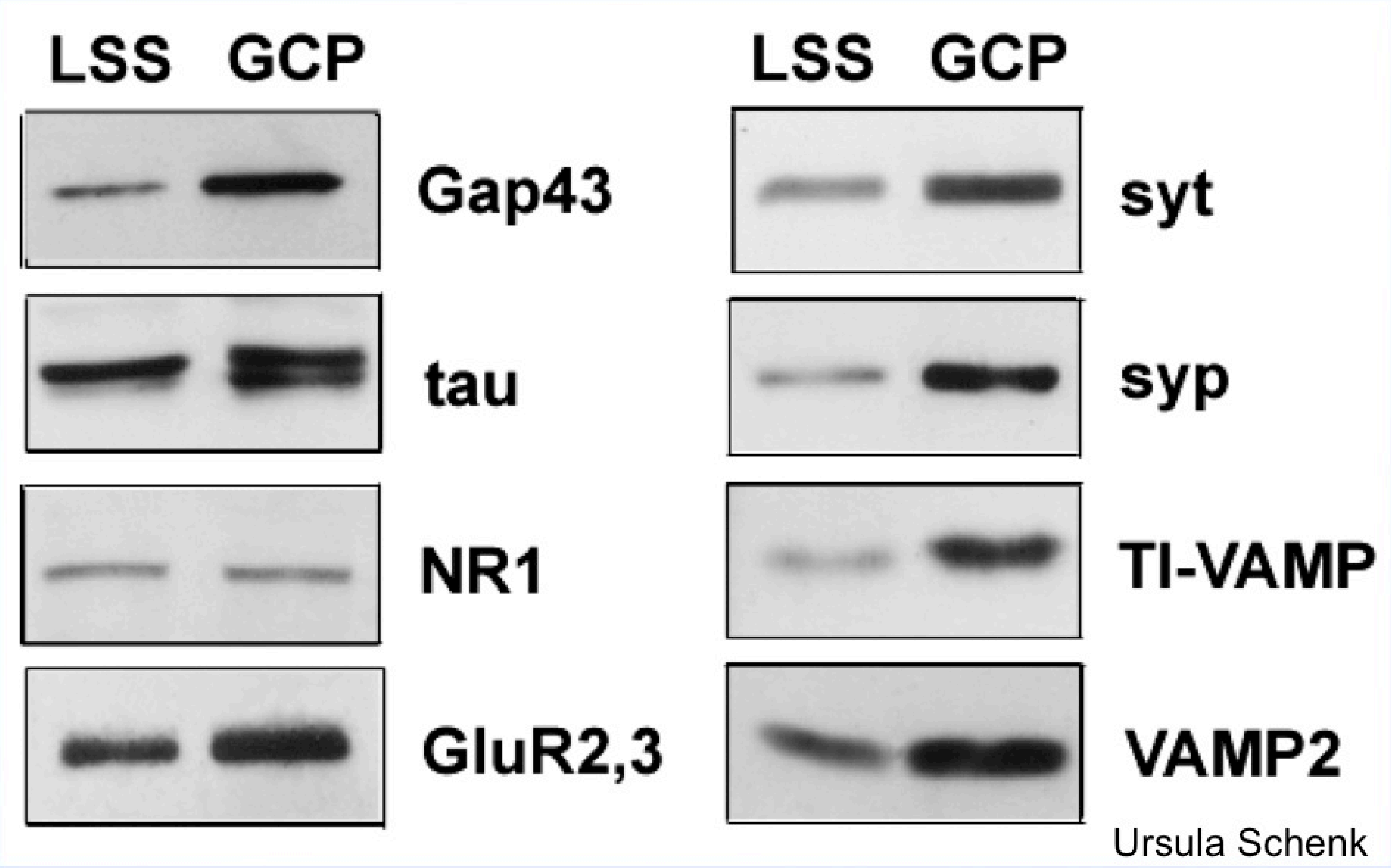


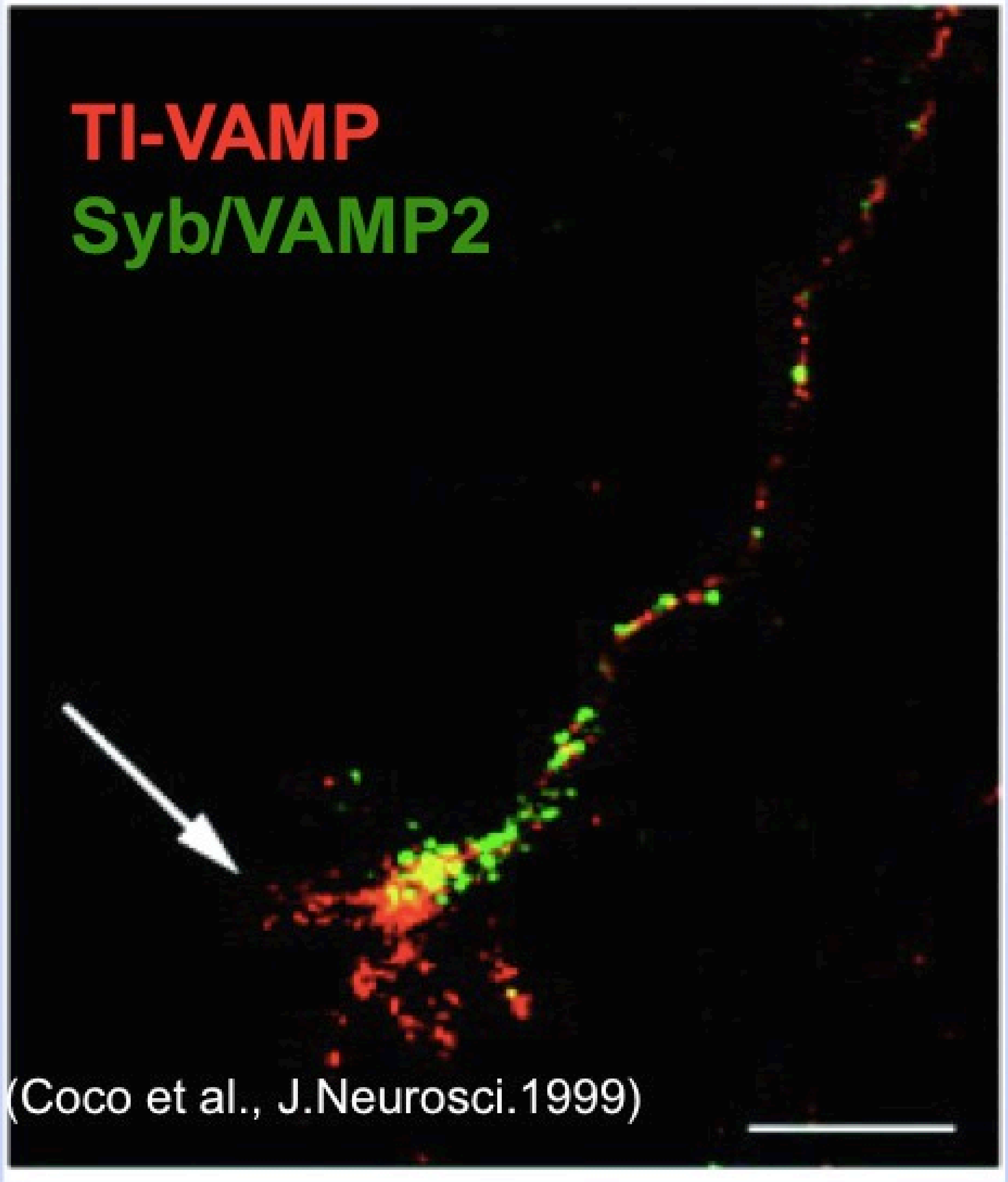


Tubulin **RFP-TI-VAMP**

Films d'Andréa Burgo

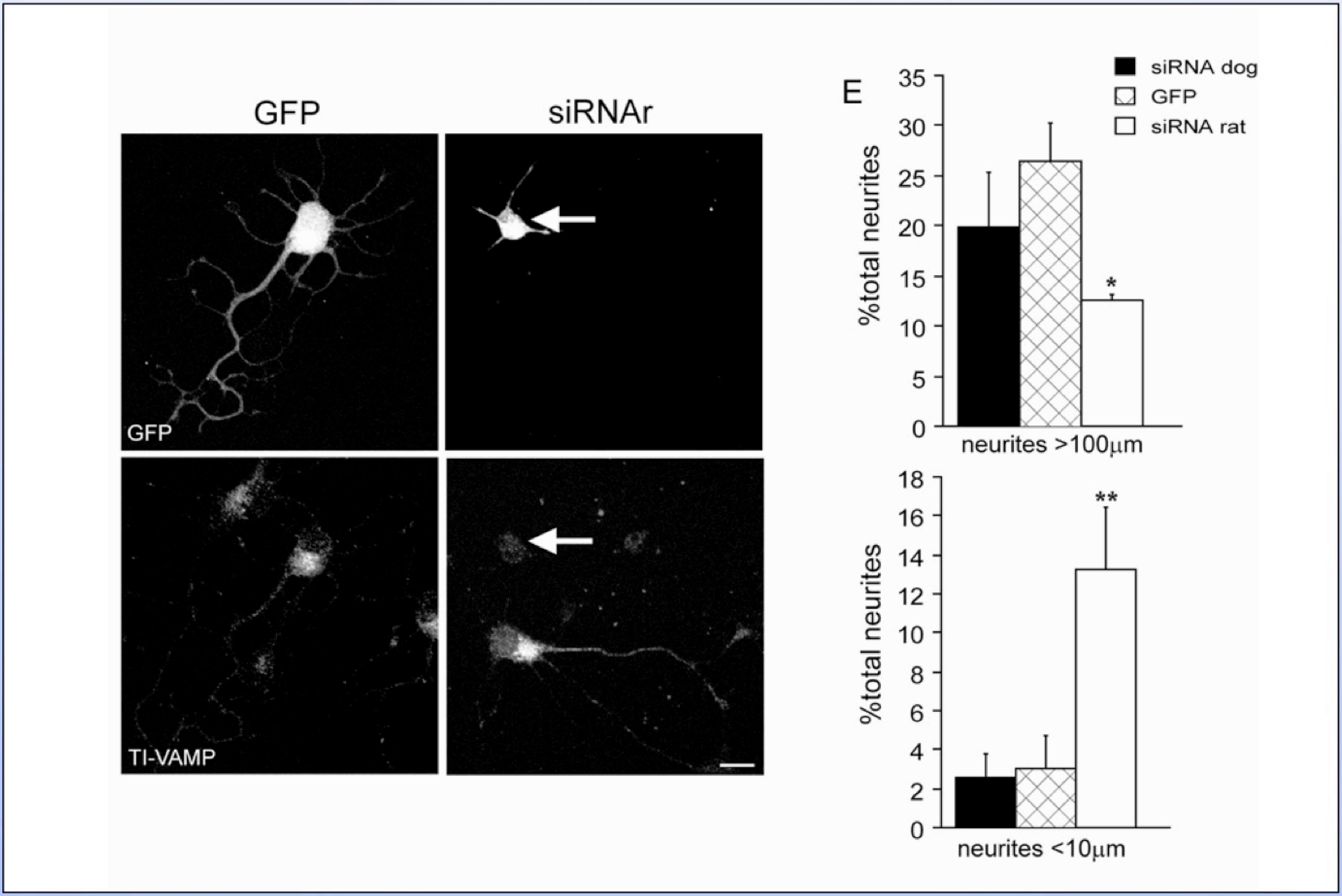
TI-VAMP dans les cônes de croissance





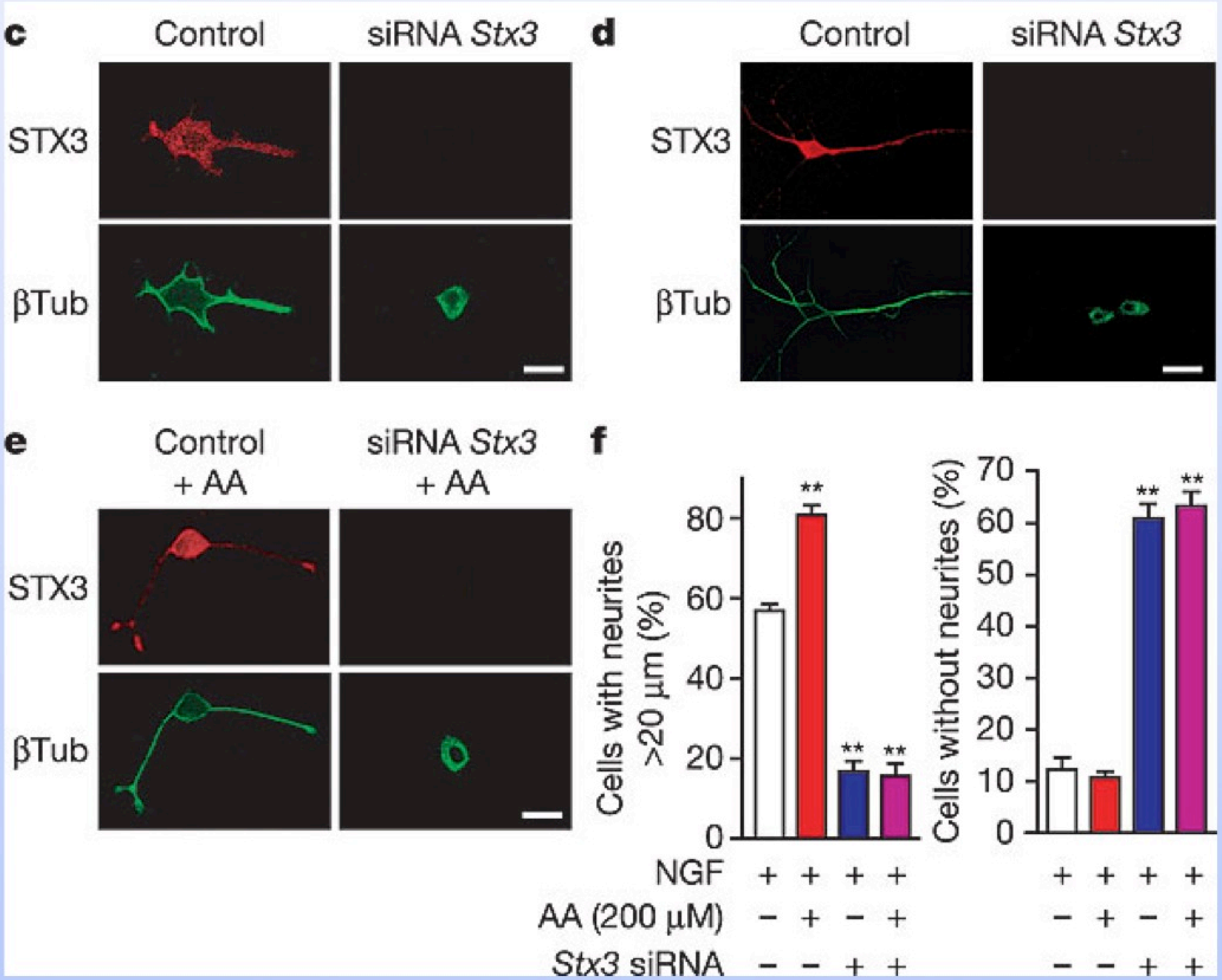
Les marqueurs présynaptiques sont enrichis dans les préparations de cones de croissance (GCP).

TI-VAMP est essentiel à la croissance neuritique



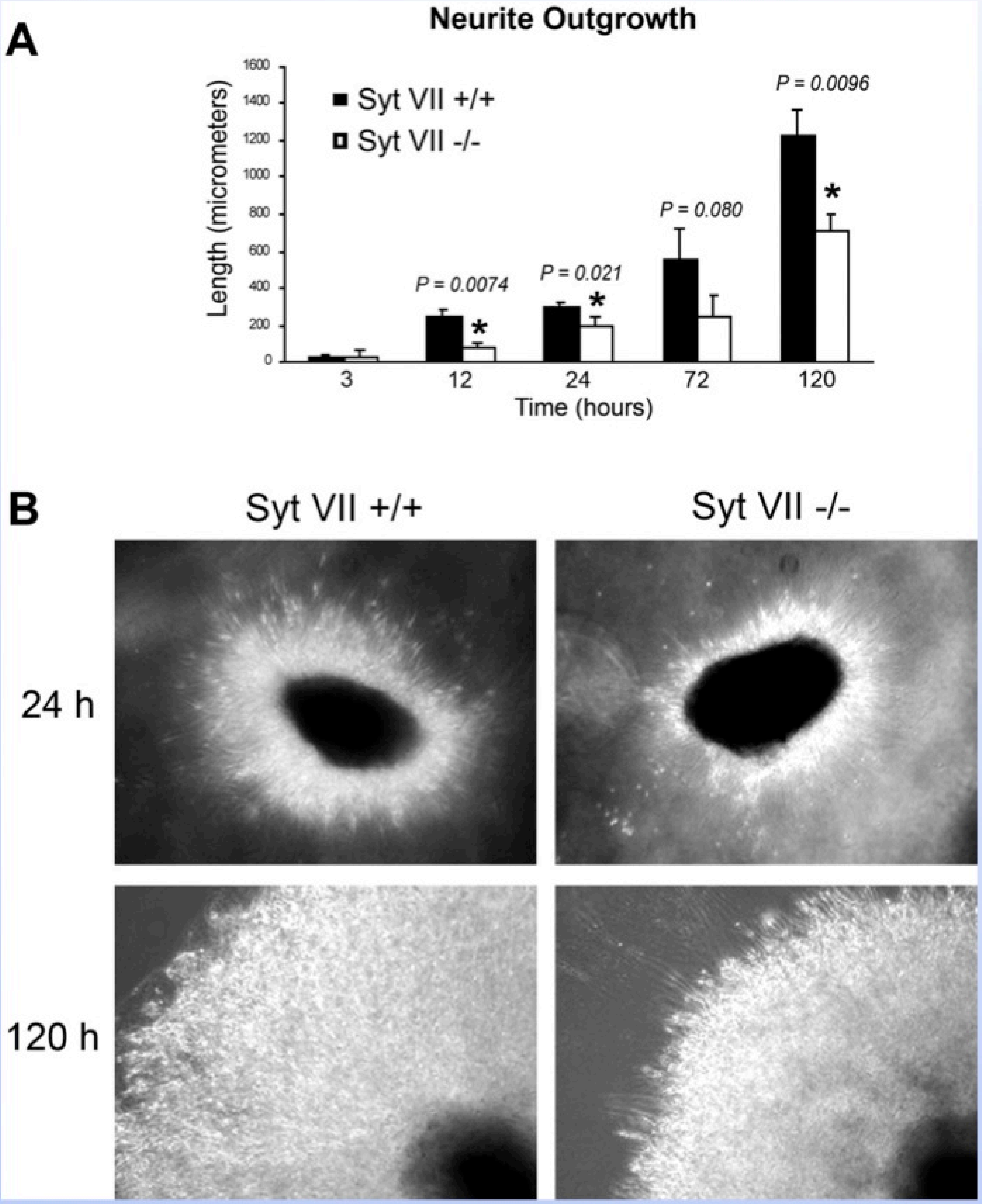
Alberts & al. MBoC 2003

Implication de la syntaxine 3 dans la croissance neuritique

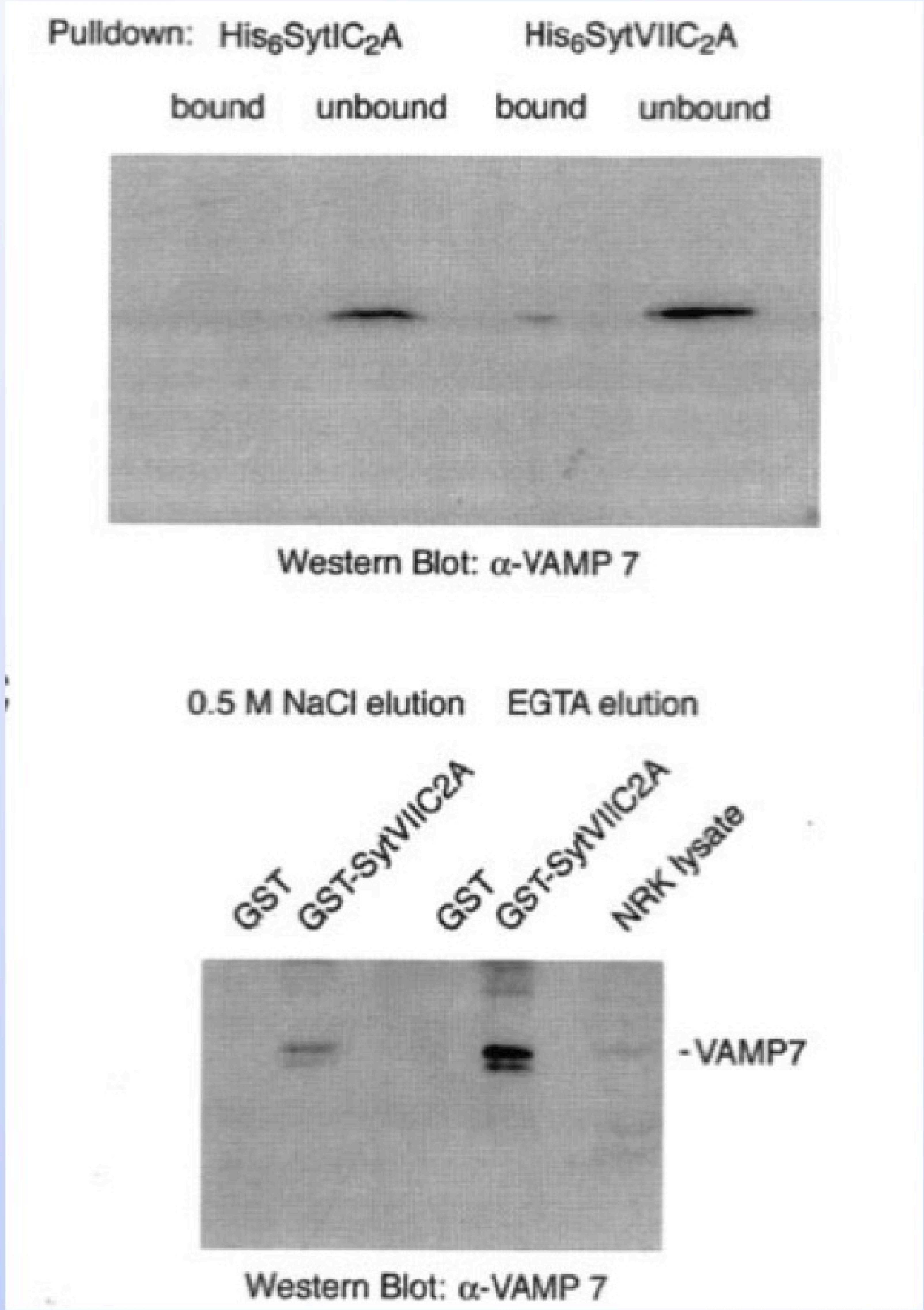


Darios & Davletov, Nature 2006

La croissance neuritique est diminuée dans les explants de ganglions spinal de souris SytVII -/-



Arantes & al J. Neurosci. 2006

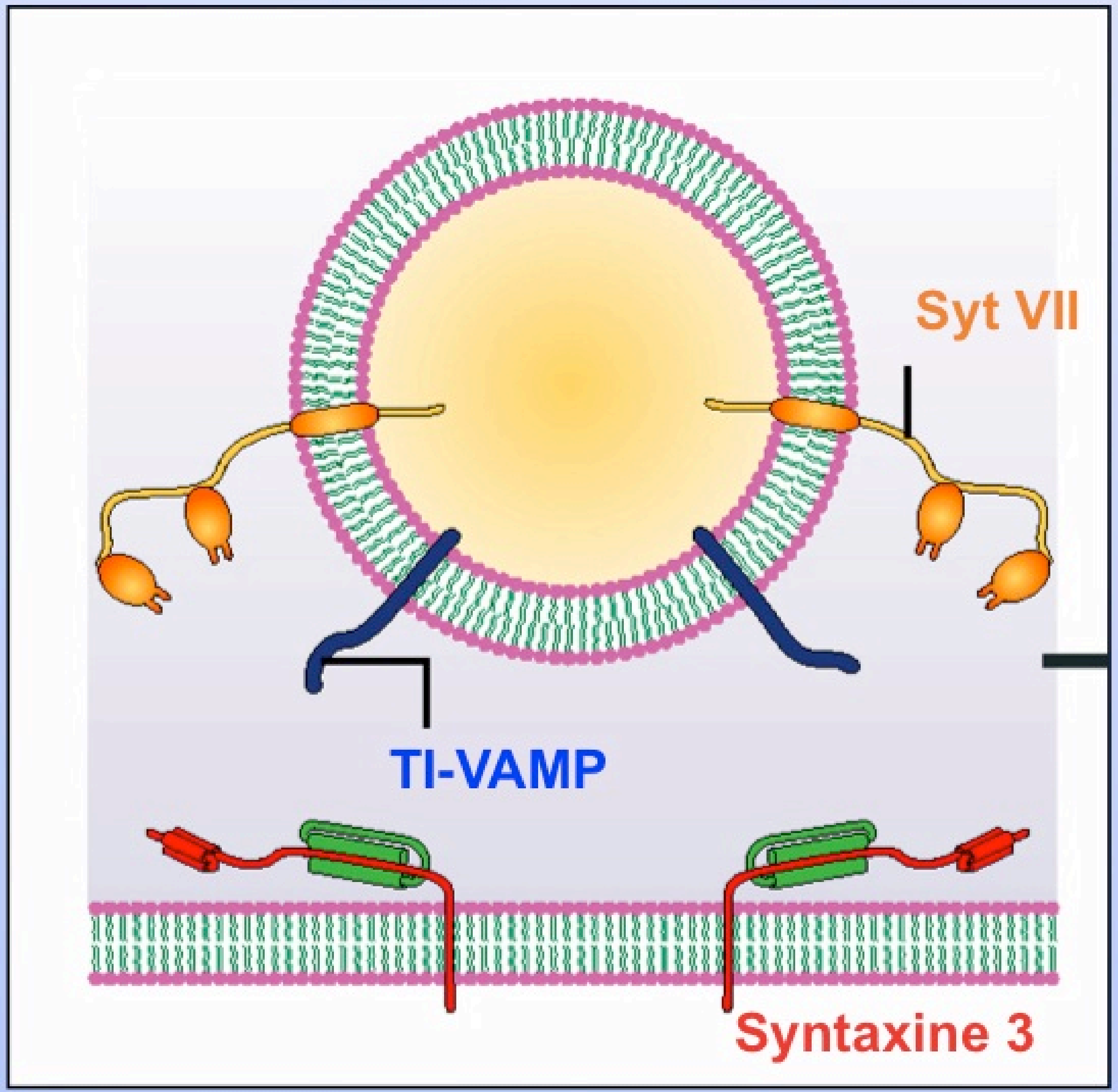


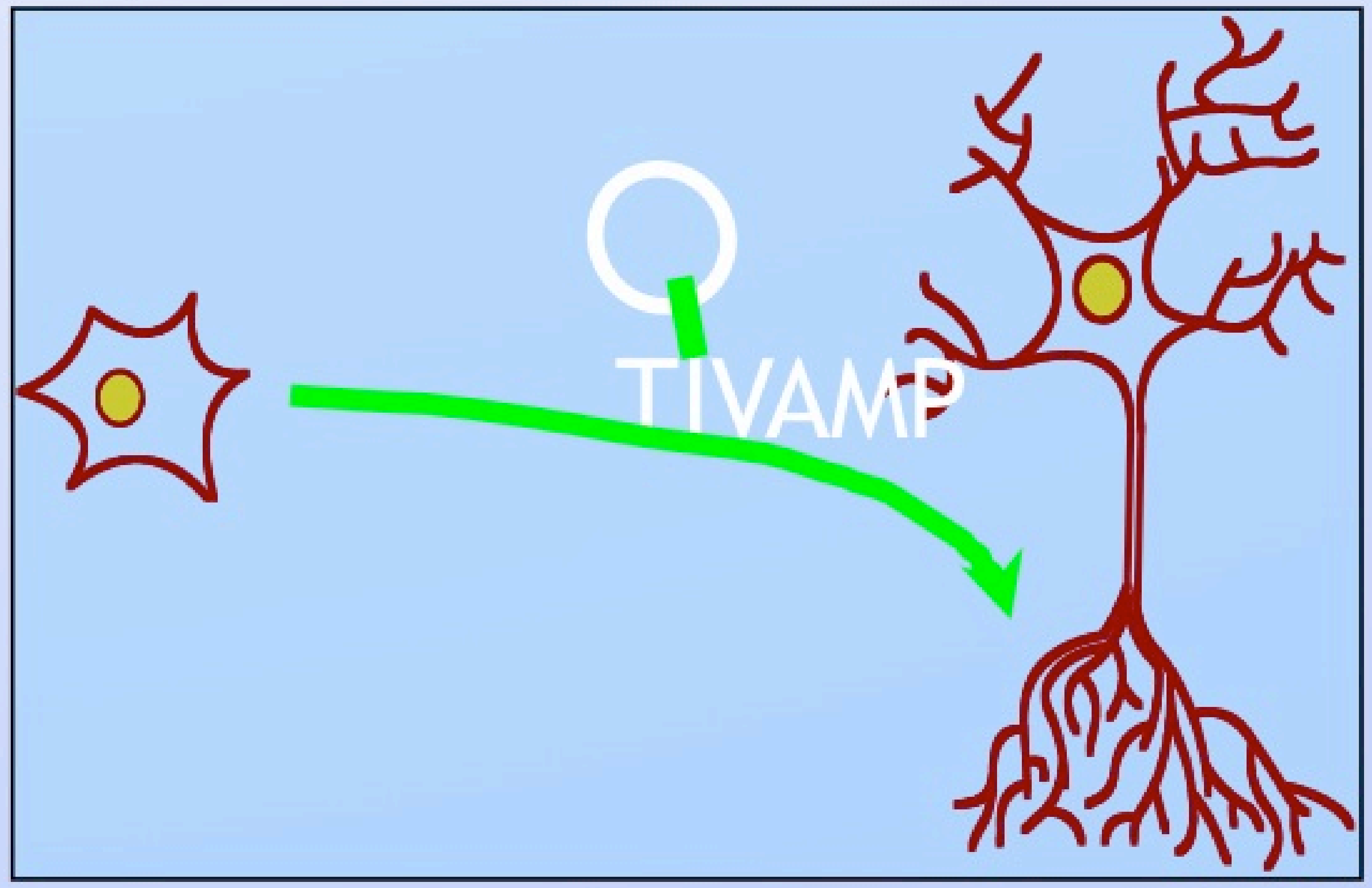
Rao & al JBC 2004

Role de l'exocytose dans la morphogenèse neuronale

Mécanisme moléculaire impliquant:

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



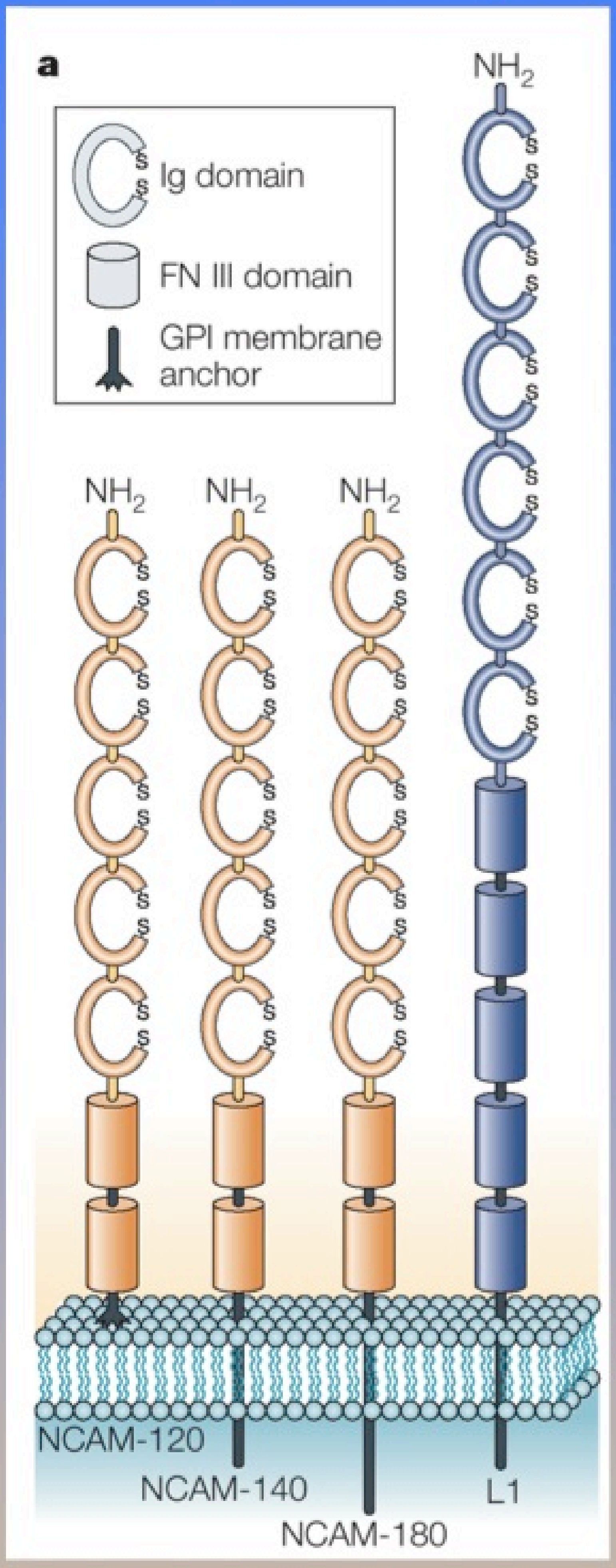


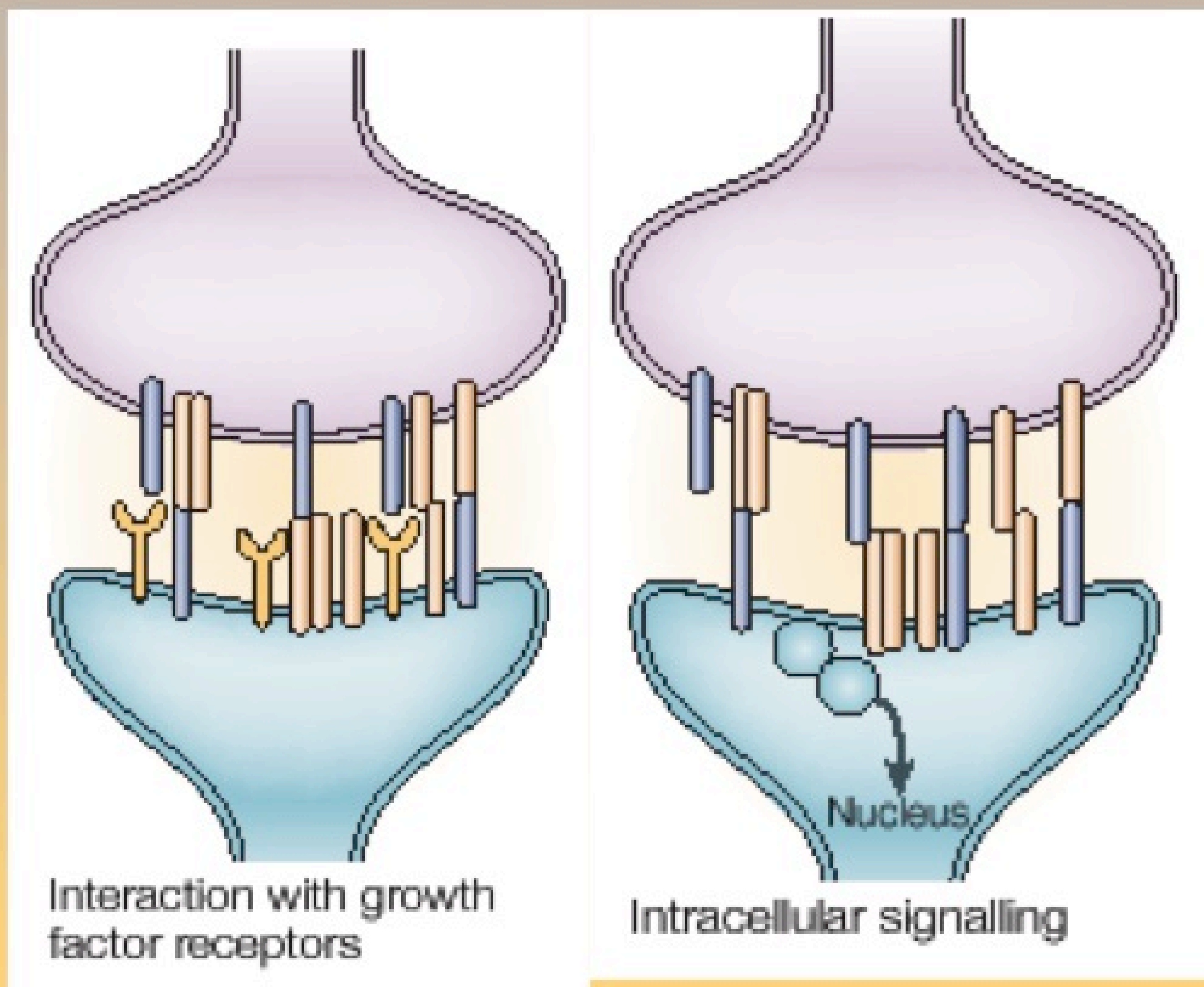
Cargo de TI-VAMP: L1

- **Appartient à la famille des Ig-CAM:**
- **Maladies associées:**
  - **Large domaine extracellulaire avec 6 motifs Ig et 5 FIII**
  - **Un segment transmembranaire helicoïdal**
  - **Un domN cytoplamique**
- **Rôle de L1:**
  - **Interactions cellulaires:**
    - **homo (L1-L1)**
    - **et hétérophilliques (L1-IgCAMs)**
  - **Liaison integrines et Recépteurs à tyrosine phosphatases.**
  - **Au cours du développement :**
    - **Différenciation cellulaire, survie.**
    - **Croissance neuritique, guidage axonal et synaptogenèse.**
  - **Chez l'adulte:**
    - **Maintient des contacts cellulaires**
    - **Plasticité synaptique et processus de mémorisation (LTP).**

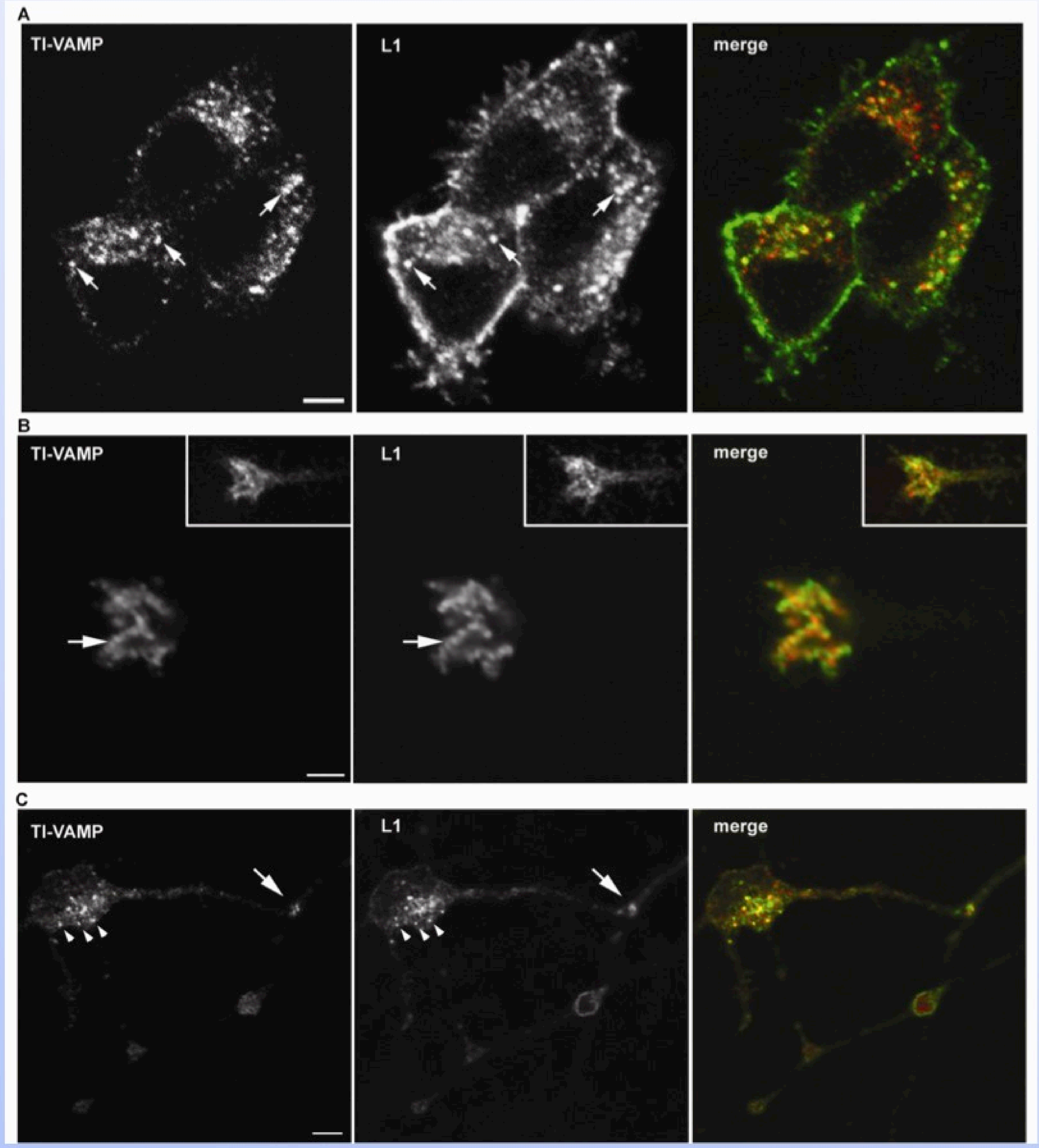
    Rôle de L1:
- **Maladies associées:**
  - **Mutations chez l'homme (MASA syndrom):**

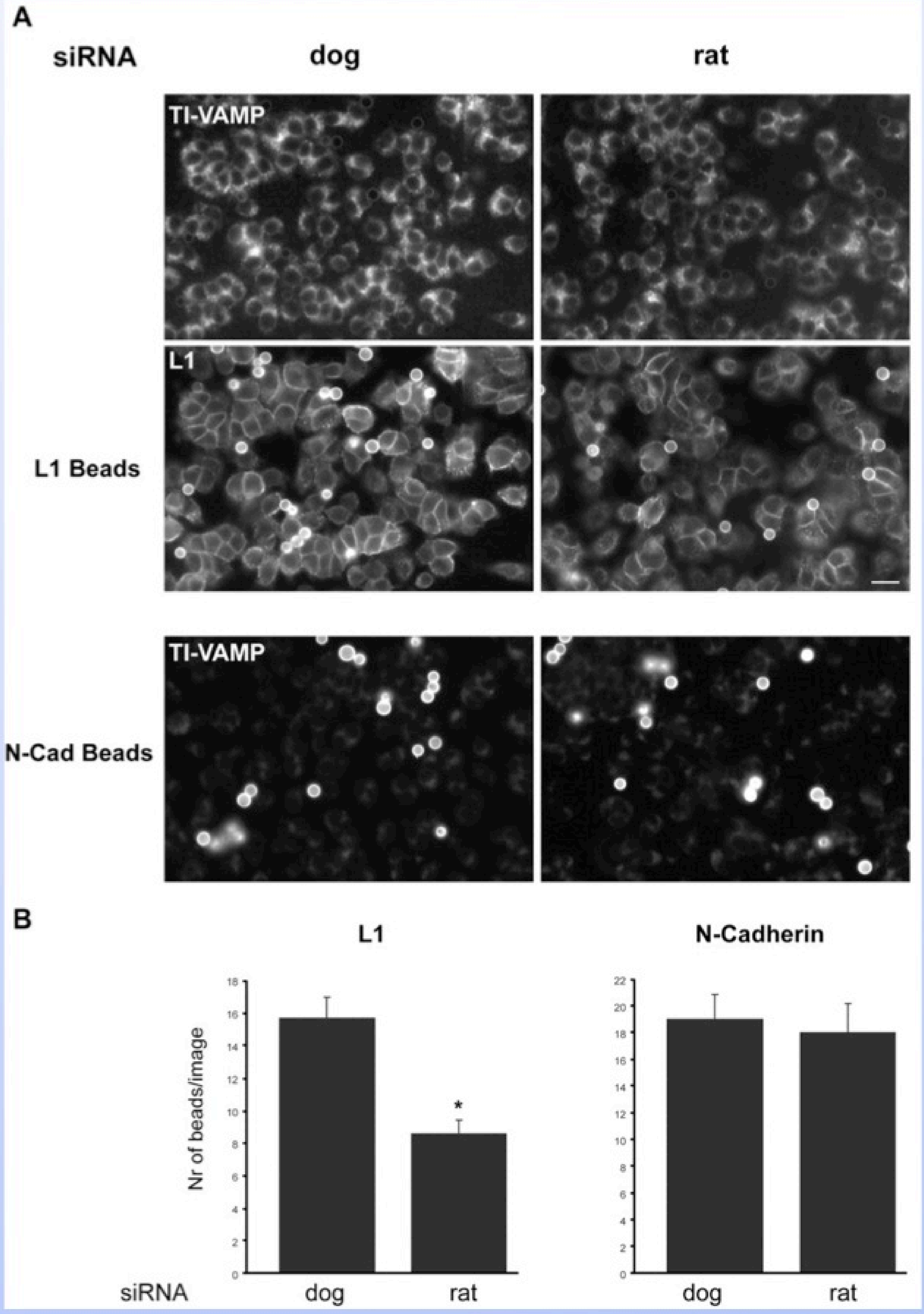
    dysfonctions neurologiques severes:
    - **Agenese du tractus corticospinal, du corps calleux**
    - **Paraplégie spastique**
    - **Retard mental**
  - **Mutation chez la drosophile (neuroglian):**
    - **lethalite embryonnaire, defaut de ciblage axonal.**





L1-CAM est une cargo de TI-VAMP

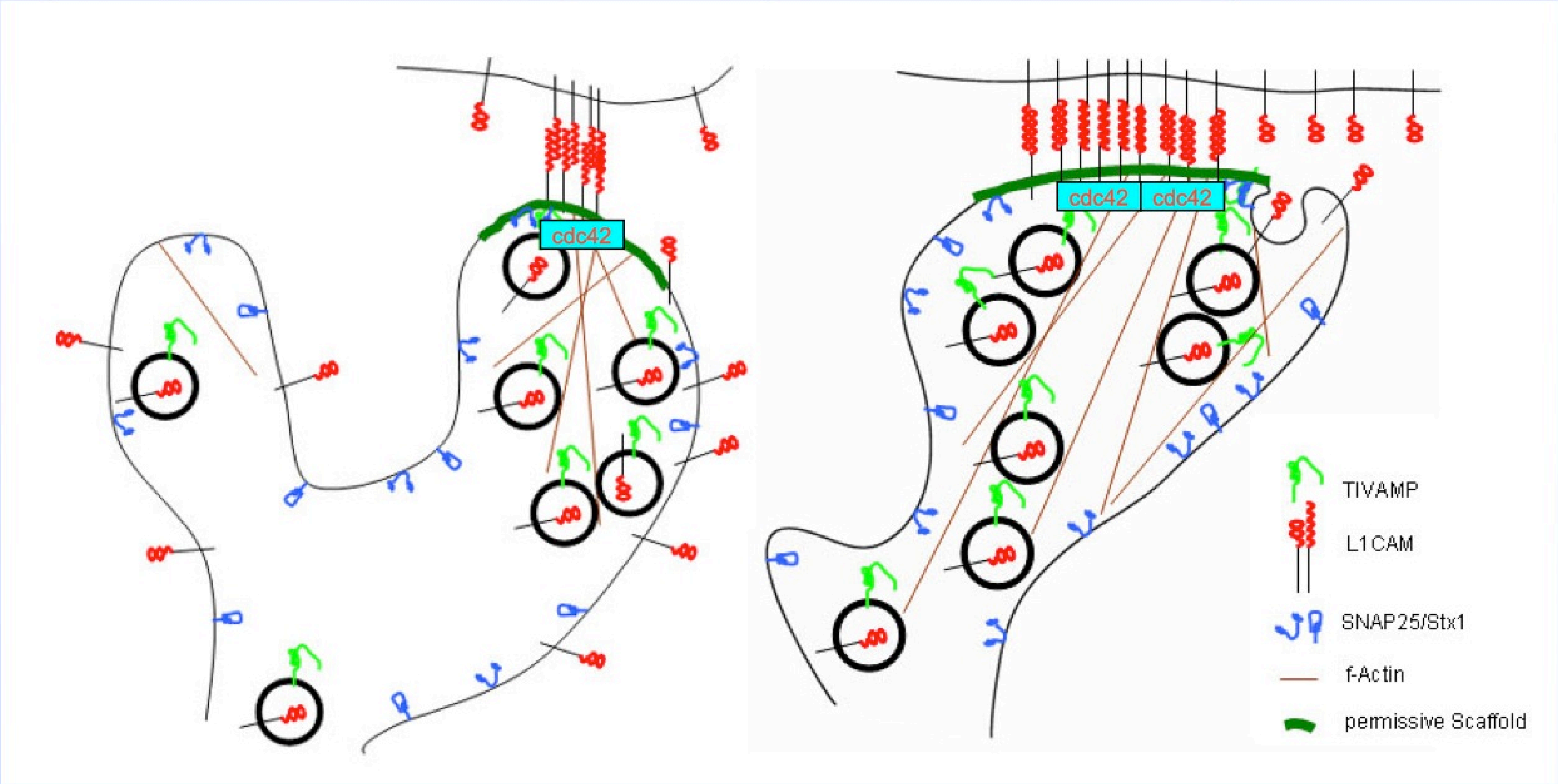




Alberts & al MBoC 2003

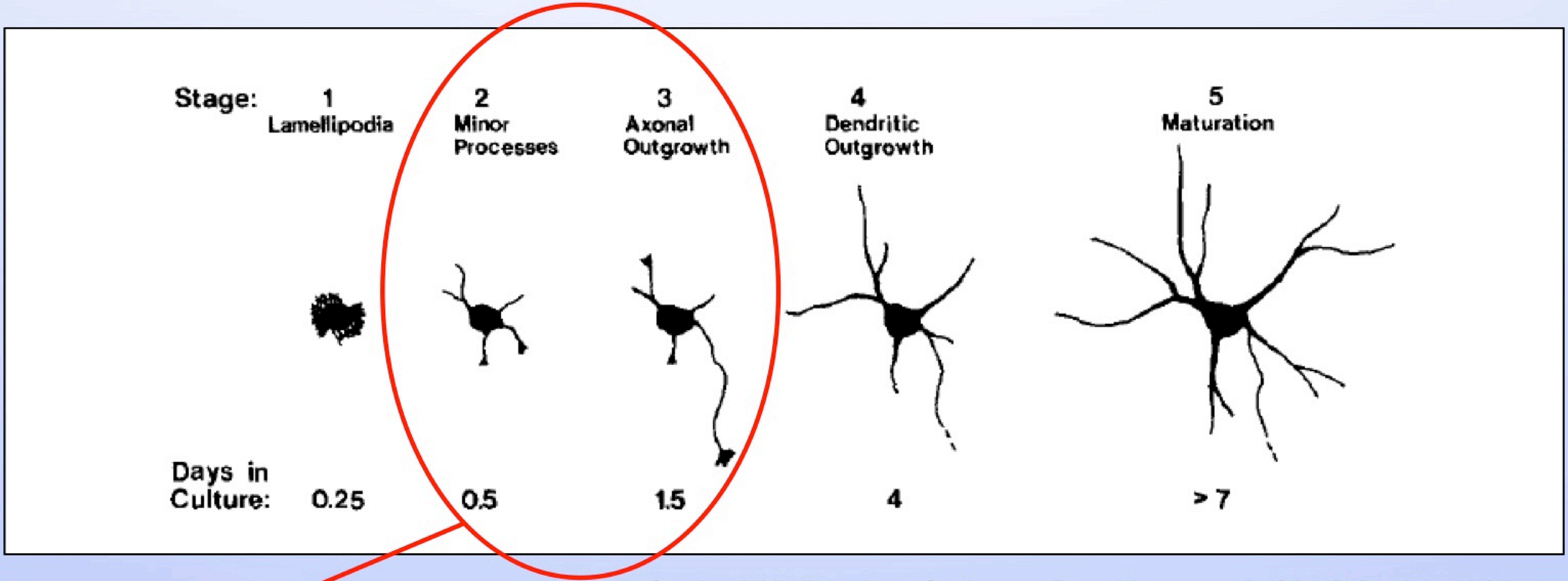
Conclusion: Integration of signaling, actin, and exocytosis in neurite outgrowth

-TI-VAMP is a vesicular SNARE that is necessary for neurite outgrowth
-TI-VAMP transports the IgCAM L1. The TI-VAMP dependent membrane trafficking regulates the stability of L1-dependent adhesive contacts
-L1 mediated adhesion induces a polarization of TI-VAMP vesicles to sites of contact
-The exocytosis of TI-VAMP is positively controlled actin dynamics and cdc42



Philipp Alberts

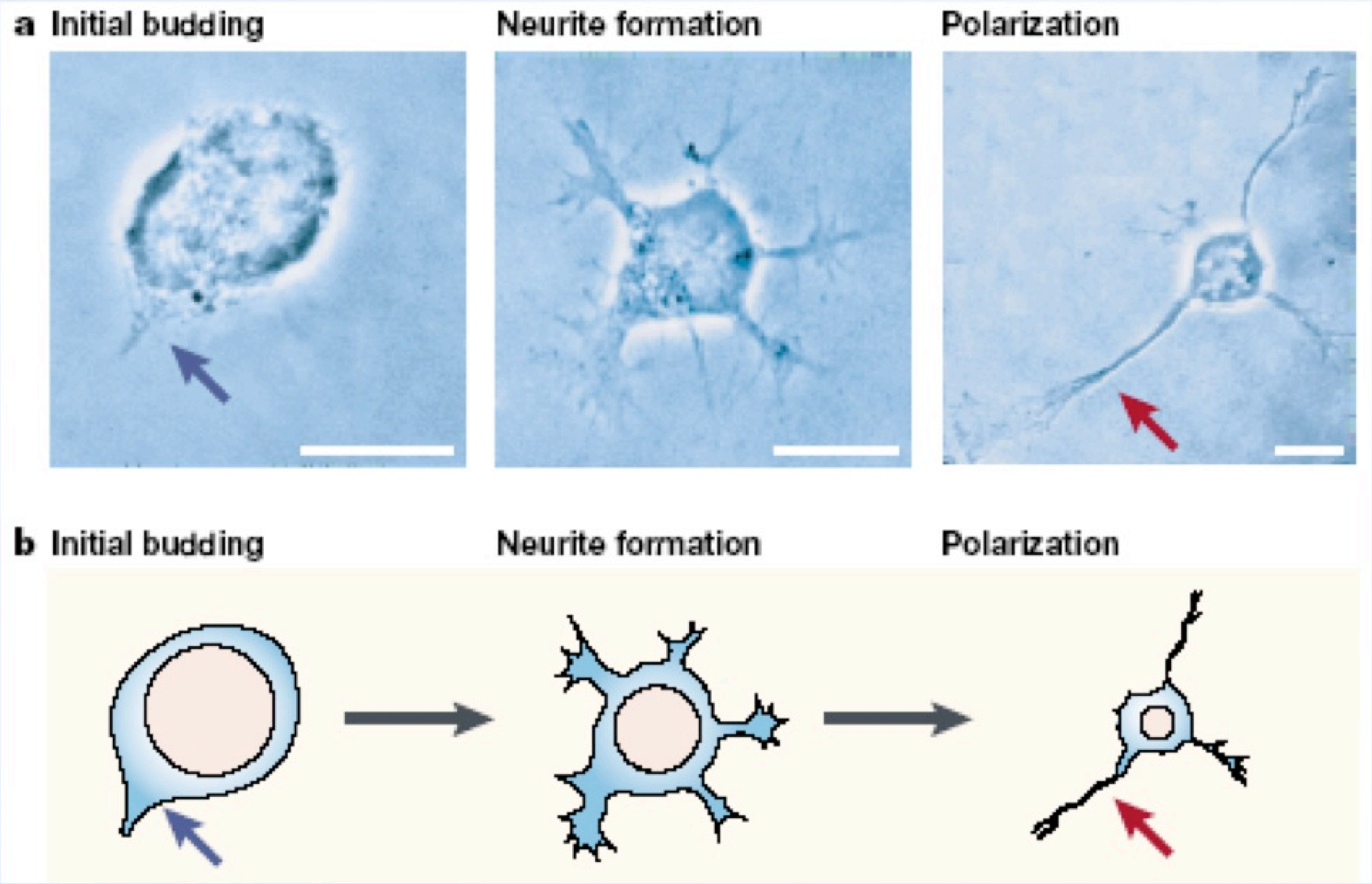
Etudes pionnières *in vitro* par Gary Banker dans les années 1980's-1990's

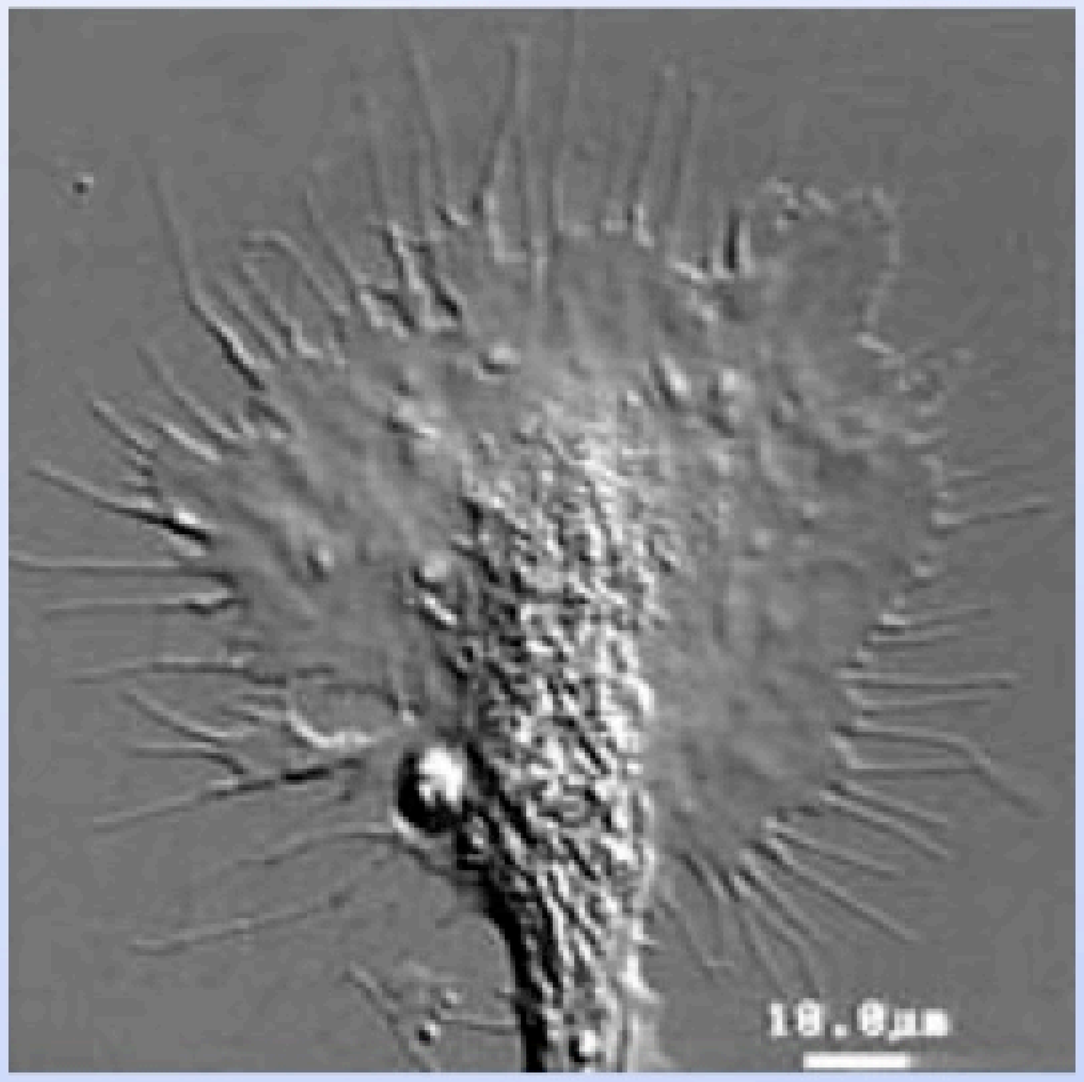


Craig AM, Banker G. Annu Rev Neurosci. 1994;17:267-310.

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



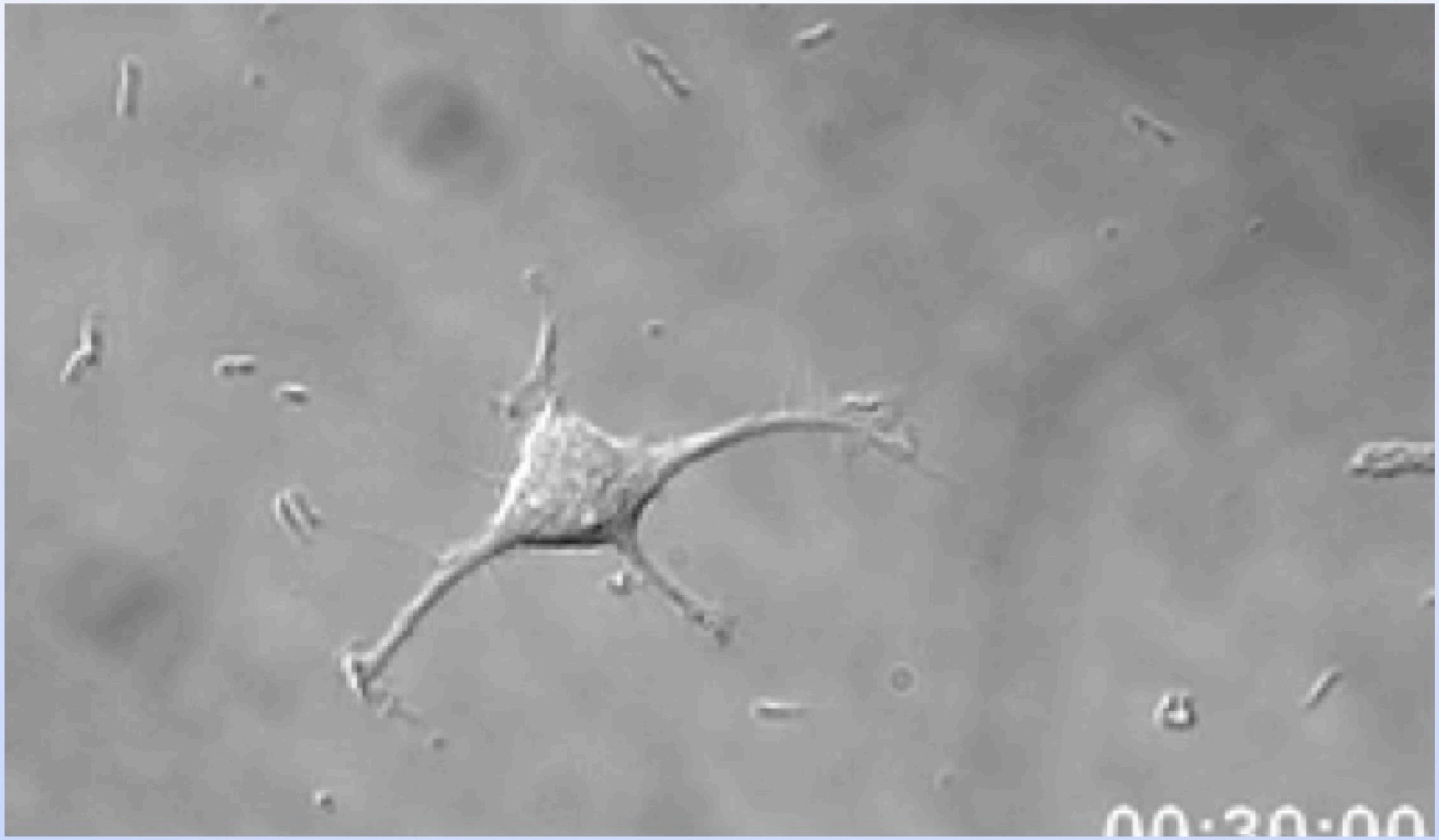


Cône de croissance

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**.

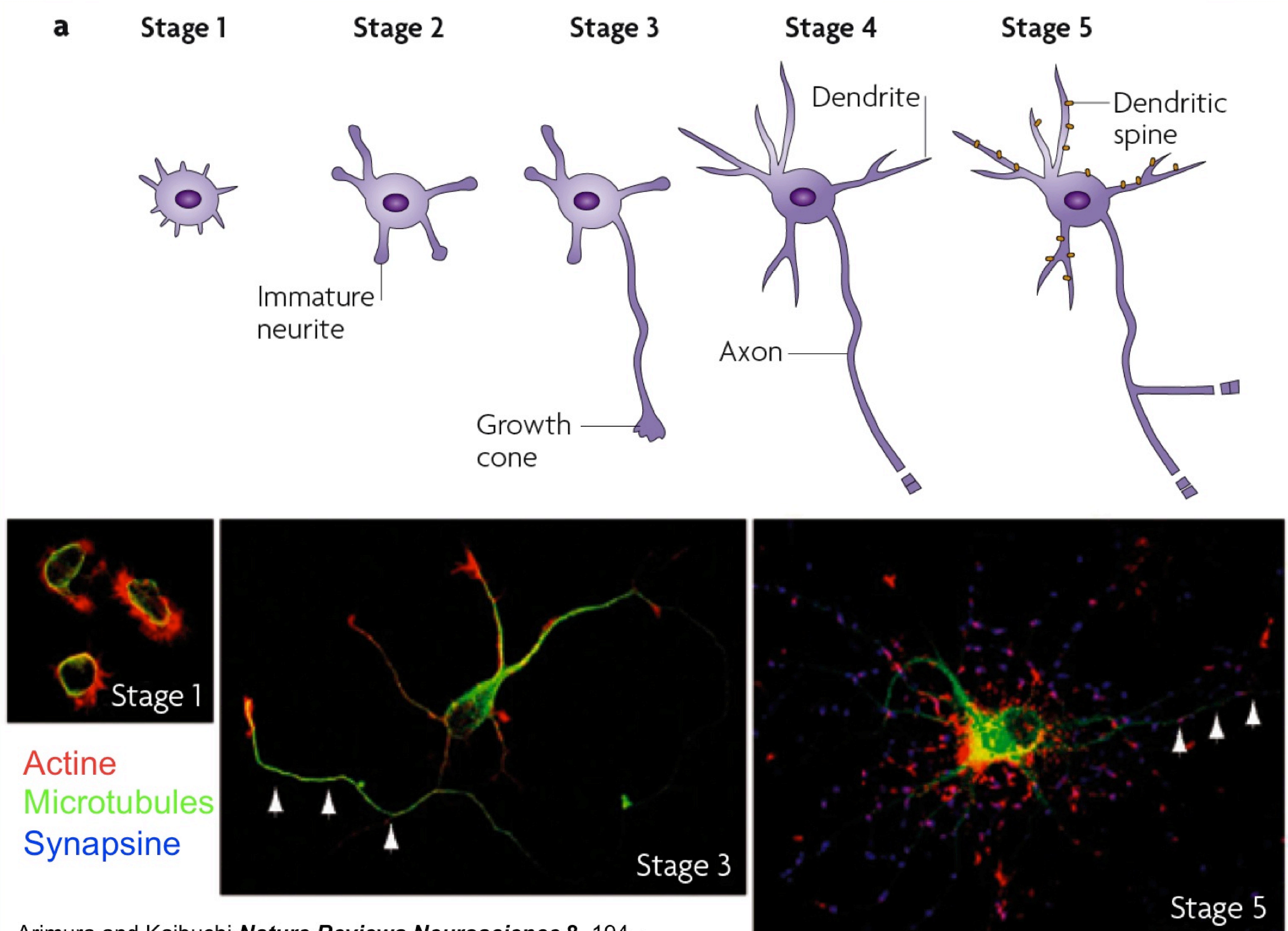
Croissance neuritique:



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)

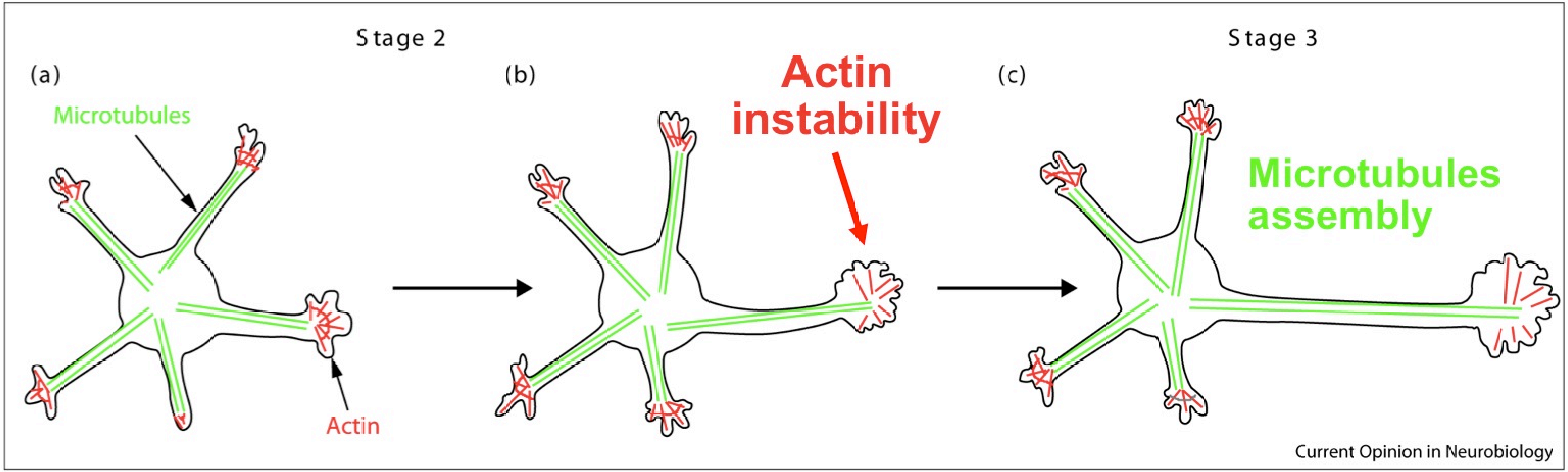


Arimura and Kaibuchi ***Nature Reviews Neuroscience* 8**, 194–205

-**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.

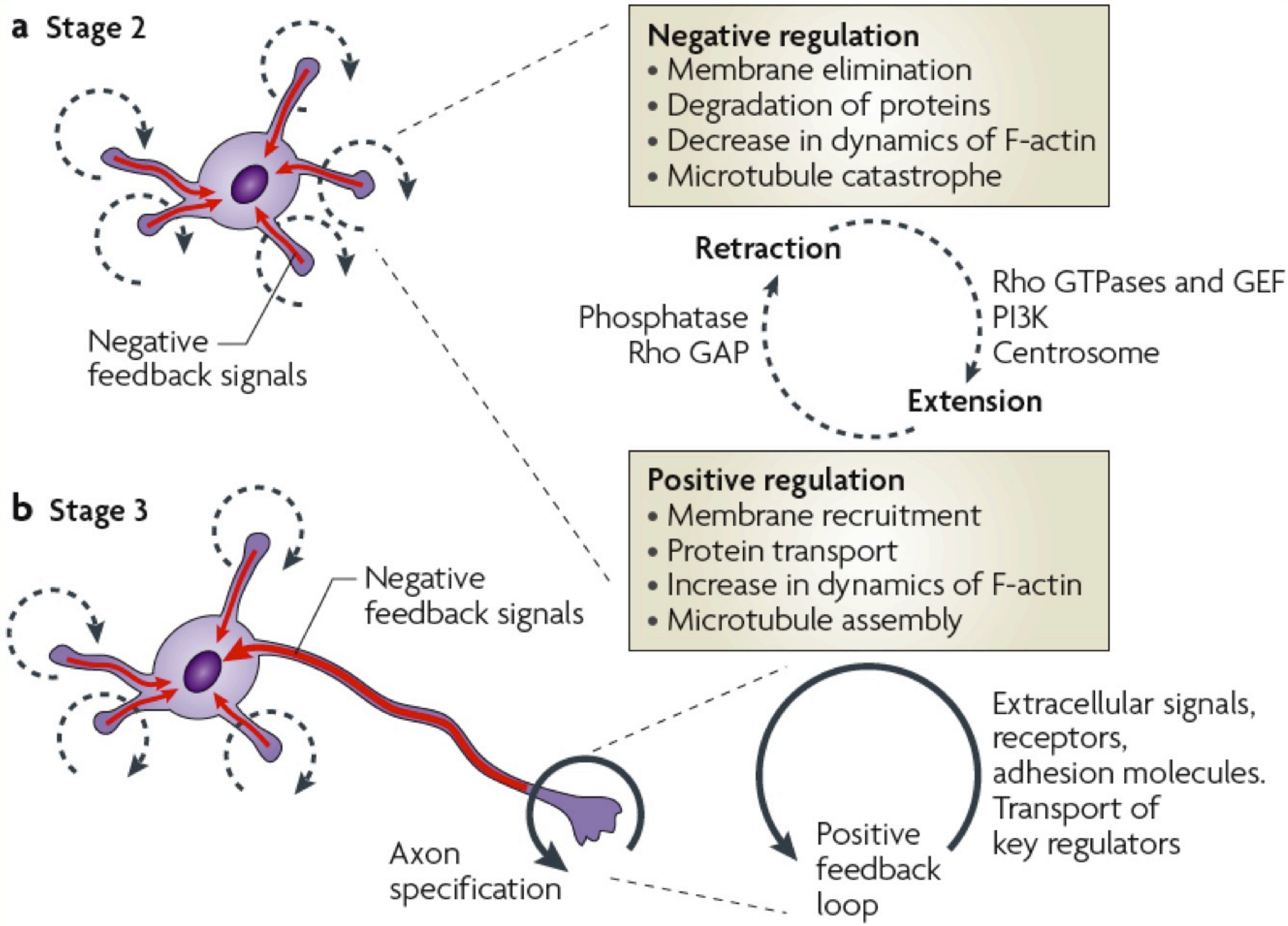


Figure 2 | **A tentative model for axon specification in neuronal polarization.**

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

lot

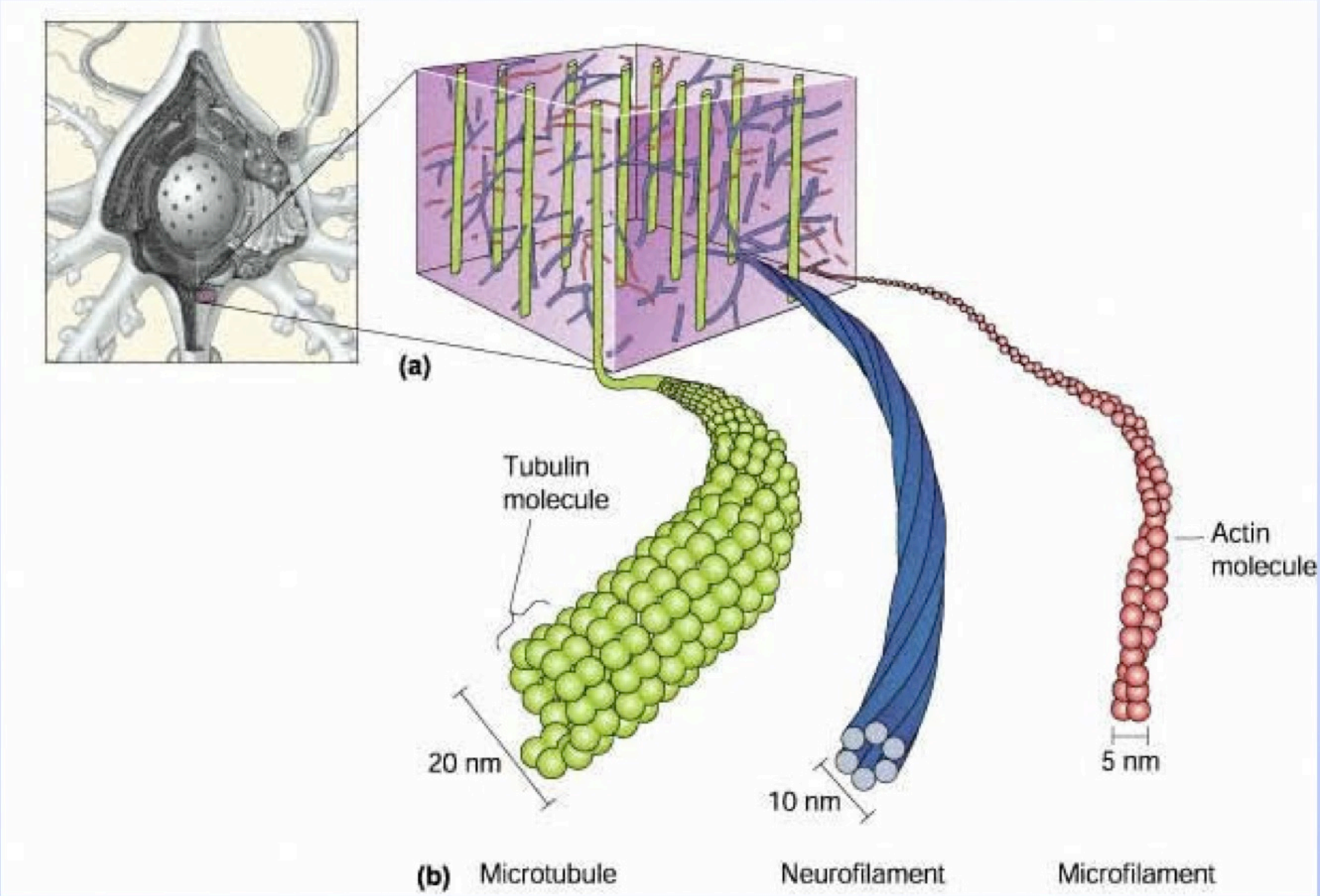
Molécules régulant la polarité

	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

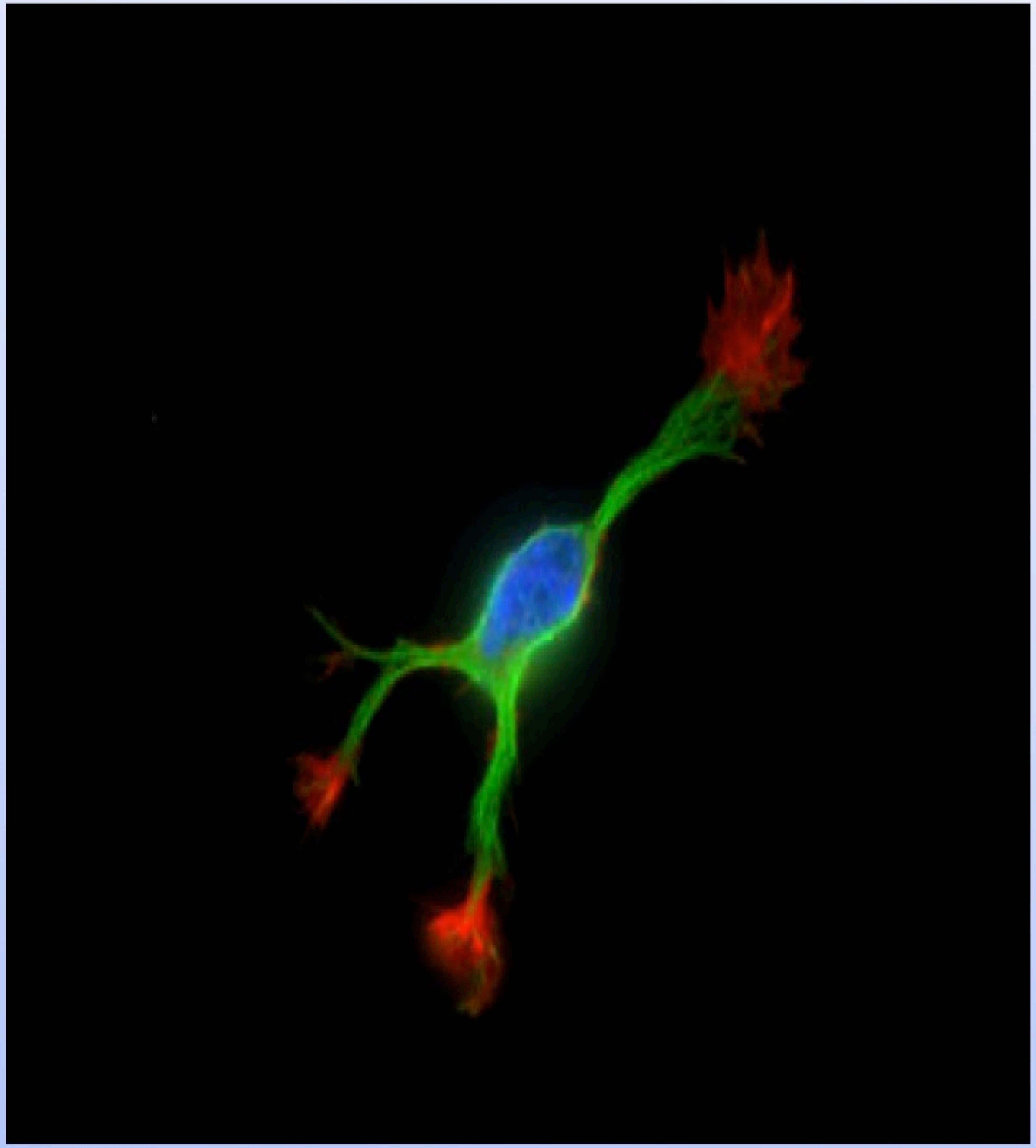
5. Le cytosquelette et la polarité neuronale



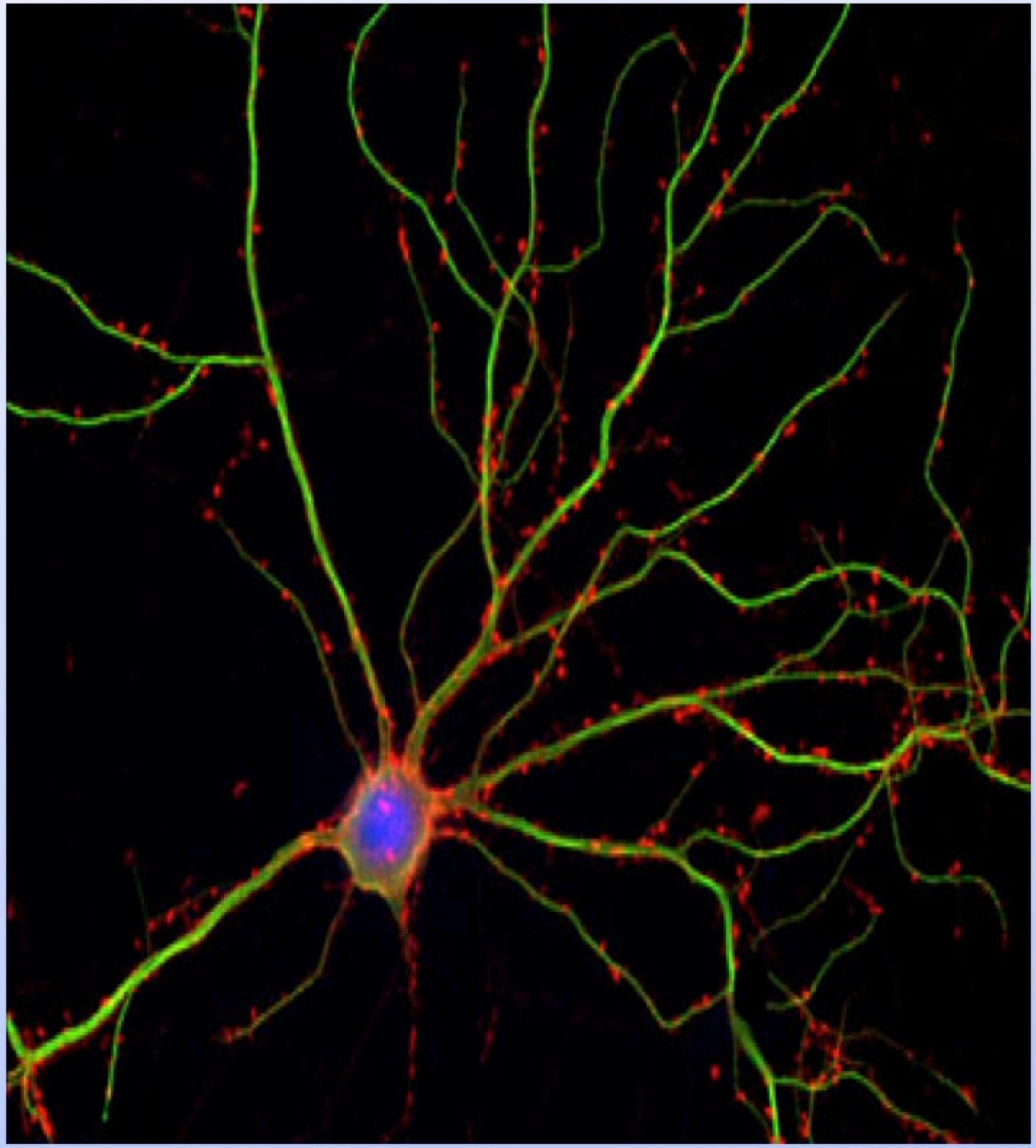
5. Cytosquelette et polarité

a) Le rôle de l'actine

HPC neuron, 24h



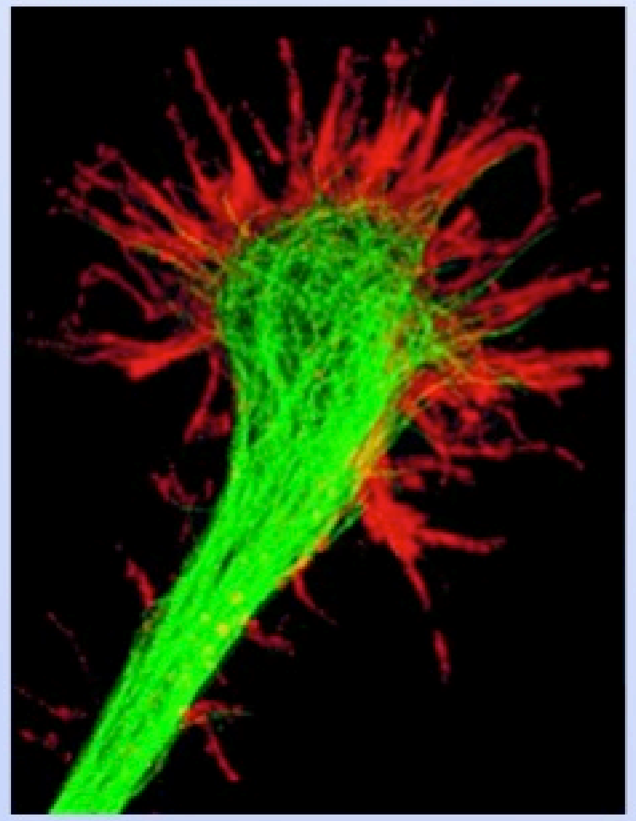
HPC neuron, 3 weeks



DNA- blue; µtubules- green; actin- red

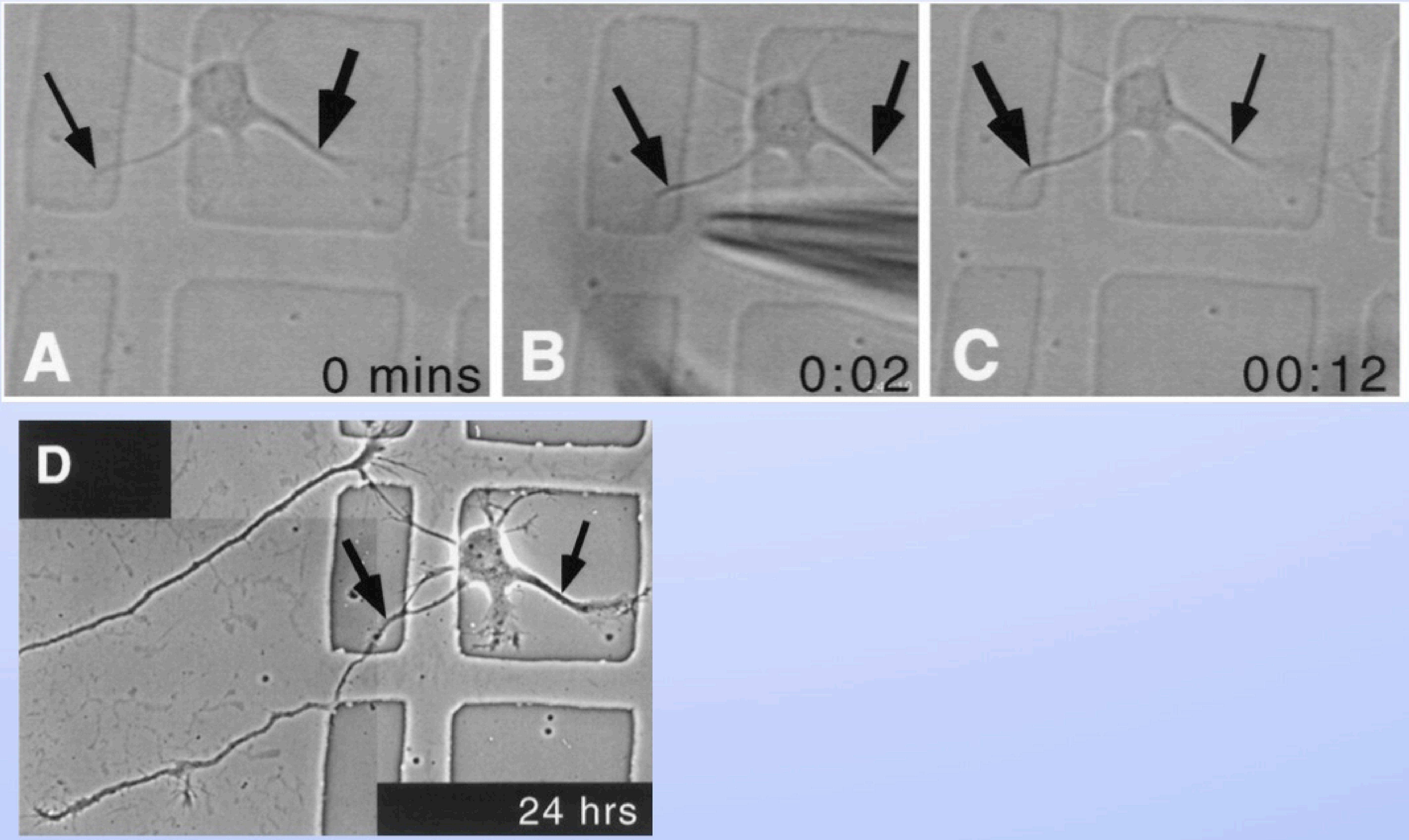
Instabilité de l'Actin dansle cone de croissance

Local perfusion of cytochalasin D onto a growth cone induces it to grow as an axon, indicating that actin destabilization is sufficient for axon formation. These data strengthen the proposed hypothesis that polarized actin-filament instability determines initial neuronal polarization



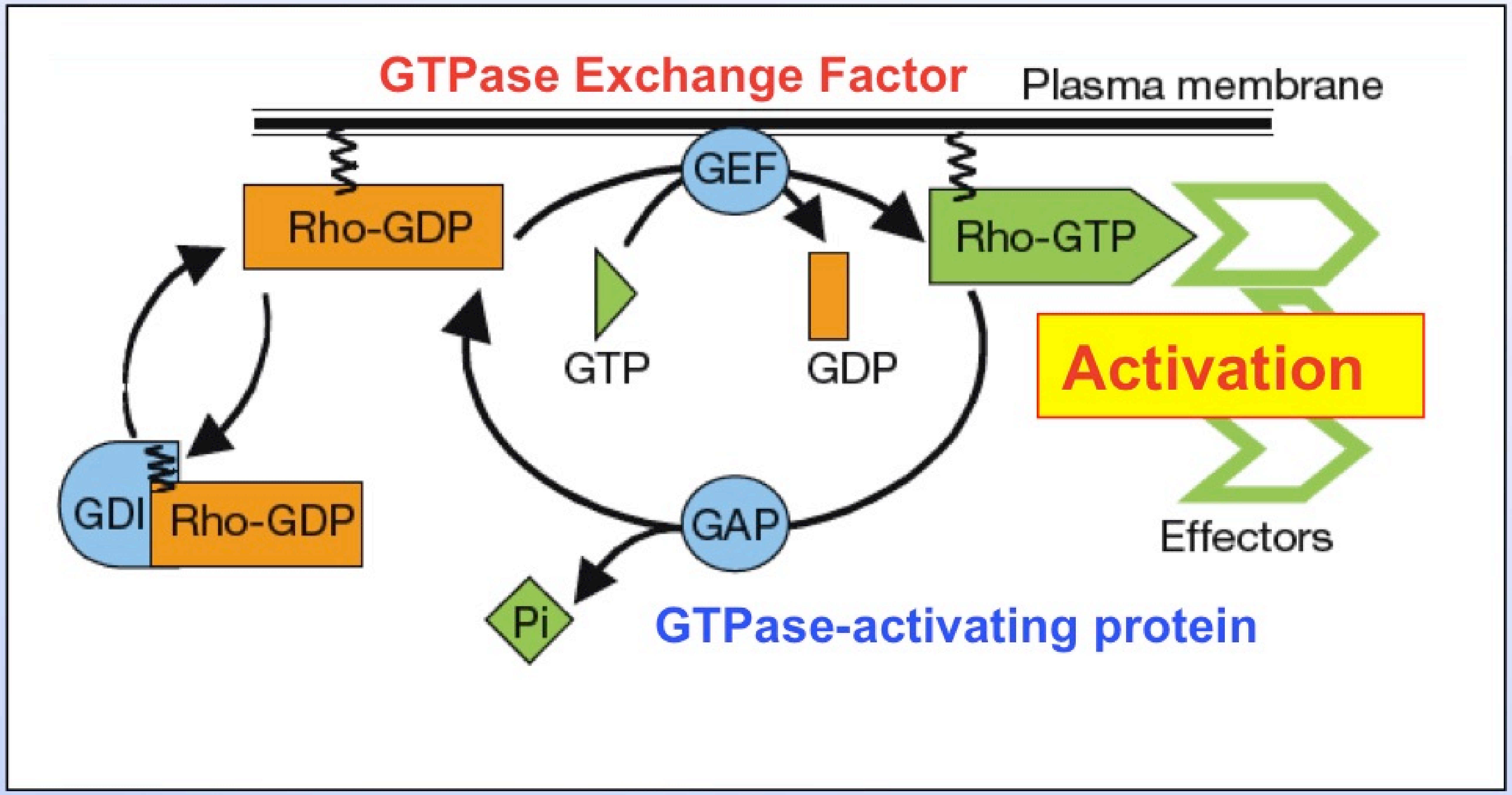
Red: actin

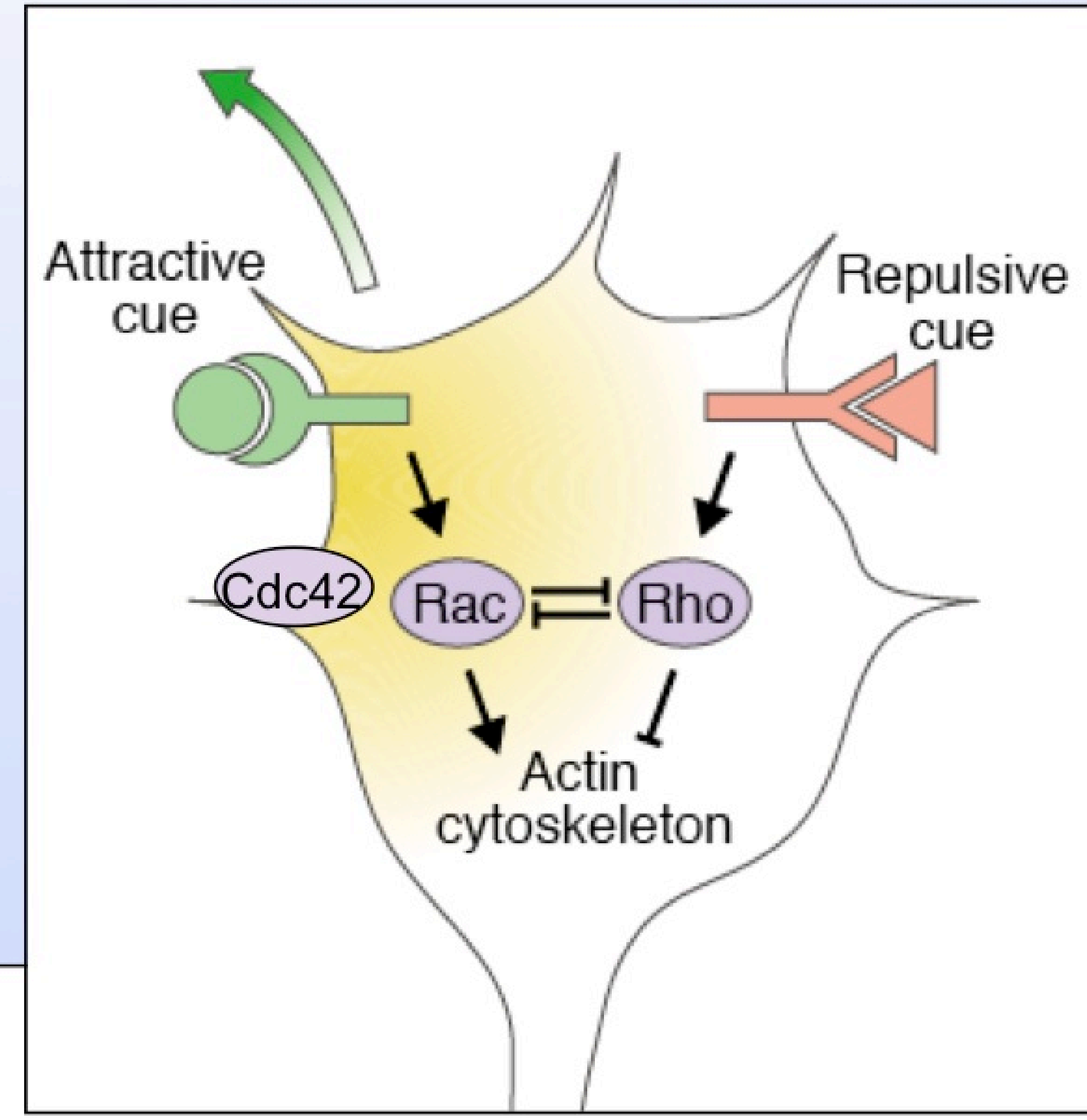
Green: microtubule

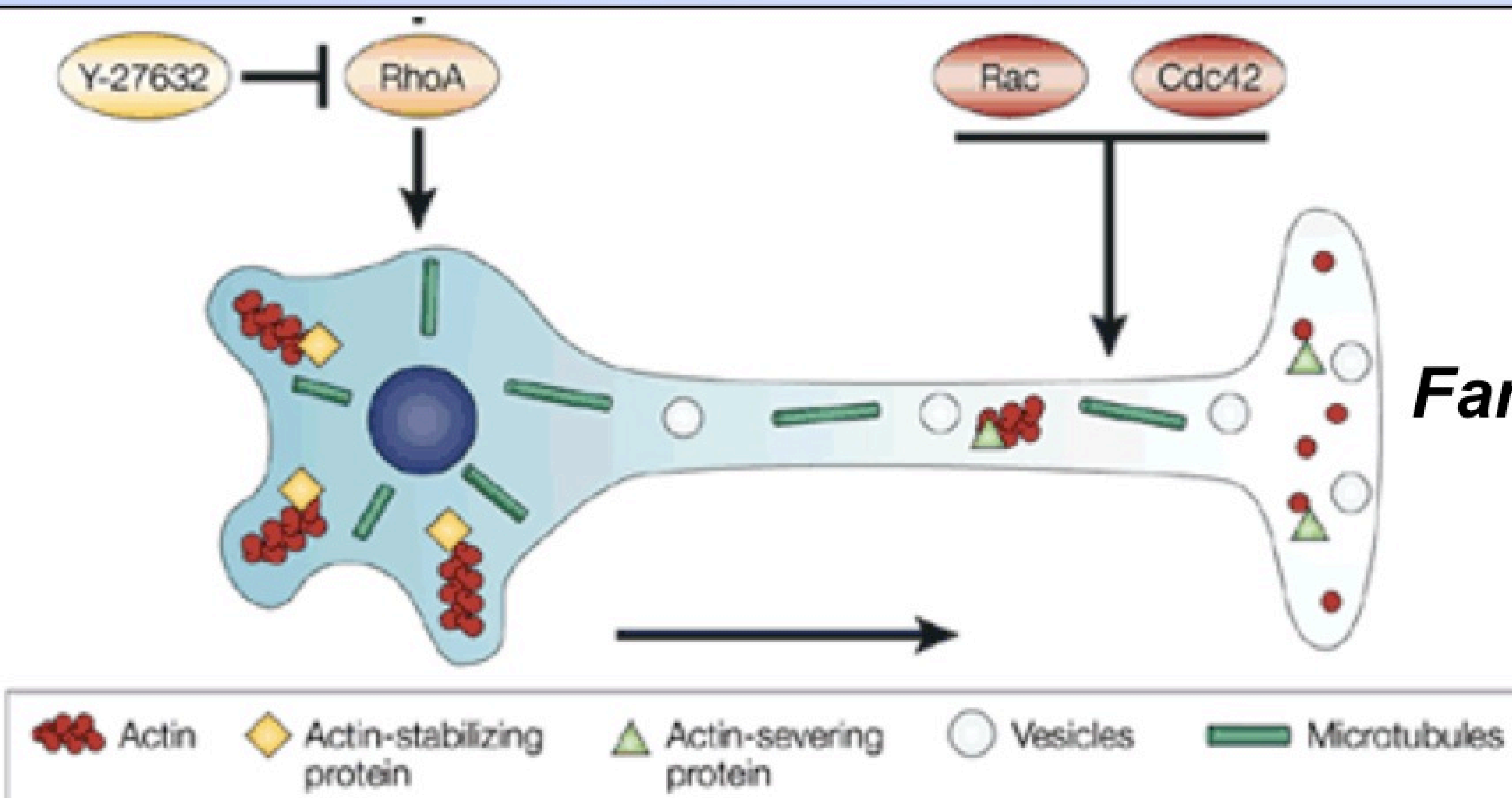


Bradke F, Dotti CG. Science. 1999 Mar 19;283(5409):1931-4.

Role du cytosquelette d'actine dans la polarité



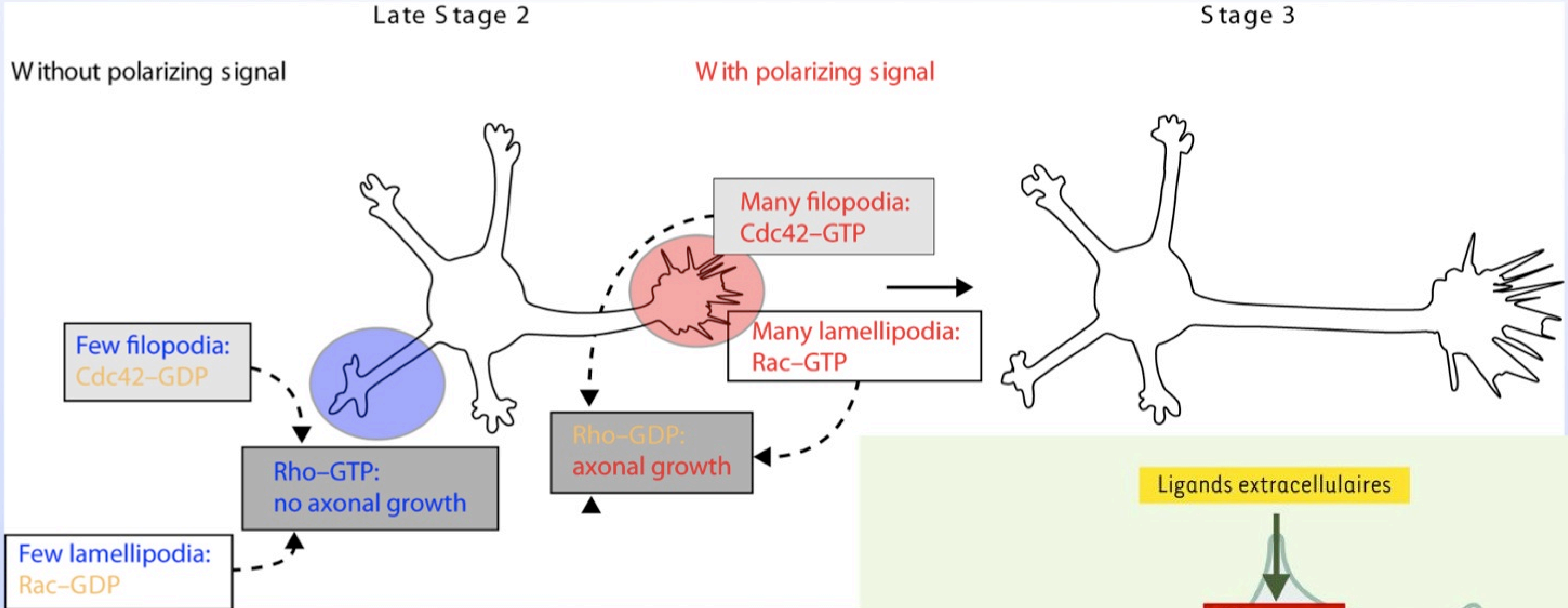




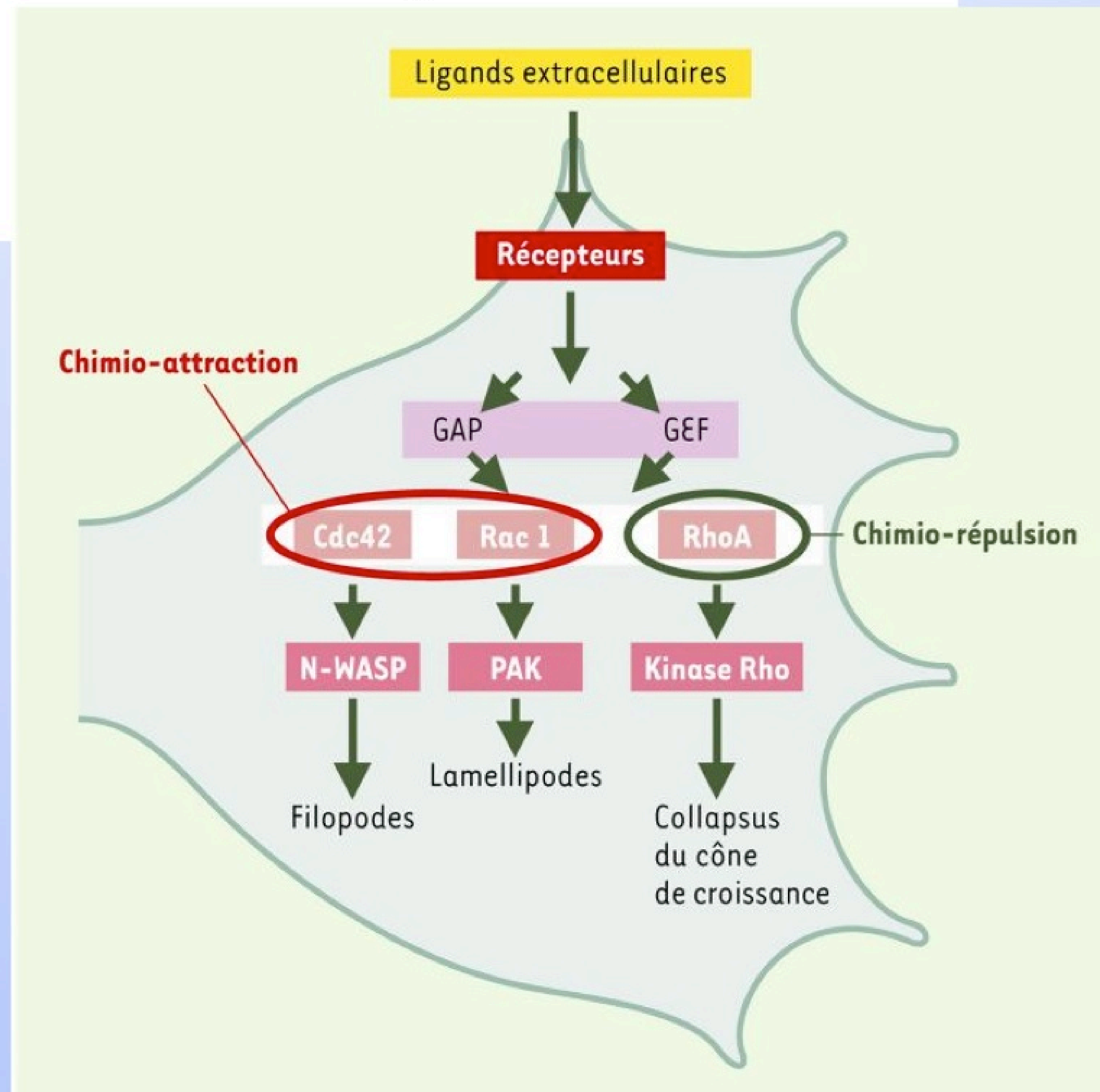
Famille des Rho GTPases:

- Rho GTPase : inhibe la croissance
- Cdc 42
- Rac1

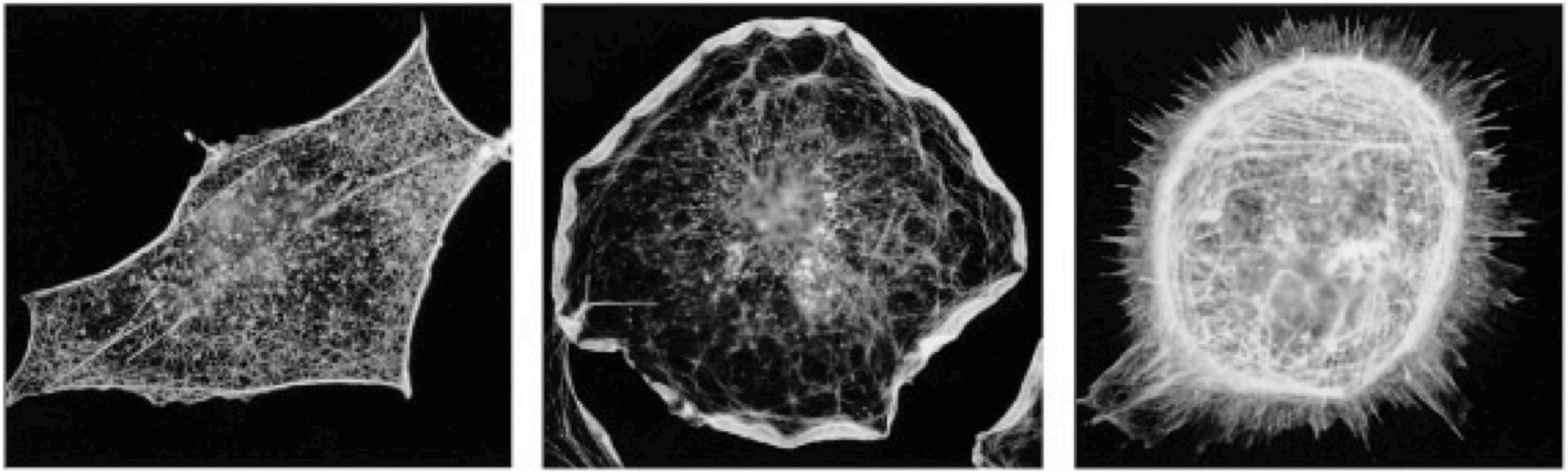
} Activent la croissance


Late Stage 2
Stage 3
Without polarizing signal
With polarizing signal
Many filopodia:
Cdc42–GTP
Many lamellipodia:
Rac–GTP
Few filopodia:
Cdc42–GDP
Rho–GDP:
axonal growth
Rho–GTP:
no axonal growth
Few lamellipodia:
Rac–GDP

Rho GTPases


Ligands extracellulaires
Récepteurs
Chimio-attraction
GAP
GEF
Cdc42
Rac 1
RhoA
Chimio-répulsion
N-WASP
PAK
Kinase Rho
Filopodes
Lamellipodes
Collapsus
du cône
de croissance

Regulation de la mophologie cellulaire par les GTPases



(A) quiescent cells (B) Rac activation (C) Cdc42 activation

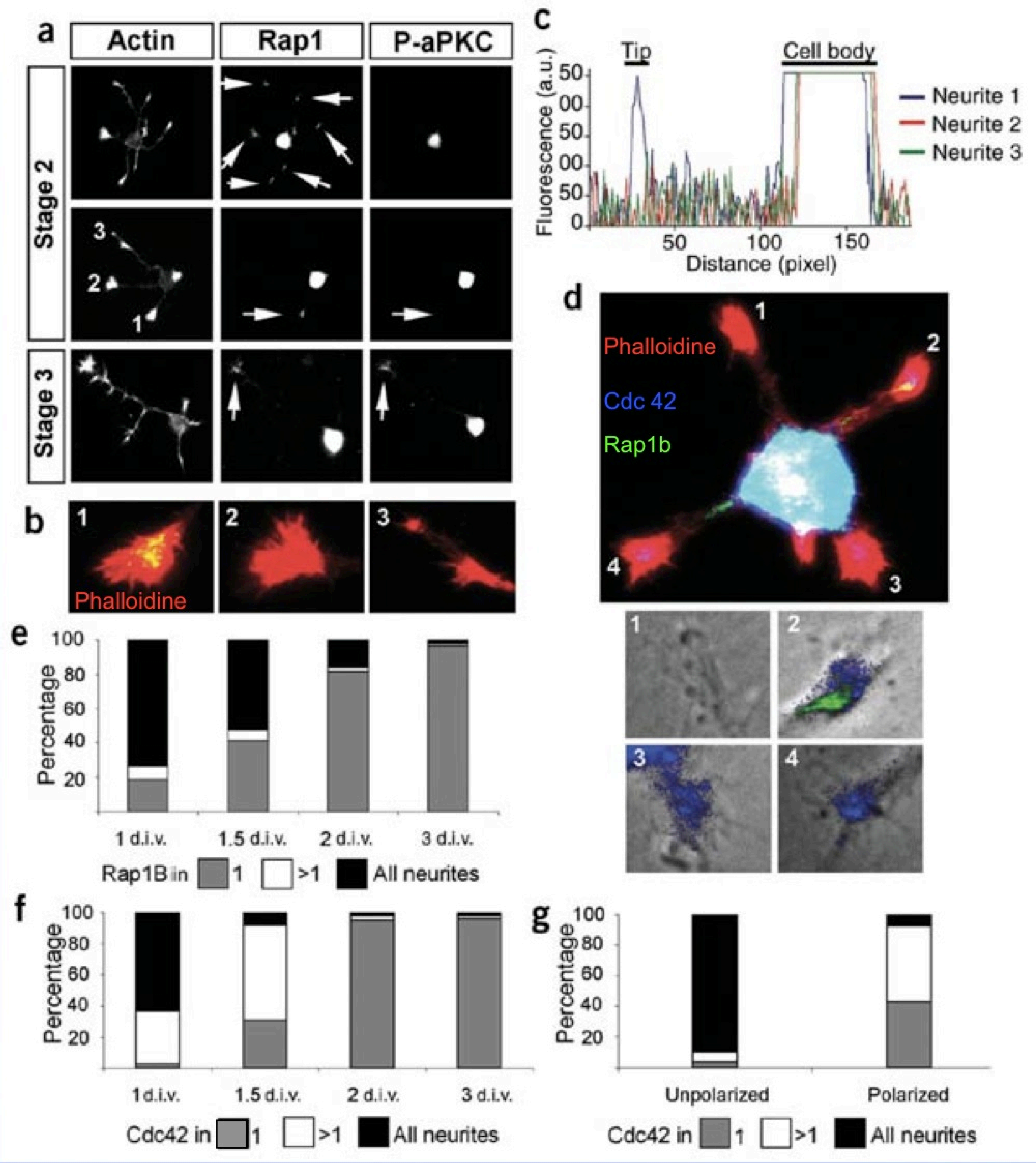
Formation de lamellipodes

Formation de filopodes

The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity.

Rap1B est localisé dans tous les neurites

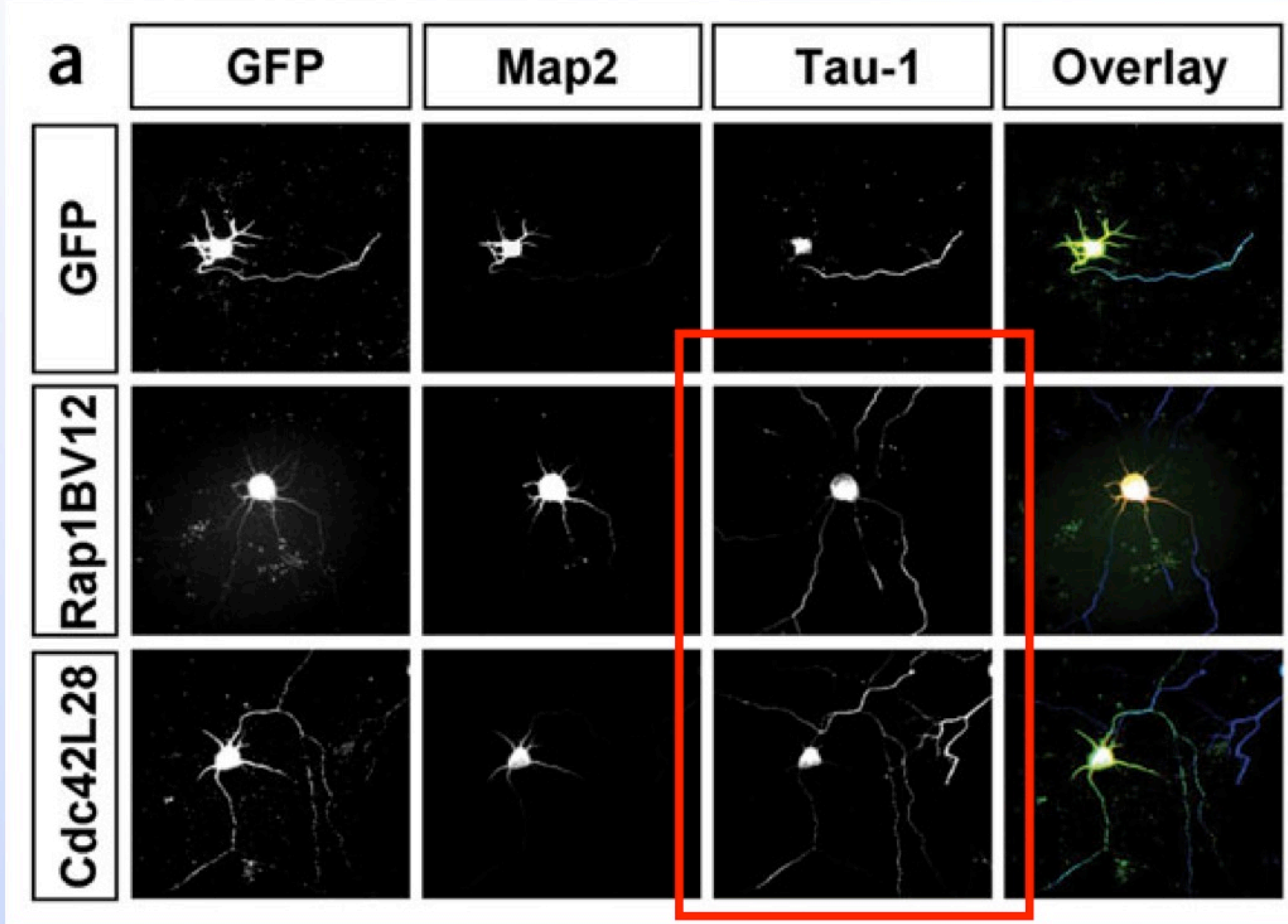
Puis il est restreint ensuite à l'extrémité de l'axone avant cdc42.



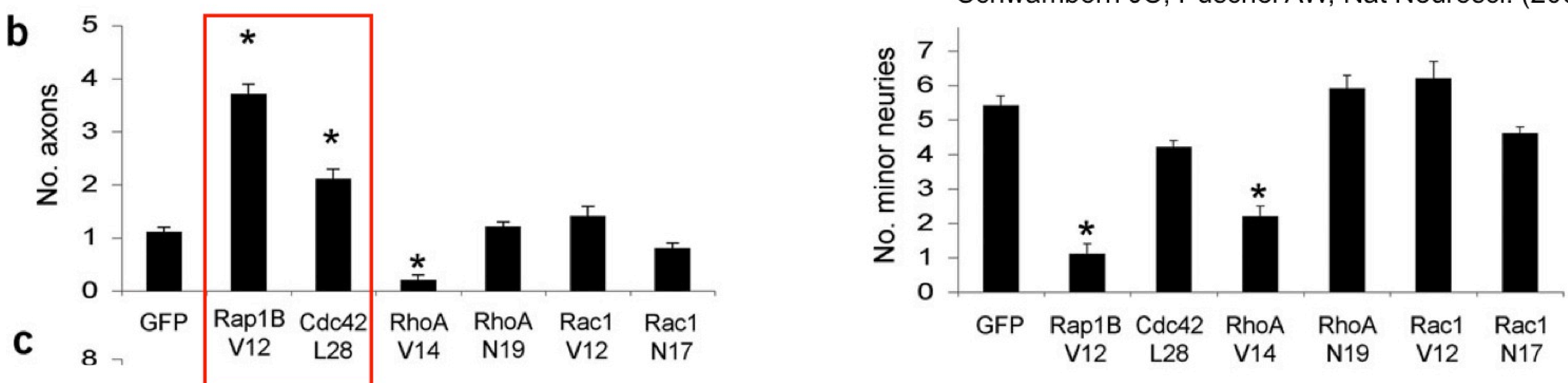
Schwamborn JC, Puschel AW, Nat Neurosci. (2004) .

The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity.

L'activation de rap1b et cdc42 induit la formation d'axones multiples.



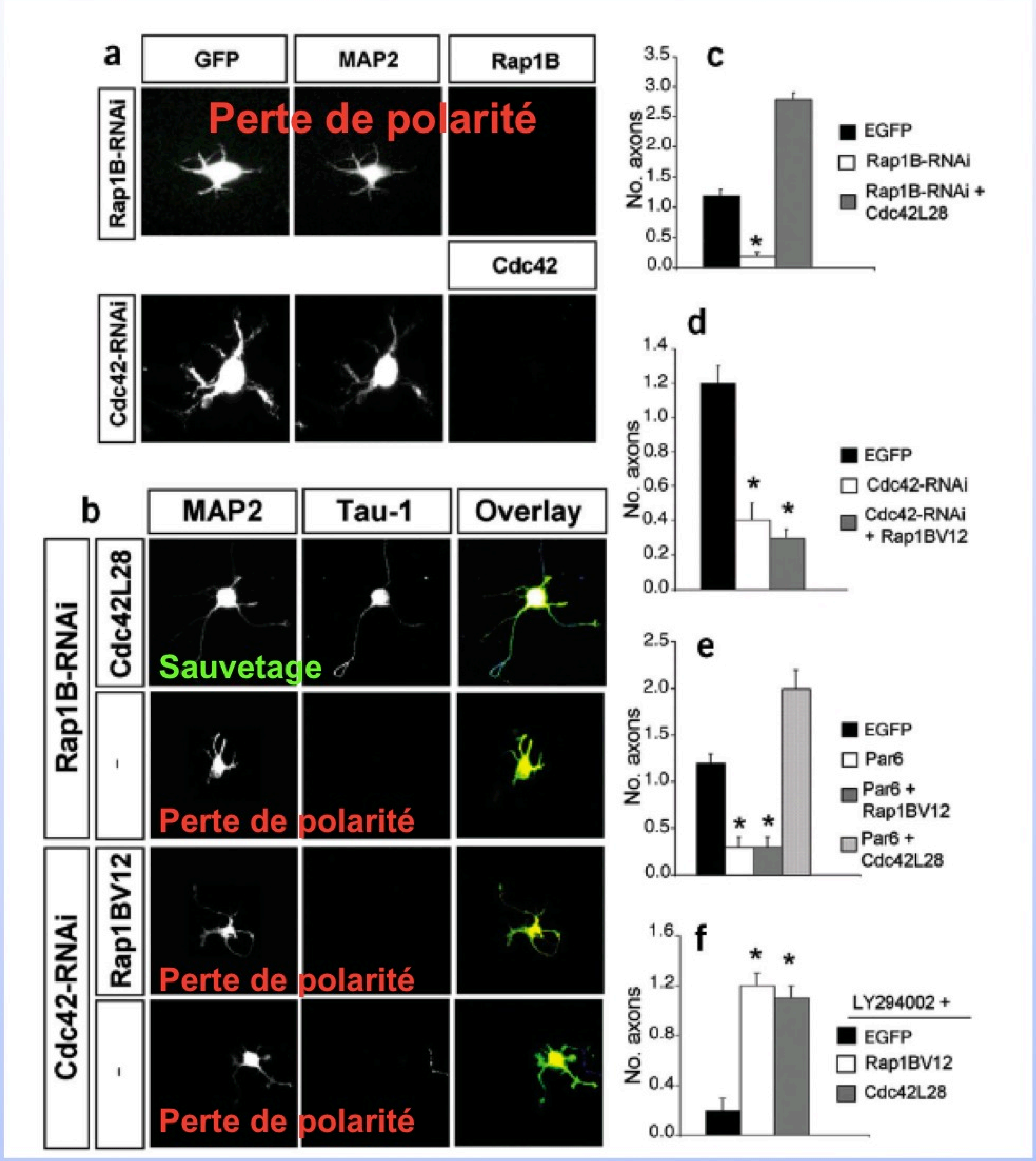
Schwamborn JC, Puschel AW, Nat Neurosci. (2004) .



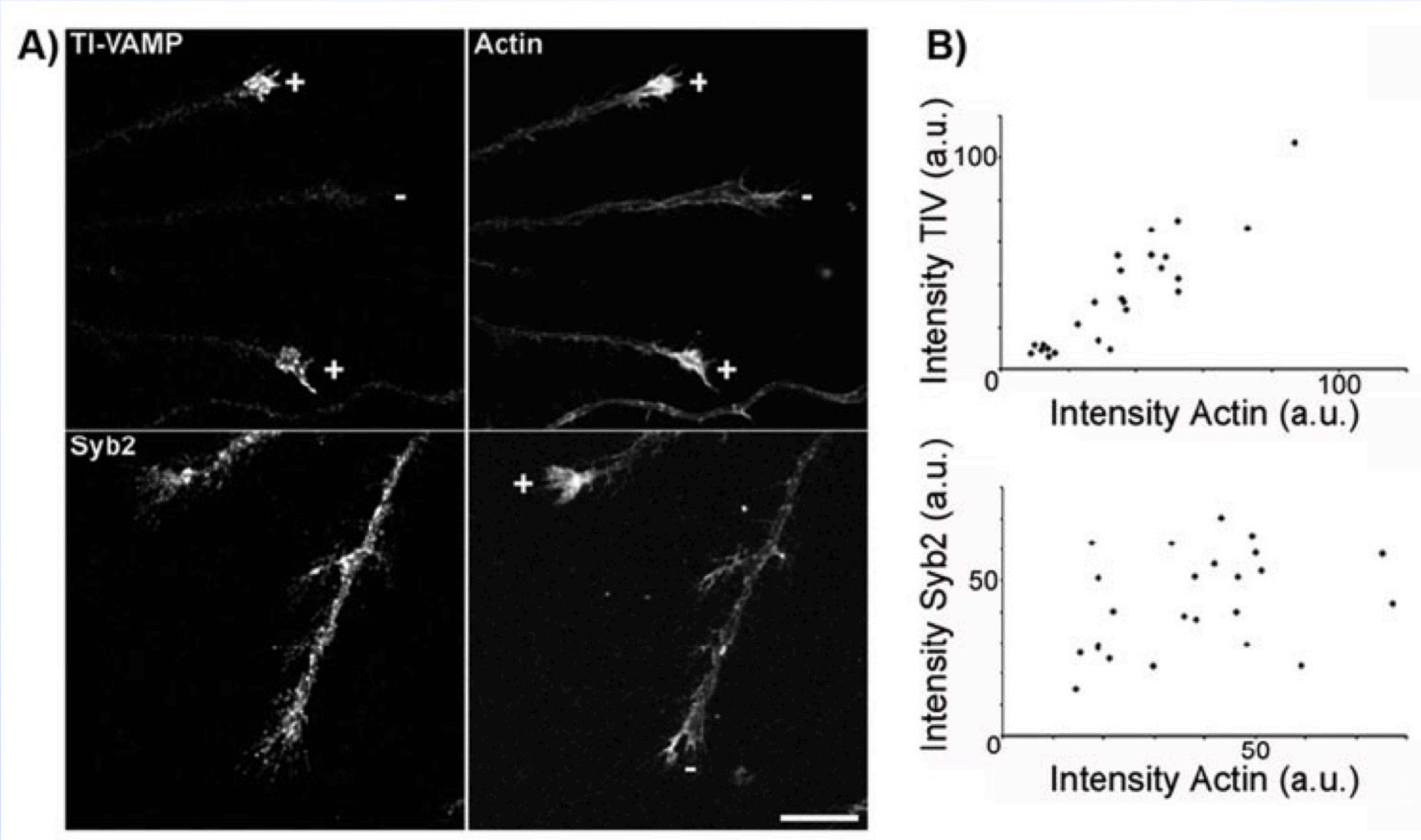
The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity.

La perte de polarité du au RNAi Rap1B est sauvé par la transfection de cdc42.
La réciproque est fausse.

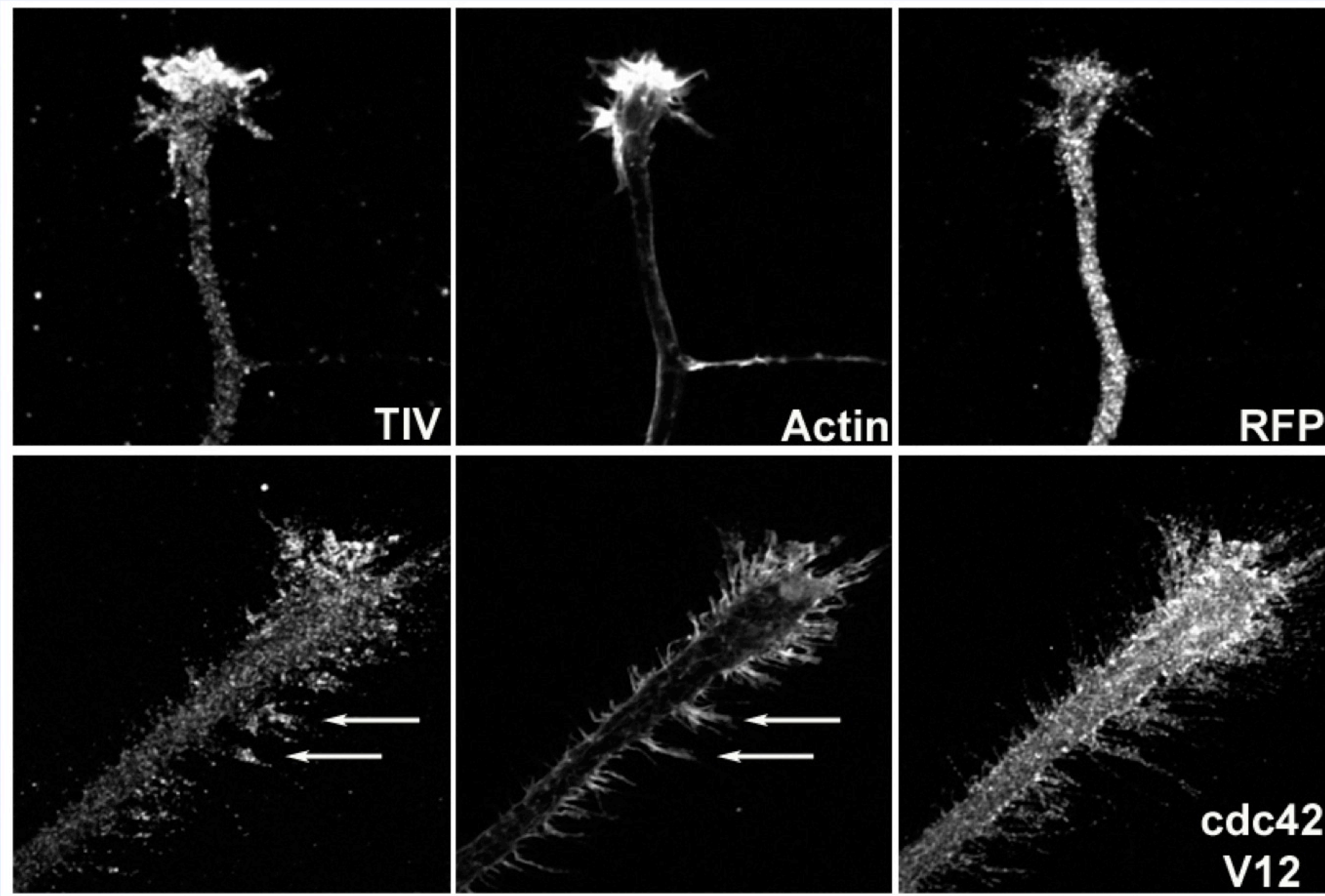
Rap1b agit donc en amont de cdc42.



TI-VAMP est associé au cône de crossance riches en actine

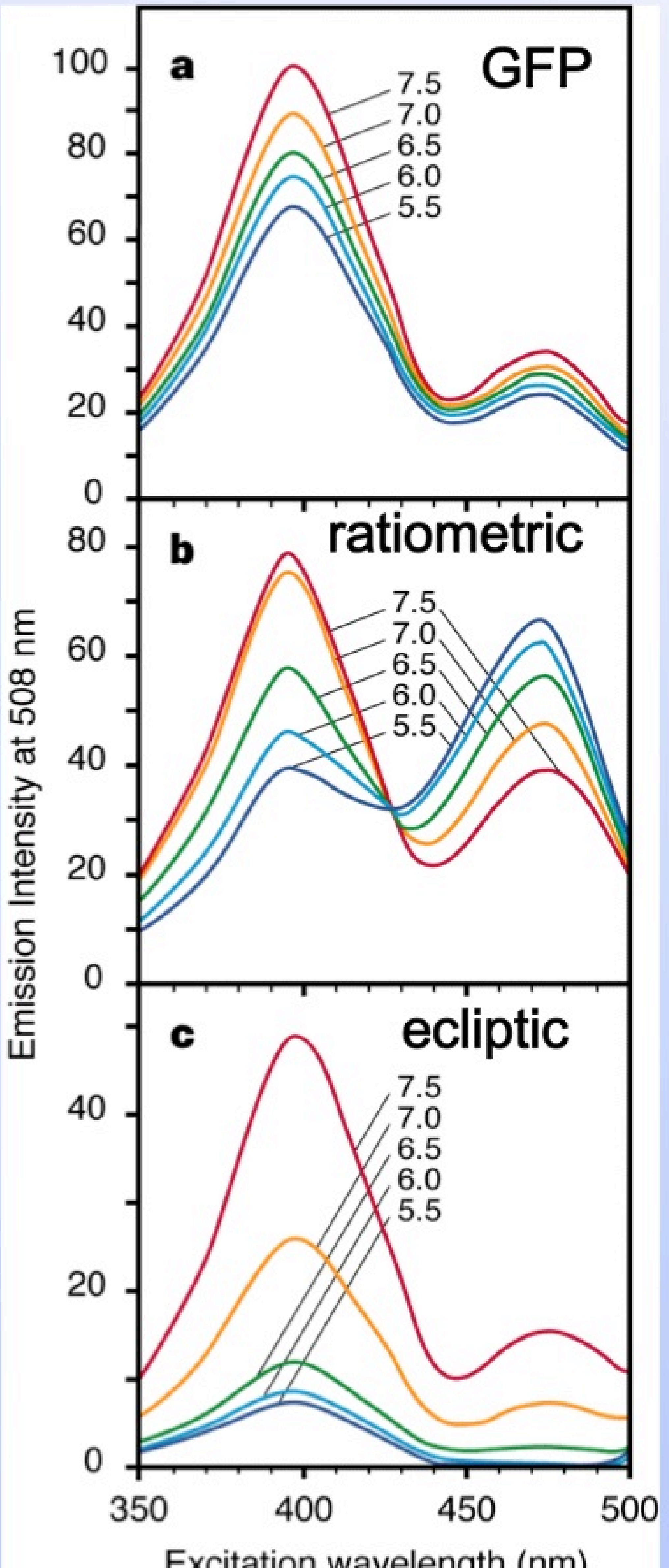


Expression of cdc42 V12 induces loss of polarisation of actin and TI-VAMP in growth cones

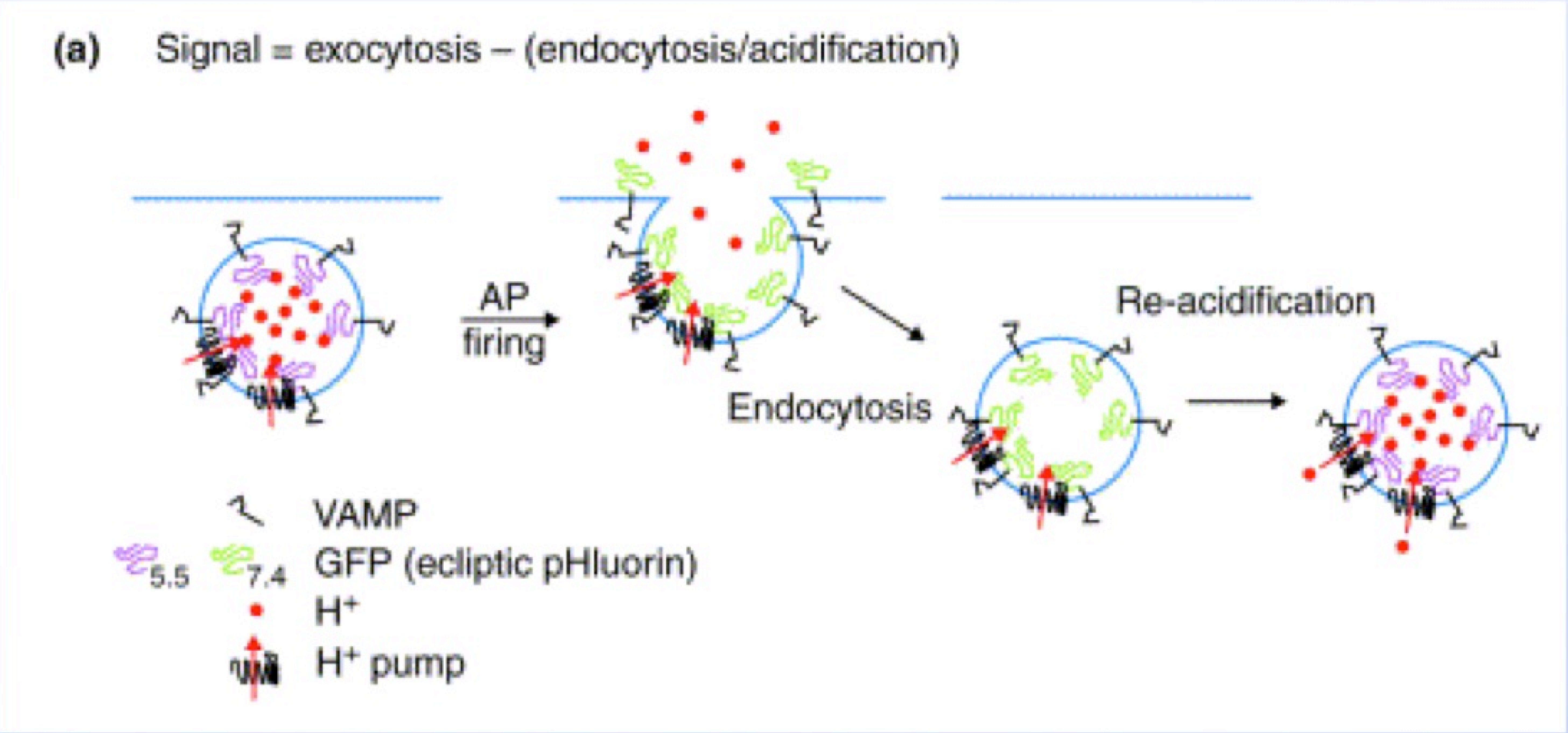


Alberts & al MBoC 2003

pHLuorins - pH-sensitive GFP variants: great tools for exocytosis

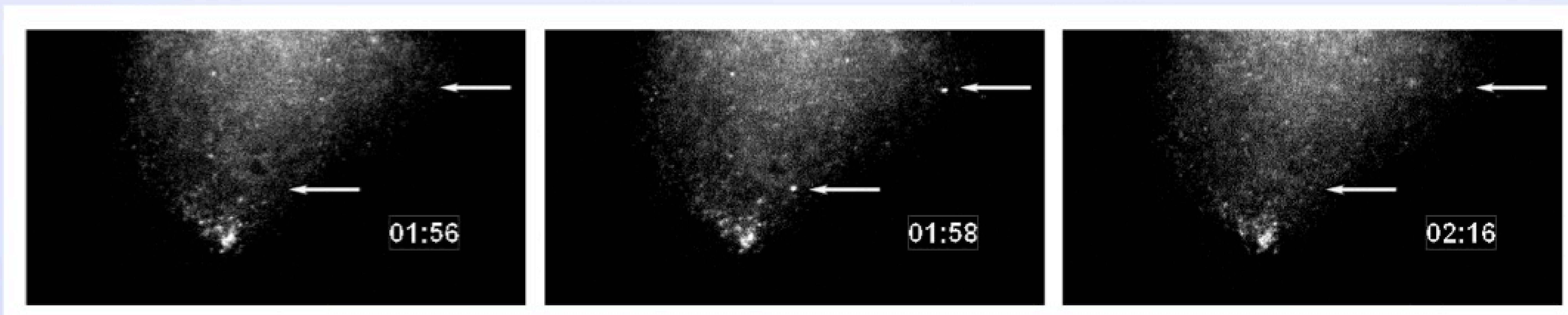


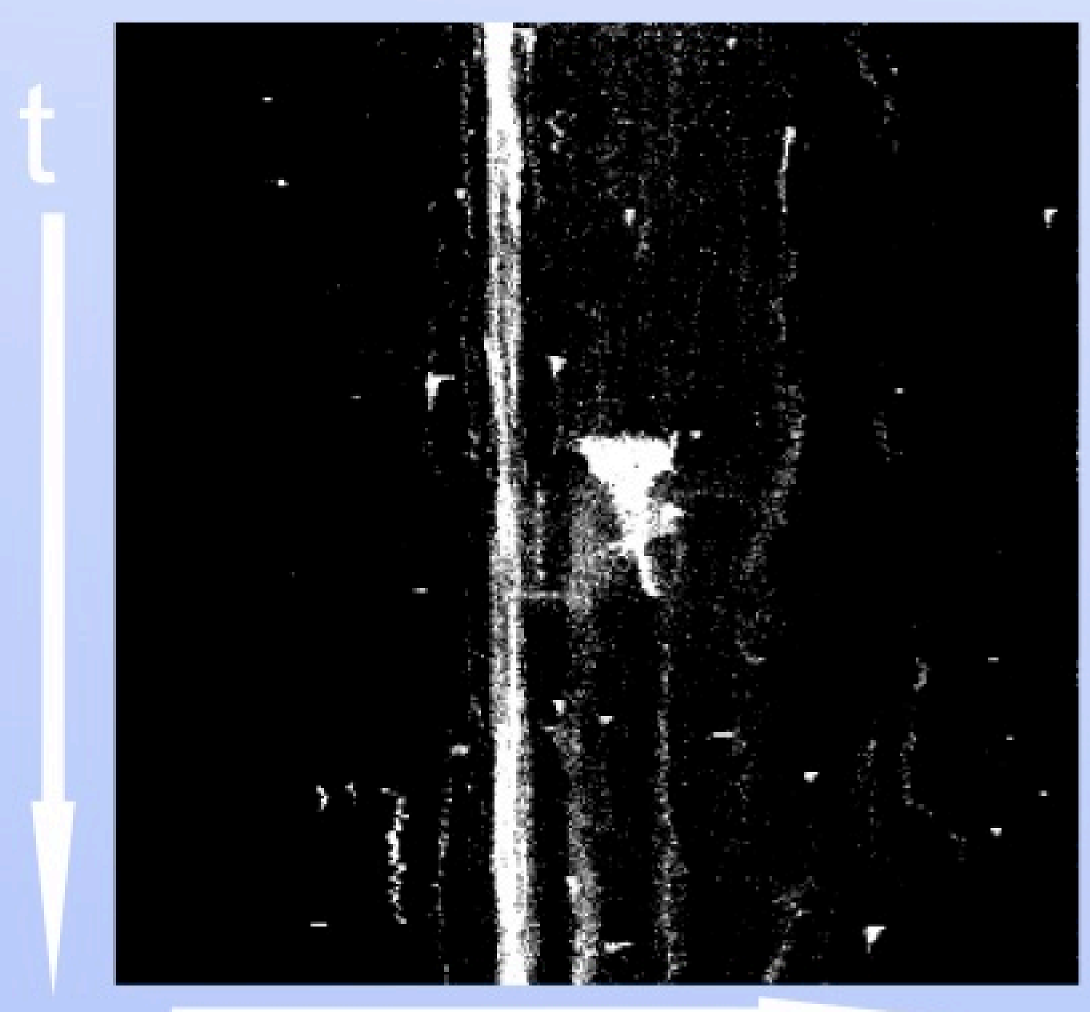
Miesenbock et al., Nature 1998



T. Ryan, Current Opinion in Neurobiology 2001

Exocytosis of TI-VAMP-pHluorin

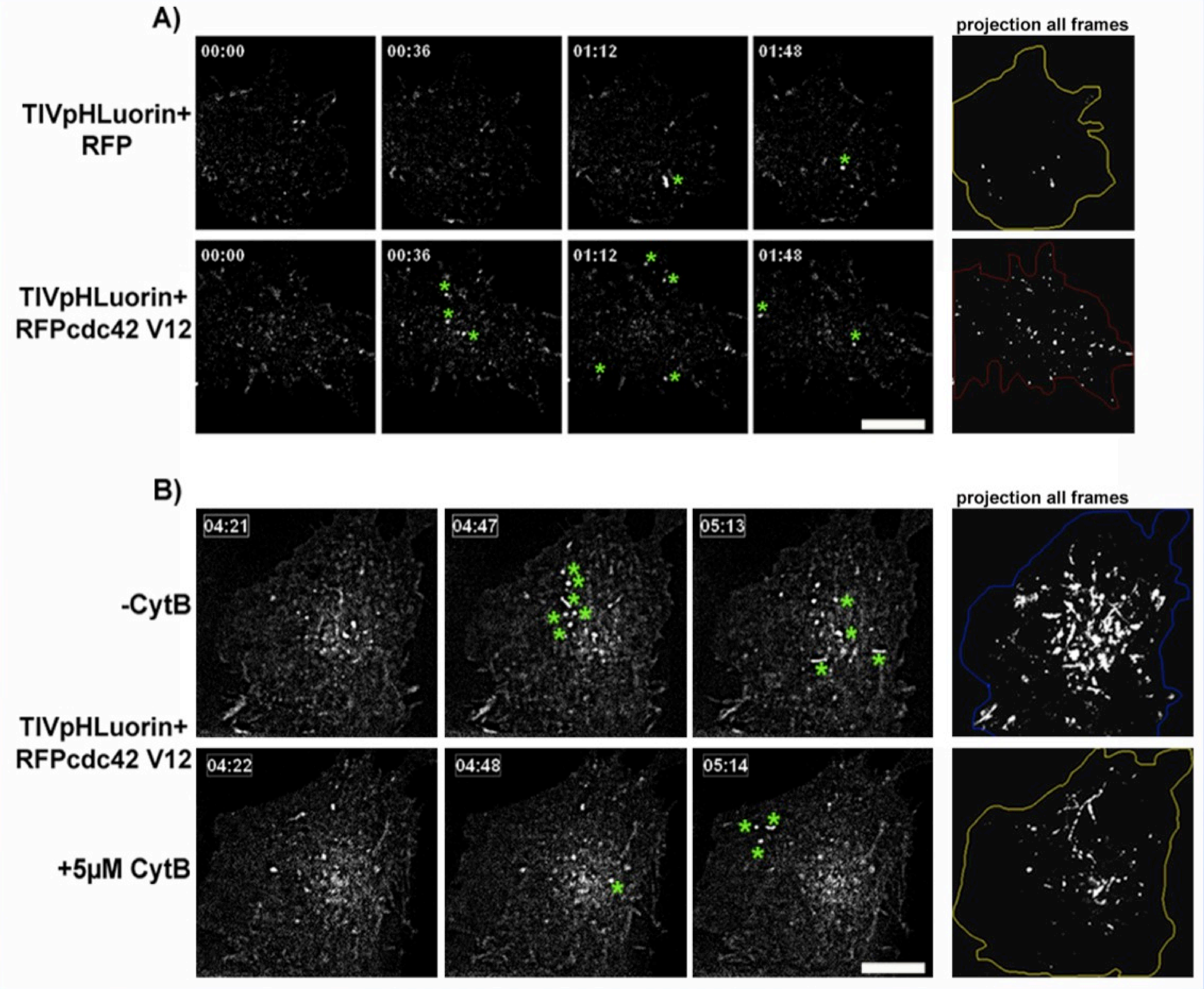




Region pixel max intensity

Philipp Alberts

Actin-dependent Cdc42-mediated activation of TI-VAMP exocytosis



Alberts & al MBoC 2006

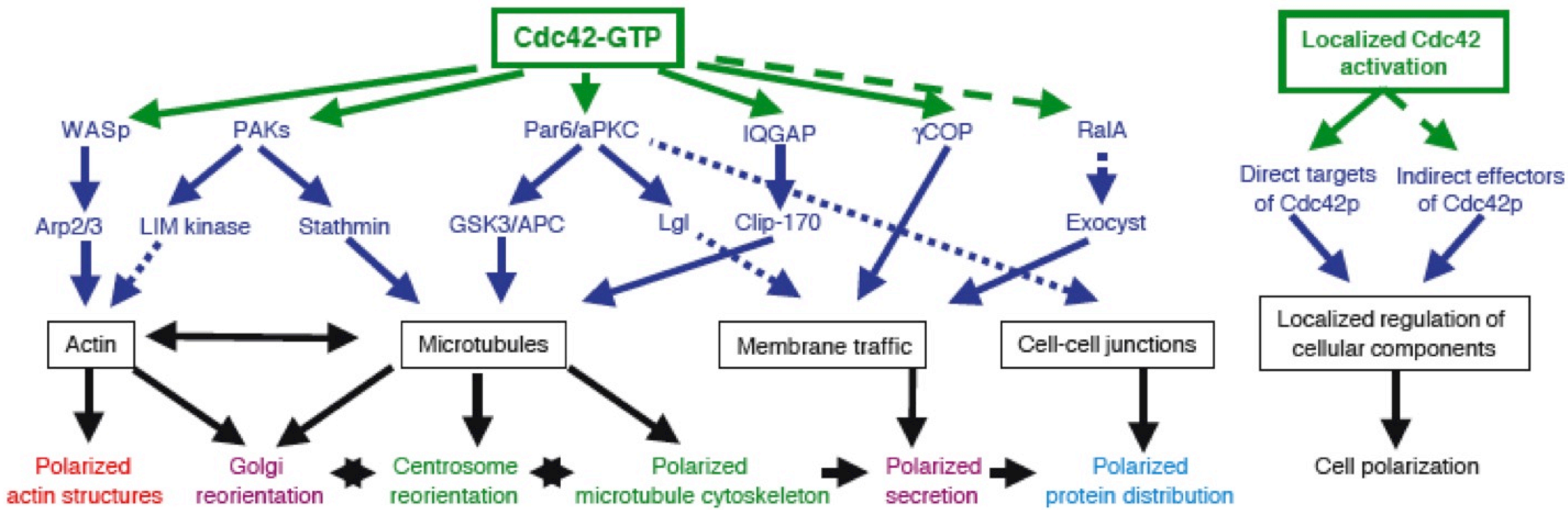
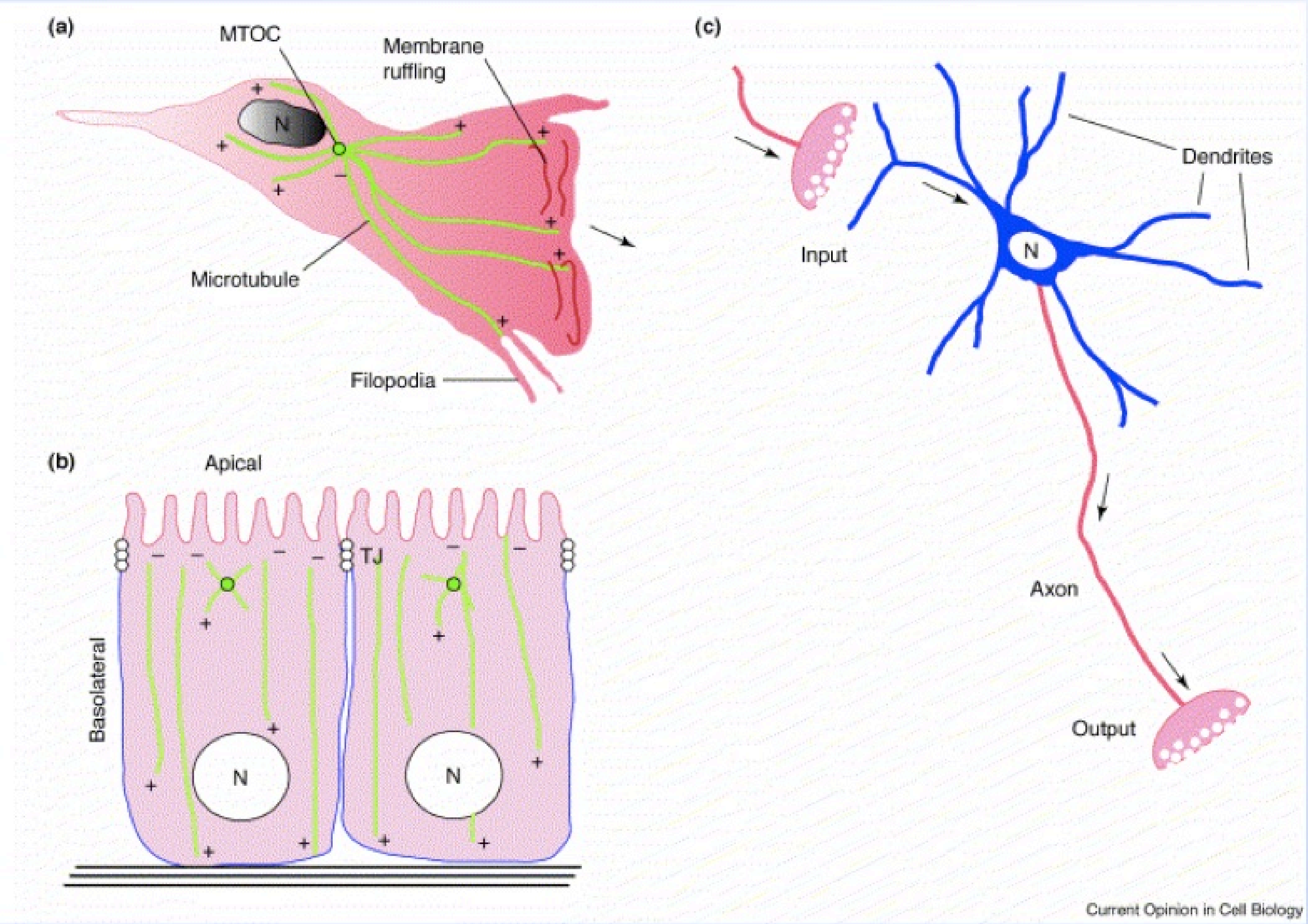
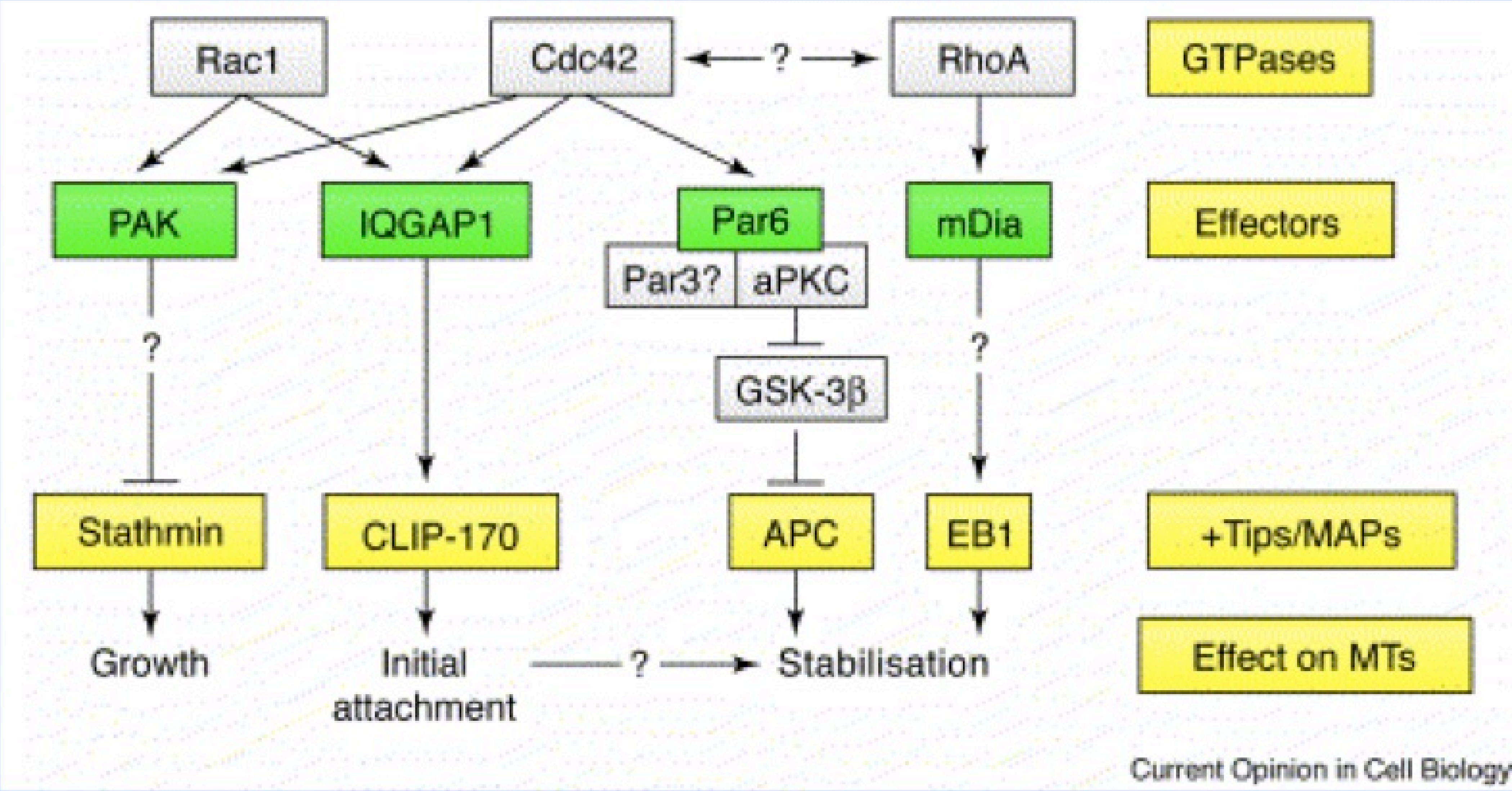


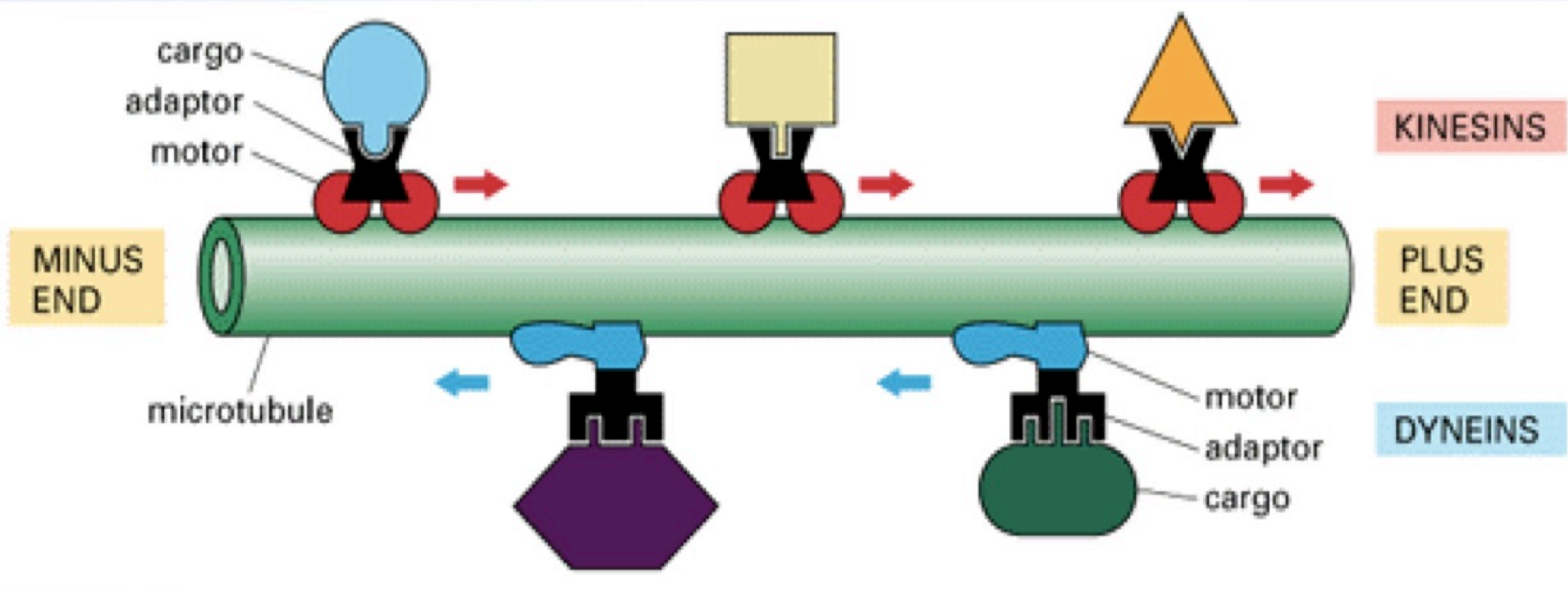
Fig. 4. Multiple signalling pathways controlled by Cdc42. Cell polarization requires the spatial and temporal regulation of several cell components. Orientation of the actin and microtubule cytoskeletons, regulation of cell contacts and organization of membrane traffic occur in concert. Multiple signalling pathways downstream of Cdc42 regulate these different cellular components (black box). These signals are transduced by different Cdc42 direct (solid line) or indirect (dotted line) effectors (blue) and involve several intermediates (blue). Cell polarization results from the localized activation of Cdc42, which leads to a localized regulation of cellular components and therefore to their asymmetric organization. The different cellular components cooperate and generate the general characteristics of cell polarization.

5. Cytosquelette et polarité

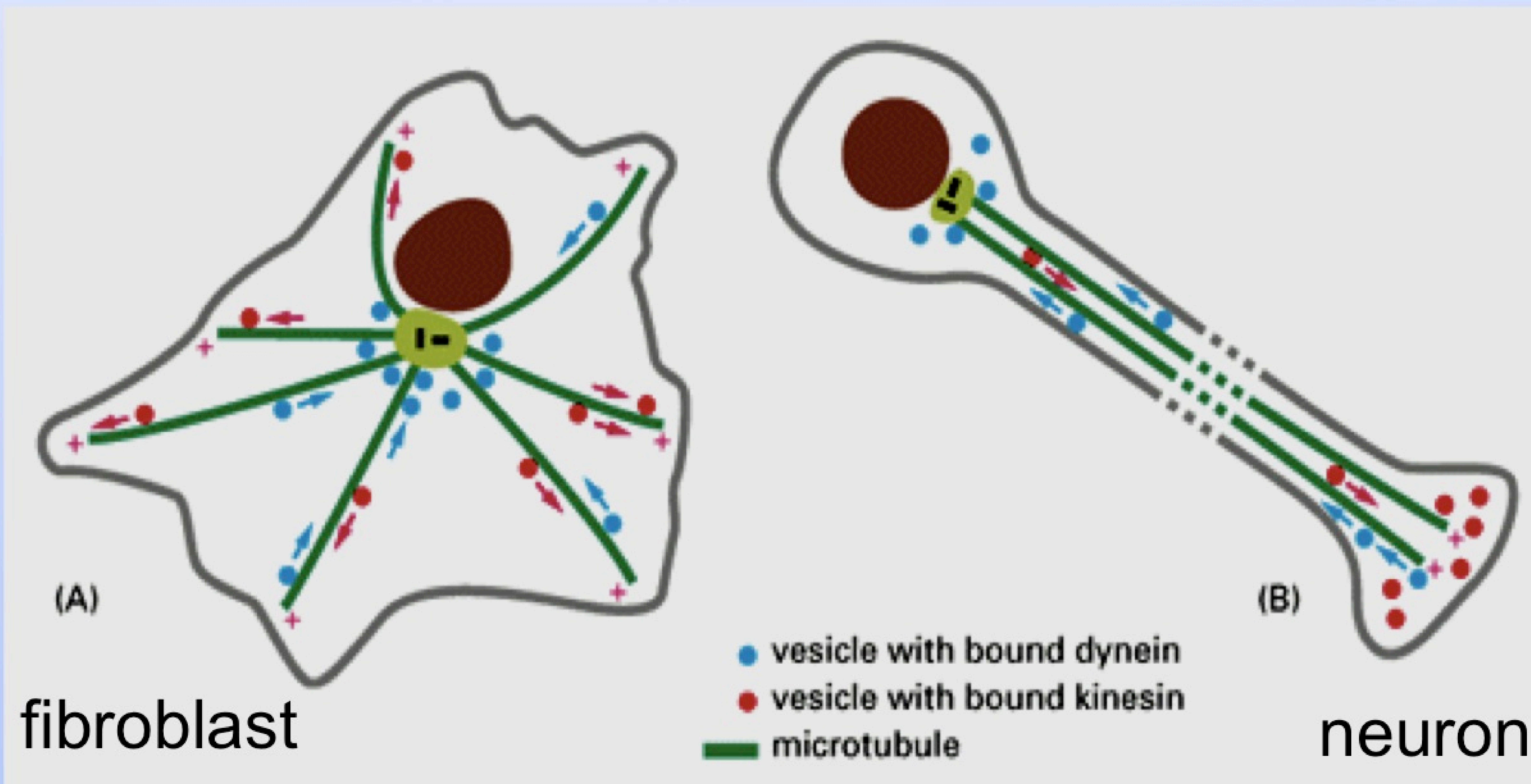
b) Le rôle des microtubules et de leur régulateurs



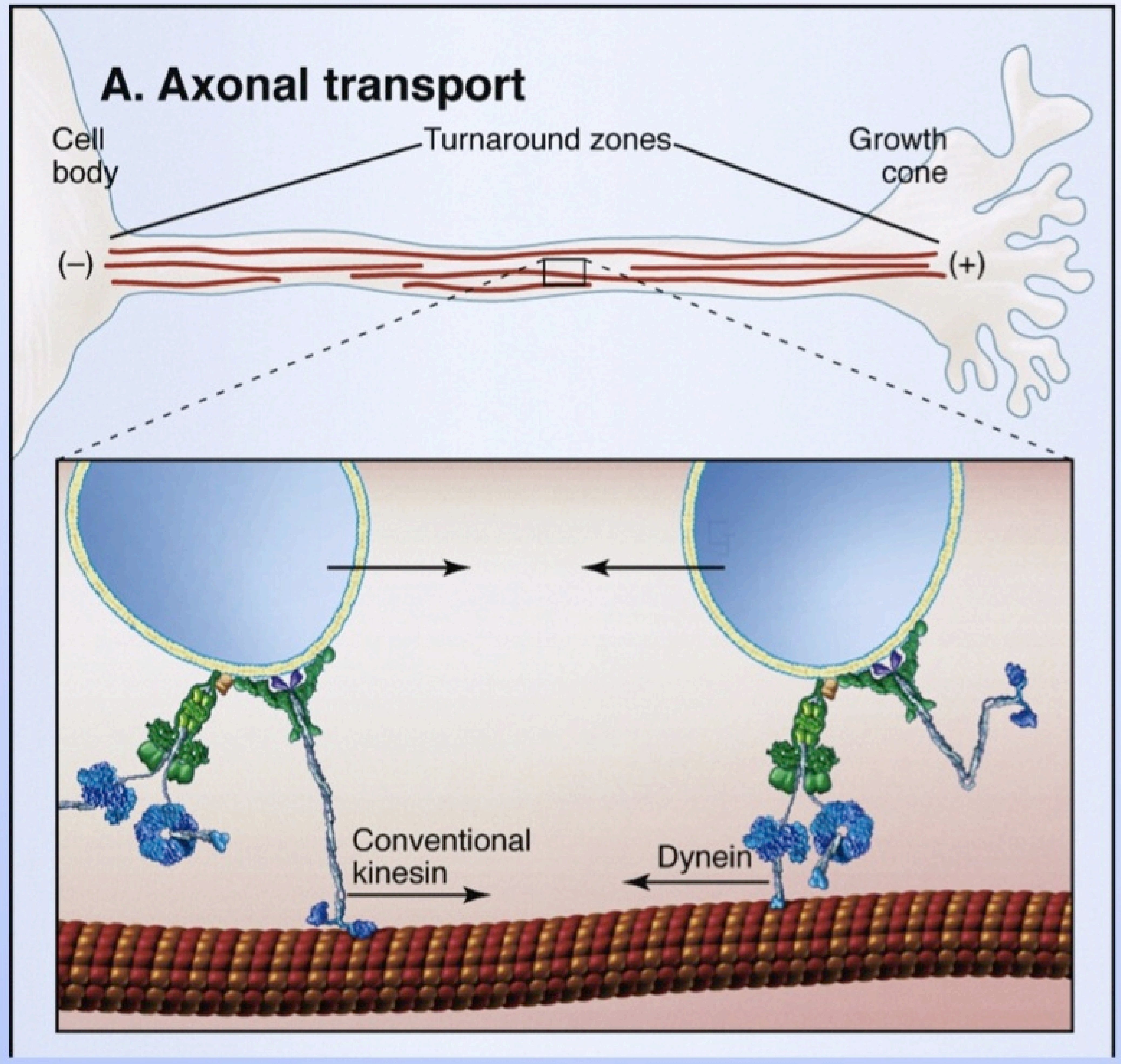




Motors carrying different cargoes in different directions

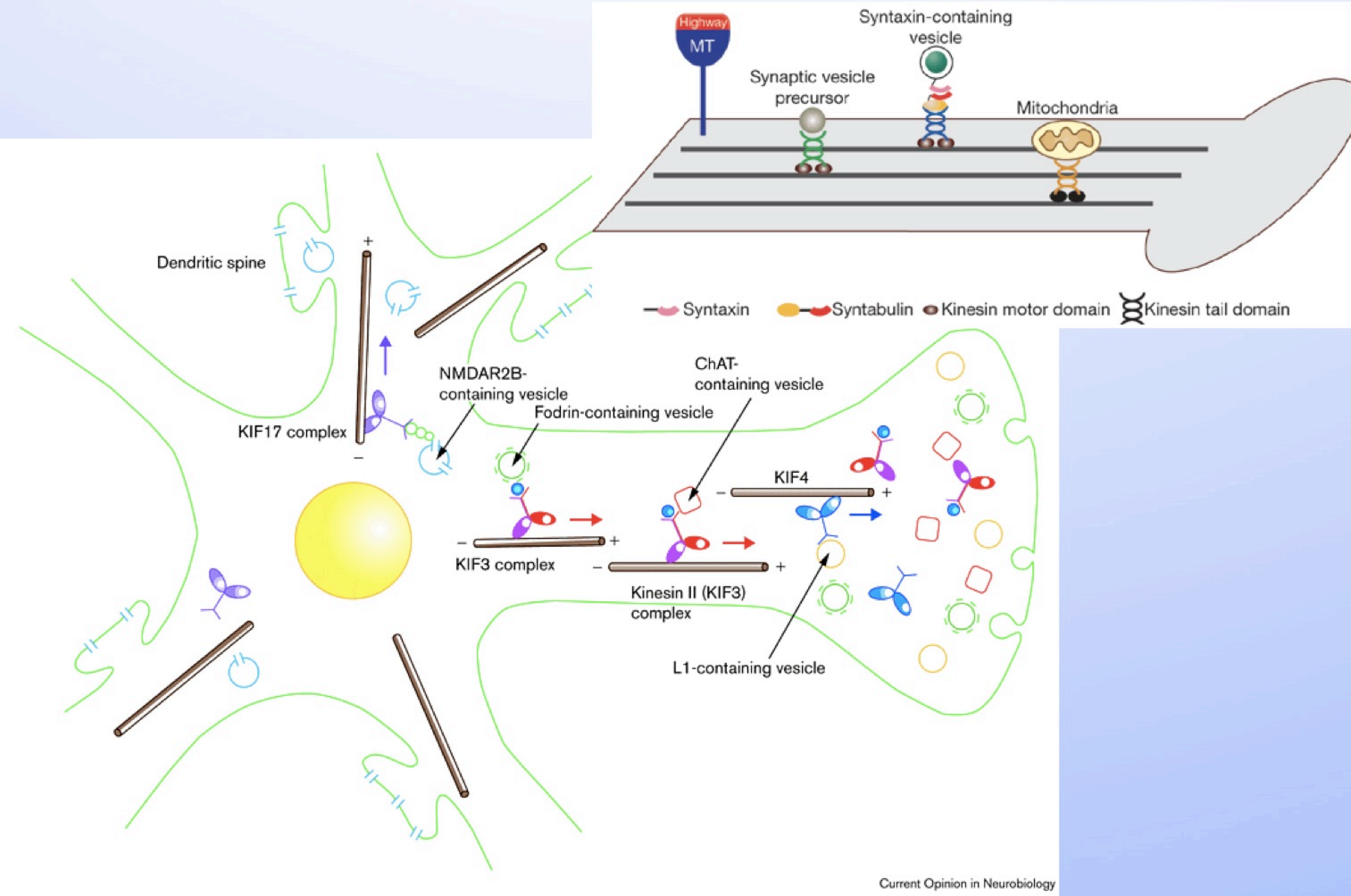


Transport Anterograde et retrograde



Cargo structures	Overall rate (pulse labeling)	Instantaneous rate (light microscopy)	Directionalit y	Duty ratio
Golgi -derived vesicles (fast anterograde)	200–400 mm/d[a] (2–5 µm/s)	1–5 µm/s[b]	Anterograde	High
Endocytic vesicles, lysosomes, autophagosomes (fast retrograde)	100–250 mm/d[a] (1–3 µm/s)	1–3 µm/s[b]	Retrograde	High
Mitochondria	<70 mm/d[c] (<0.8 µm/s)	0.3–0.7 µm/s[d]	Bidirectional	Intermediate
Microfilaments, cytosolic protein complexes (slow component b)	2–8 mm/d[e] (0.02–0.09 µm/s)	Unknown	Unknown	Unkn own
Microtubules, neurofilaments (slow component a)	0.2–1 mm/d[e] (0.002–0.01 µm/s)	0.3–1 µm/s[f]	Bidirectiona l	Low

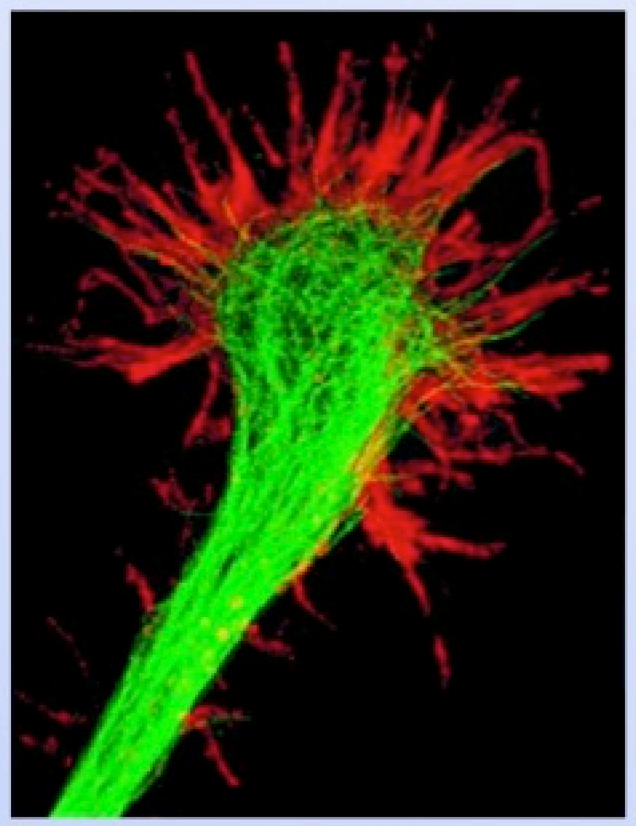
Role des kinesines

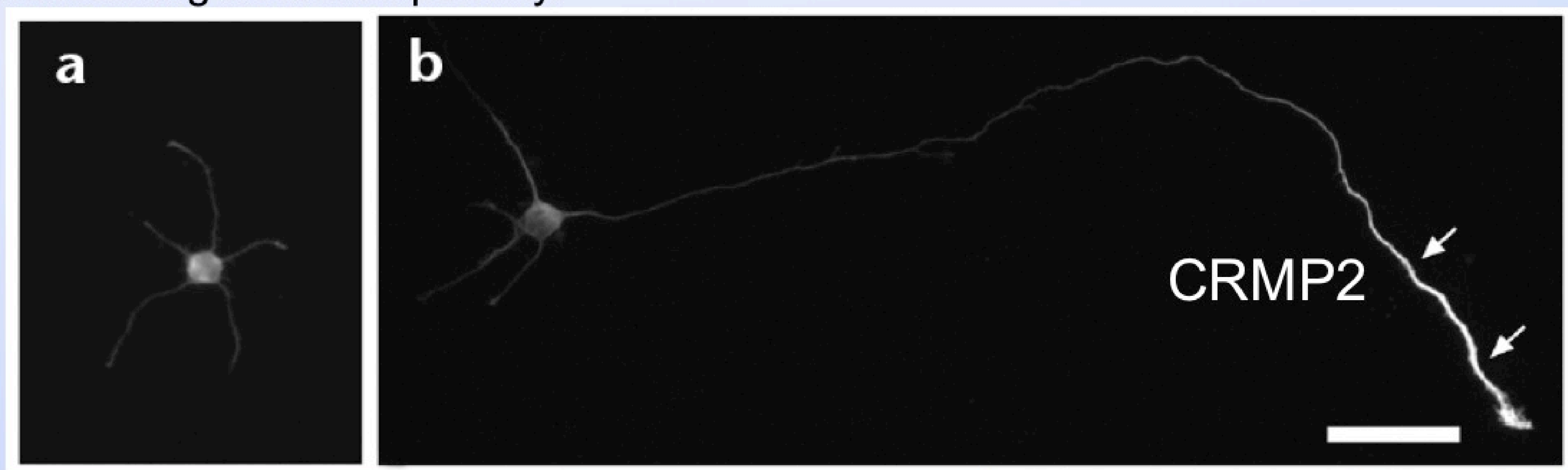


Microtubule stabilization by CRMP2

(Collapsin Response Mediator Protein 2 /unc33)

In cultured hippocampal neurons, one axon and several dendrites differentiate from a common immature process. Here we found that CRMP-2/TOAD-64/Ulip2/DRP-2 (refs. 2-4) level was higher in growing axons of cultured hippocampal neurons, that overexpression of CRMP-2 in the cells led to the formation of supernumerary axons and that expression of truncated CRMP-2 mutants suppressed the formation of primary axon in a dominant-negative manner. Thus, CRMP-2 seems to be critical in axon induction in hippocampal neurons, thereby establishing and maintaining neuronal polarity.



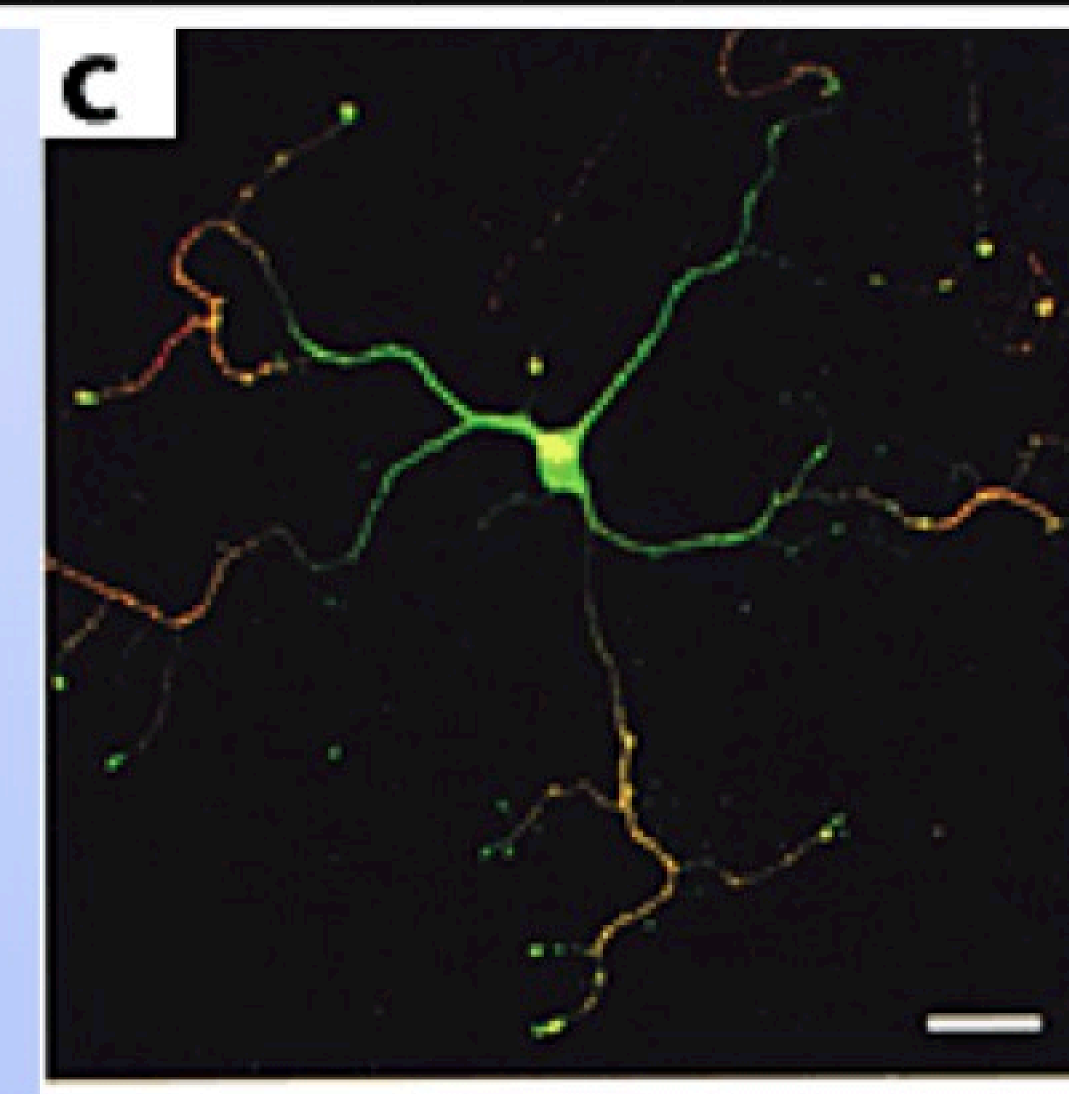


Red: actin

Green: microtubule

CRMP2 interagit avec les hétérodimères de tubuline et favorise l'assemblage des microtubules in vitro.

Surexpression de CRMP2: conversion de dendrites en axone surnuméraires.



Inagaki et al., Nat Neurosci. 2001 Aug;4(8):781-2.

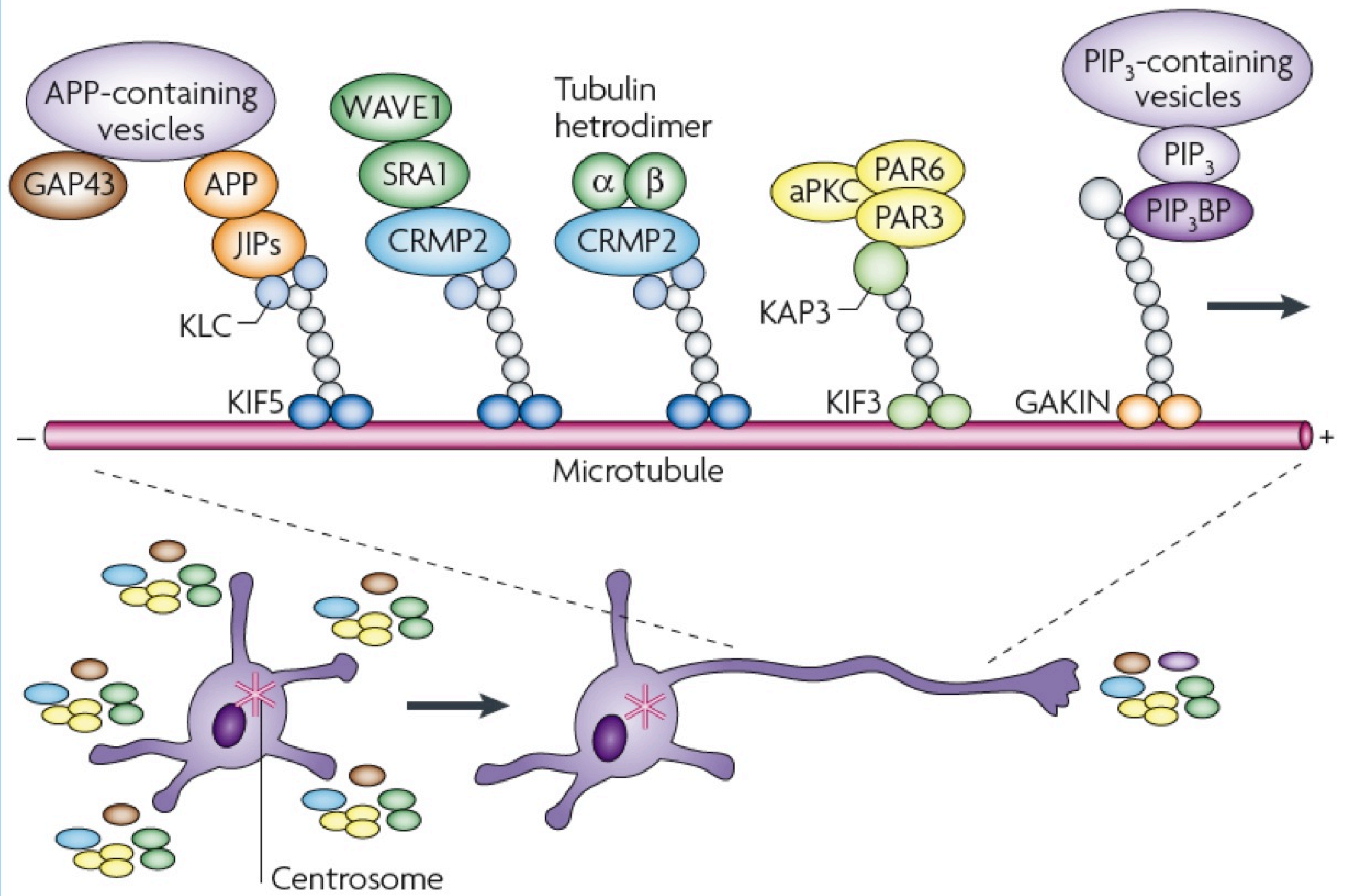
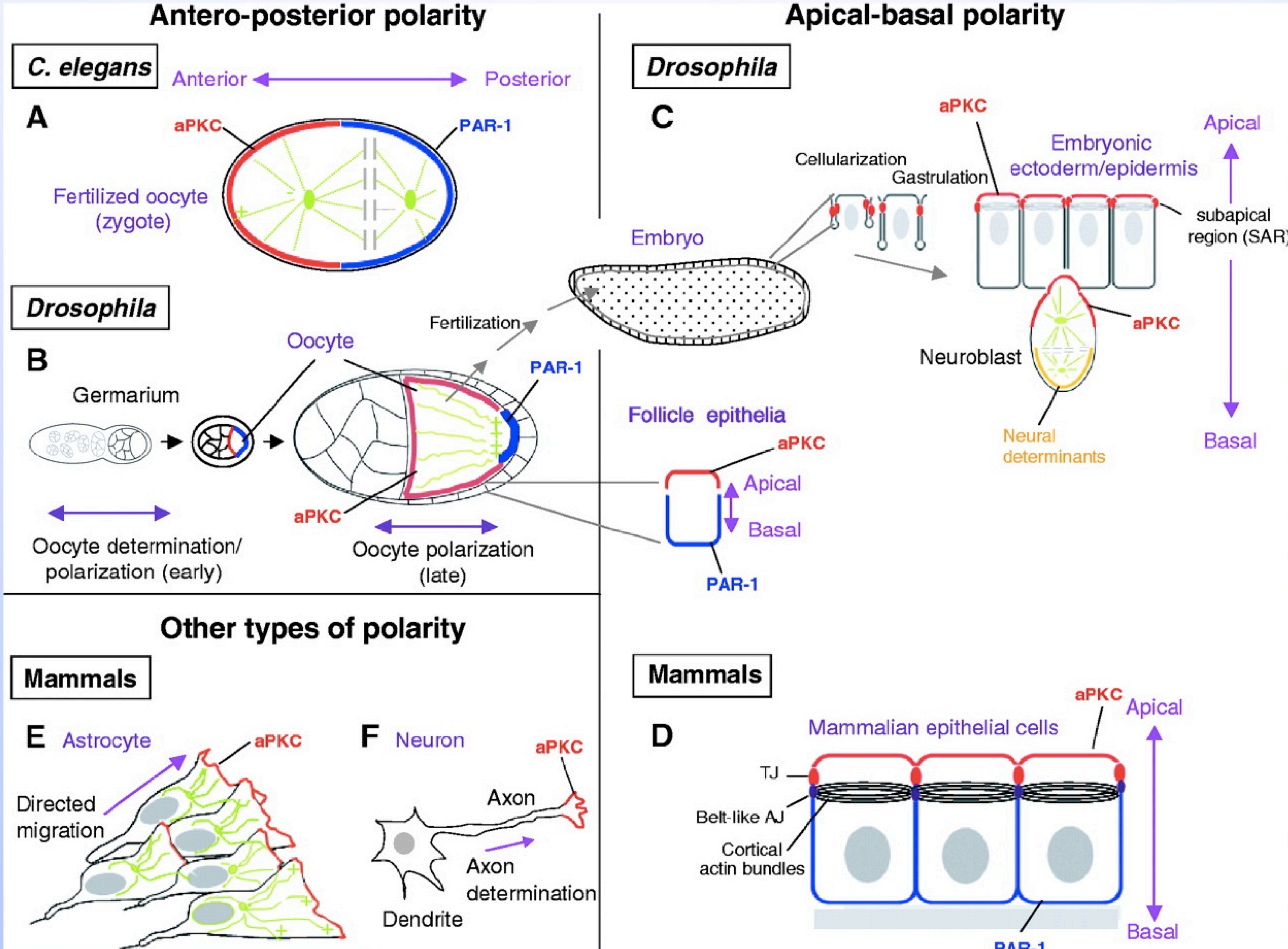


Figure 4 | **Transport of key regulators in axon formation by kinesin.** When one

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

Various types of cell polarity in which the PAR-aPKC system is involved

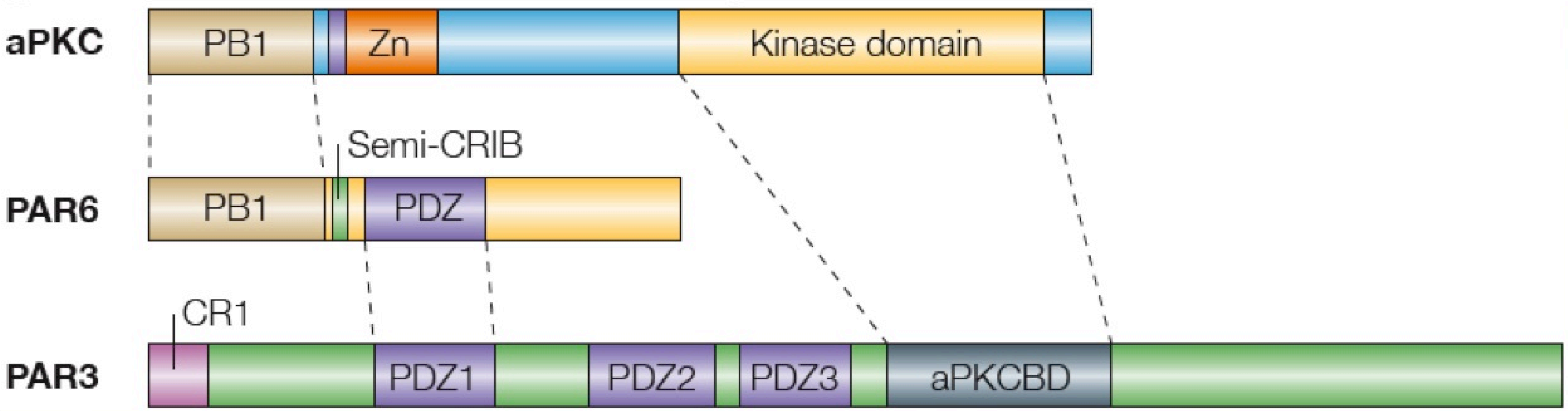


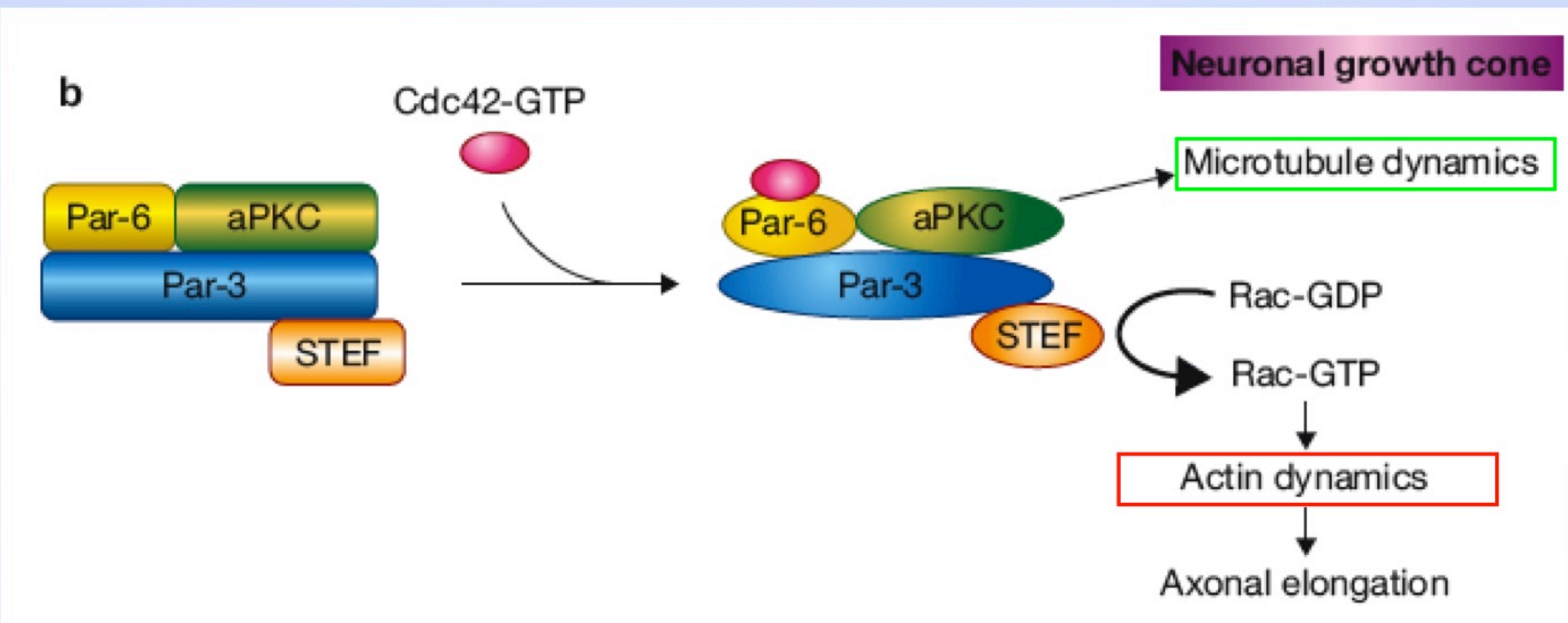
Journal of Cell Science

Suzuki, A. et al. J Cell Sci 2006;119:979-987

Table 1. Localization of proteins of the PAR-aPKC complex in different species

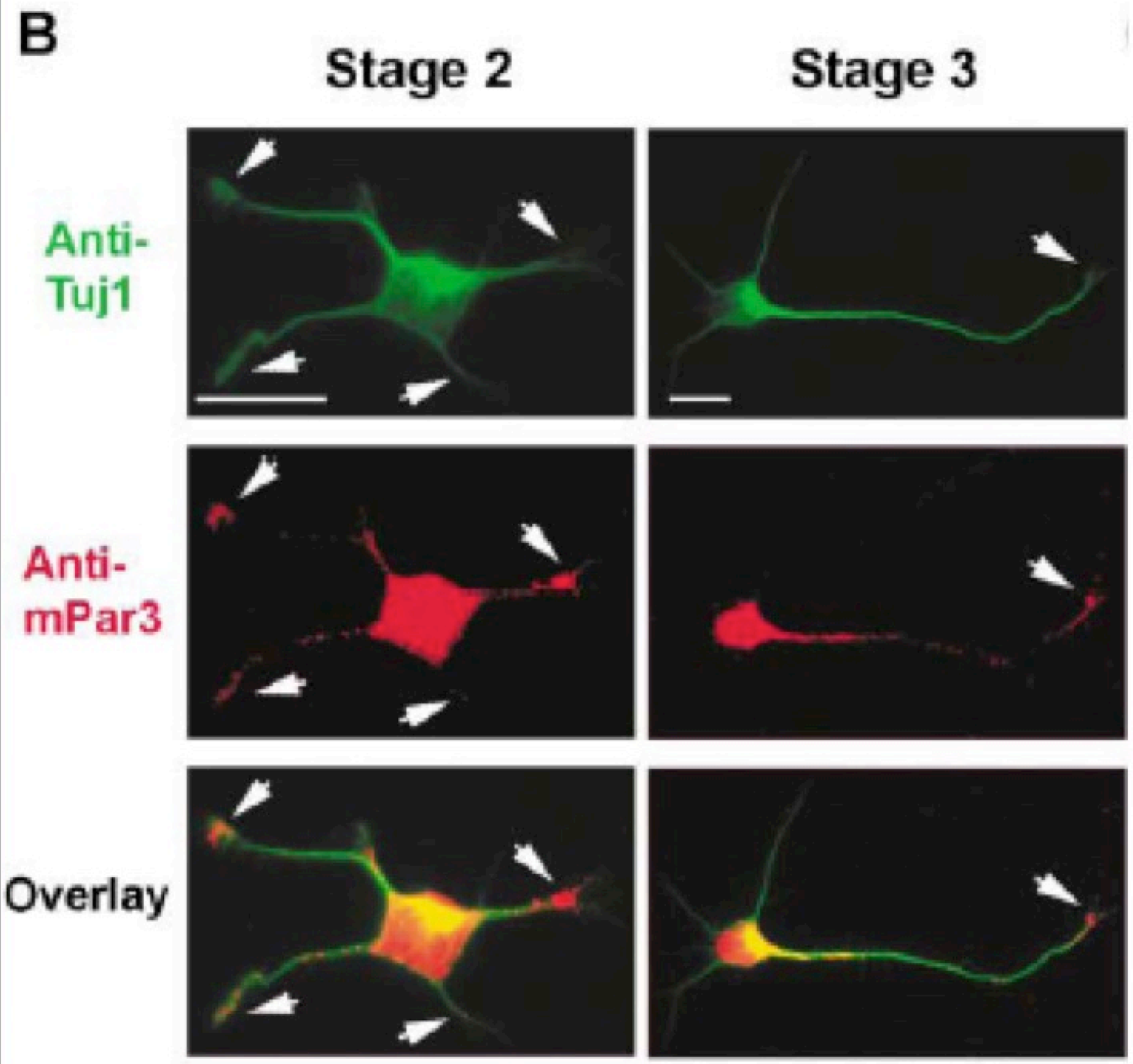
				Localization				
				C. elegans	*Drosophila*			Mammal
S. pombe	*C. elegans*	*Drosophila*	Mammal	zygote	late oocyte	epithelia	neuroblast	epithelia
–	PAR-3	Bazooka	PAR-3/ASIP*	Anterior cortex	Anterior cortex	SAR/apical membrane	Apical cortex	TJ†/apical membrane
–	PAR-6	PAR-6	PAR-6α,β,γ					
–	PKC-3	aPKC	aPKCλ,ζ					



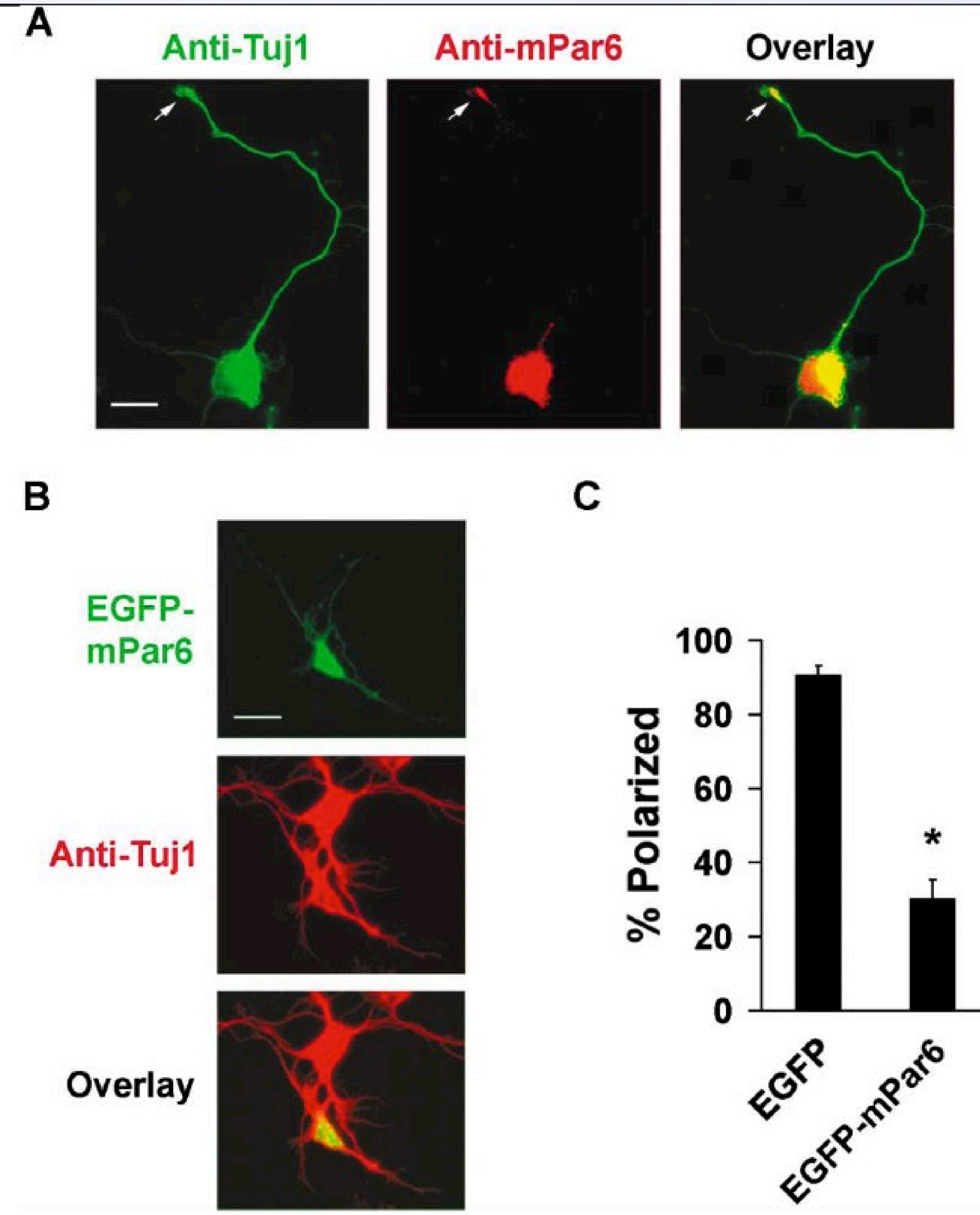


The evolutionary conserved polarity protein:

Par3 and Par 6 complex and PI3K



Expression à l'extrémité de l'axone

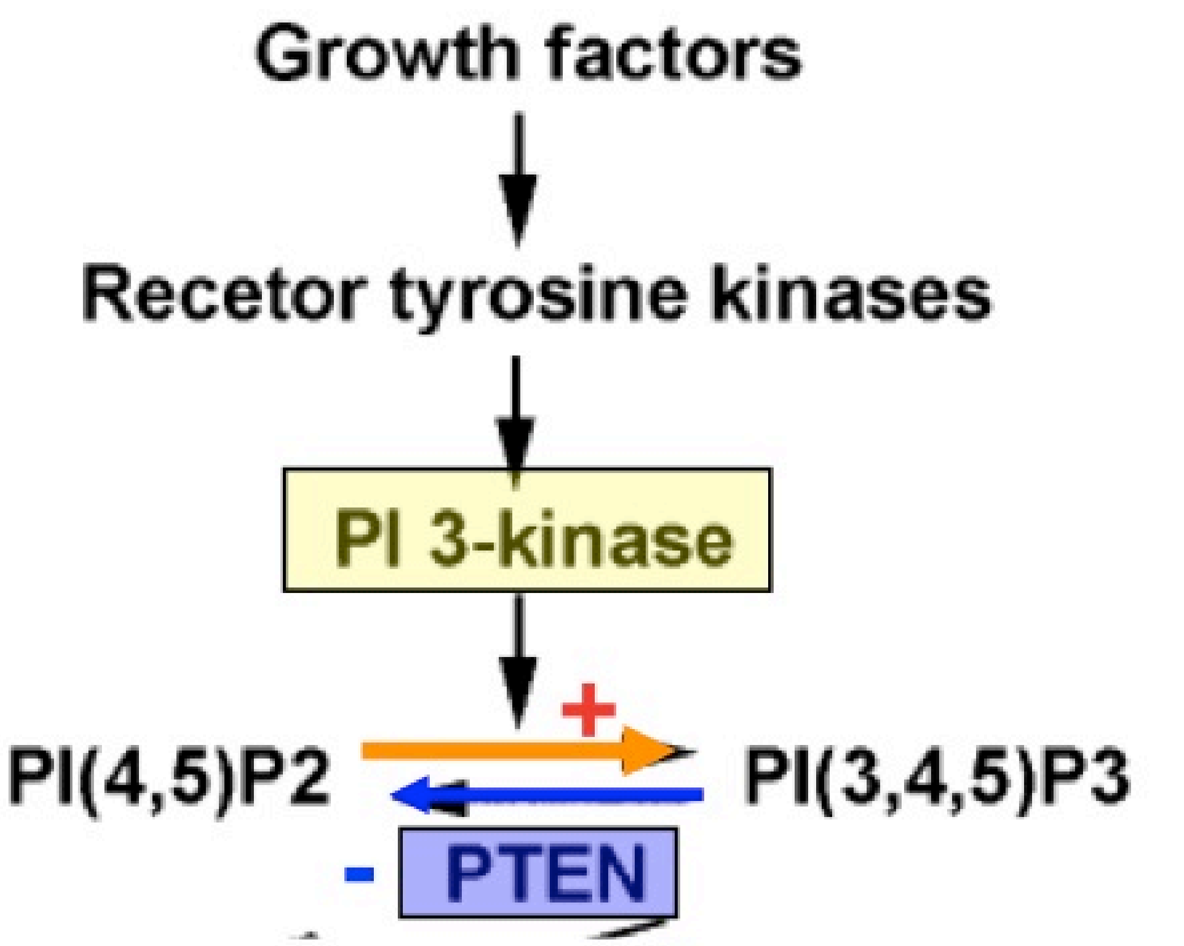


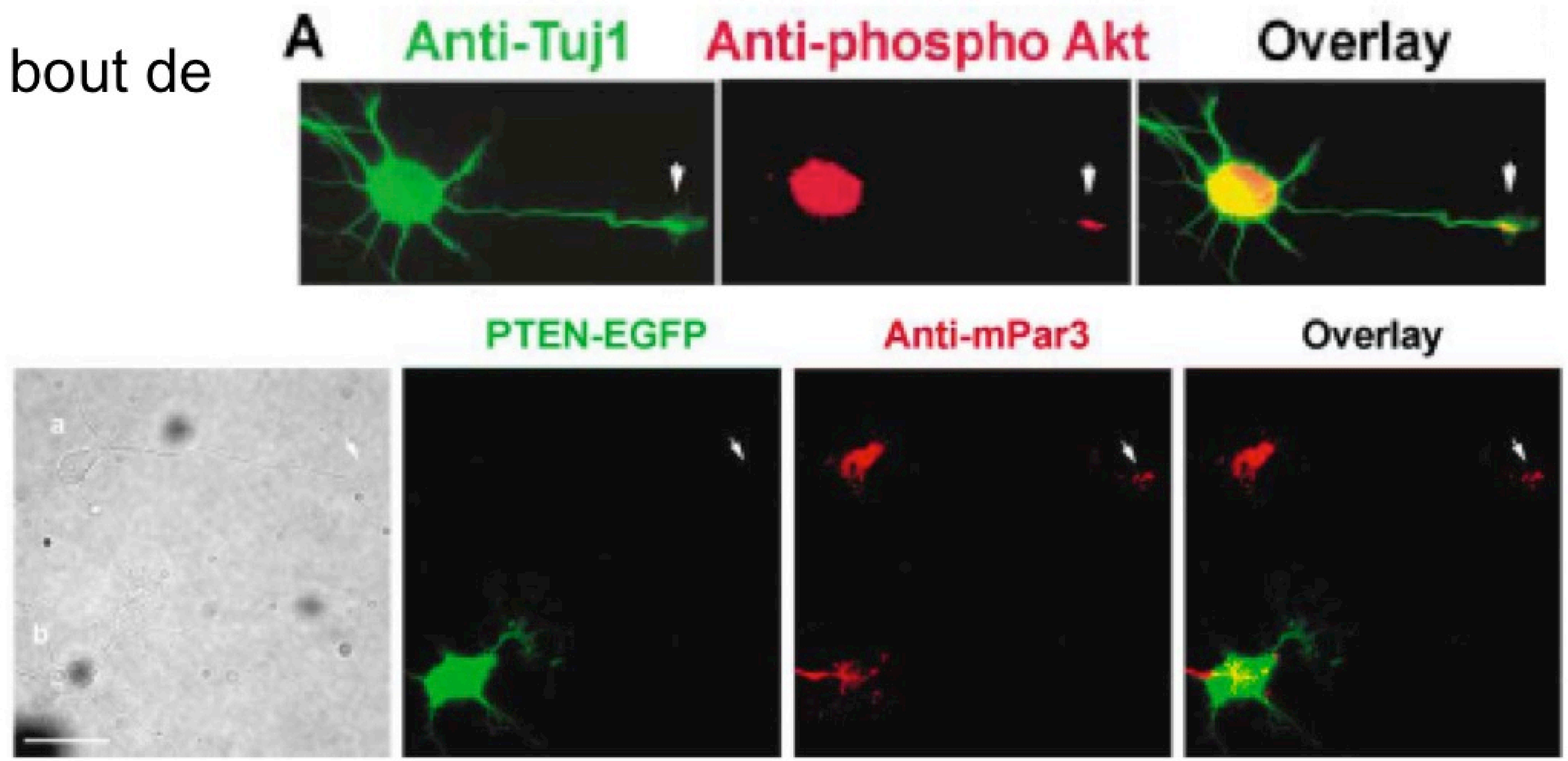
Surexpression Par6: perte de l'axone, neurites multiples: perte de polarité

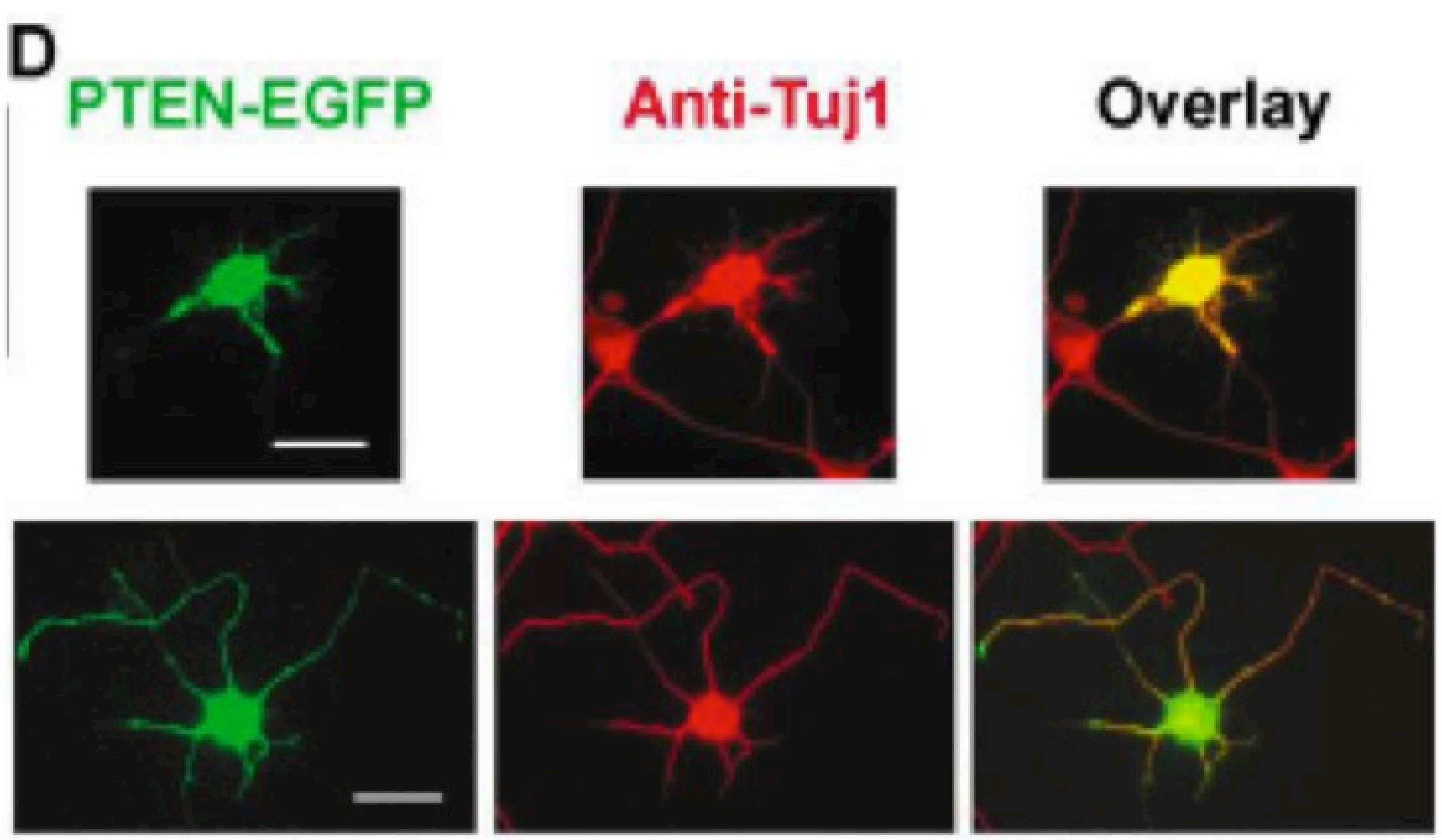
Shi SH, Jan LY, Jan YN. Cell. 2003 Jan 10;112(1):63-75.

The evolutionary conserved polarity protein: Par3 and Par 6 complex and PI3K

PI3K phosphoryle AKT au bout de l'axone







Les neurones exprimant PTEN, expriment par3 dans le soma et pas au bout de l'axone.

Les neurones exprimant PTEN, ne possède pas d'axone unique mais de multiples prolongements.

Shi SH, Jan LY, Jan YN. Cell. 2003 Jan 10;112(1):63-75.

Cell (2003), Vol. 112, 63–75.
Hippocampal Neuronal Polarity Specified by Spatially Localized mPar3/mPar6 and PI 3-Kinase Activity

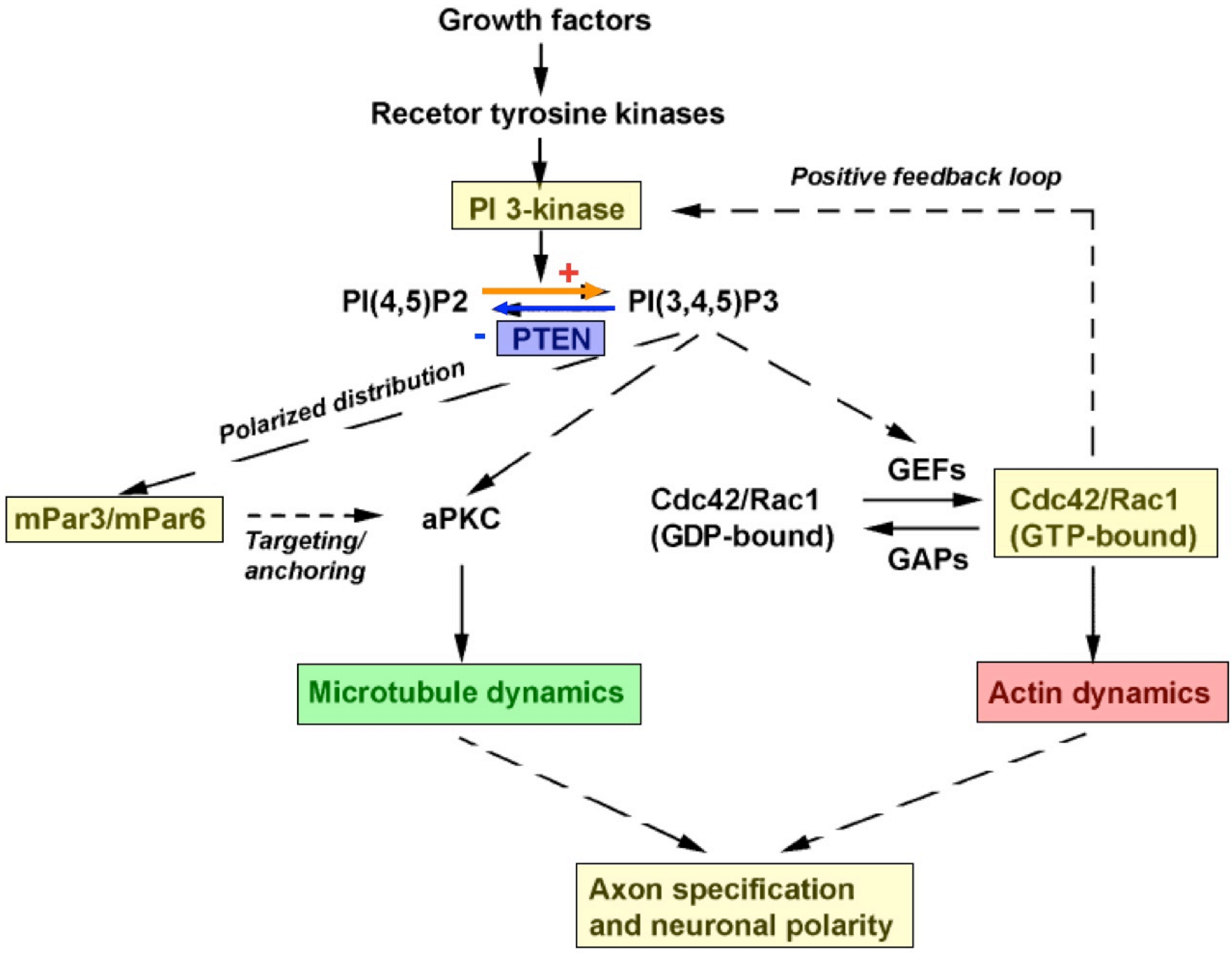


Figure 8. A Model for the Signaling Processes that Specify Neuronal Polarity

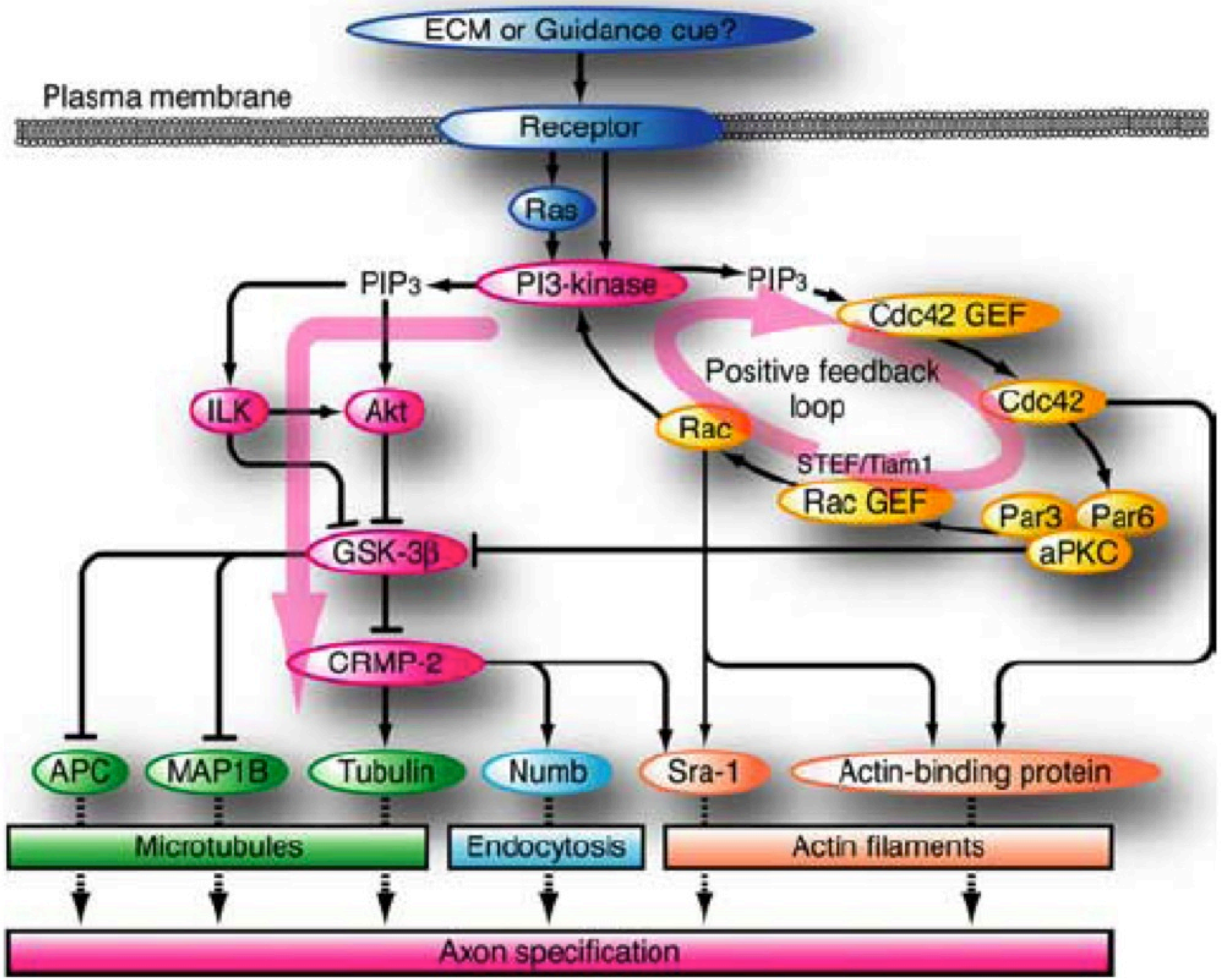
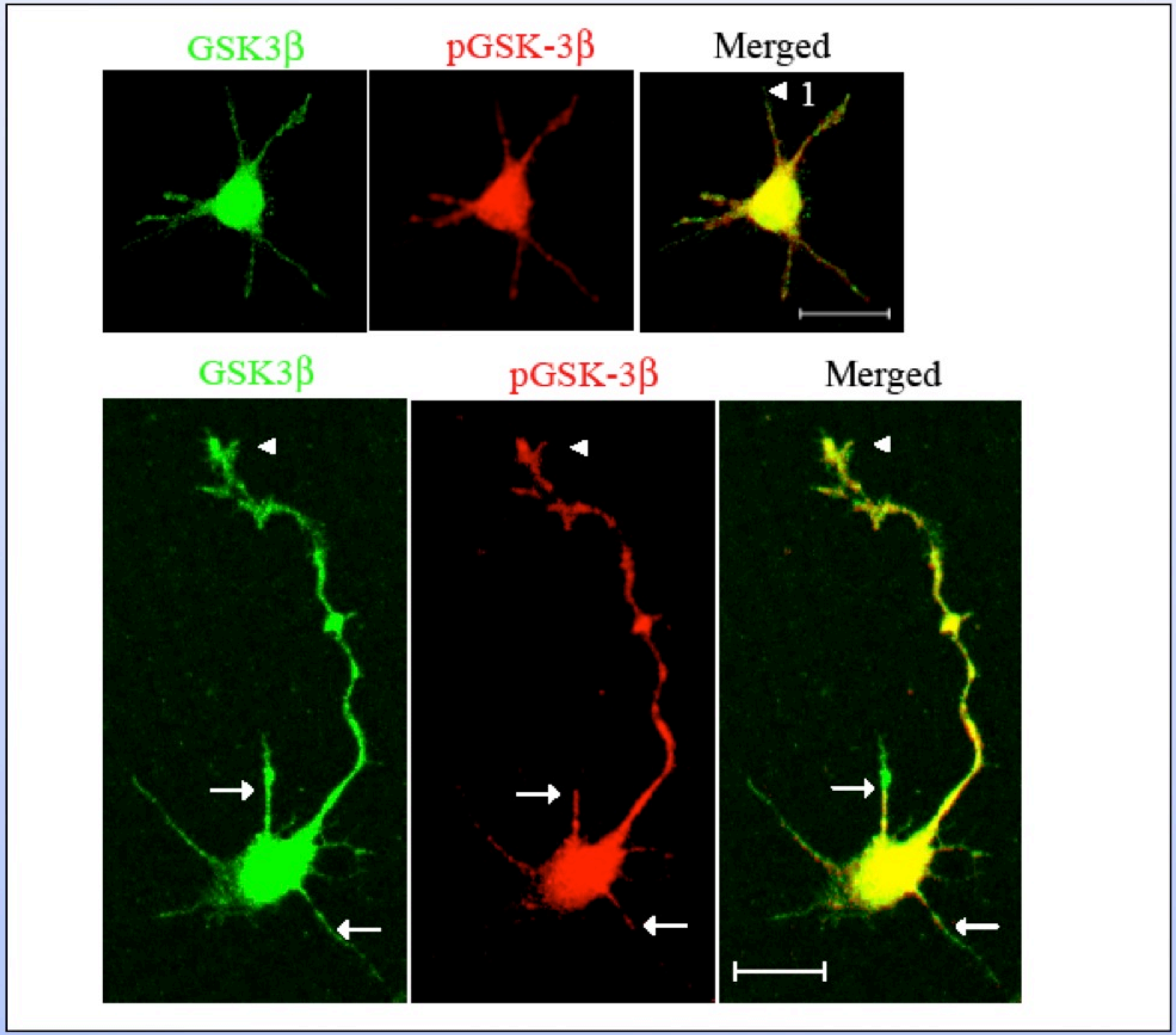


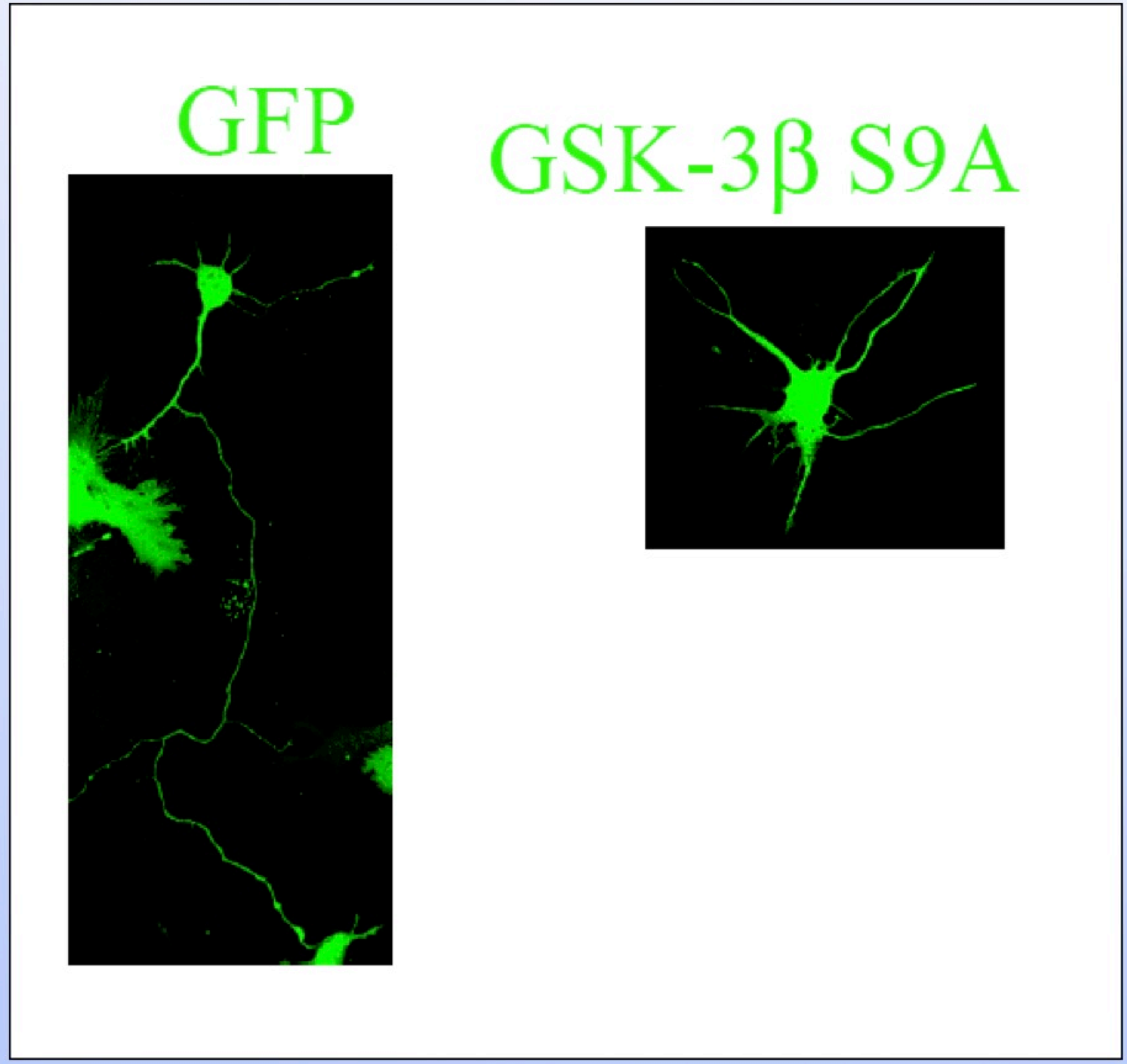
Figure 2. Signaling cascades in axon specification. In one immature neurite (the future axon), the extracellular matrix (ECM) activates PI3-kinase through interaction with adhesion molecules or receptors, thereby producing PIP_3. Accumulated PIP_3 drives two major signaling cascades: the Akt/GSK-3β/CRMP-2 pathway and the positive feedback loop composed of Cdc42, the Par complex, and Rac1. These signaling cascades regulate cytoskeletons, endocytosis, protein trafficking, and transcriptions to promote neurite elongation and to determine axon or dendrite fate.

GSK-3β in neuronal polarity

Polarized inactivation of GSK-3β (phosphorylation) in axon



Inhibition de l'induction de l'axone par un mutant de GSK-3 beta constitutivement actif: GSK-3 beta S9A

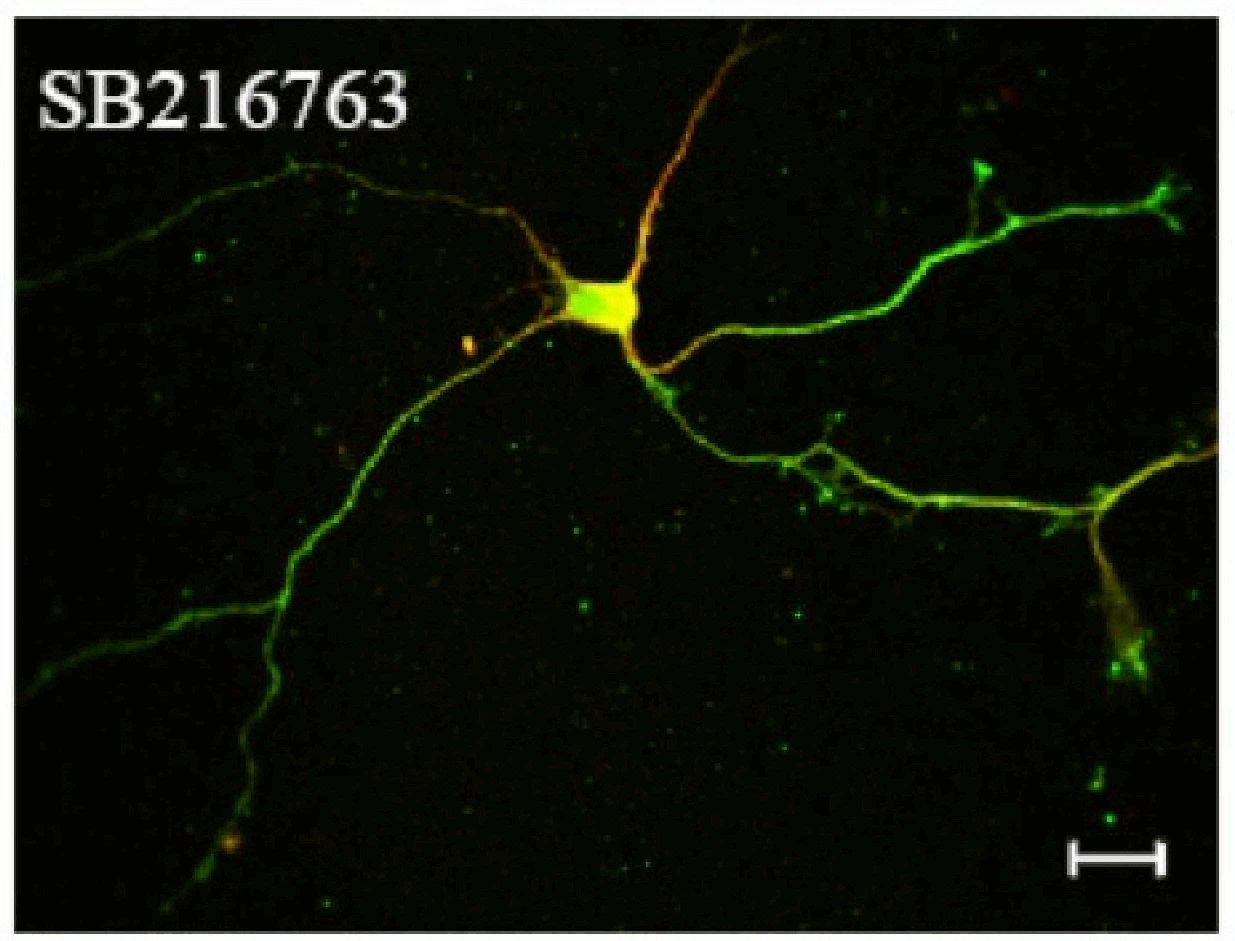


L'activation prolongée de la GSK3b inhibe la formation de l'axone.

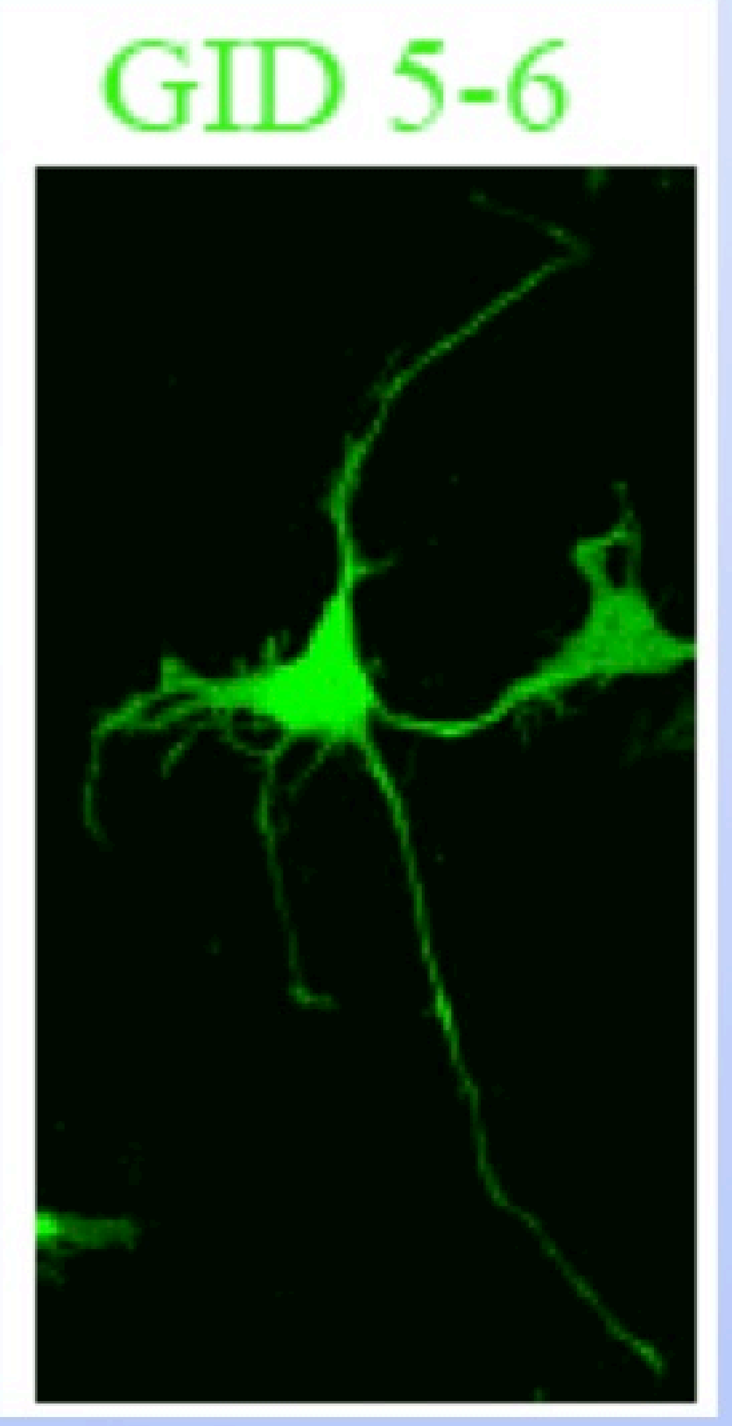
L'inhibition de l'activité de la GSK-3 beta induit la formation de multiples axones.

Inhibiteurs chimiques

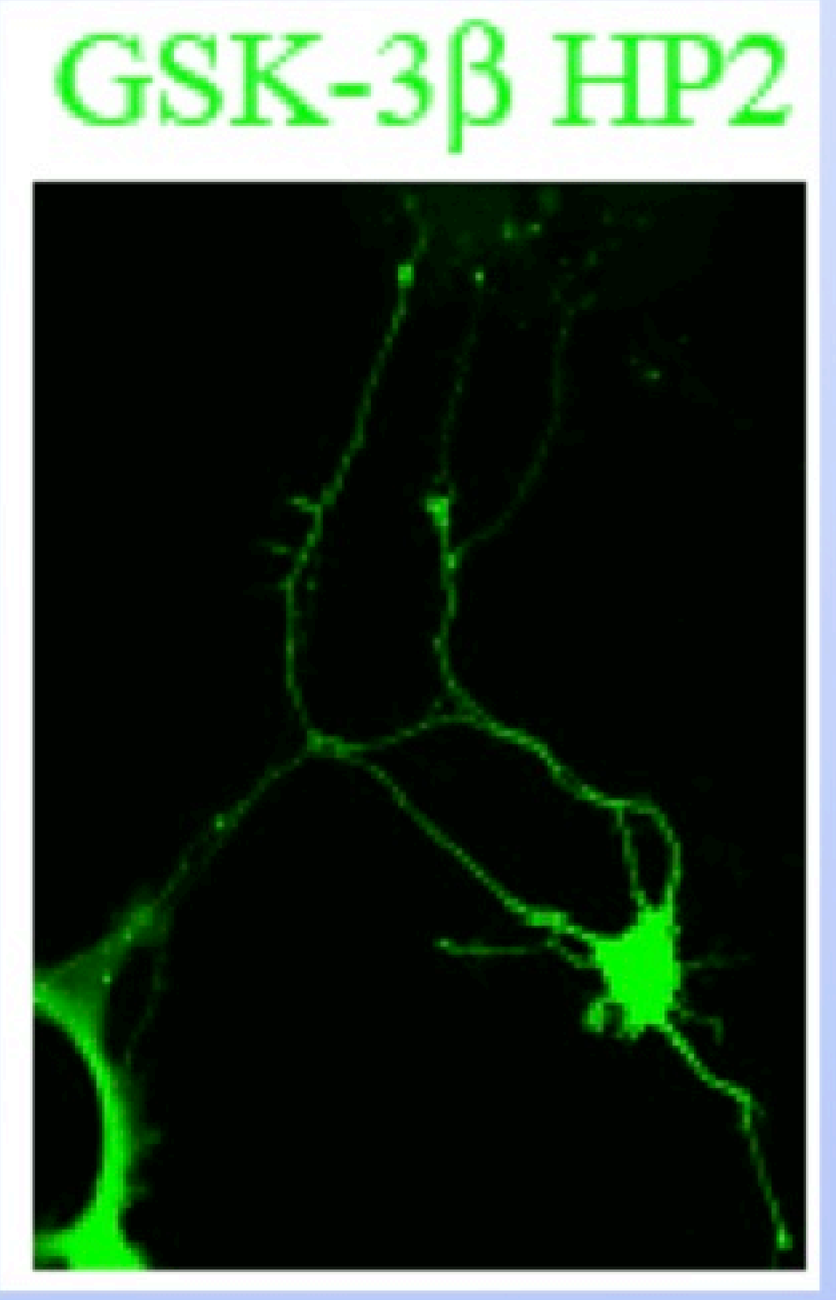
ou transfection de la forme active d'Akt.



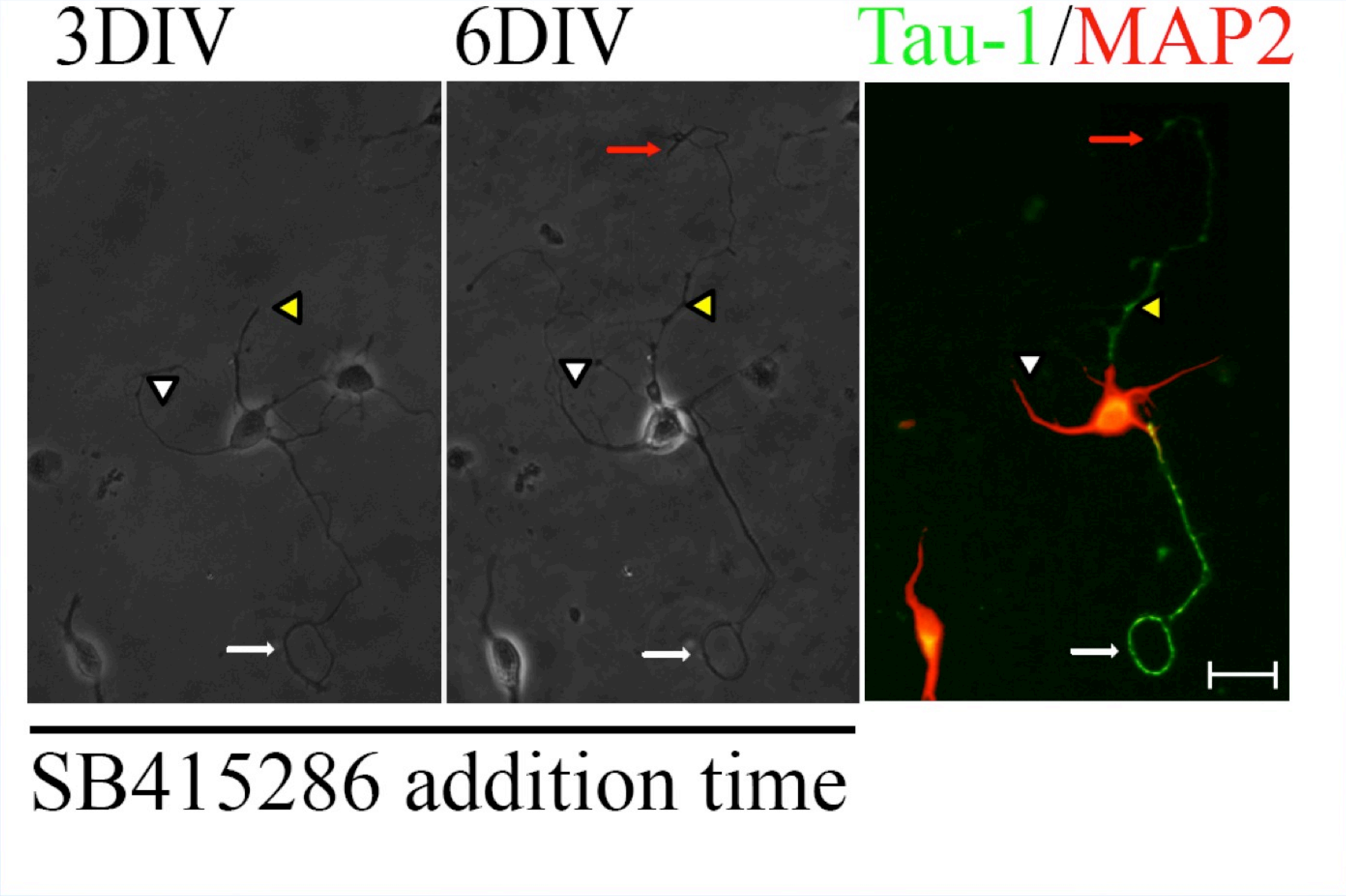
Peptide inhibitor



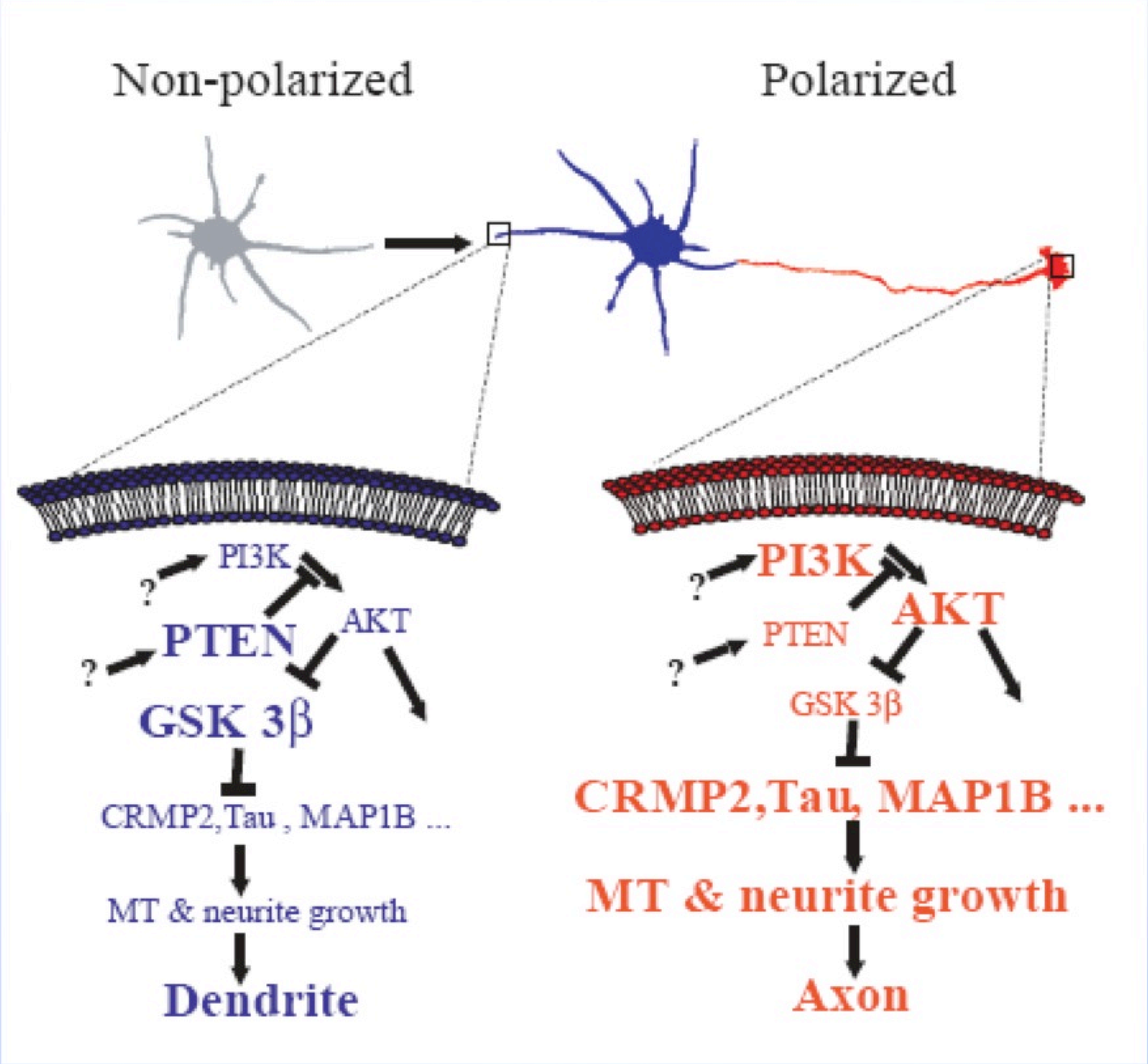
RNAi



L'inhibition de GSK3 au stade 3 convertit des dendrites en axone.



Modele



Lors de la croissance neuritique du futur axone, la PI3K phosphoryle le PIP3 et active Akt qui va inhiber GSK3beta. Cela résulte en la phosphorylation de CRMP2 et qui promeut la formation de microtubules et la croissance axonale.

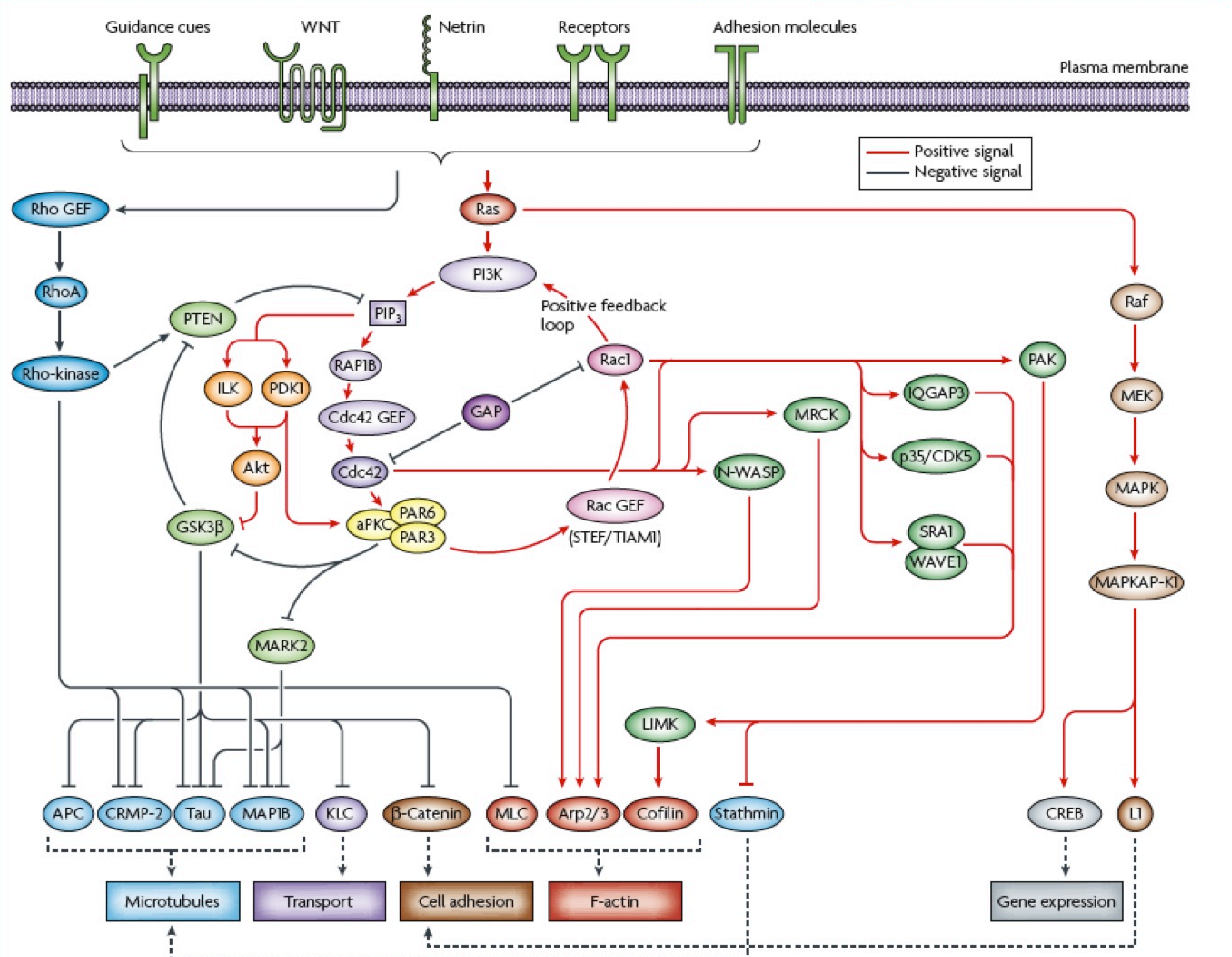
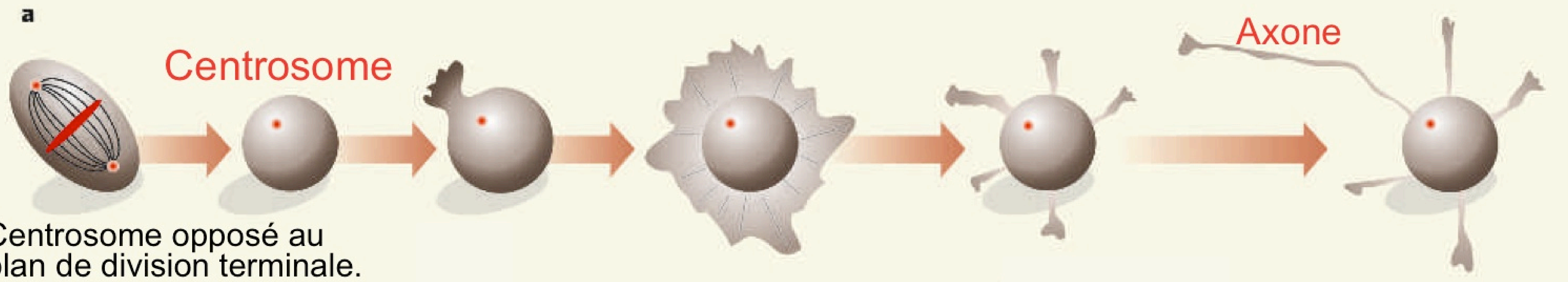
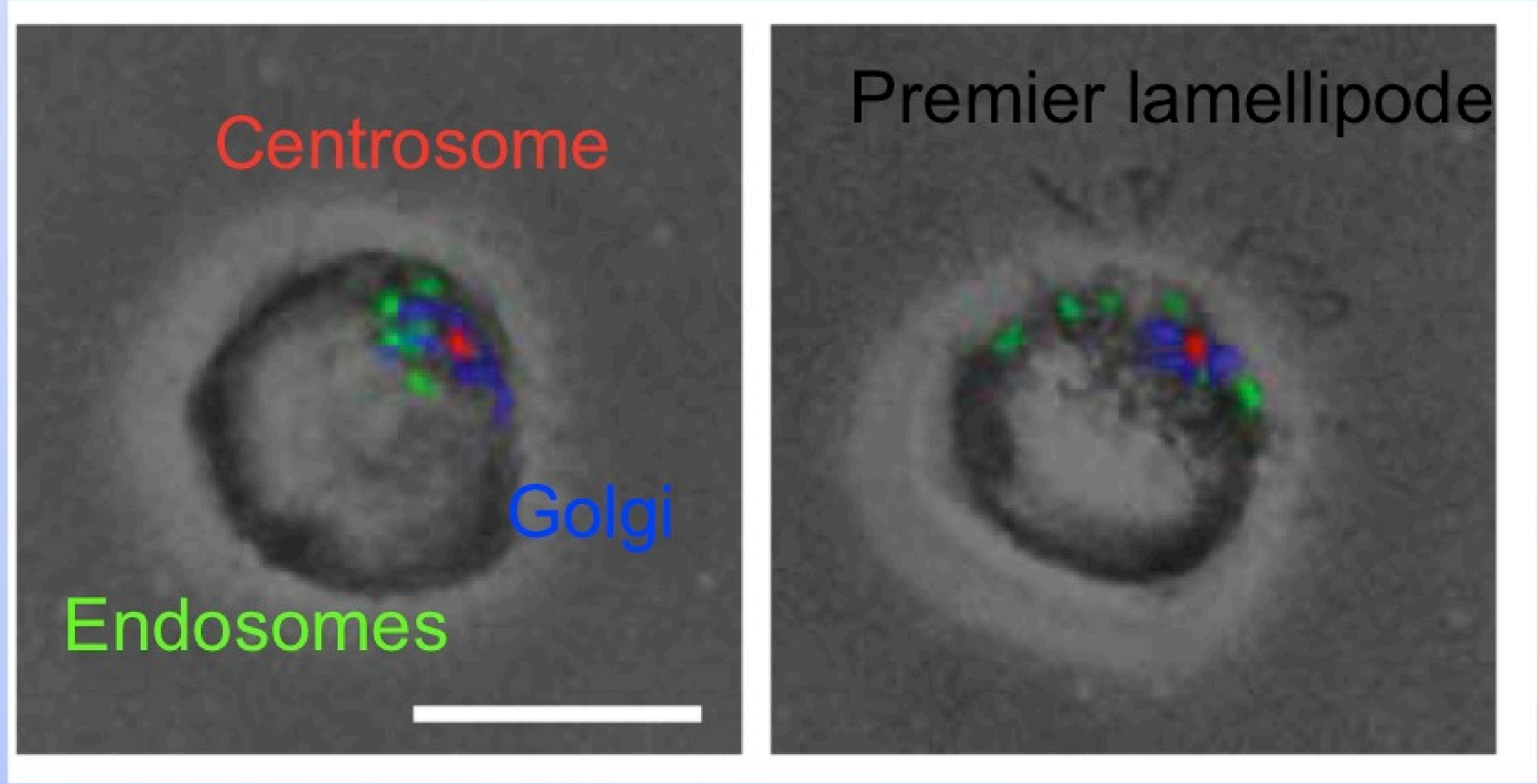


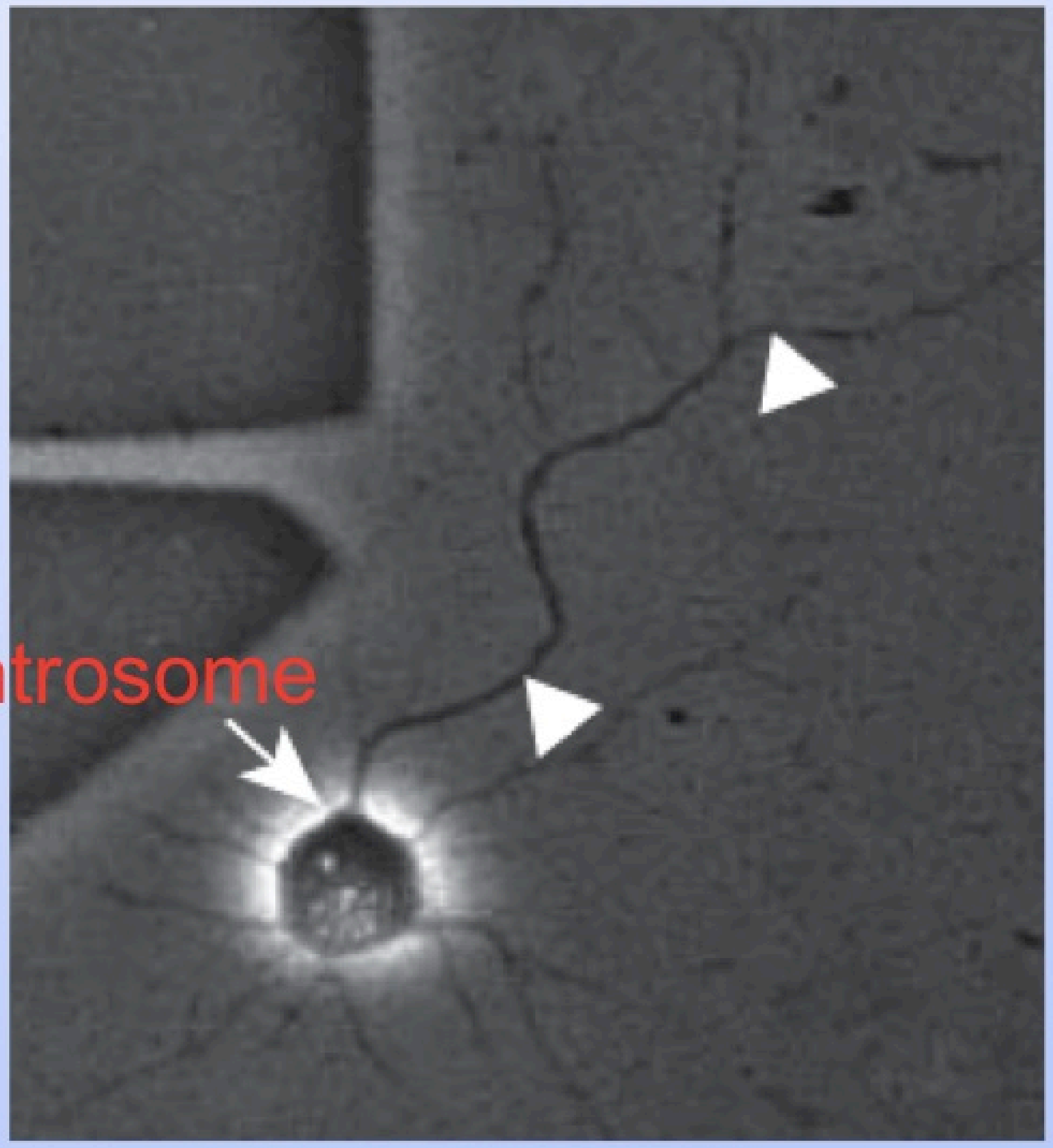
Figure 3 | **Simplified model of positive and negative signals.** In response to the activation of cell surface receptors by

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

La localisation du centrosome détermine la polarisation d'un neurite en axone.

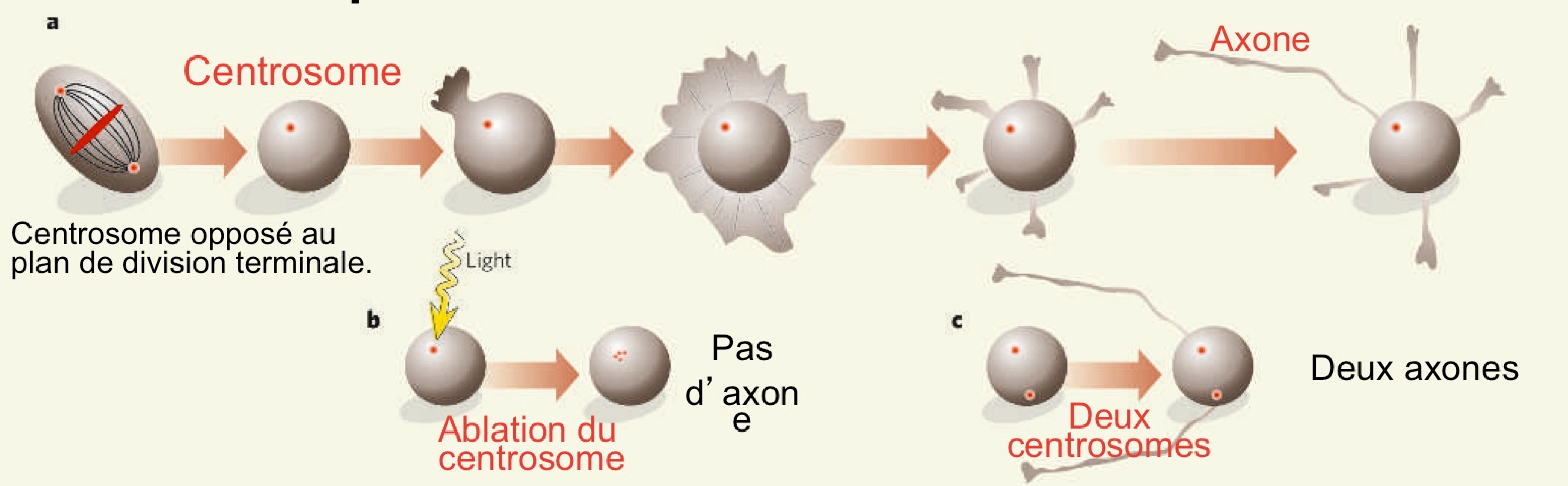


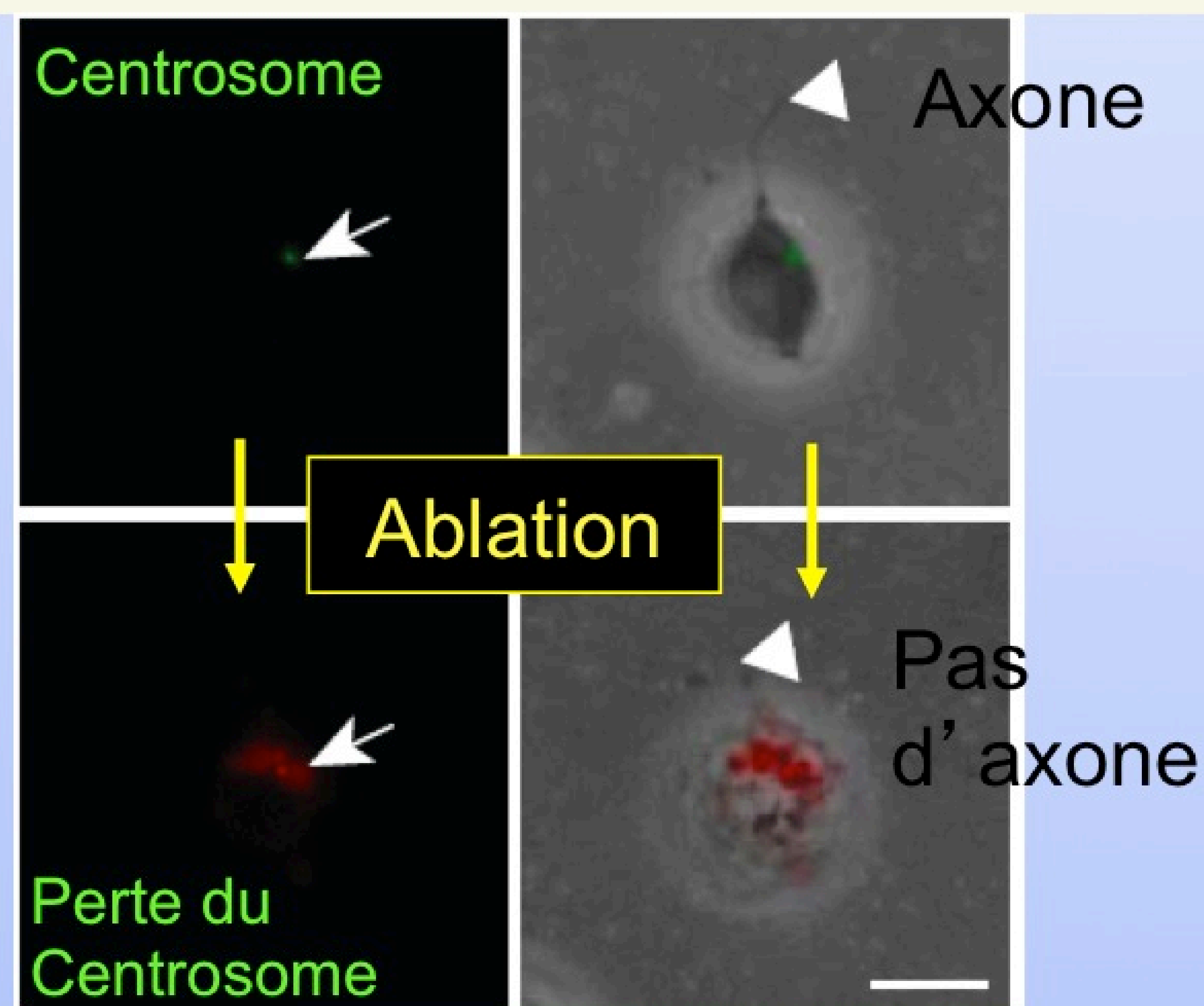


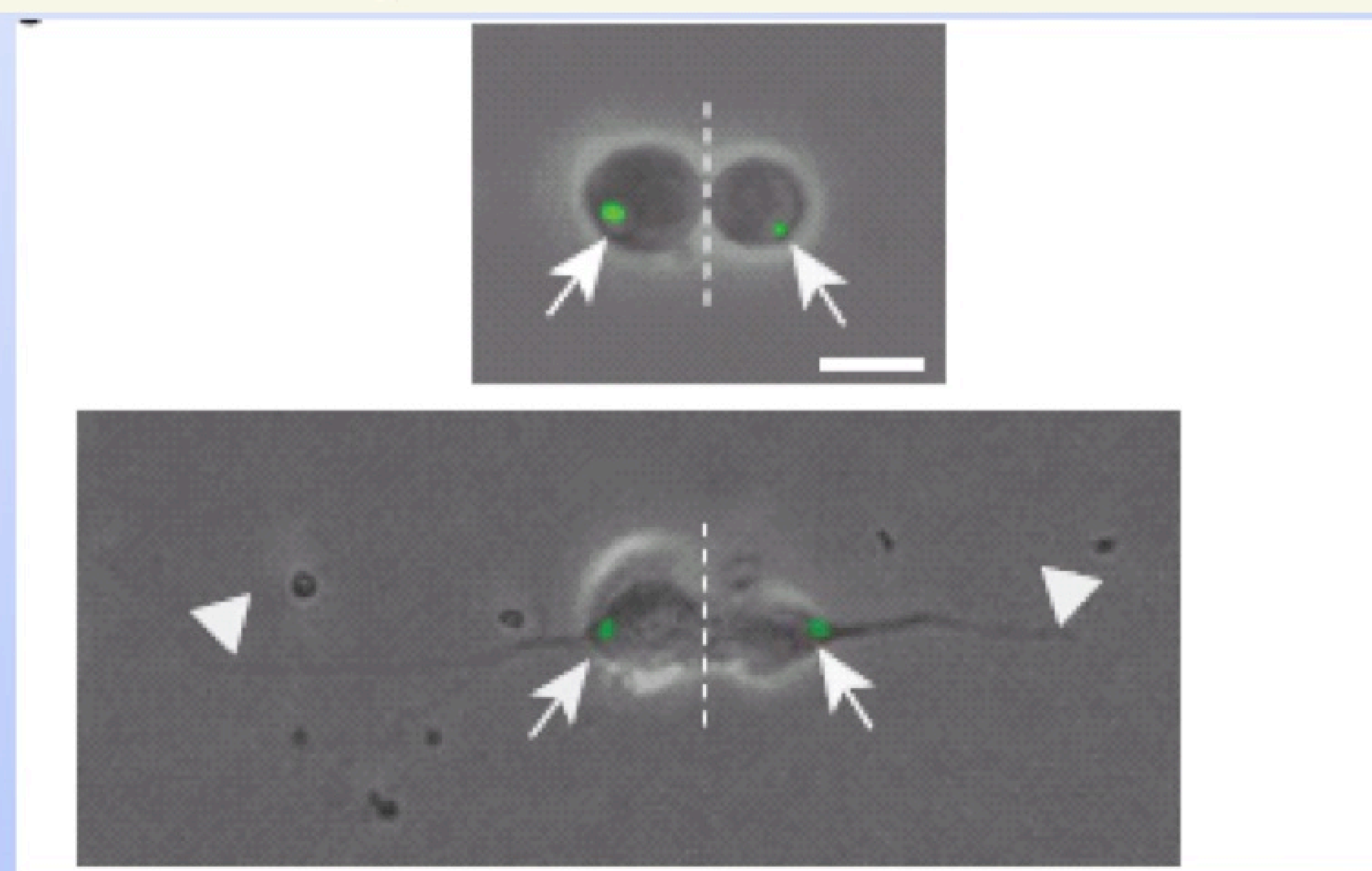


Froylan Calderon de Anda, & **Carlos G. Dotti** Nature (2005)

La localisation du centrosome détermine la polarisation d'un neurite en axone.





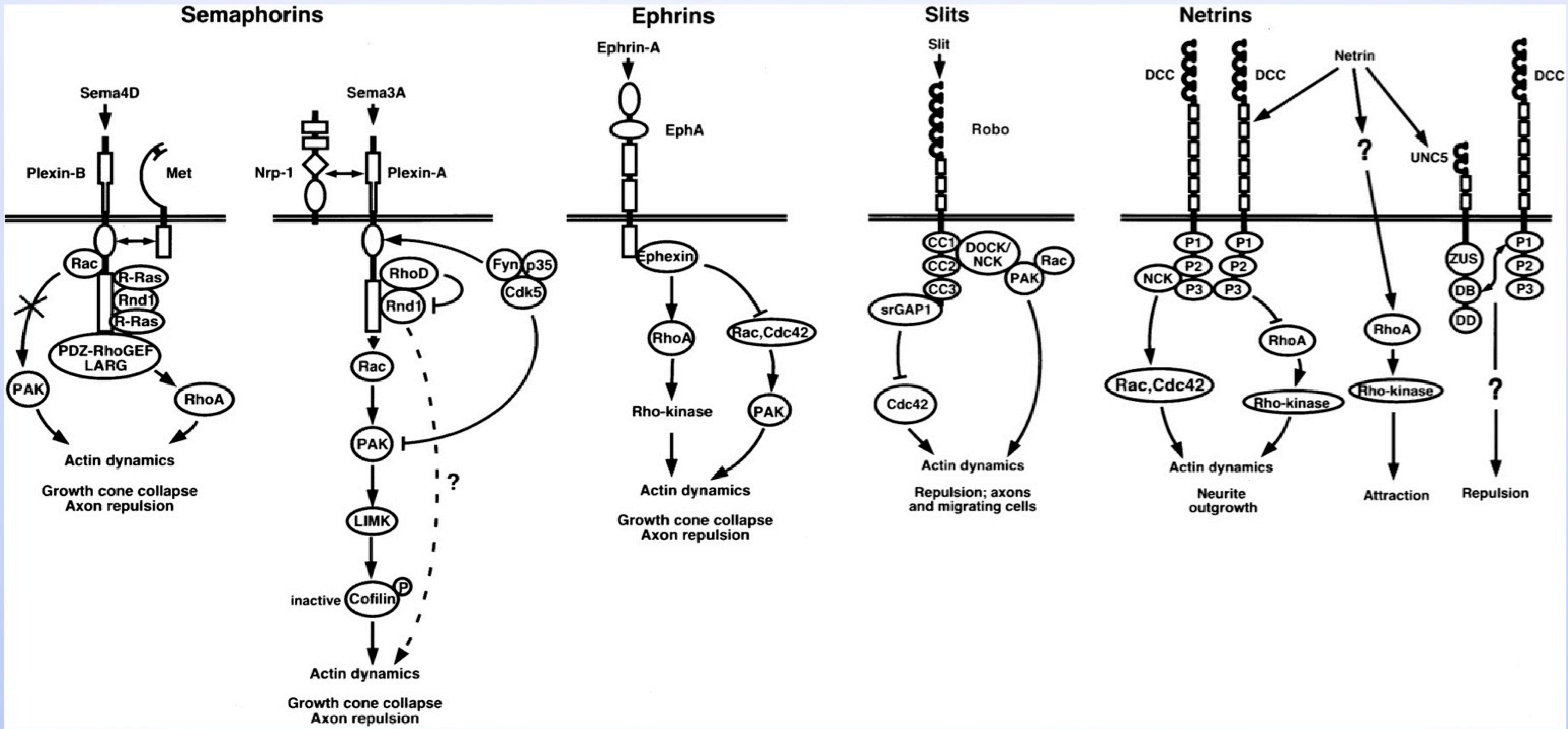


Froylan Calderon de Anda, & **Carlos G. Dotti** Nature (2005)

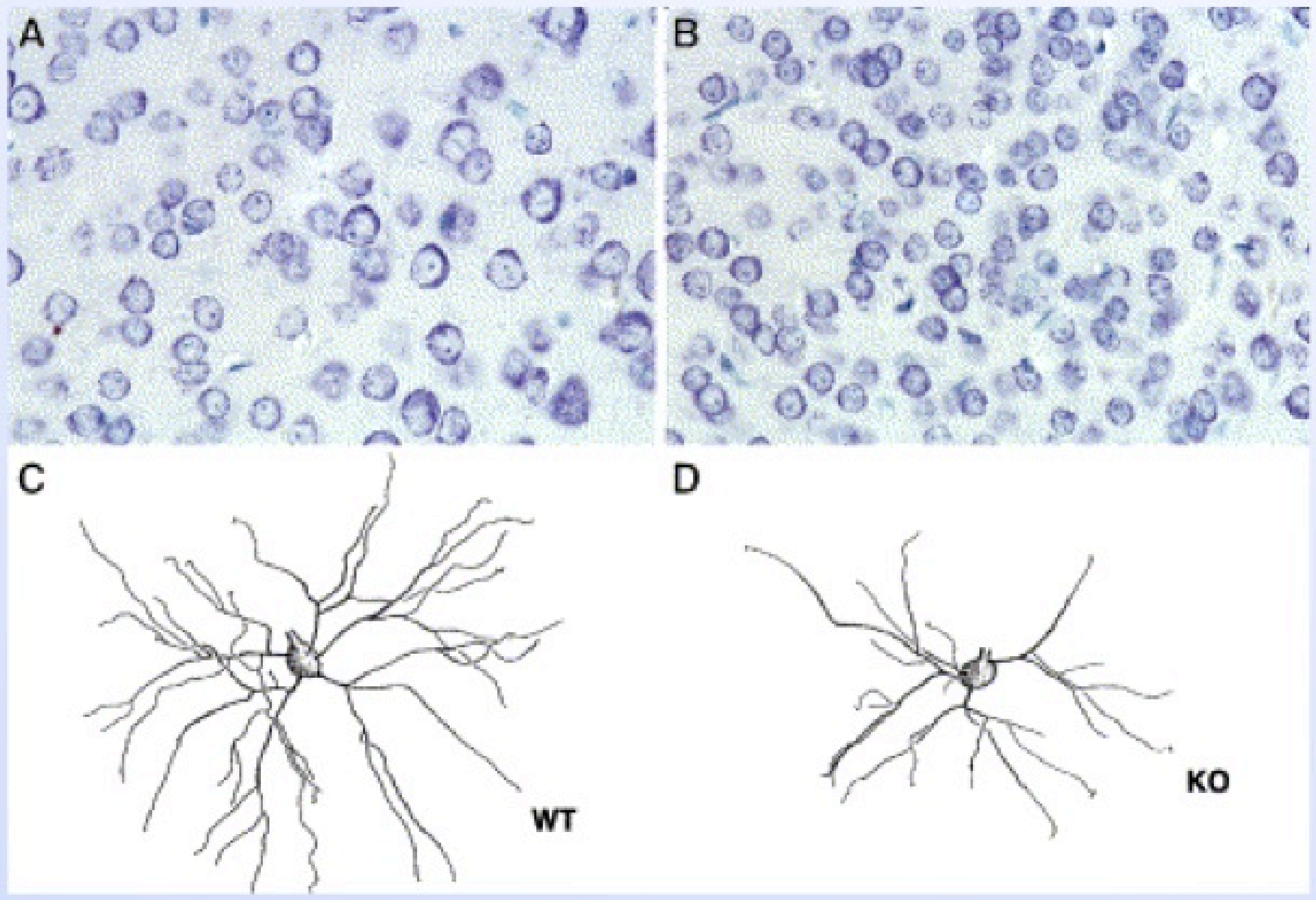
La polarité cellulaire peut etre régulée par des molécules extracellulaire agissant sur des récepteurs présents sur les cônes de croissance.

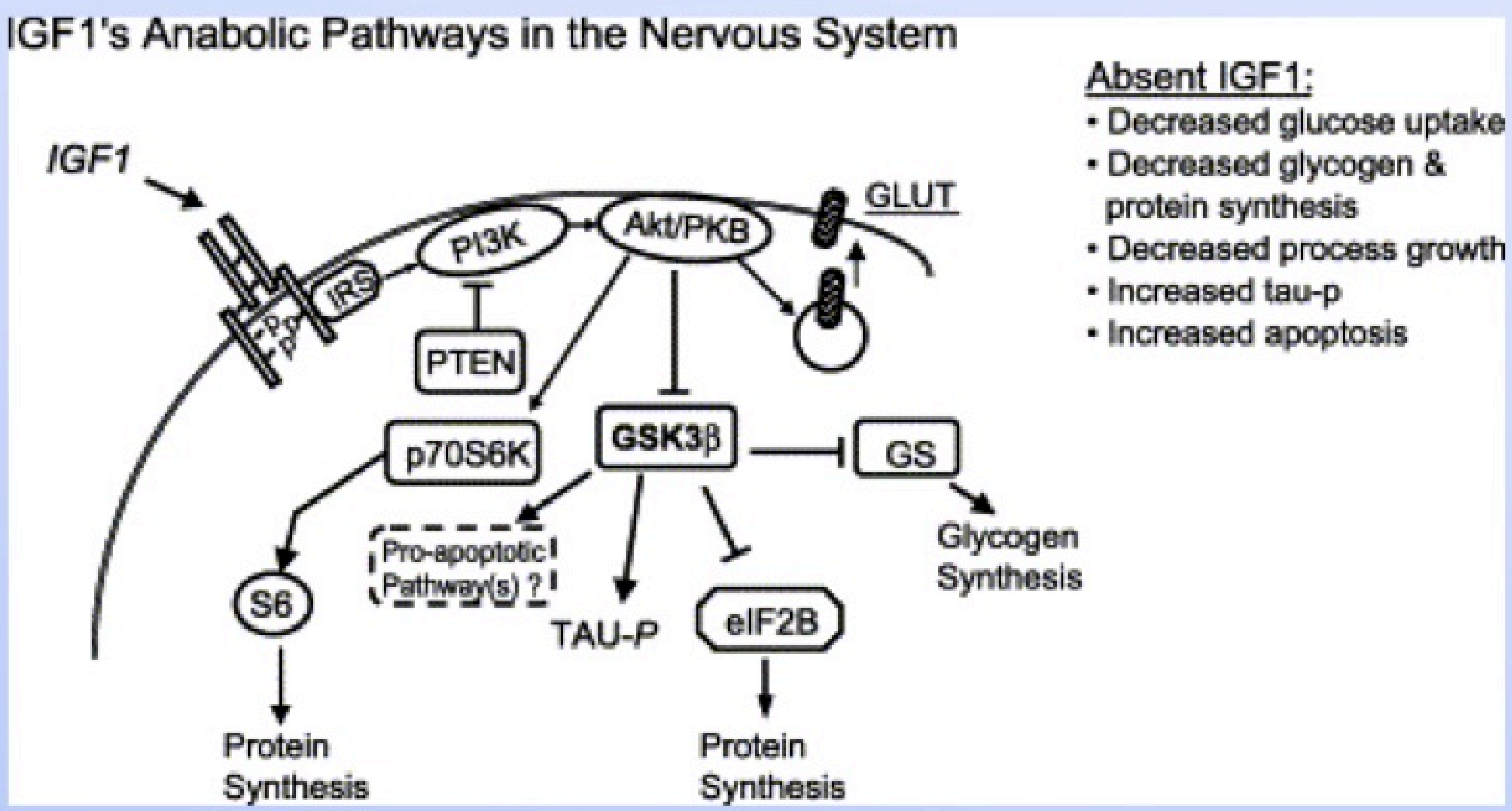
1. Récepteurs membranaires (GPCRs, RTKs) détectant un signal asymétrique extérieur.
2. Les récepteurs activent les GTPase Rho.
3. Les protéines Rho induisent des changement de cytosquelette induce cytoskeletal changes at the leading

Signalisation et croissance



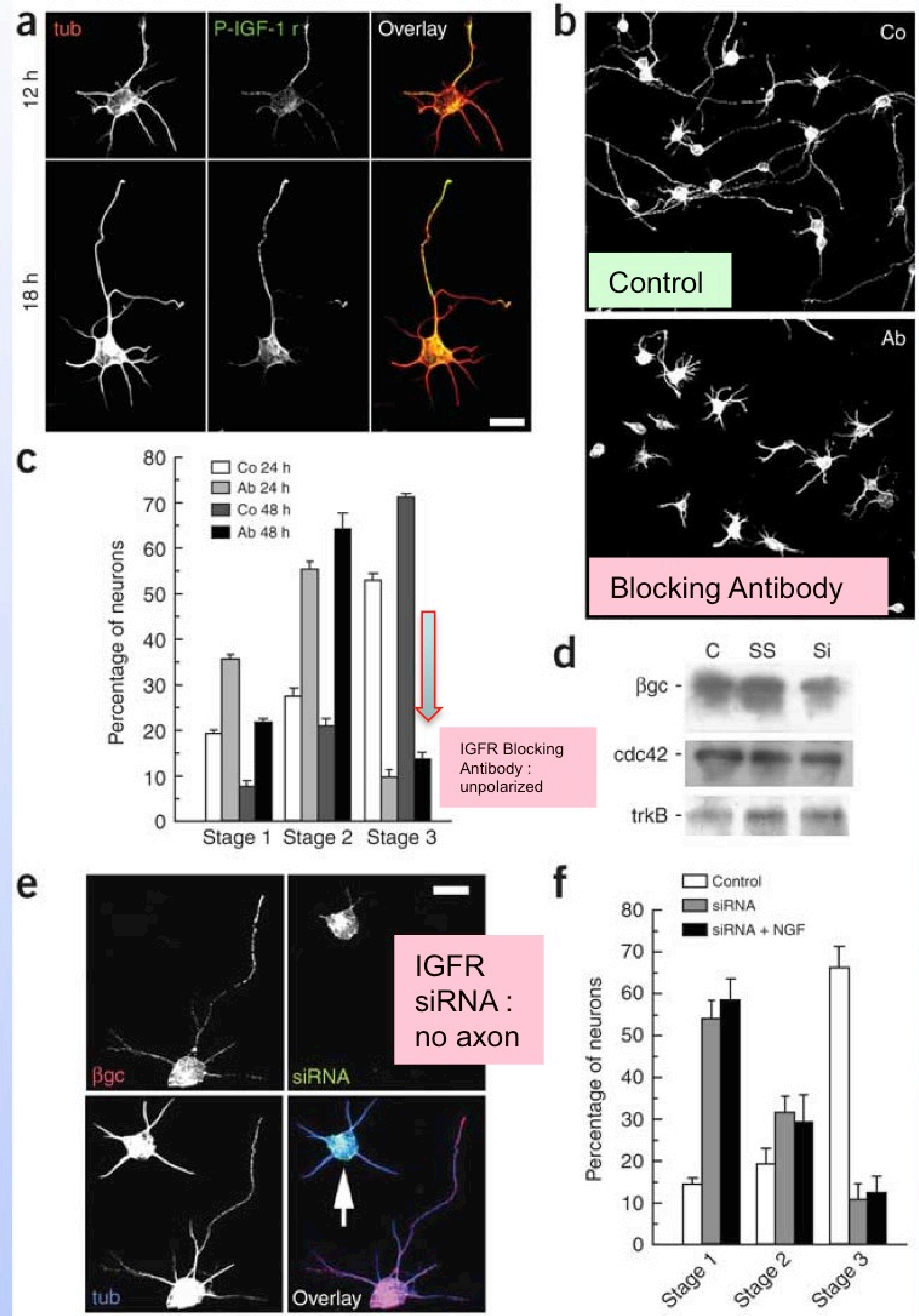
IGF1R?





IGF-1 receptor is essential for the establishment of hippocampal neuronal polarity

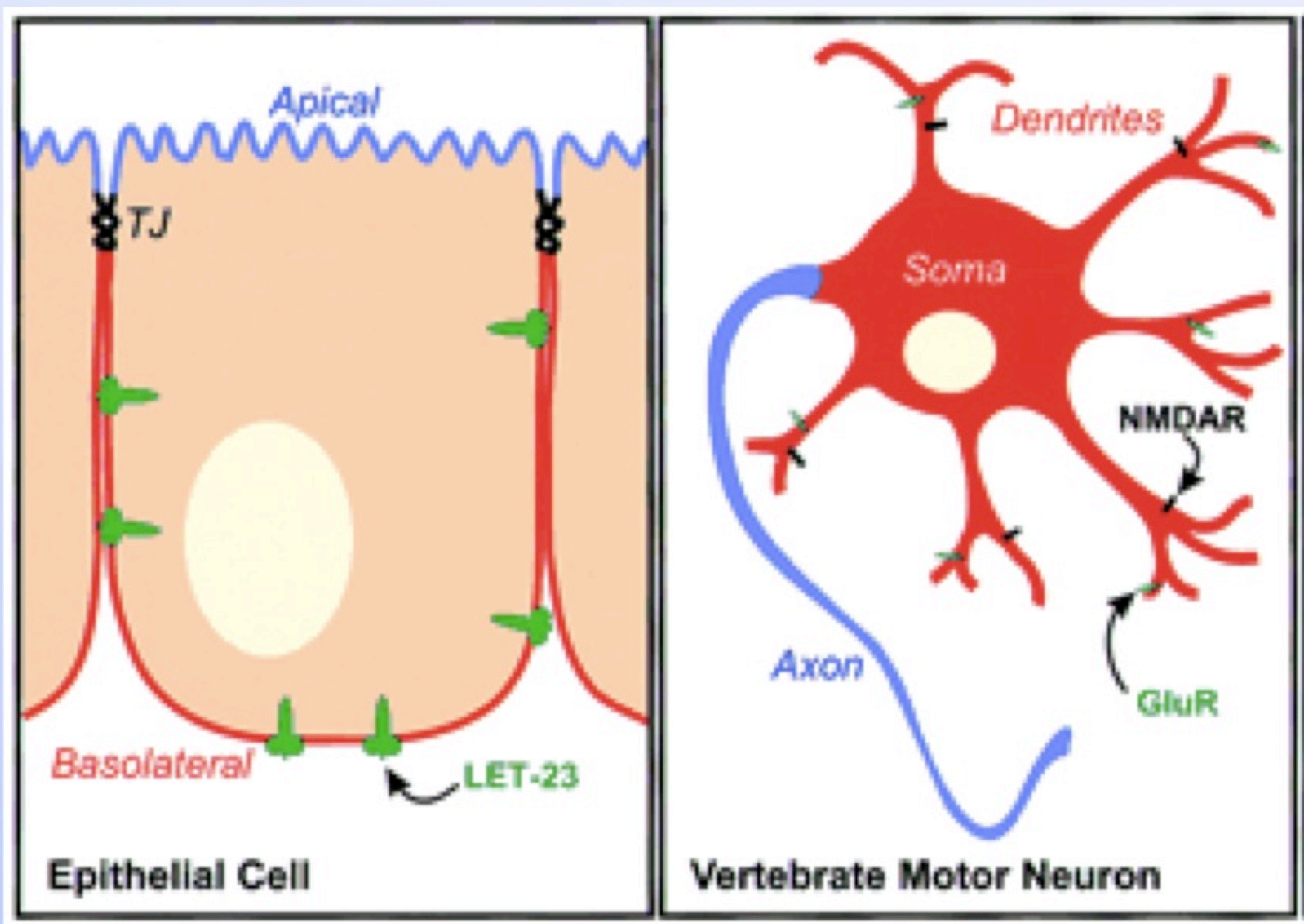
Lucas Sosa, Sebastian Dupraz, Lisandro Laurino, Flavia Bollati, Mariano Bisbal, Alfredo Cáceres, Karl H Pfenninger & Santiago Quiroga Nature Neuroscience 2006



Reducing IGF-1R function by incubation with blocking antibody
or by siRNA silencing prevents axon formation in rat hippocampal neurons

6. La métaphore épithéliale imparfaite

APICAL~AXON



BASOLATERAL~DENDRITE

Polarized Sorting of Viral Glycoproteins to the Axon and Dendrites of Hippocampal Neurons In Culture
C. G. Dotti and K. Simons Cell, Vol. 62, 63–72, July, 1990

TfR:

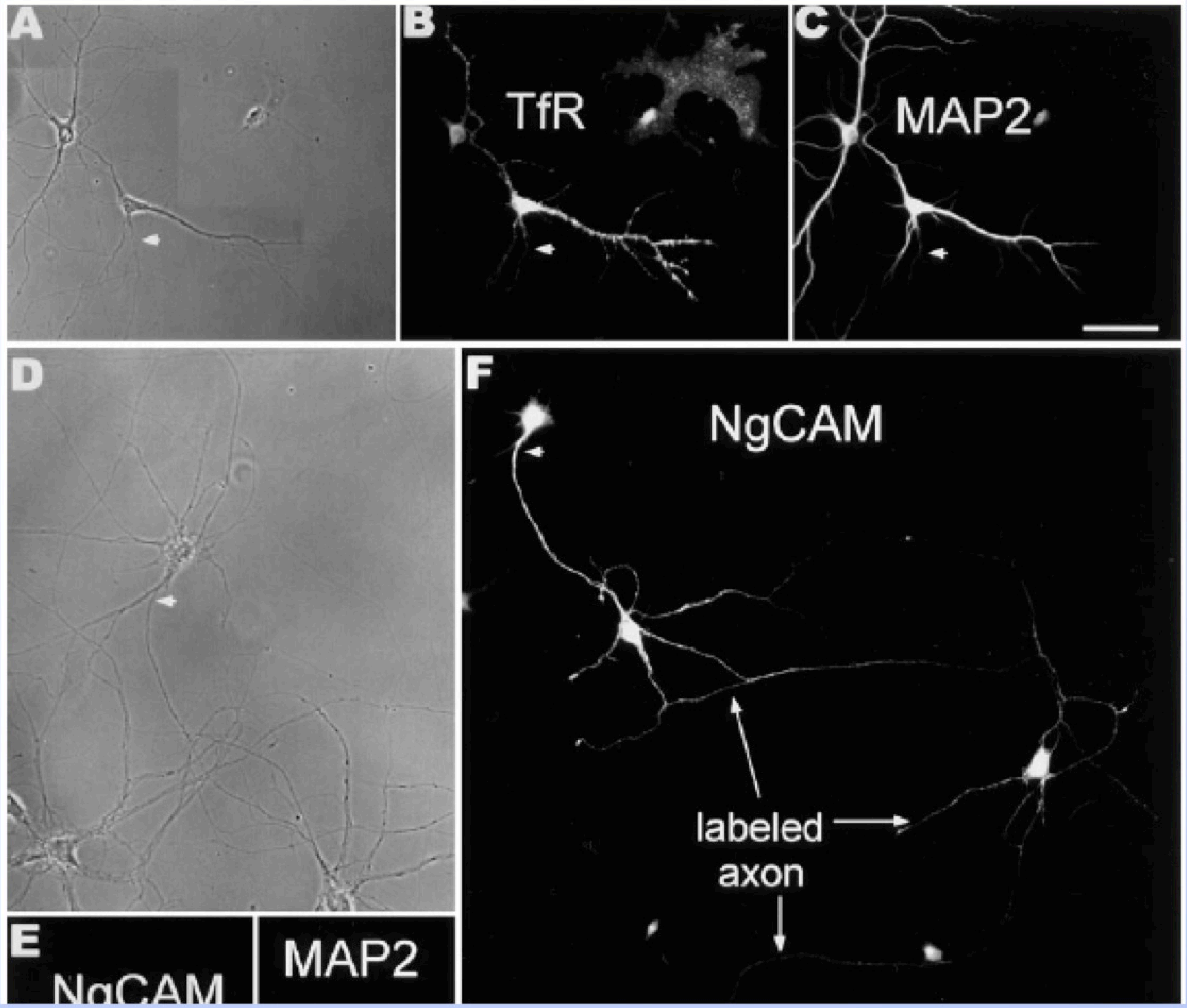
Epithelial: basolateral

Neurone: dendrite

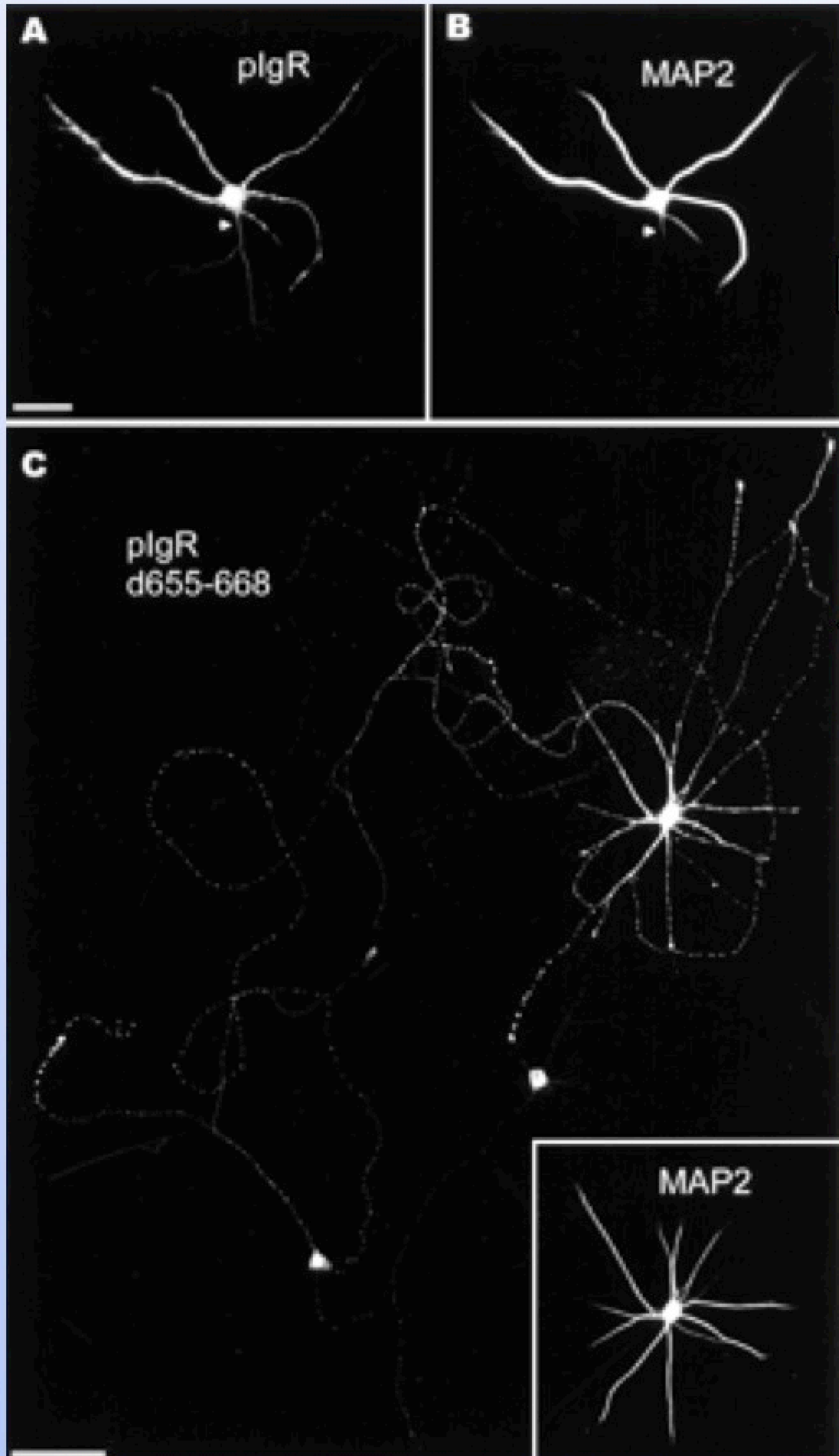
NgCAM:

Epithelial: apical

Neurone: axonal



Jareb, Mark and Banker, Gary (1998).
The Polarized Sorting of Membrane Proteins Expressed in Cultured Hippocampal Neurons Using Viral Vectors. *Neuron* 20:855



Expression dendritique

Mutation du motif basolatéral

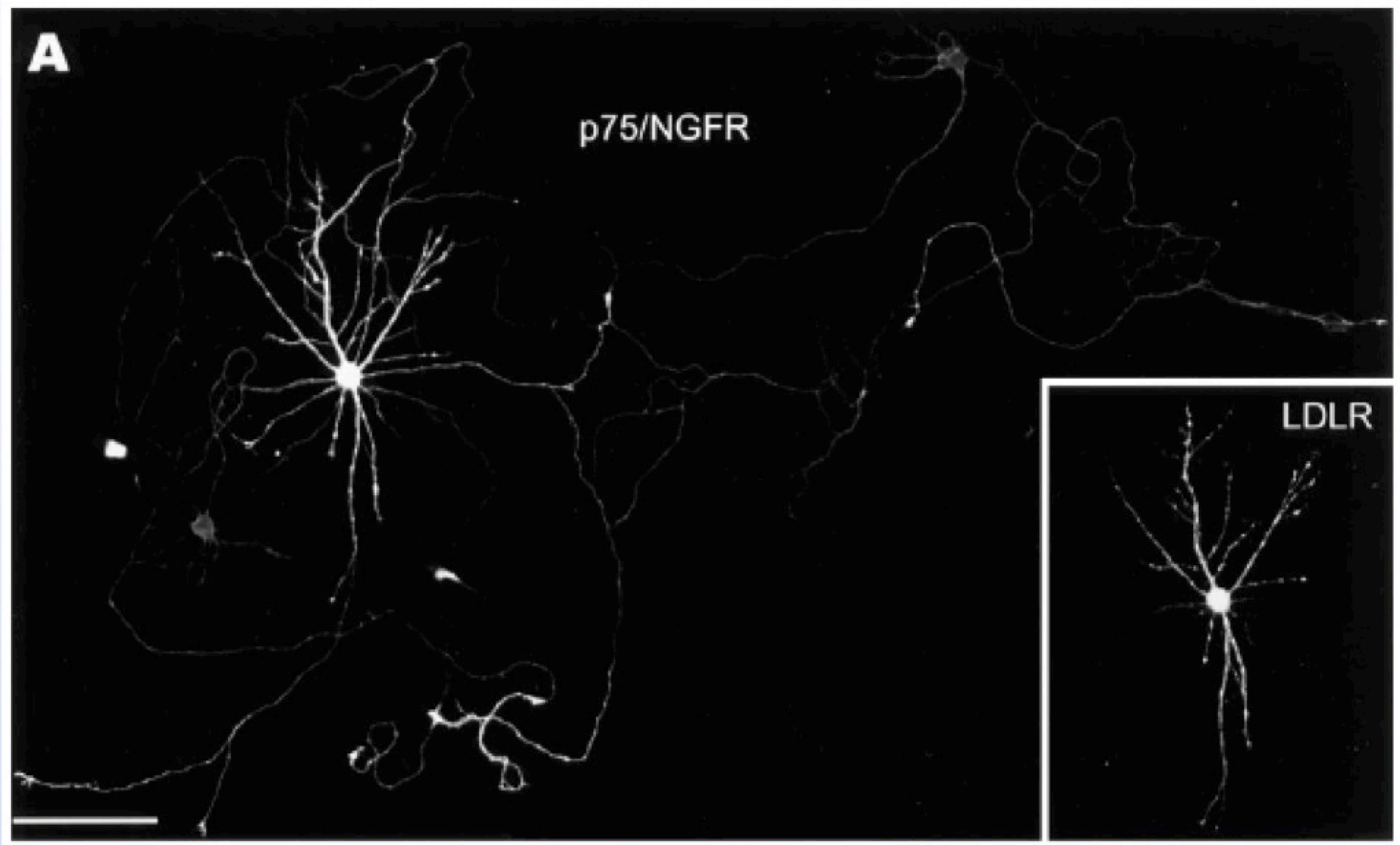
Expression dendritique + axonale

La mutation du motif basolatéral épithéliale induit une perte de localisation polarisée.

Jareb, Mark and Banker, Gary (1998). The Polarized Sorting of Membrane Proteins Expressed in Cultured Hippocampal Neurons Using Viral Vectors. *Neuron* 20:855

Contre-exemple:

Des protéines exprimées dans le pole apical des cellules épithéliales se retrouve dans les dendrites des neurones.

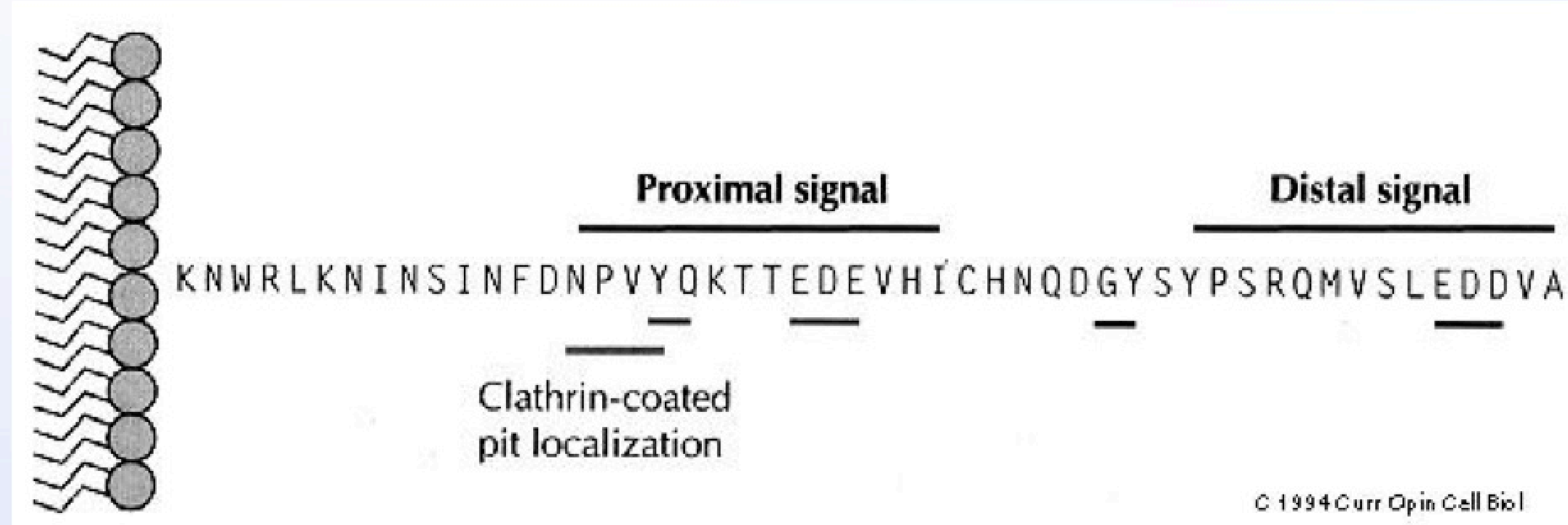


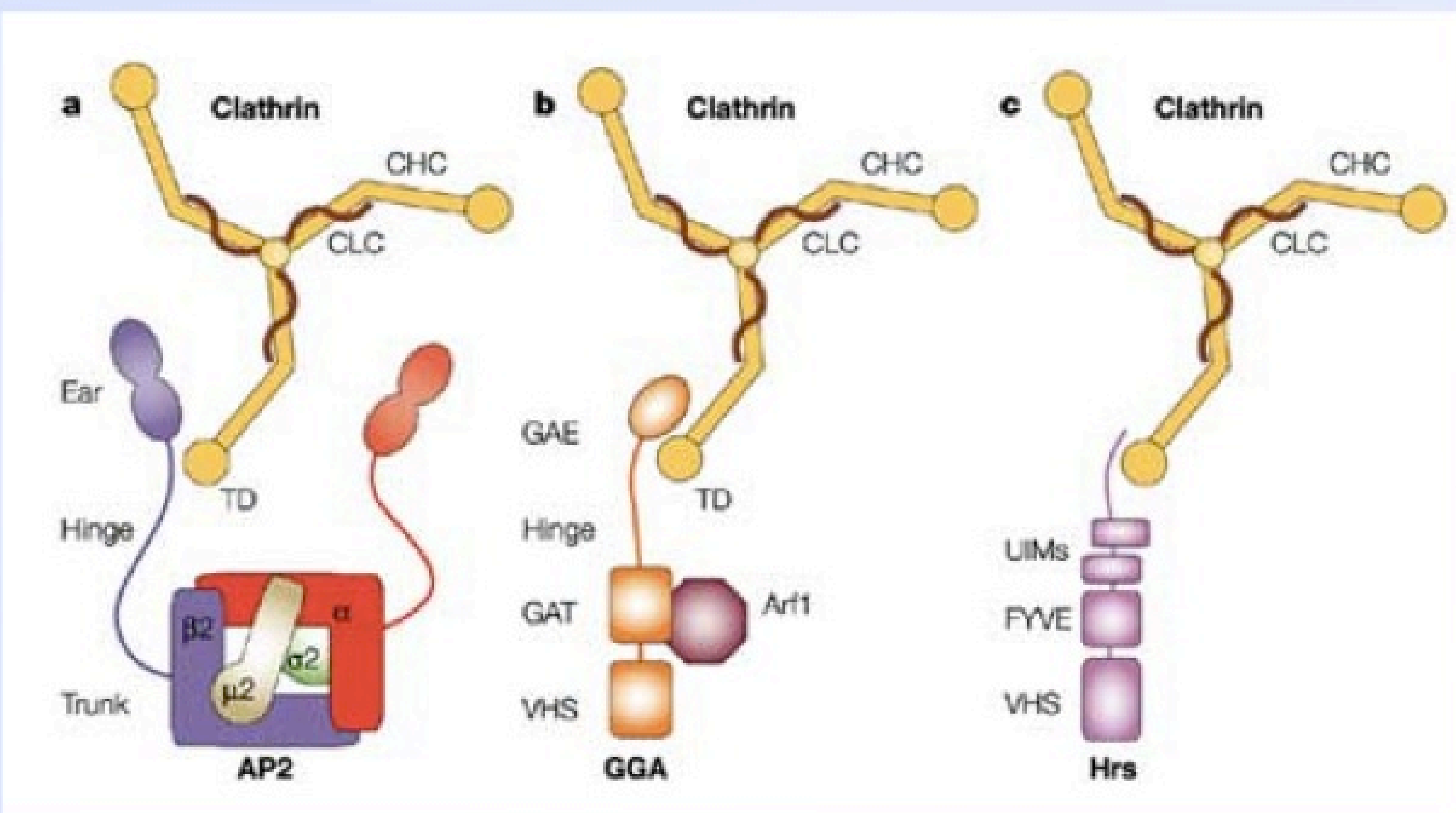
Les signaux basolatéraux semblent conservés des cellules épithéliales aux neurones,

mais les neurones possèdent des signaux axonaux propres.

Jareb, Mark and Banker, Gary (1998). The Polarized Sorting of Membrane Proteins Expressed in Cultured Hippocampal Neurons Using Viral Vectors. *Neuron* 20:855

motifs de ciblages





Major sorting signal and adaptor interactions	
SORTING SIGNAL	**BINDS TO:**
Tyr-X-X-hydrophobic (YXXØ)	μ subunits of adaptor complexes
Asn-Pro-X-Tyr (NPXY)	Other accessory proteins (ARH, Dab2)
[Asp/Glu]-X-X-X-Leu-[Leu/Ile] (Dileucine)	GGA, Others?
Monoubiquitin	Other accessory proteins (Eps 15)

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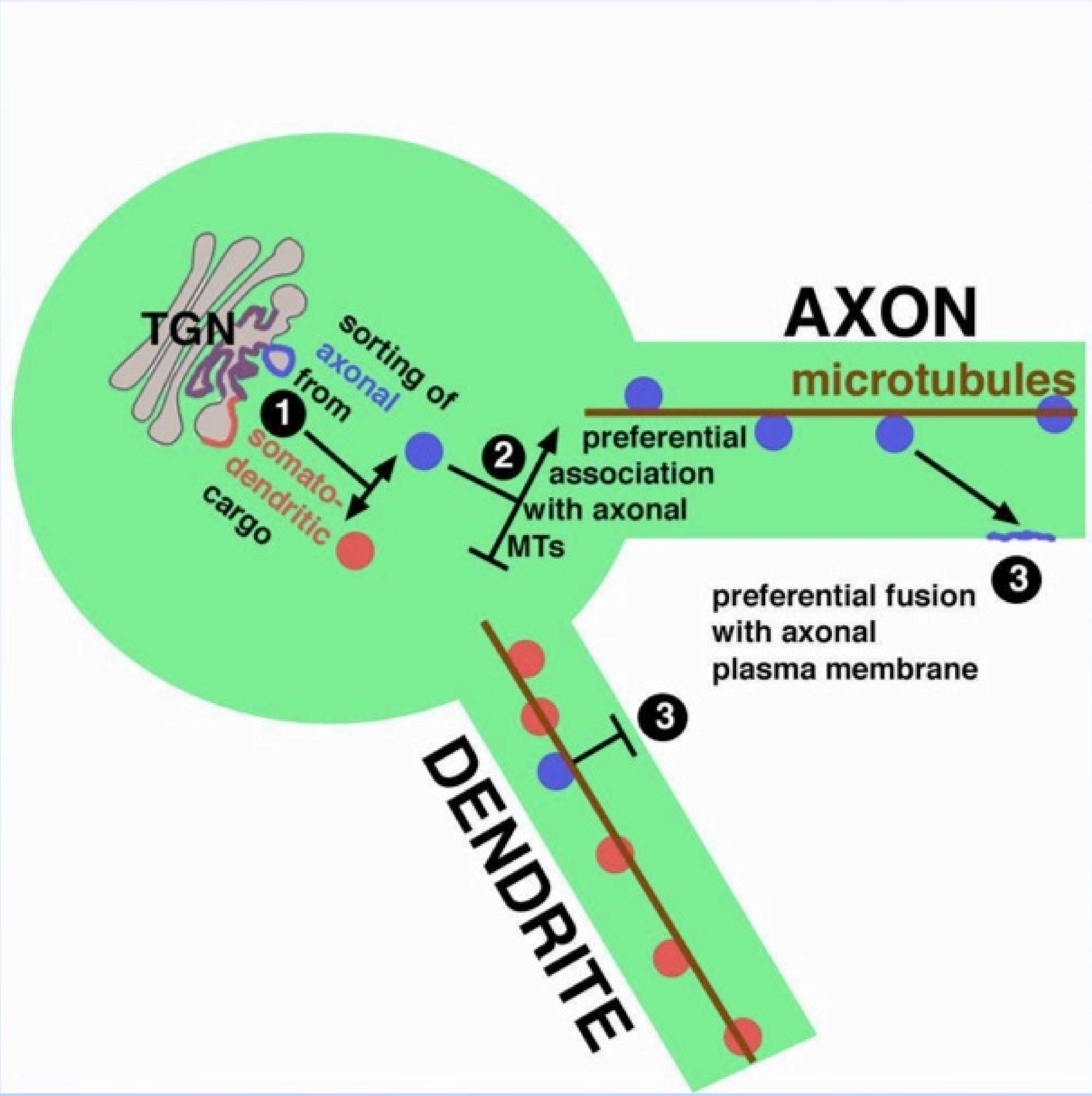
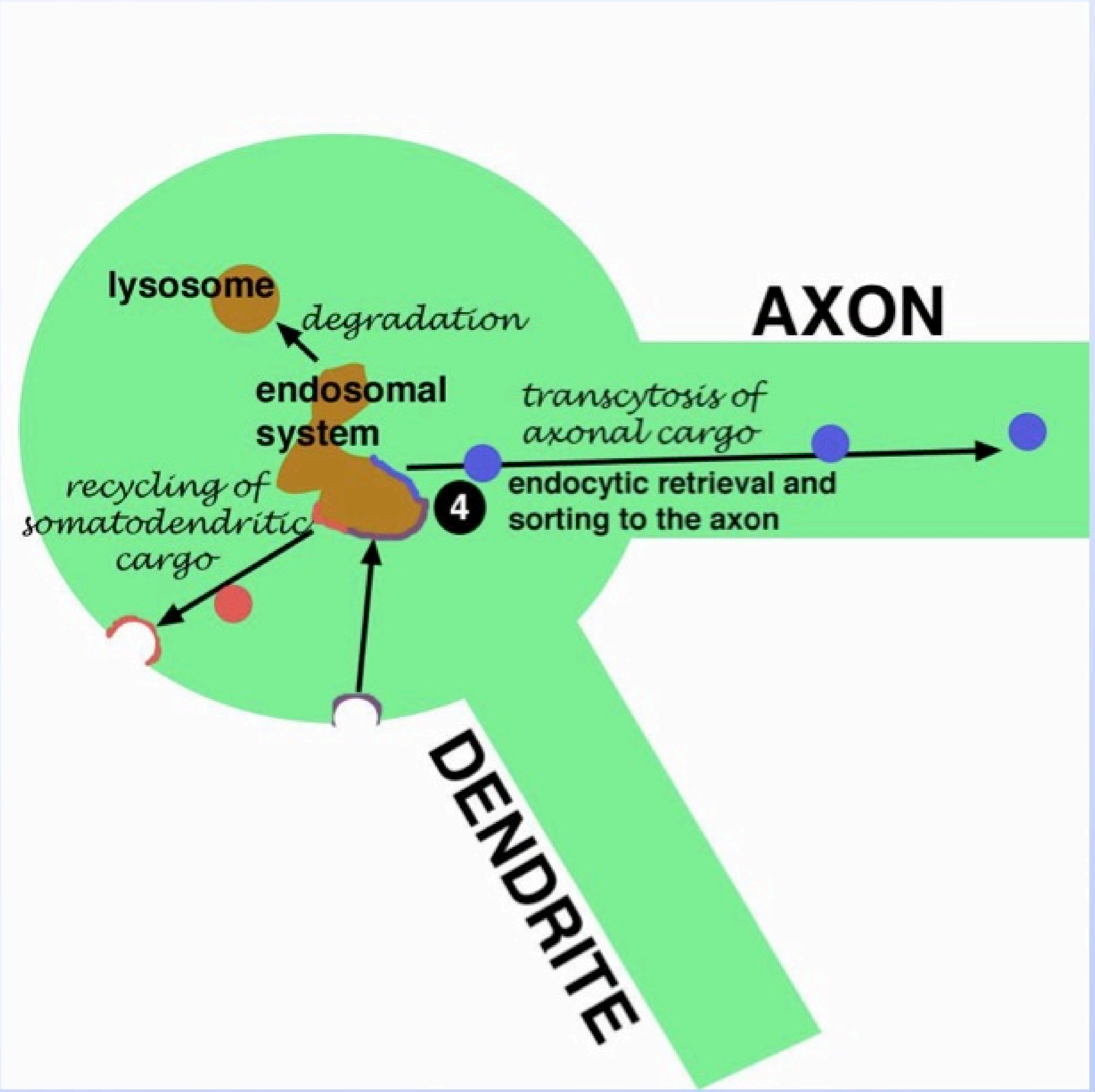
Type	Location	Representative examples
Basolateral sorting signals		
YXX¯	Cytoplasmic	Inßuenza virus hemagglutinin (Tyr mutant) Vesicular stomatitis virus glycoprotein
LL/IL	Cytoplasmic	FcRII-B2 receptor
Others	Cytoplasmic	Transferrin receptor LDL receptor distal domain Polymeric immunoglobulin receptor
Apical sorting signals		
GPI-anchor	Membrane, luminal leaßet	Placental alkaline phosphatase Thy-1
N-glycosylation	Luminal	gp-80 Erythropoietin
Transmembrane domain	Membrane	Inßuenza virus neuraminidase Inßuenza virus hemagglutinin
Late endosomes/lysosomes		
Mannose-6-phosphate	Luminal	Cathepsin B Cathepsin D
GYXX¯	Cytoplasmic	Lamp-1, Lamp-2 CD63
MHC class II compartments		
LI, ML	Cytoplasmic	Invariant chain (Ii)
Regulated secretory vesicles		
Conformation-dependent motif	Loop stabilized by one disulÞde bridge	Chromogranin B Pro-opiomelanocortin
Selective aggregation	Luminal	

Sequences are given in the single letter amino acid code; ¯ is a b ulky hydrophobic amino acid.

Transport sélectif ou rétention ?

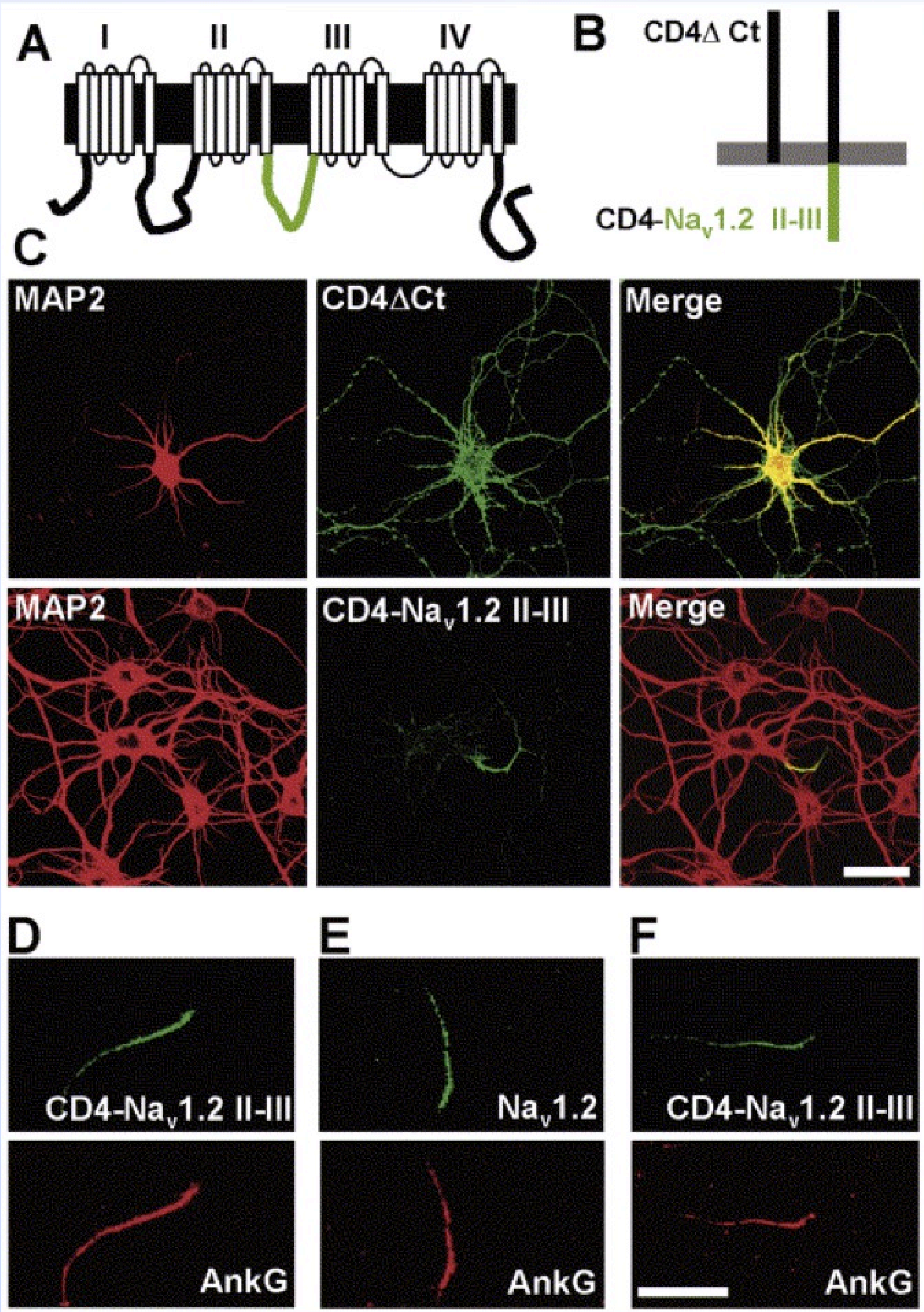


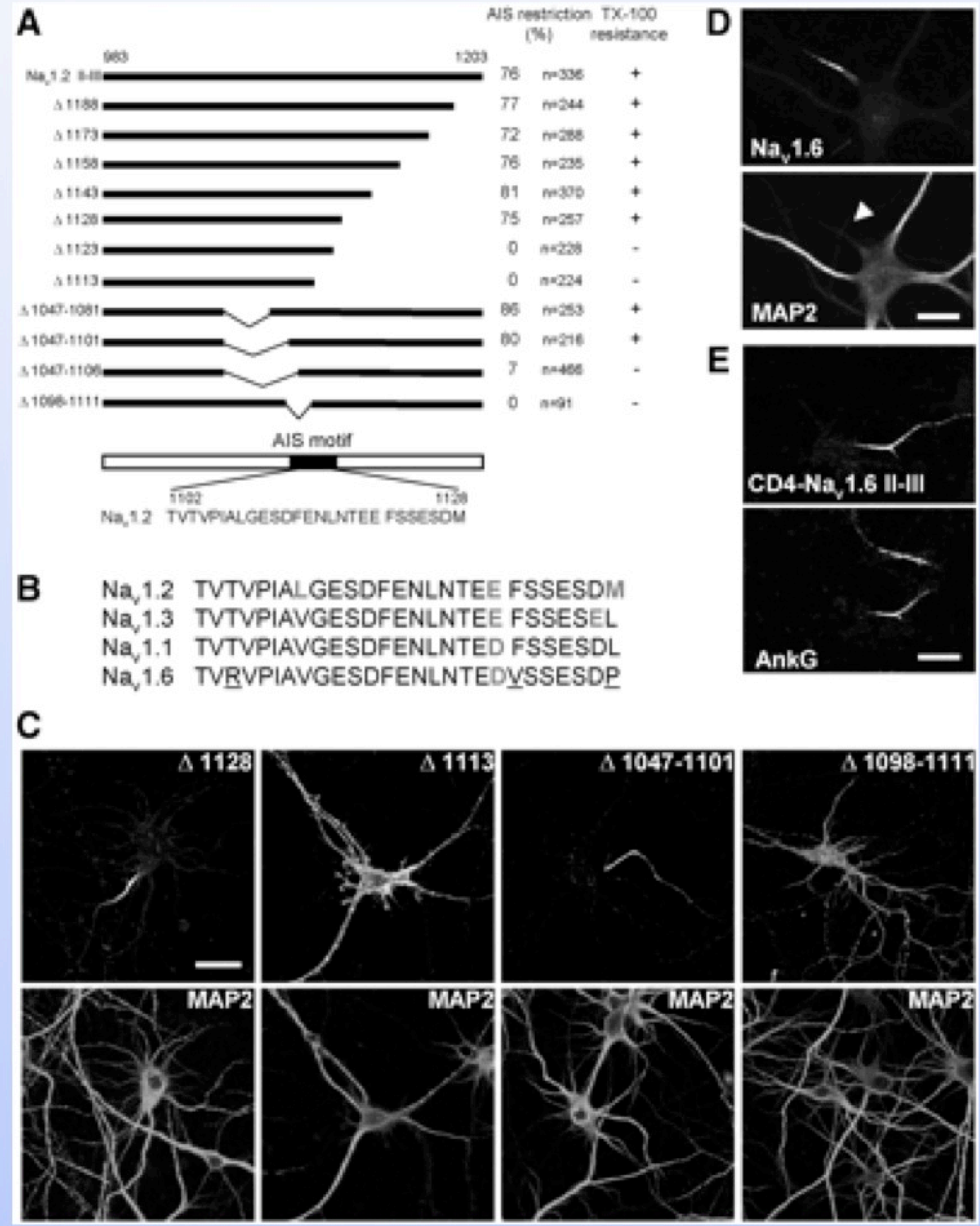


RTf

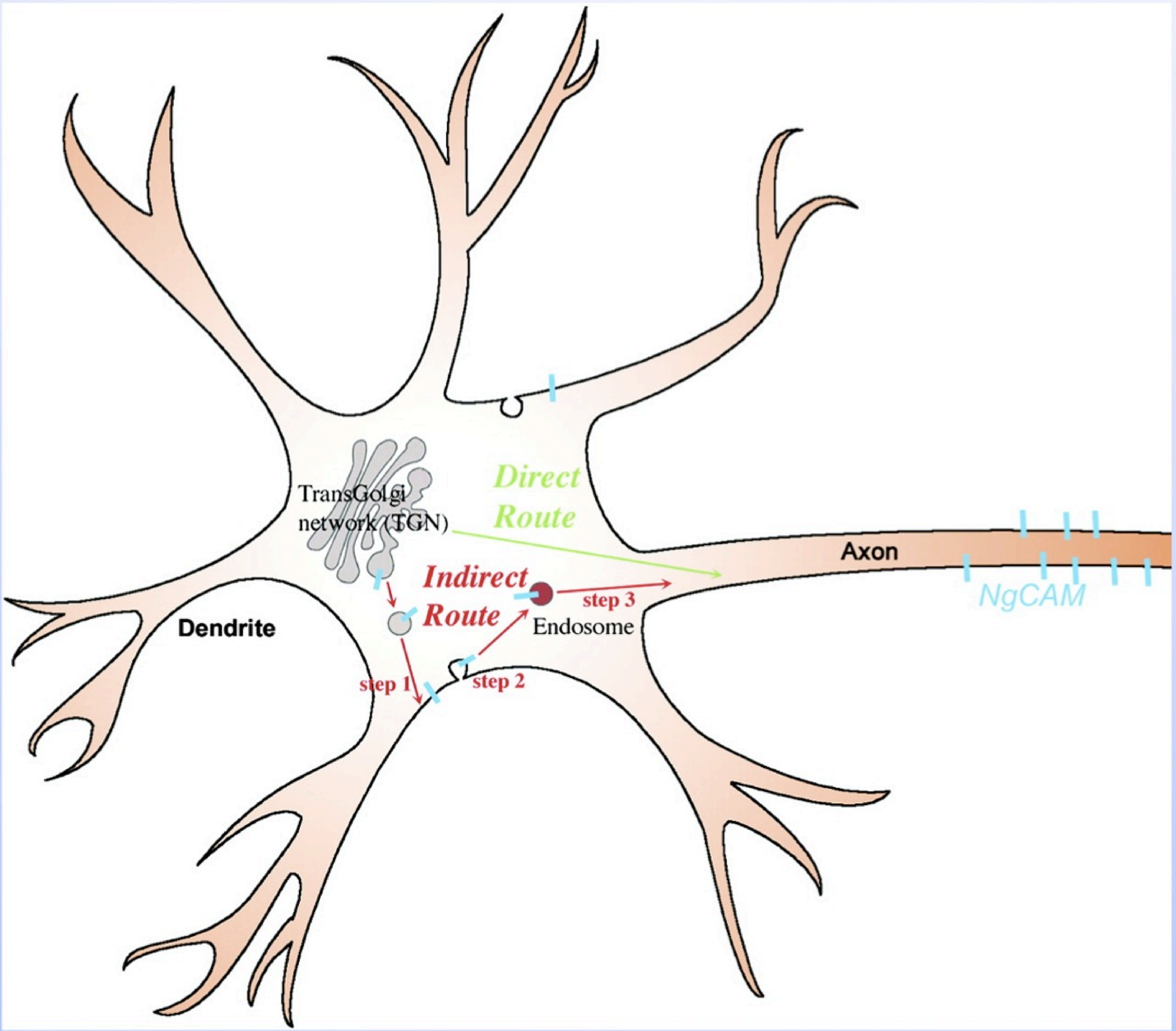
NgCAM dendritique
puis NgCAM axonal

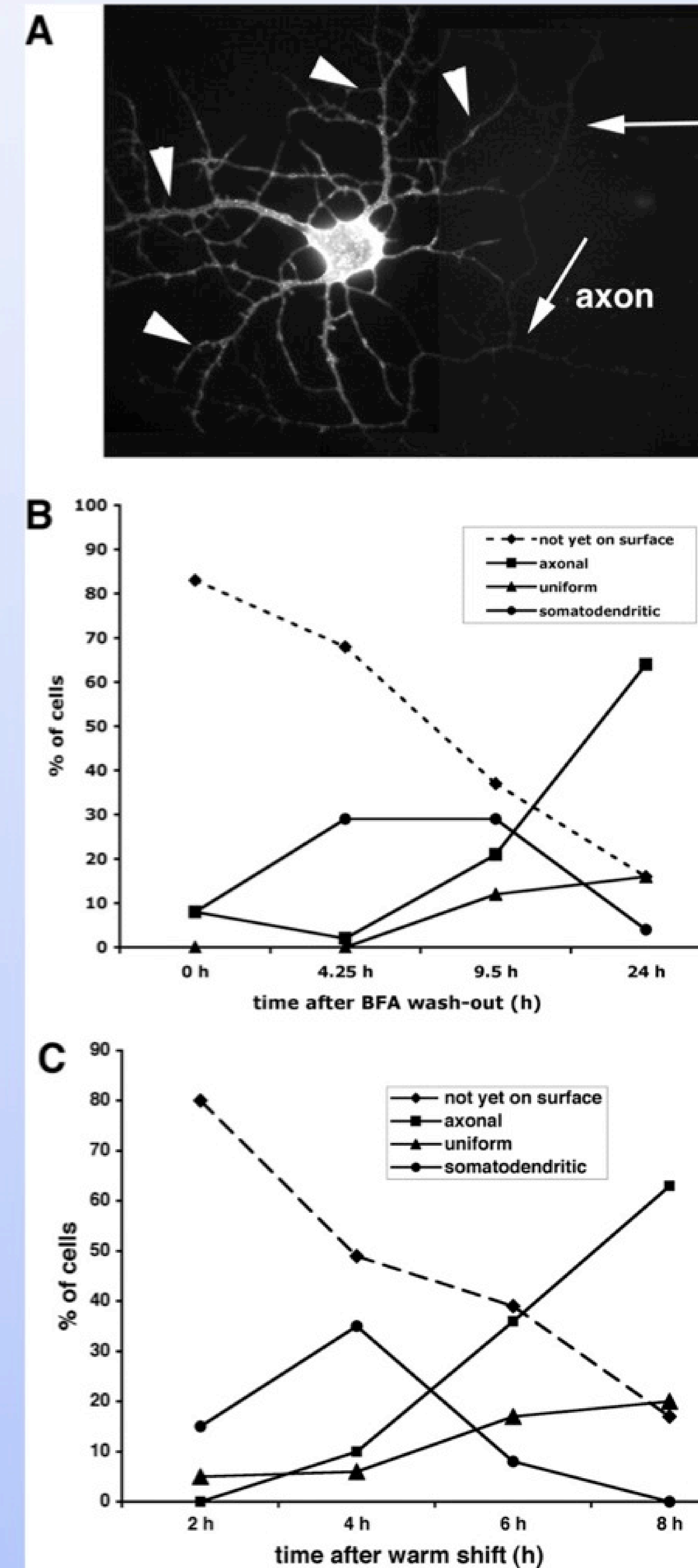
Example de signal: pour rejoindre le segment initial de l'axone (AIS) par l'équipe de B. Dargent & coll.



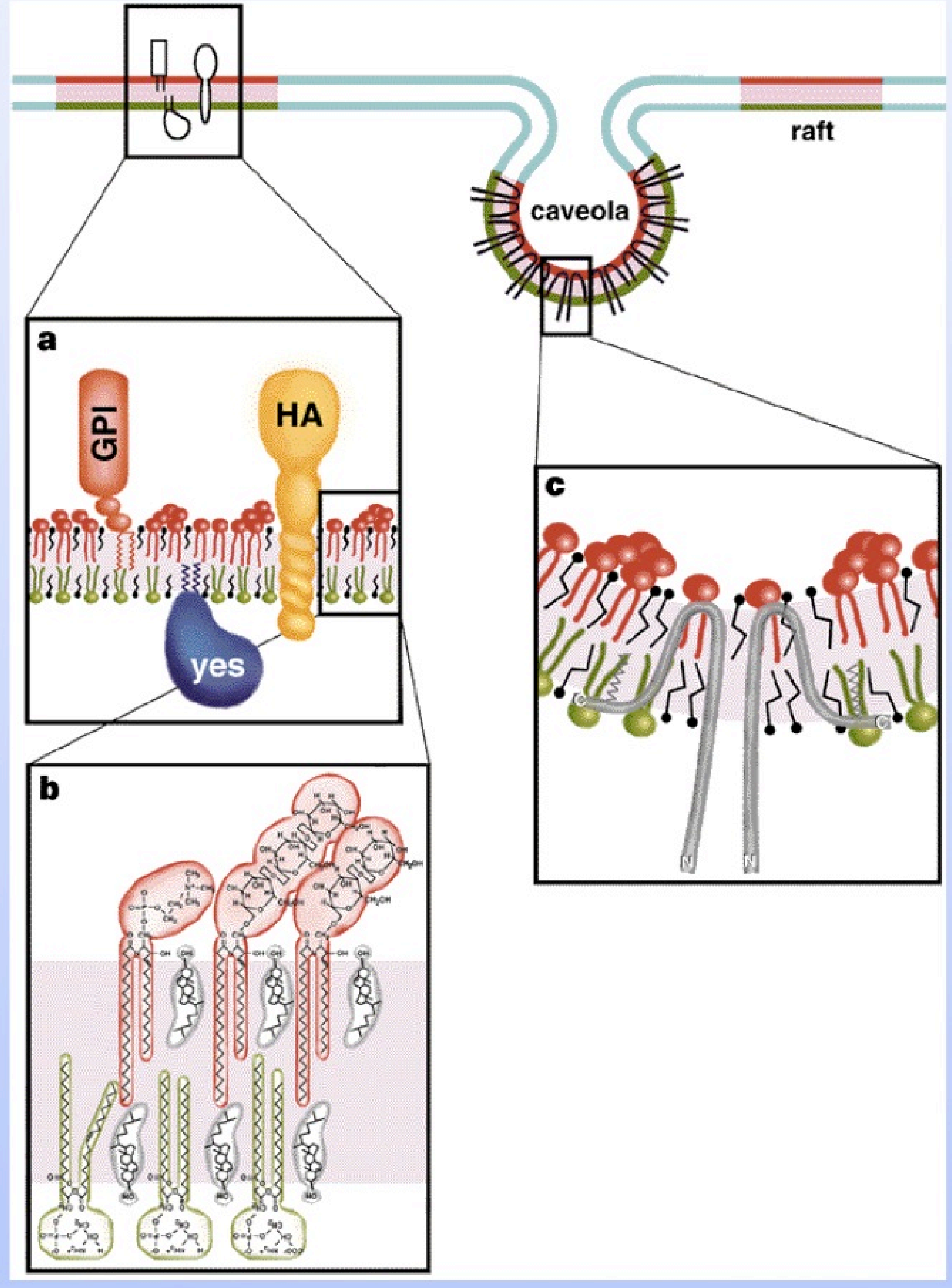


Transcytosis of NgCAM in neurons

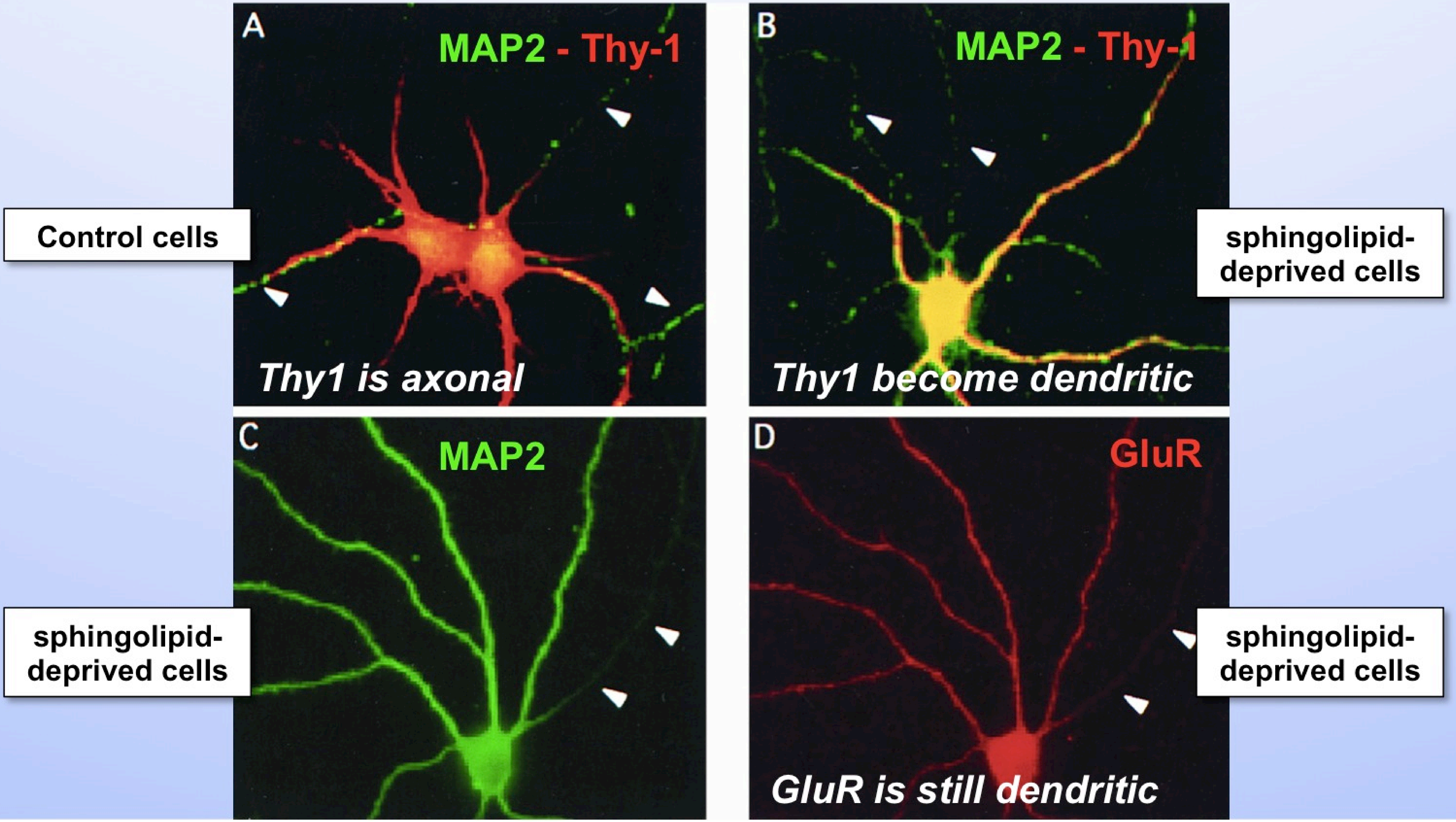




Raft: axonal signals?



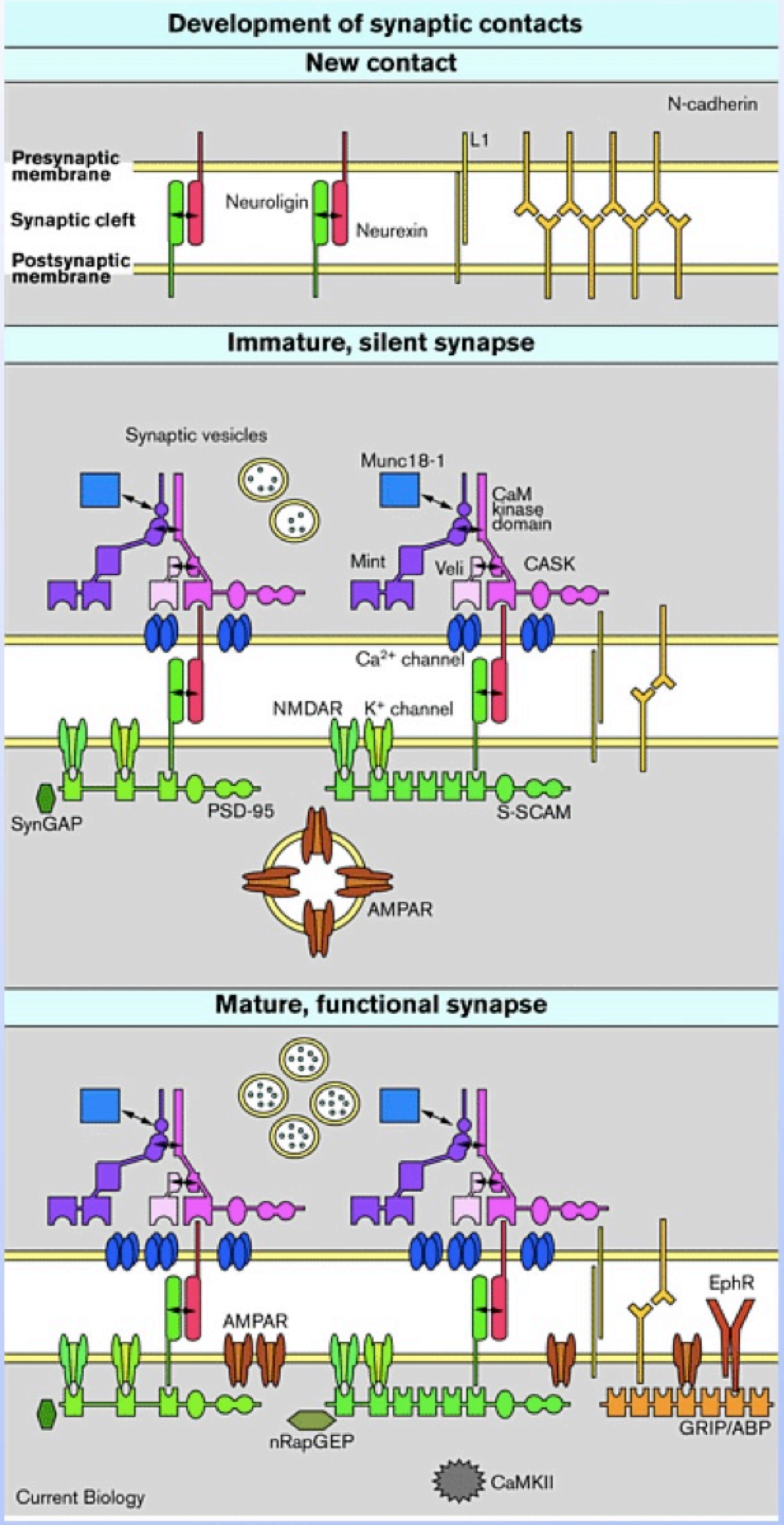
Missorting of the axonal Thy-1 but not of a dendritic membrane protein occurred in sphingolipid-deprived cells. These results indicate that neurons sort a subset of axolemmal proteins by a mechanism that requires the formation of protein-lipid rafts. The involvement of rafts in axonal membrane sorting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



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

PNAS

8. Formation of the synapse



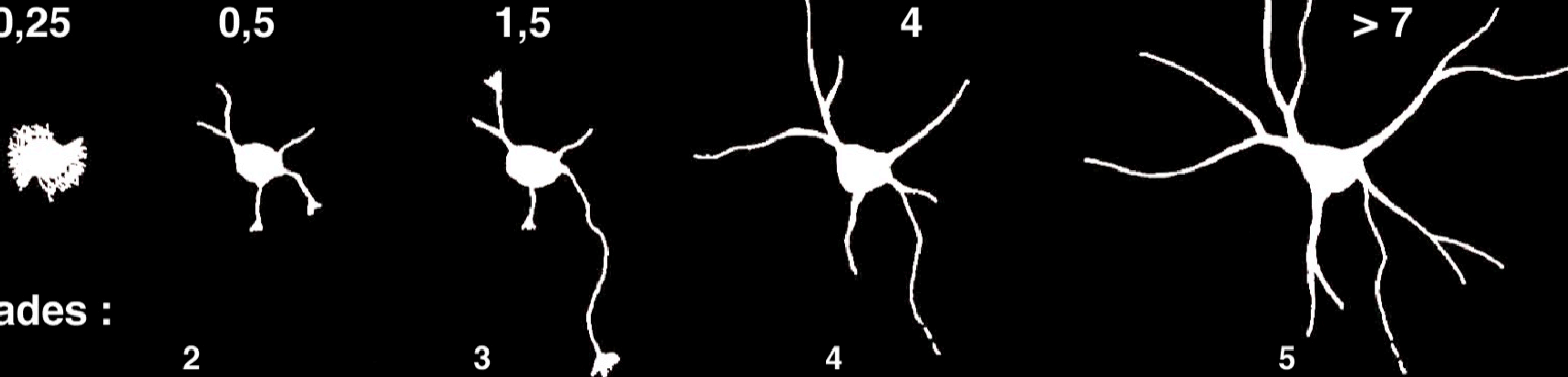
Axonal outgrowth and polarisation

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**



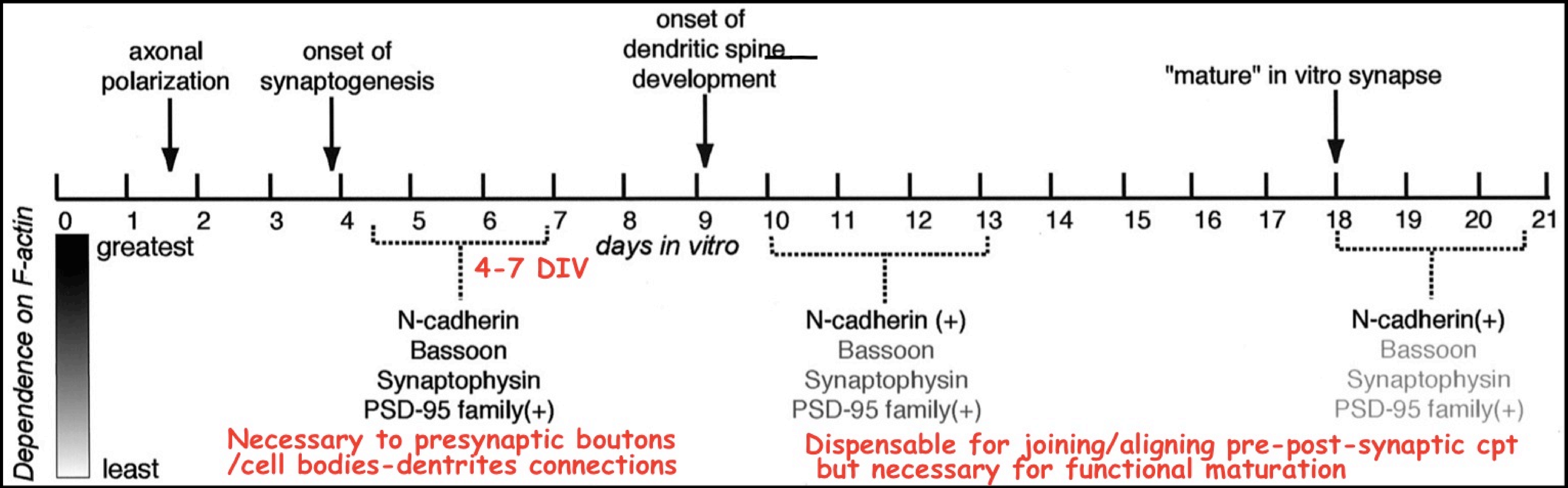
Stades :

1 Lamelipodes
2 Neurites mineurs
3 Croissance axonale
4 Croissance dendritique
5 Maturation

DIV 18-21

Phase contrast microscopy (Goslin et al., 1977)

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

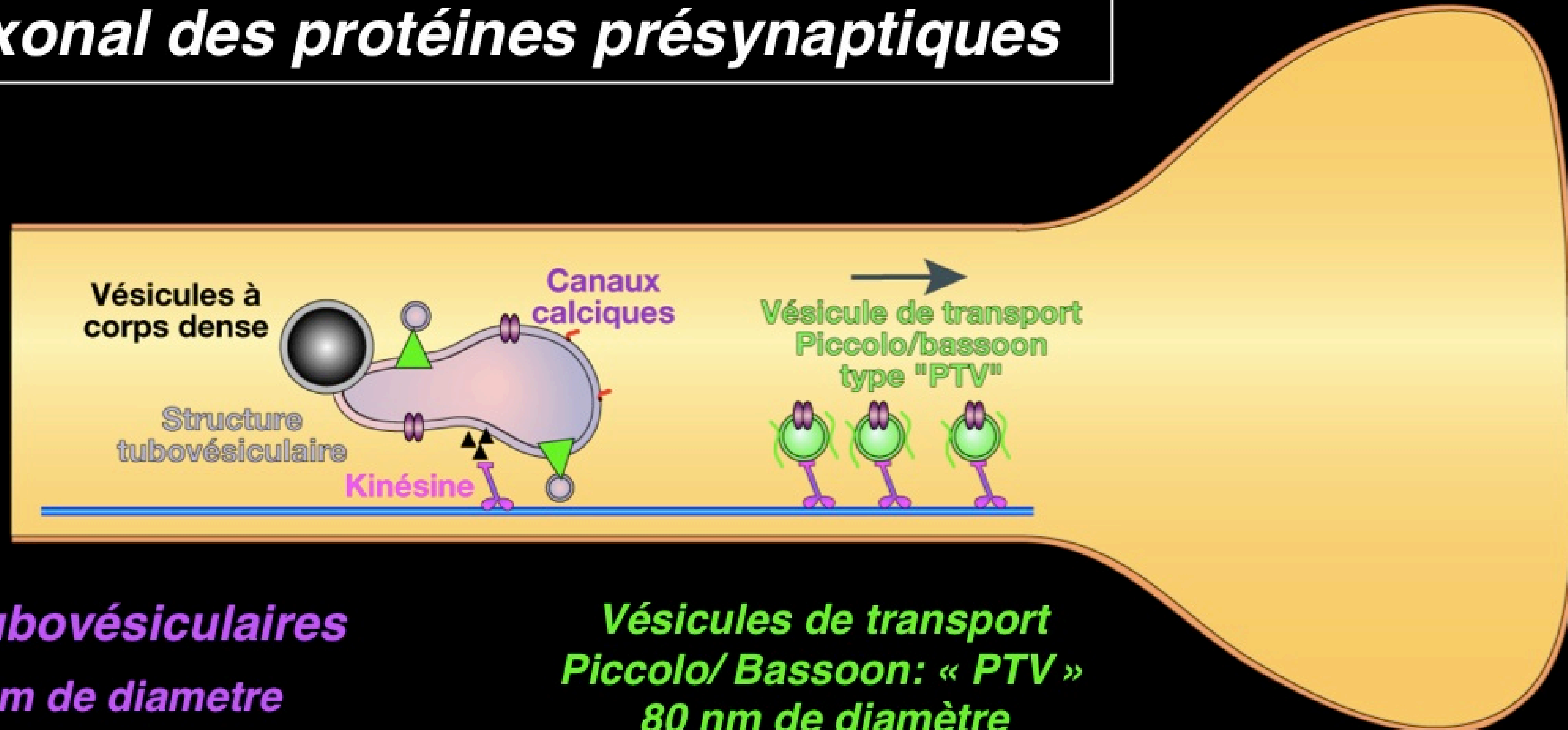


a role in directing pre- and postsynaptic specializations ?

a role in dentritic spine morphology ?

J. Neurosci. 2001;21:6991

Transport axonal des protéines présynaptiques



Structures tubovésiculaires

500-1500 nm de diametre

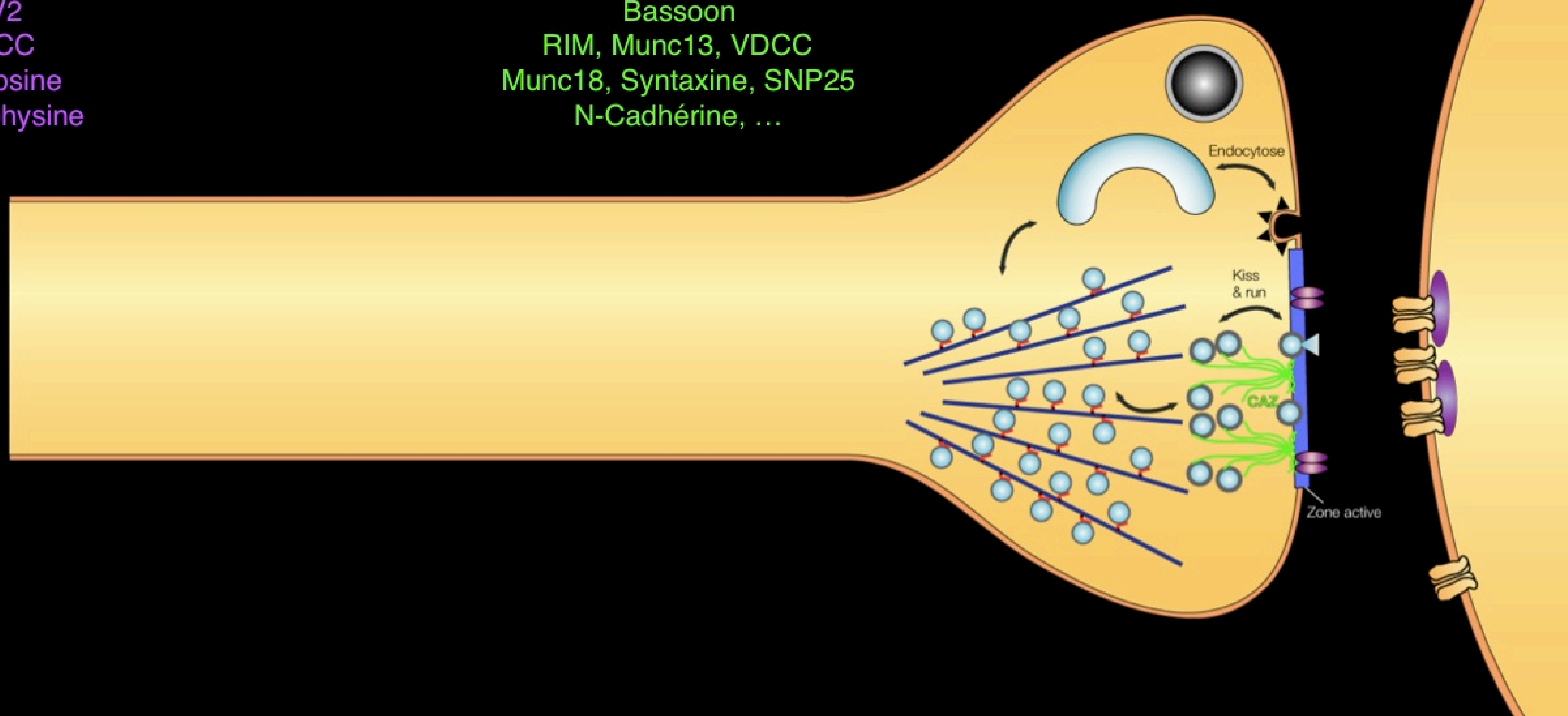
Ahmari et coll., Nat Neur, 2000.

VAMP
SV2
VDCC
Synapsine
amphiphysine

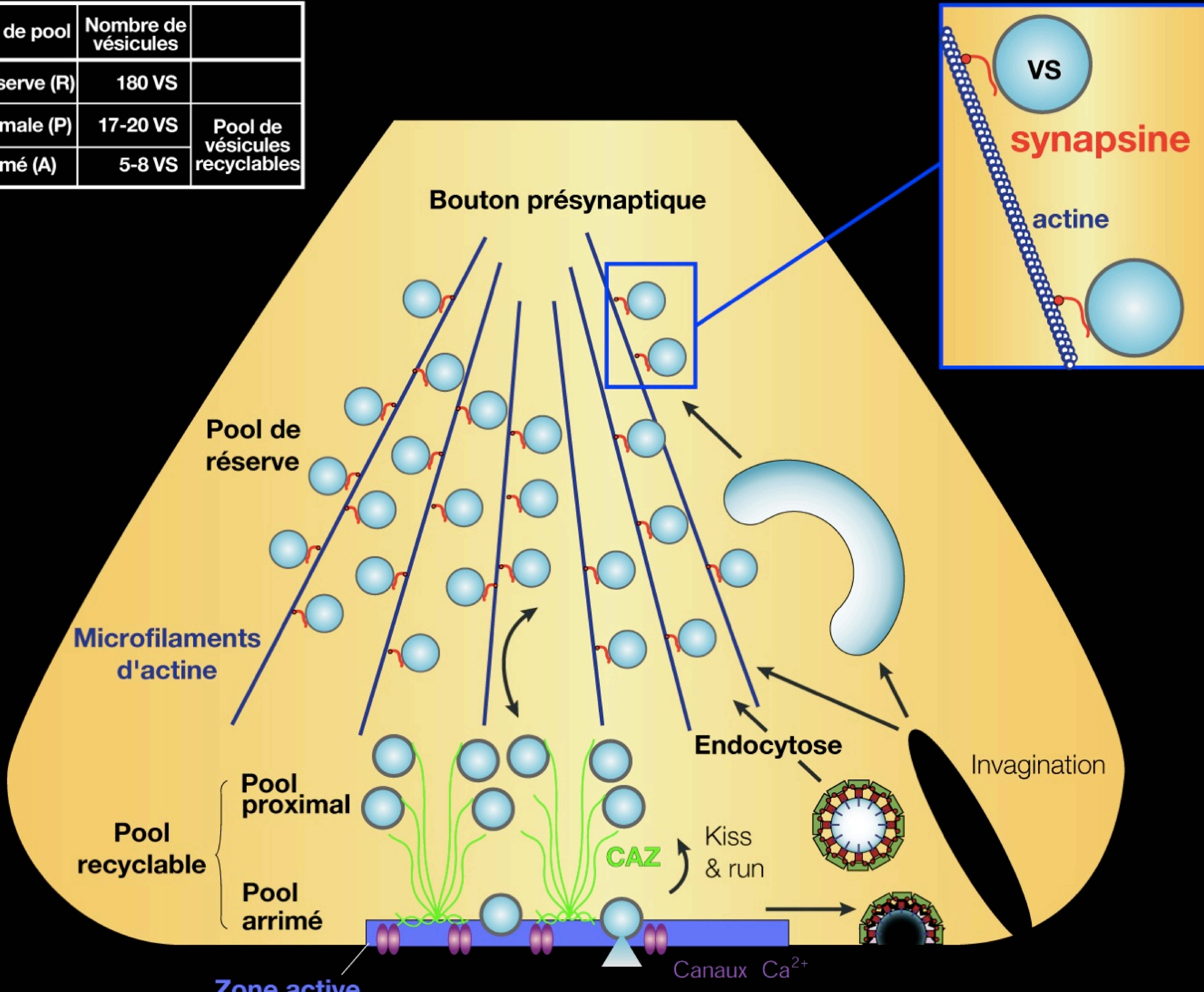
Vésicules de transport Piccolo/ Bassoon: « PTV »
80 nm de diamètre

Zhai et al., Neuron, 2001

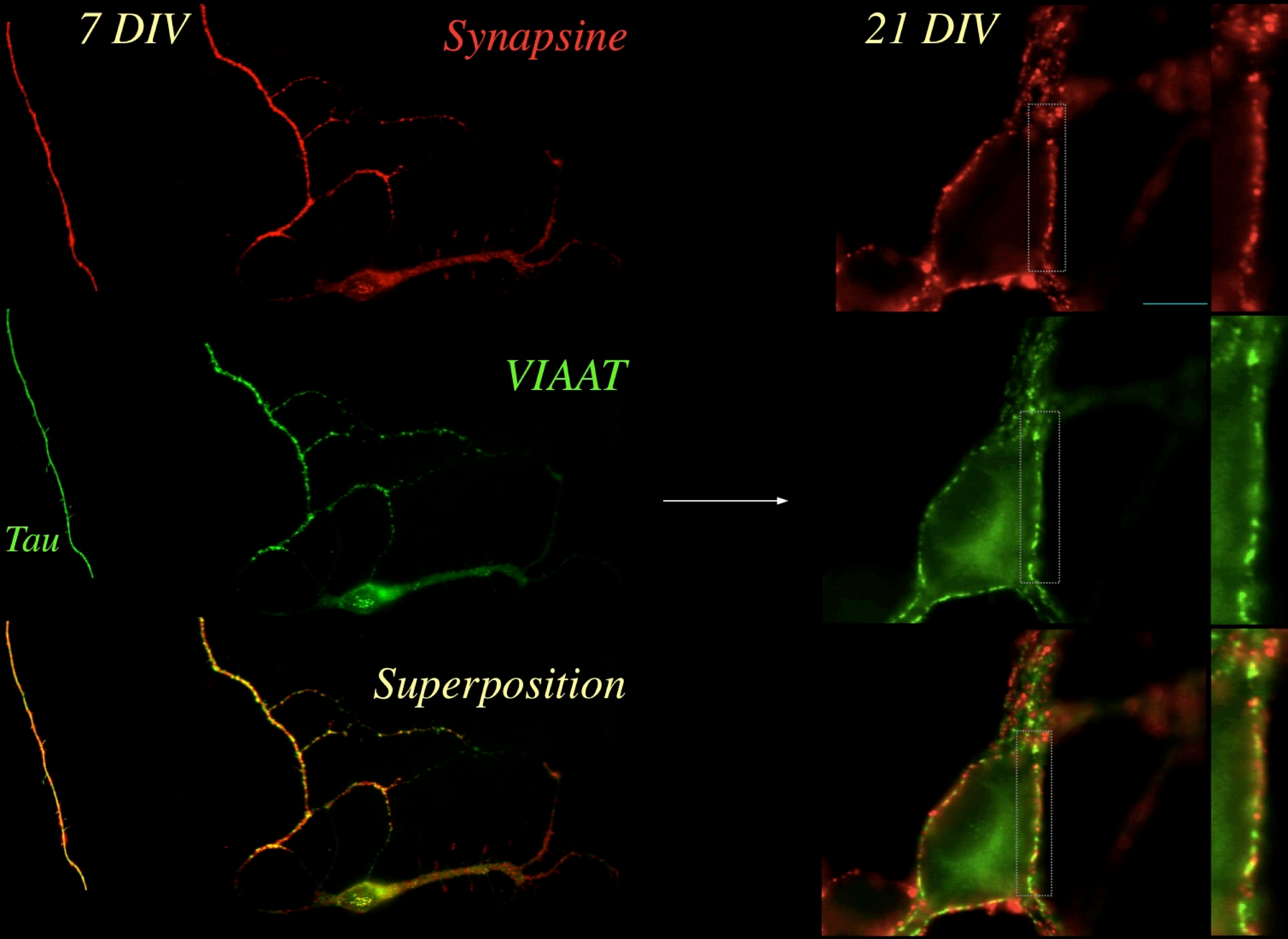
Piccolo
Bassoon
RIM, Munc13, VDCC
Munc18, Syntaxine, SNP25
N-Cadhérine, …



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.



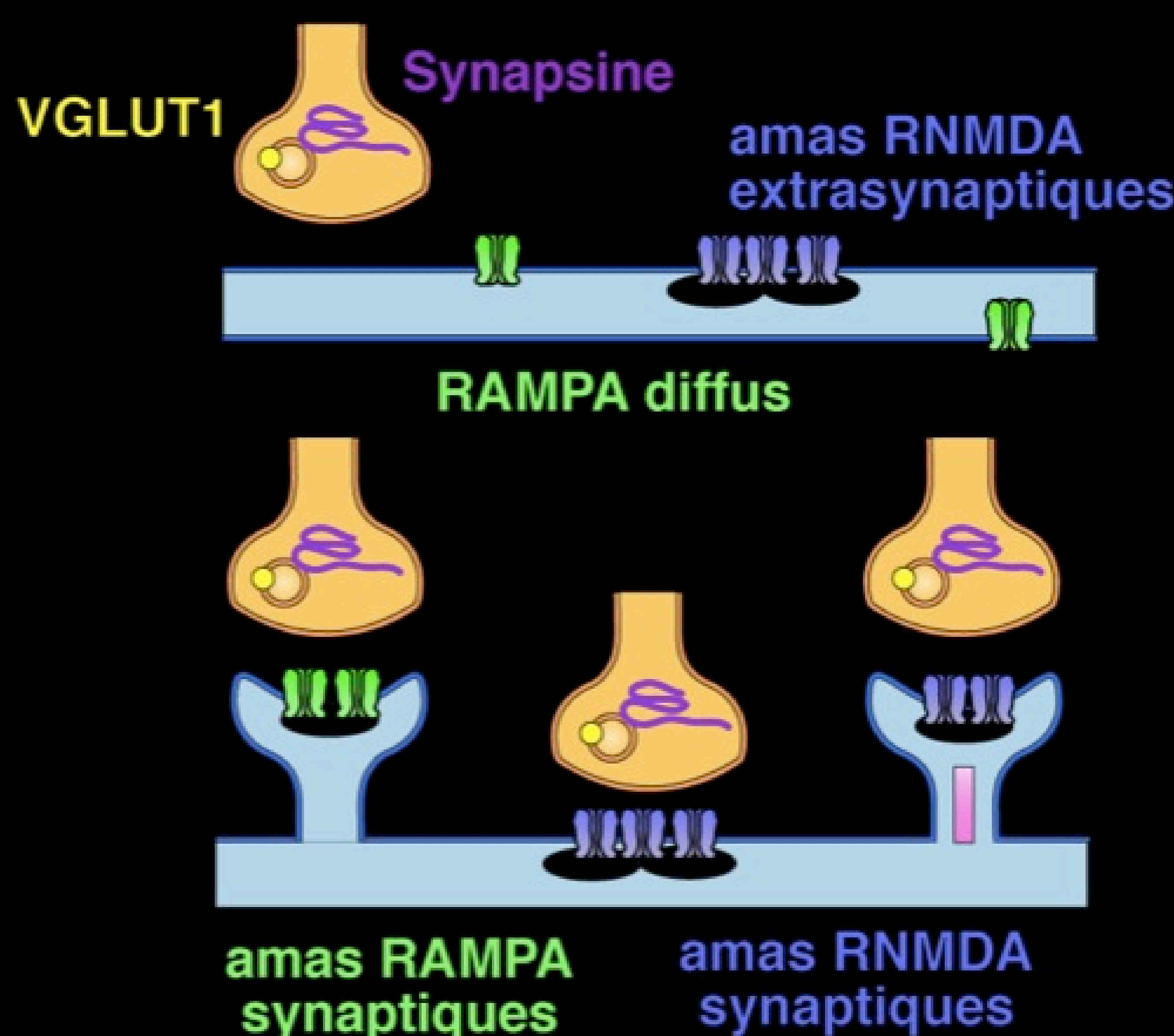
VIAAT: Expression diffuse dans l'axone puis formation d'amas dès 10 jours de culture.

Ciblage des protéines post-synaptiques

Synapse excitatrice hippocampe

Laboratoire A.M. Craig

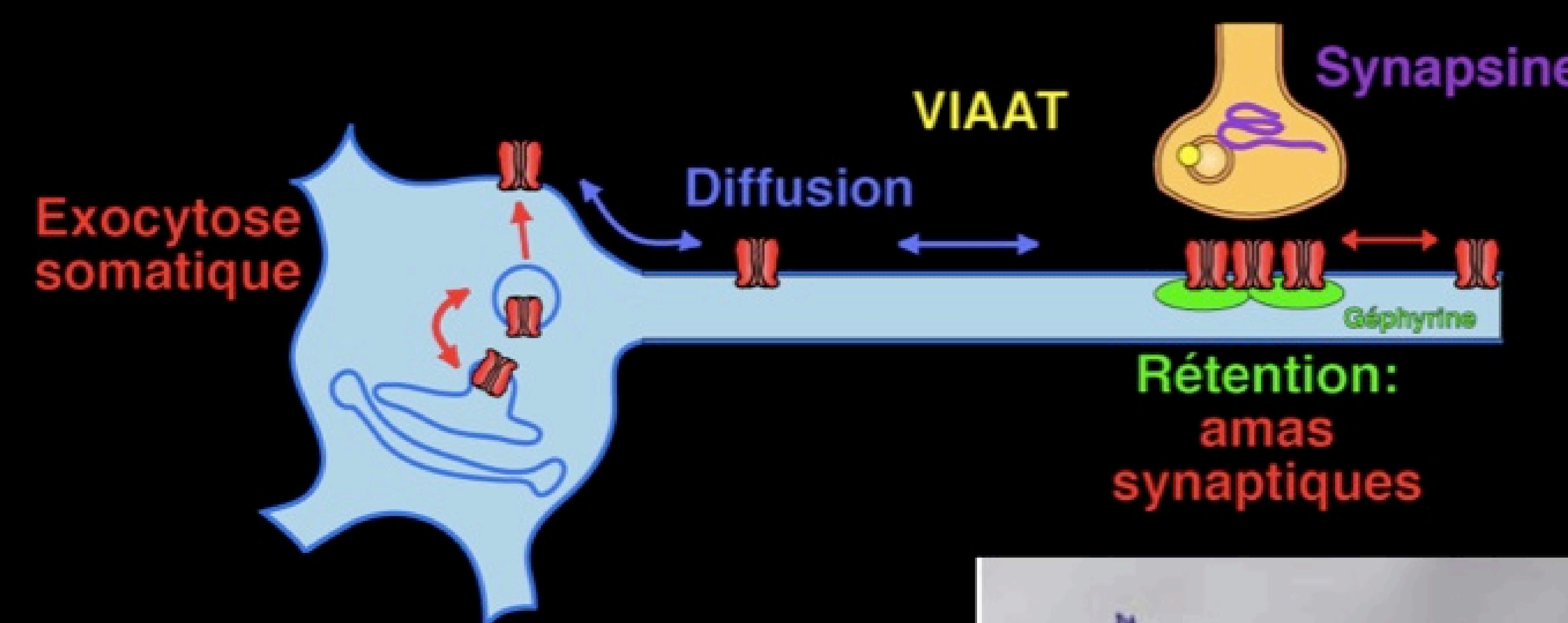
Rao et al., J. Neurosci., 2000
Rao et al., J. Neurosci., 2000

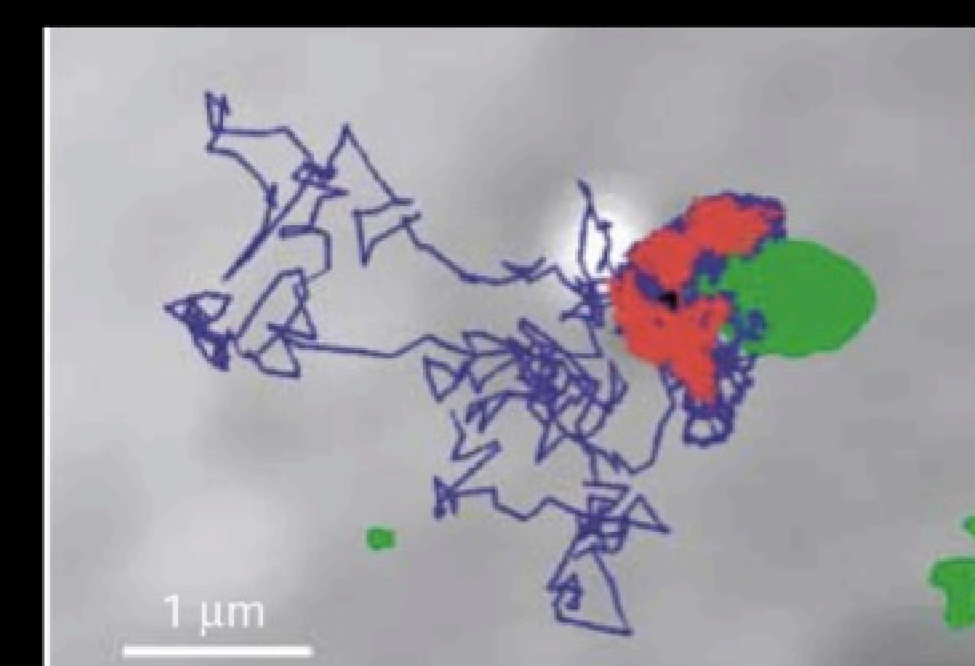


Synapse inhibitrice moelle épinière

Laboratoire A.Triller

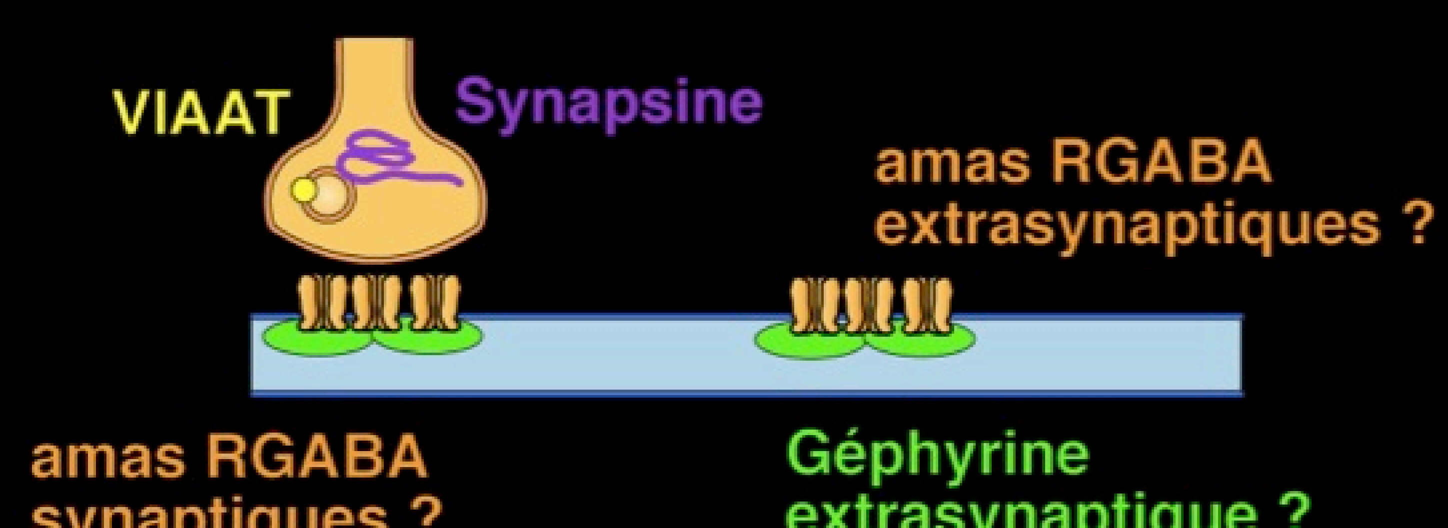
Rosenberg et al., J. Neurosci, 2000
Meier et al, JCS, 2000
Meier et al., Nat. Neur., 2001





Synapse inhibitrice hippocampe

?



Importance de l'établissement de 2 compartiments distincts:

Axonal <> Somatodendritique

- Passage stade 2 à 3
- Instabilité de l'actine
- Assemblage des microtubules
- Activation de la voie PI3K-AKT-GSK3b-CRMP2
- Différents moyens d'atteindre le compartiment: transport sélectif, ou tri par endocytose
- Formation de contacts polarisés: les synapses….

Si questions ultérieures: lydia.danglot@inserm.fr