MCDB 153

Neural Development

Axonal Outgrowth and Guidance
Lecture Set 4

Where have we been so far and where are we going?

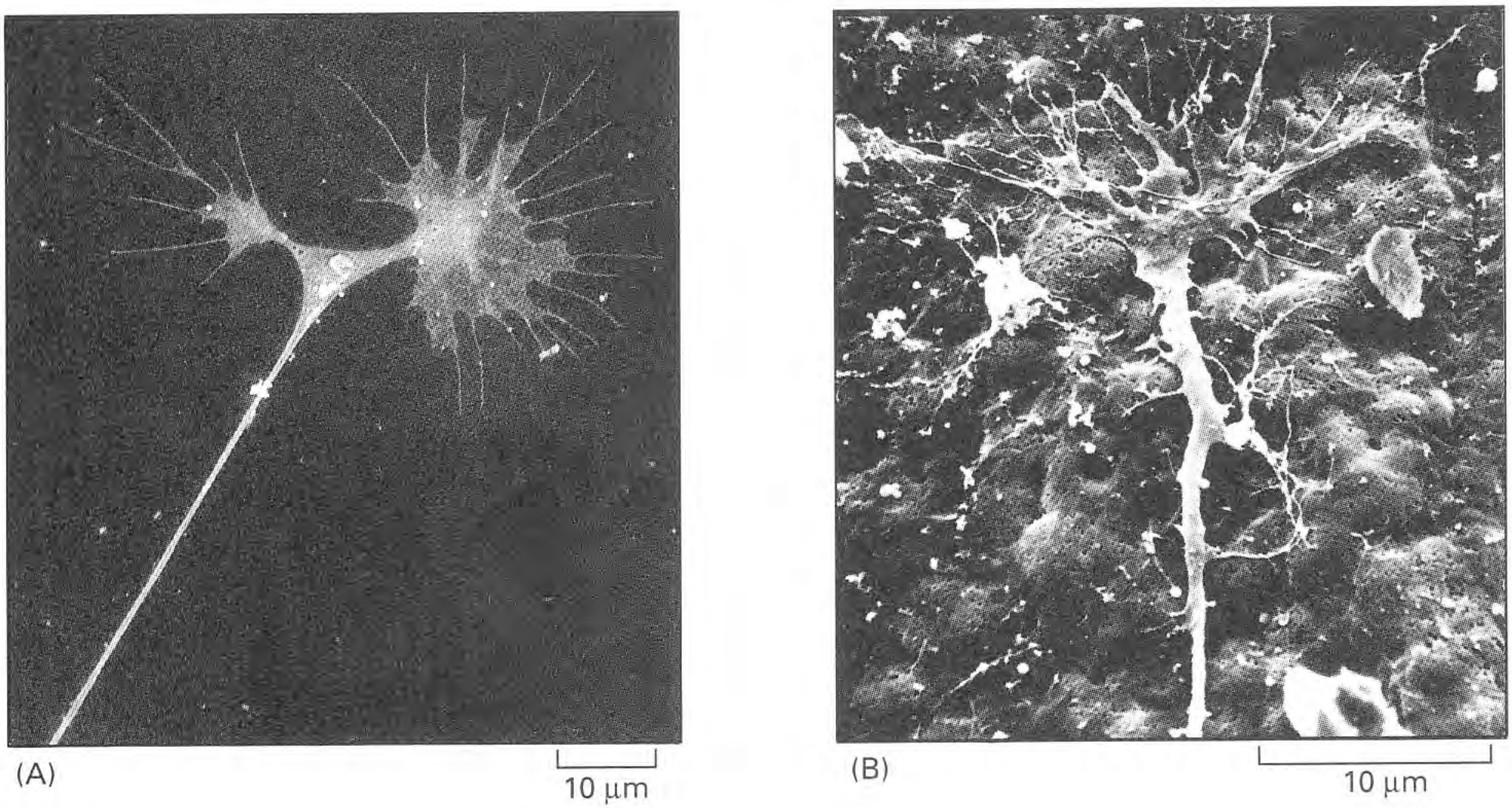
Fertilization --> Blastula --> Gastrula --> Neurula

- Neuroblast Proliferation
- Neuroblast Differentiation

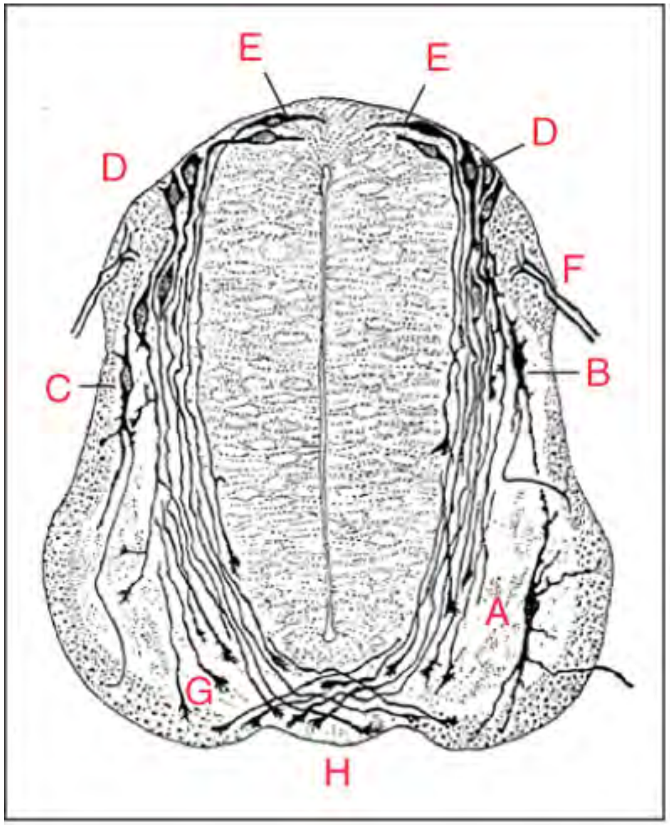
- Neuroblast Migration
  - Navigation
  - Mechanical Movement
- Axon Outgrowth
  - Navigation
  - Mechanical Movement

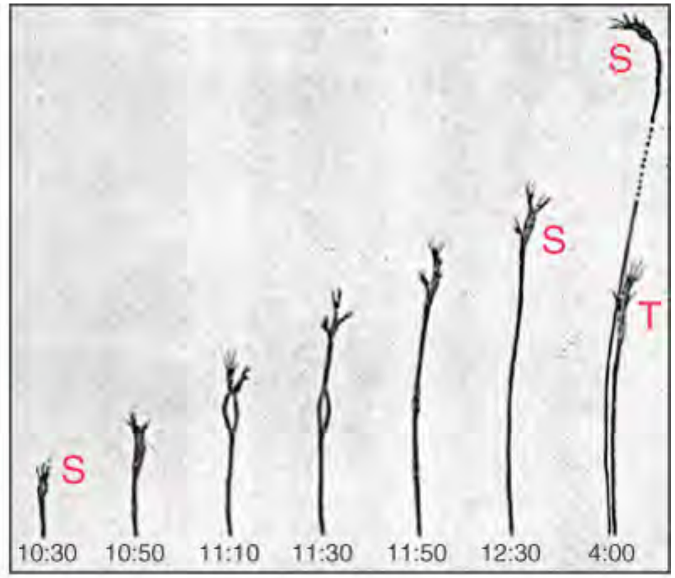
- Synaptogenesis
- Programmed Cell Death
- Synaptic Rearrangements
- Active Cell Maintenance
- Neurodegeneration

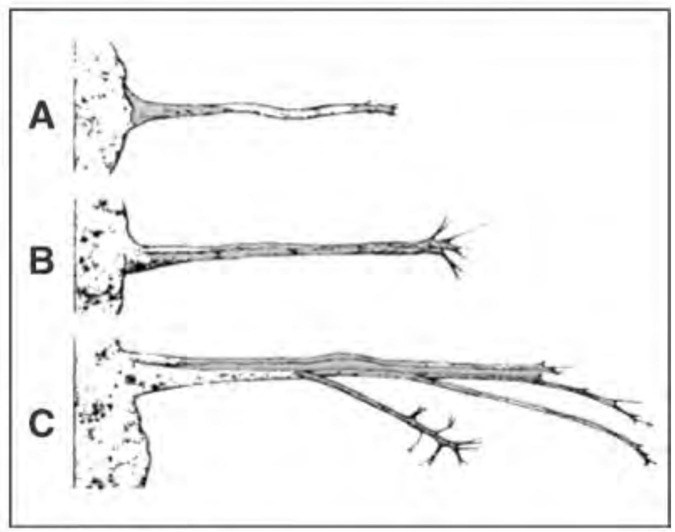
Growth Cones



Early drawings by Ramon y Cajal (A), Speidel (B) and Harrison[C]

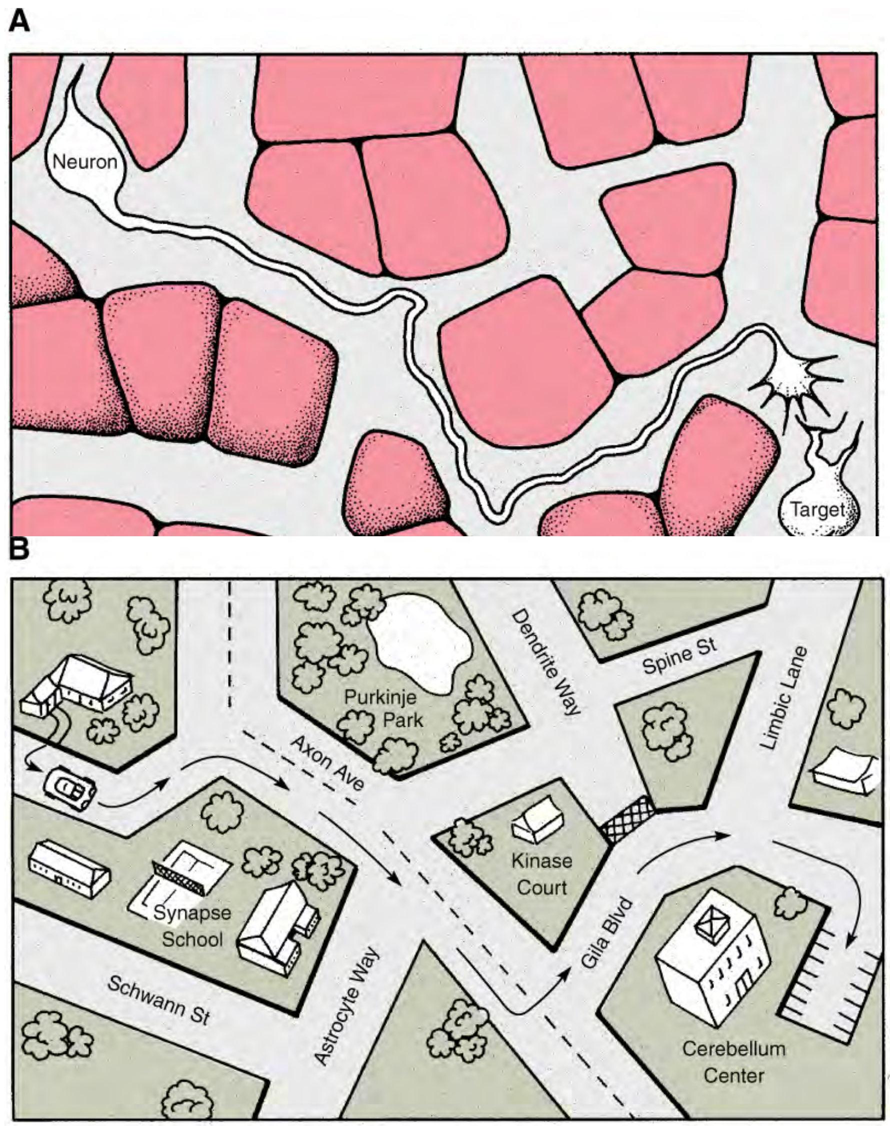






(From Ramon y Cajal, 1890; Harrison, 1910; Speidel, 1941)

Cell Migration/Axonal Outgrowth and Pathfinding/Navigation: What is the engine and what are the road signs?



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Cell/Growth Cone Movement involves reaching forward, grabbing the surface, pulling and reaching forward again - tension is generated

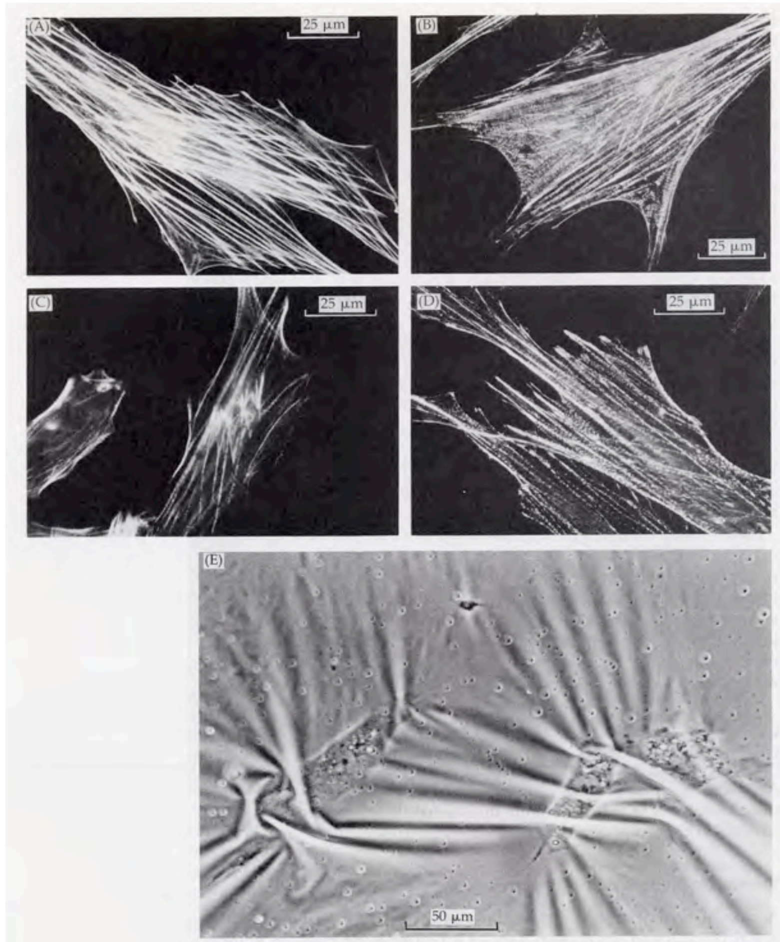
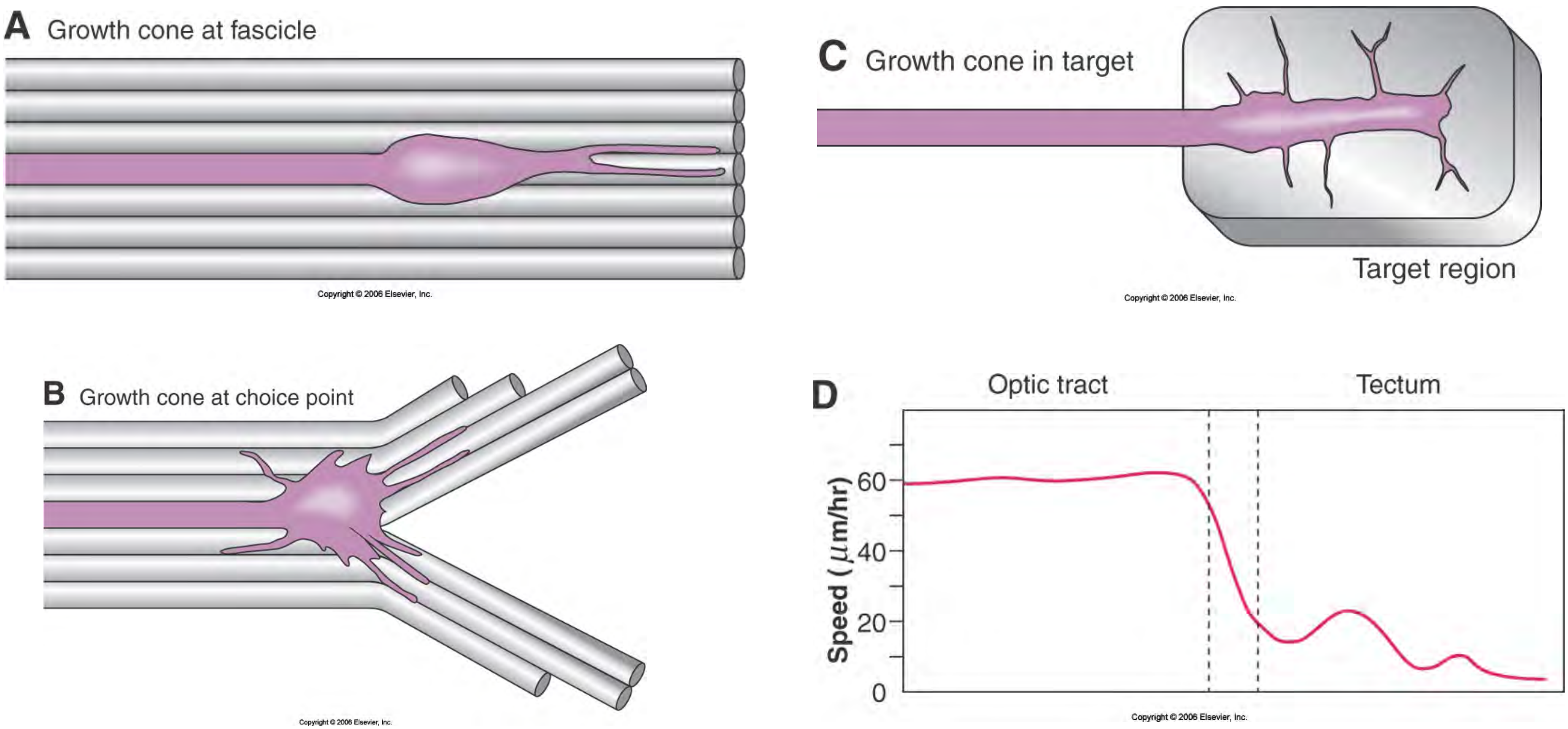
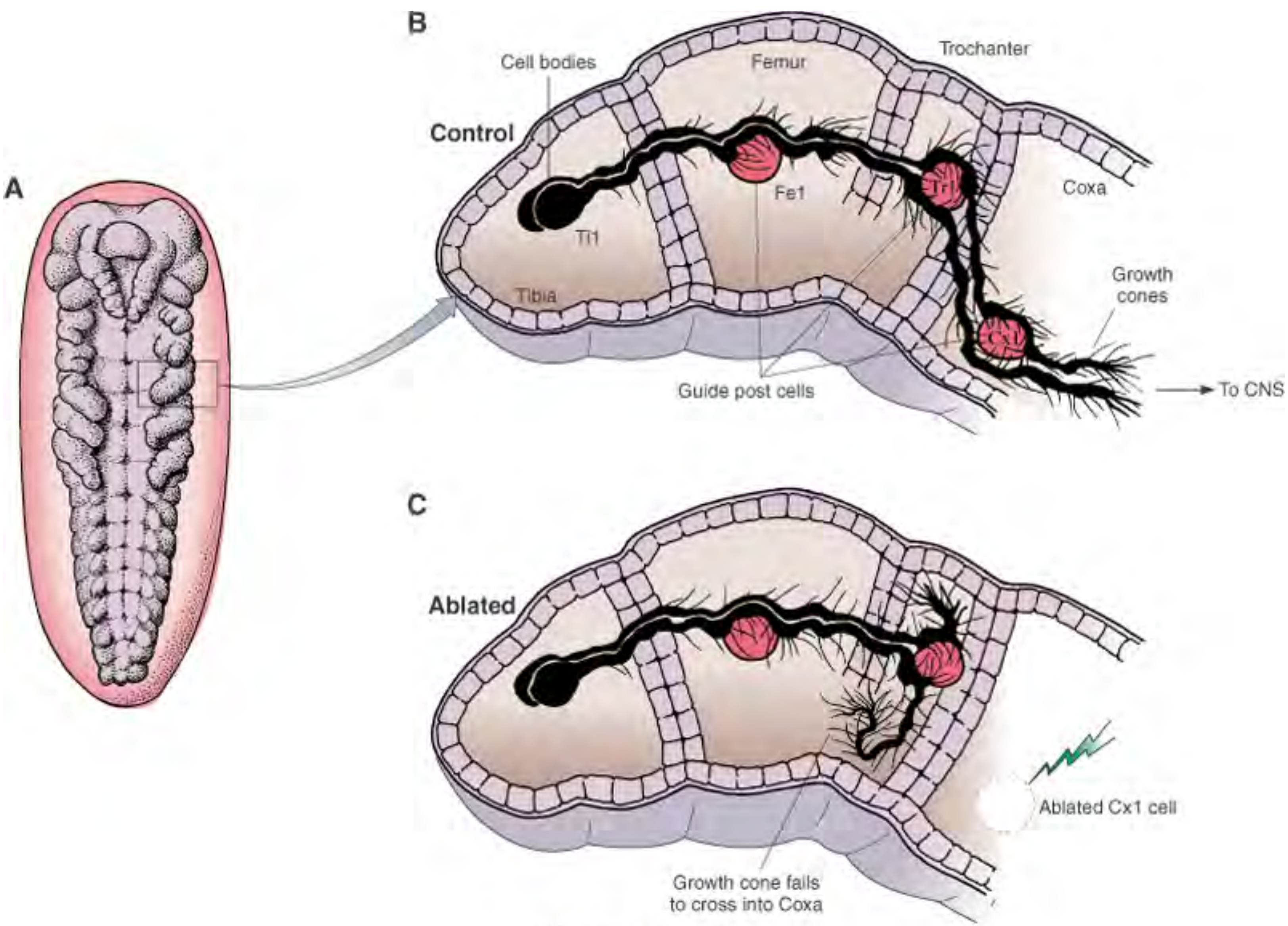


FIGURE 2. *Cultured fibroblasts stained with fluorescent antibodies to various contractile proteins. (A) Actin. (B) Myosin. (C) Tropomyosin. (D) α-Actinin. (E) Fibroblasts cultured on a thin film of silicone rubber demonstrate directly the traction generated during cell movement; note the wrinkles produced in the rubber film. (A–D from Abercrombie, 1982; courtesy of M. Osborne; E from Harris, 1982.)*

Different shapes and speeds of growth cones in different environments

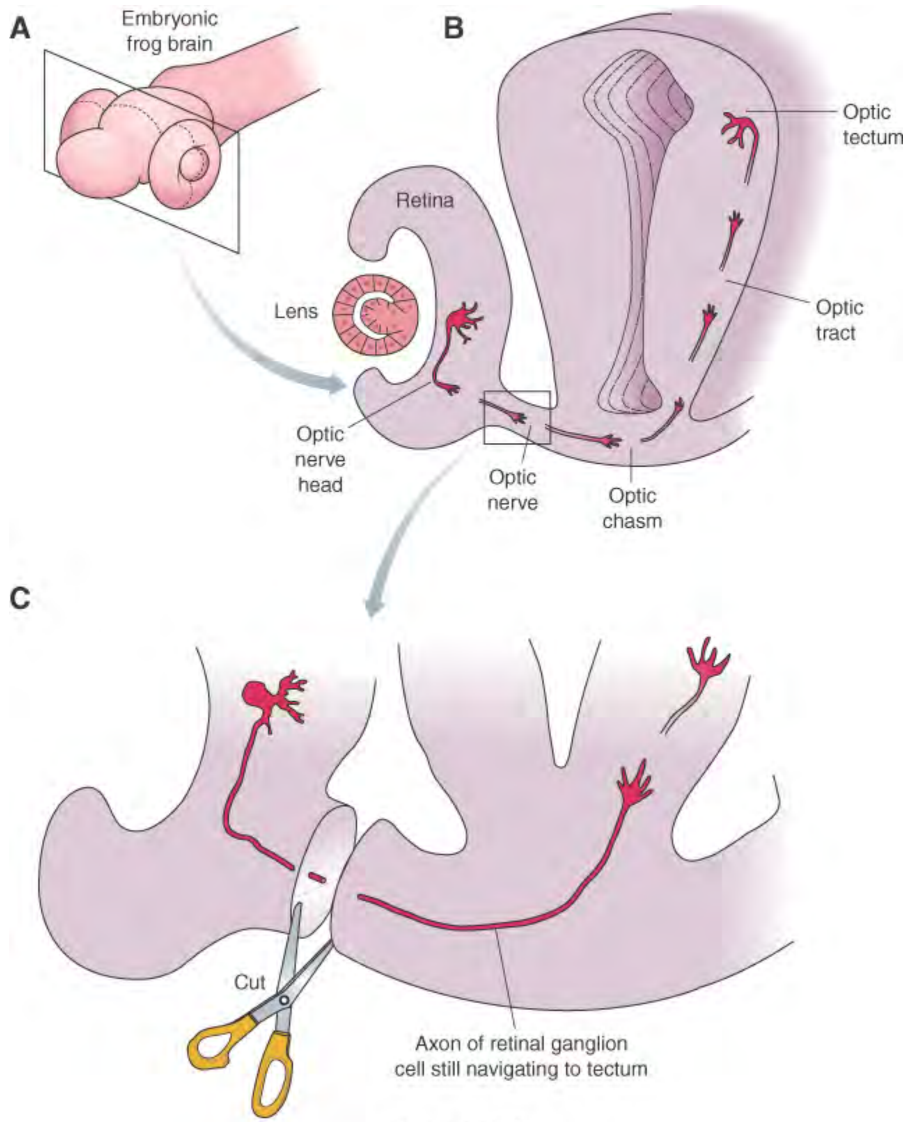


Important Navigational “Roadsigns”: guidepost/stepping stone cells



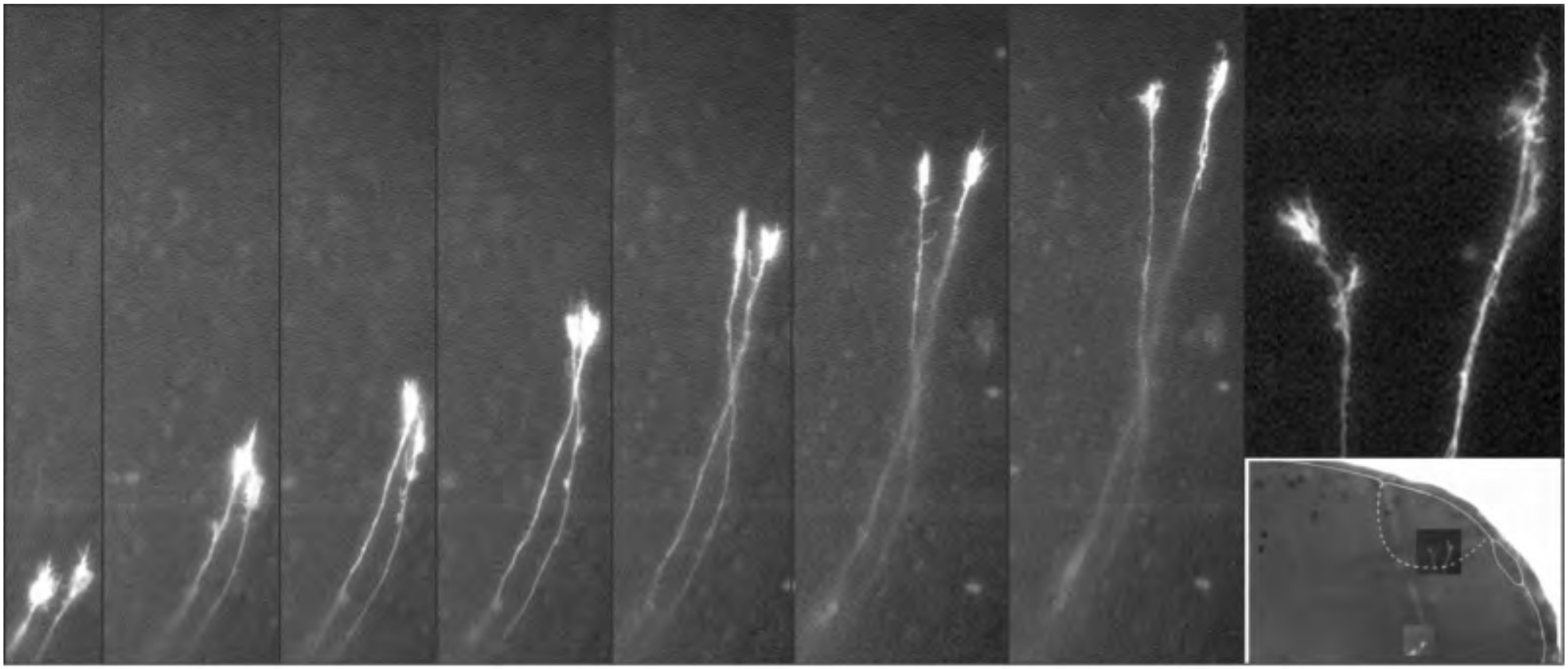
(After Bentley and Caudy, 1983)

Axons grow from retina to tectum using growth cones to navigate (and doesn't need the cell body….at least for a while!)



(After Harris et al., 1987)

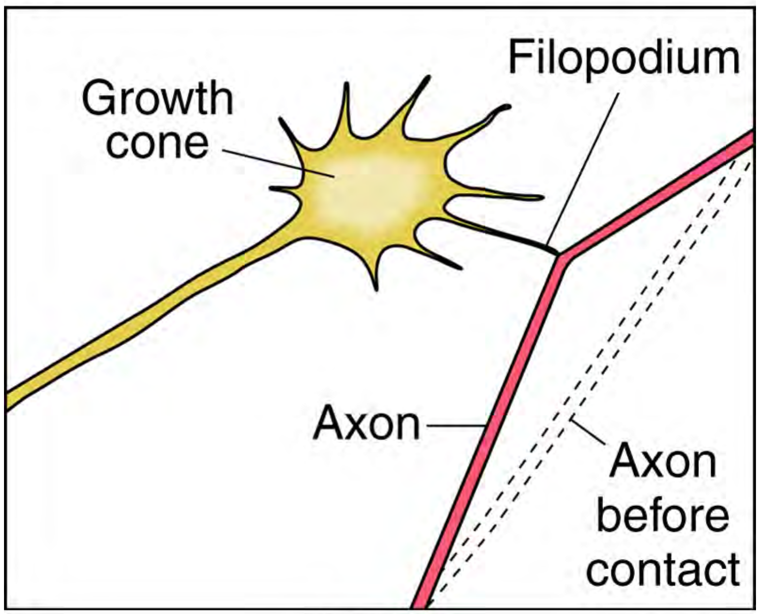
Time lapse images of two neurons growing in optic tract



(Courtesy of Sonia Witte and Christine Holt)

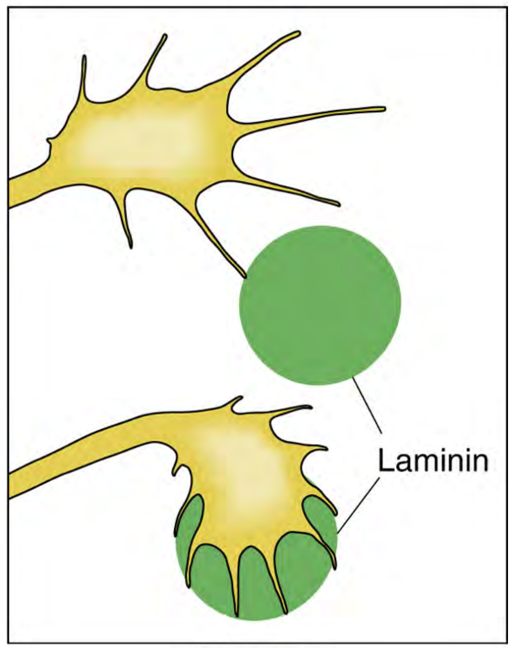
Single filopodia can direct growth cones

A



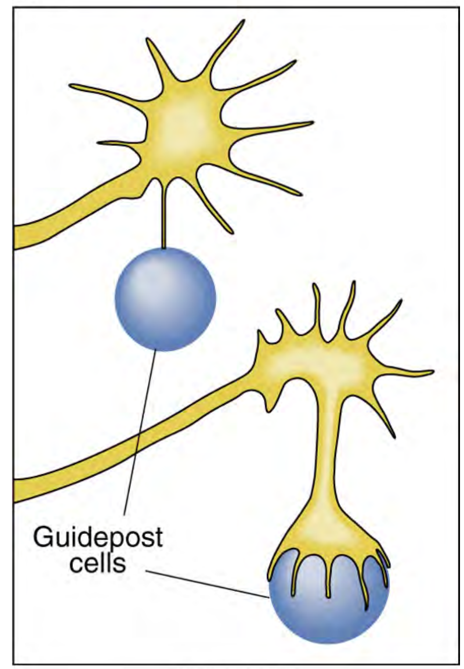
(After Mitchison and Kirschner, 1988)

B



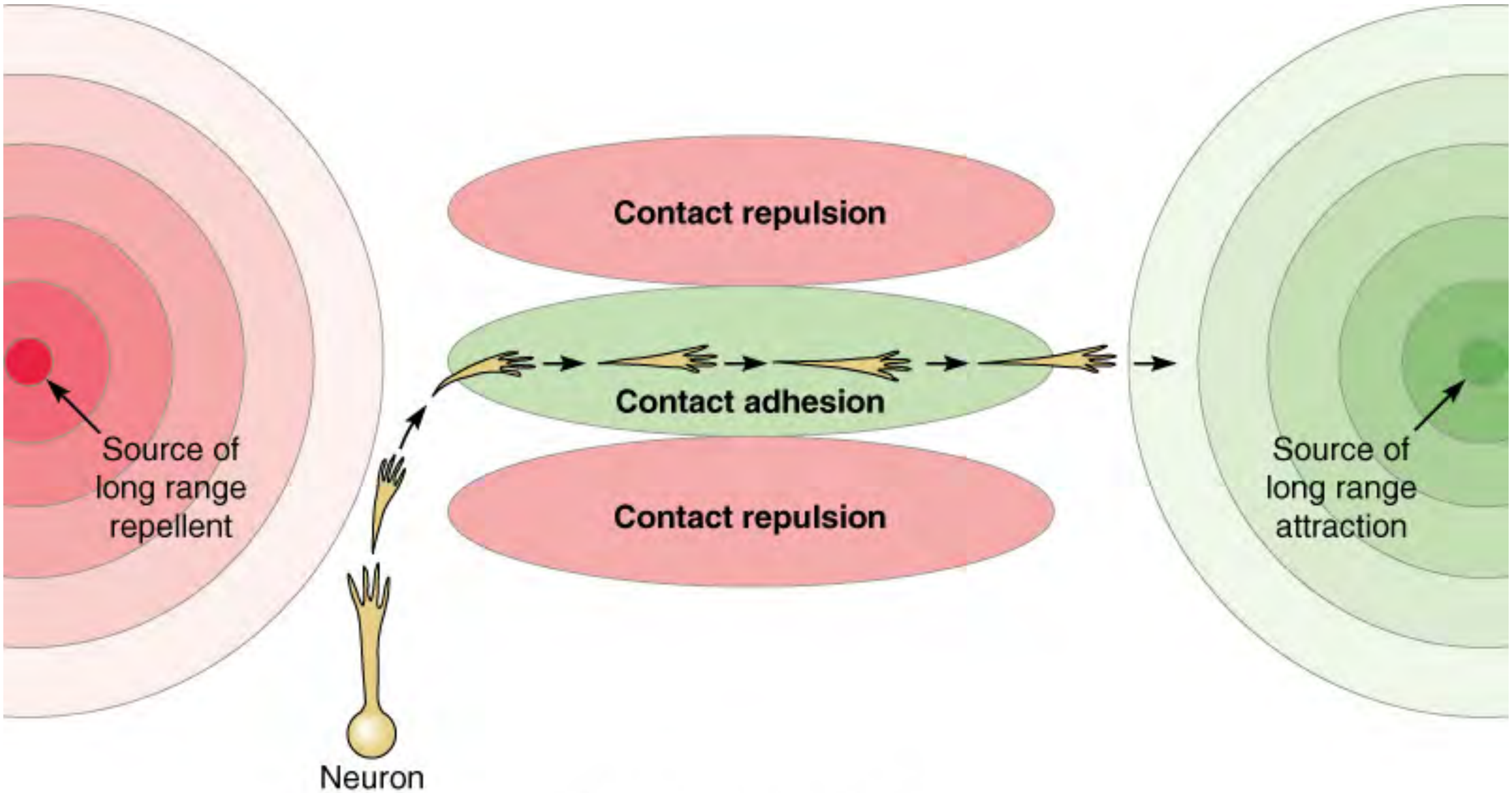
(After Mitchison and Kirschner, 1988)

C



(After Mitchison and Kirschner, 1988)

Short and long-range guidance cues

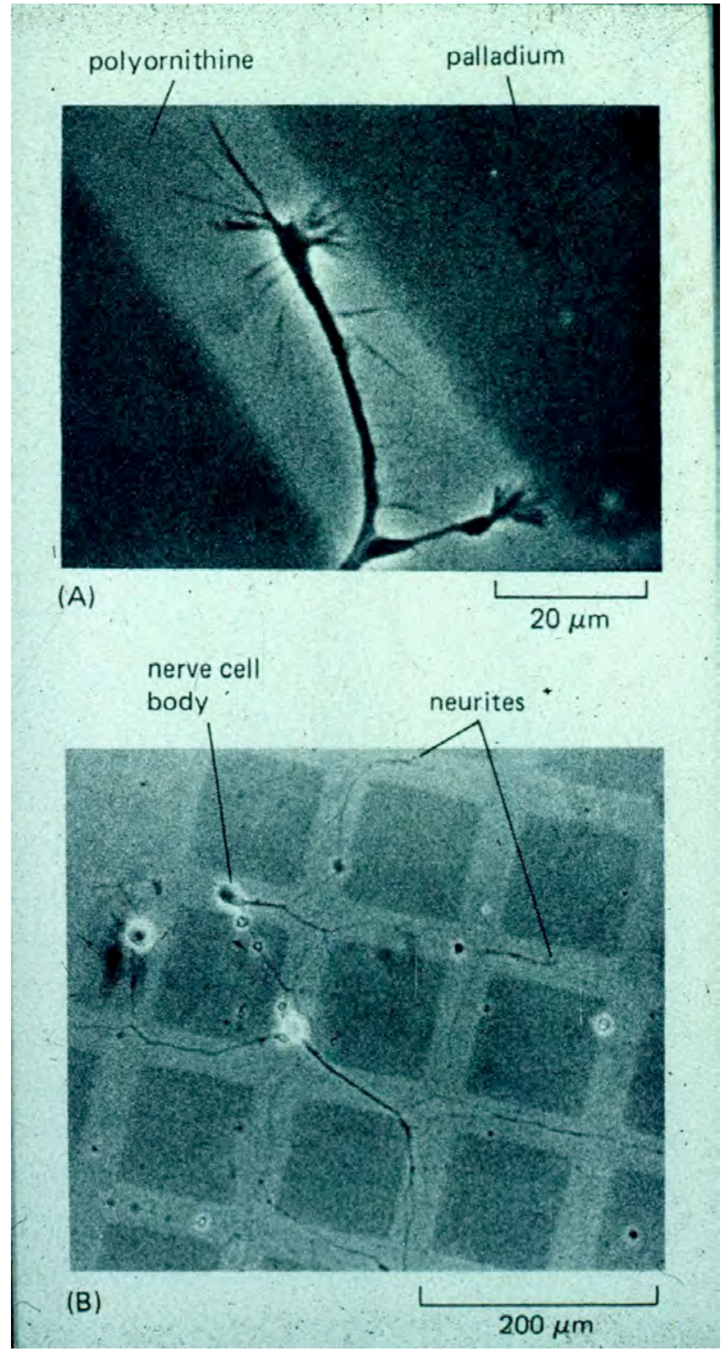


(After Tessier-Lavigne and Goodman, 1996)

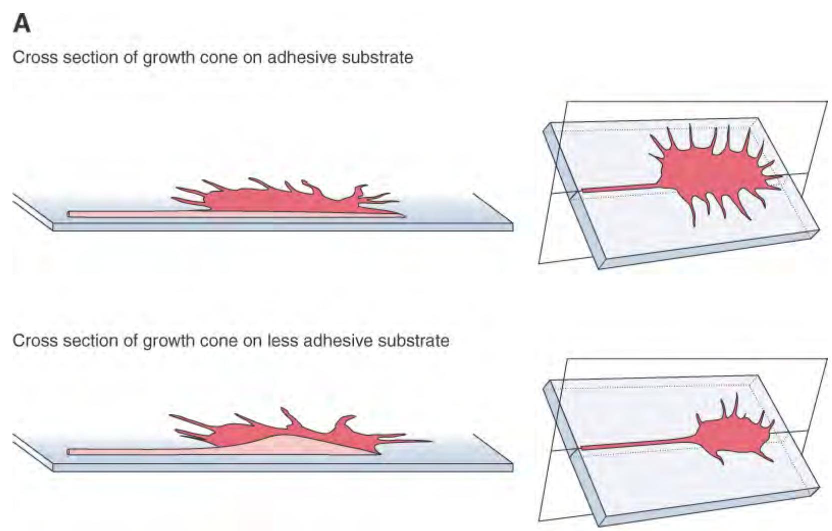
Two Hypothetical Mechanisms for Cell/Growth Cone Navigation

1. Guidance by Differential Preferences for Insoluble Roadways.
    - growing axons encounter many different microenvironments;
    - growth cones are attracted to some and repelled by others (some are neutral)
    - attractants are like “molecular roadways”
    - repellants can be viewed as “guardrails on a road”

Growth cones preferentially migrate on the most adhesive surface.



Growth cones and adhesion



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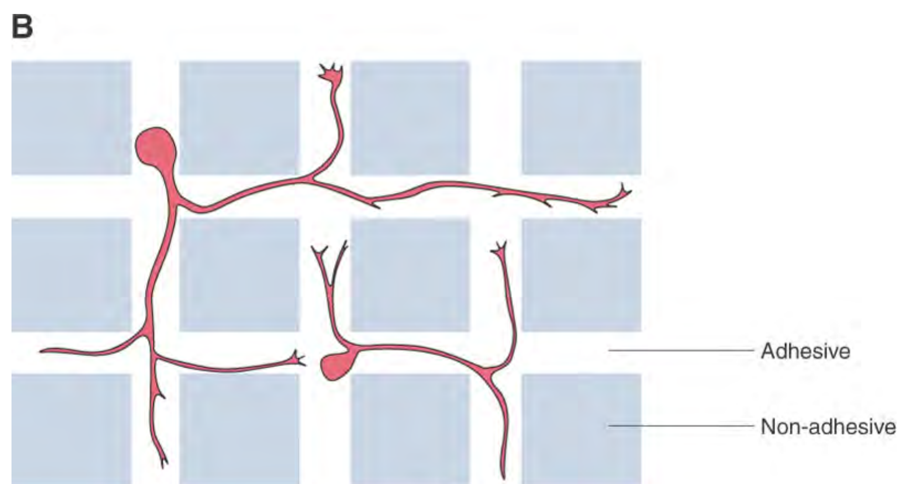

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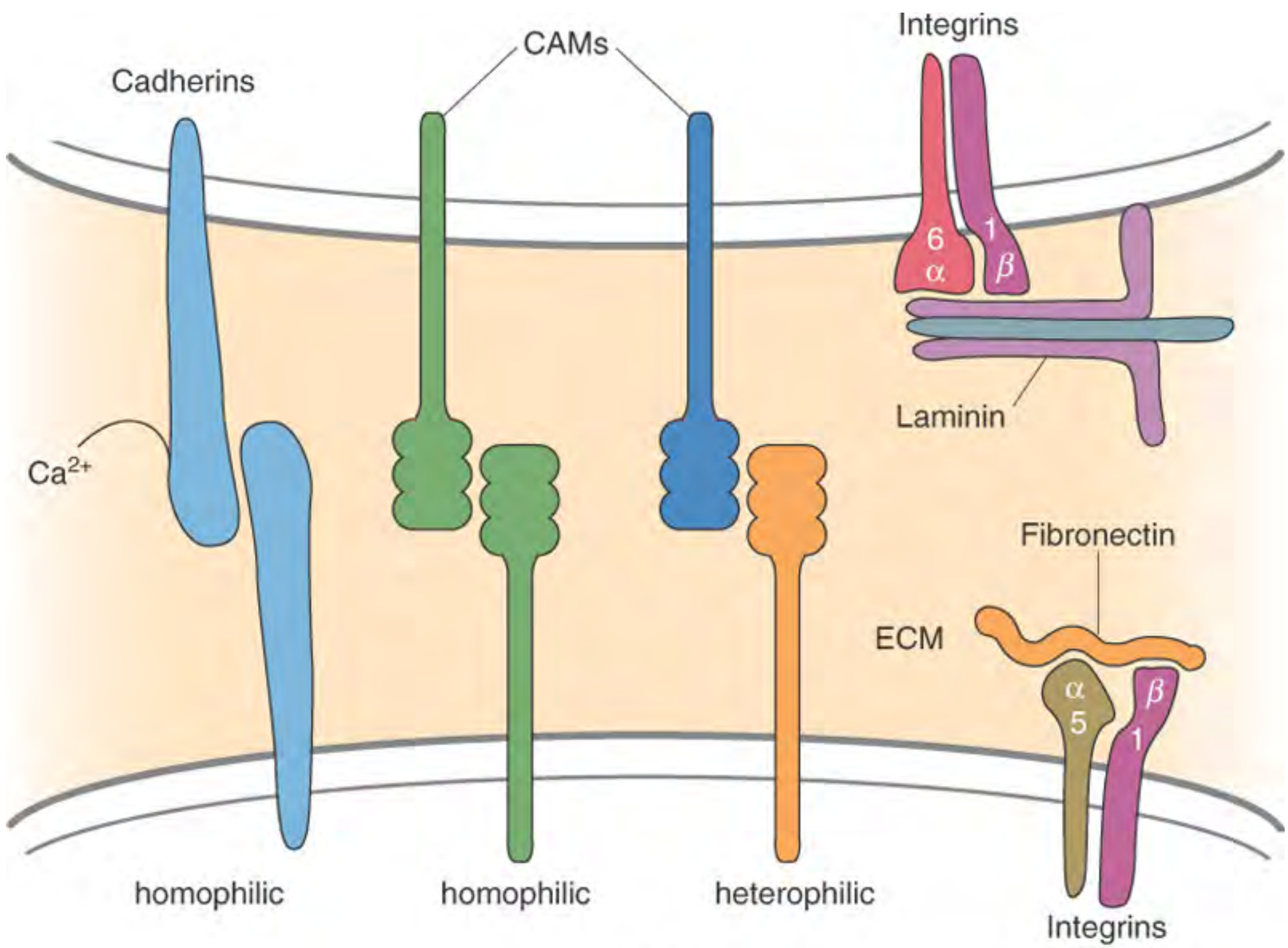
Examples of adhesion molecules expressed on growth cones (all associate intracellularly with cytoskeletal elements)



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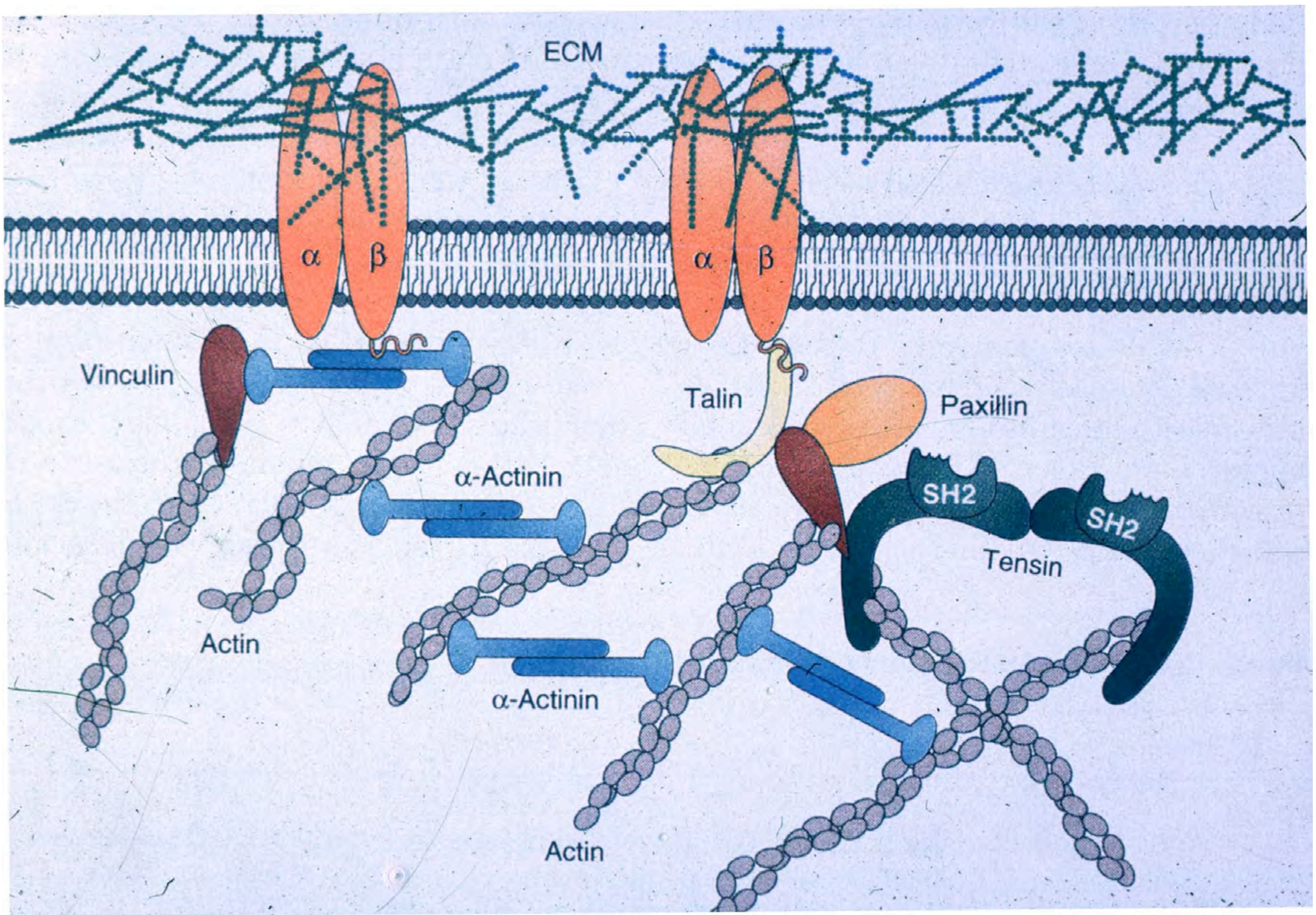
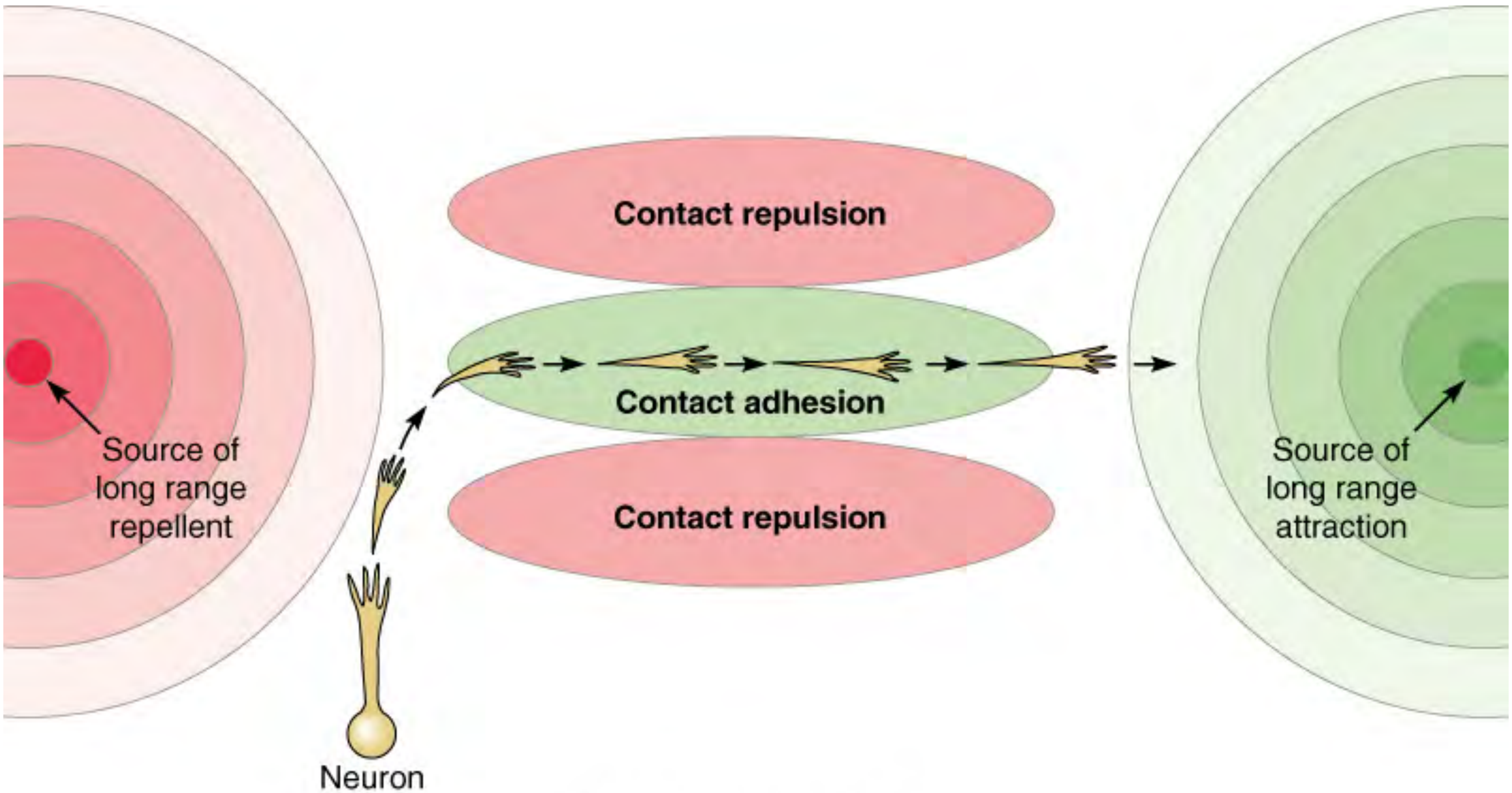
Integrins “integrate” the extracellular matrix with the cytoskeleton



Short and long-range guidance cues; How about diffusable gradients?



(After Tessier-Lavigne and Goodman, 1996)

Two Hypothetical Mechanisms for Cell/Growth Cone Navigation

1. Guidance by Differential Preferences for Insoluble Roadways.
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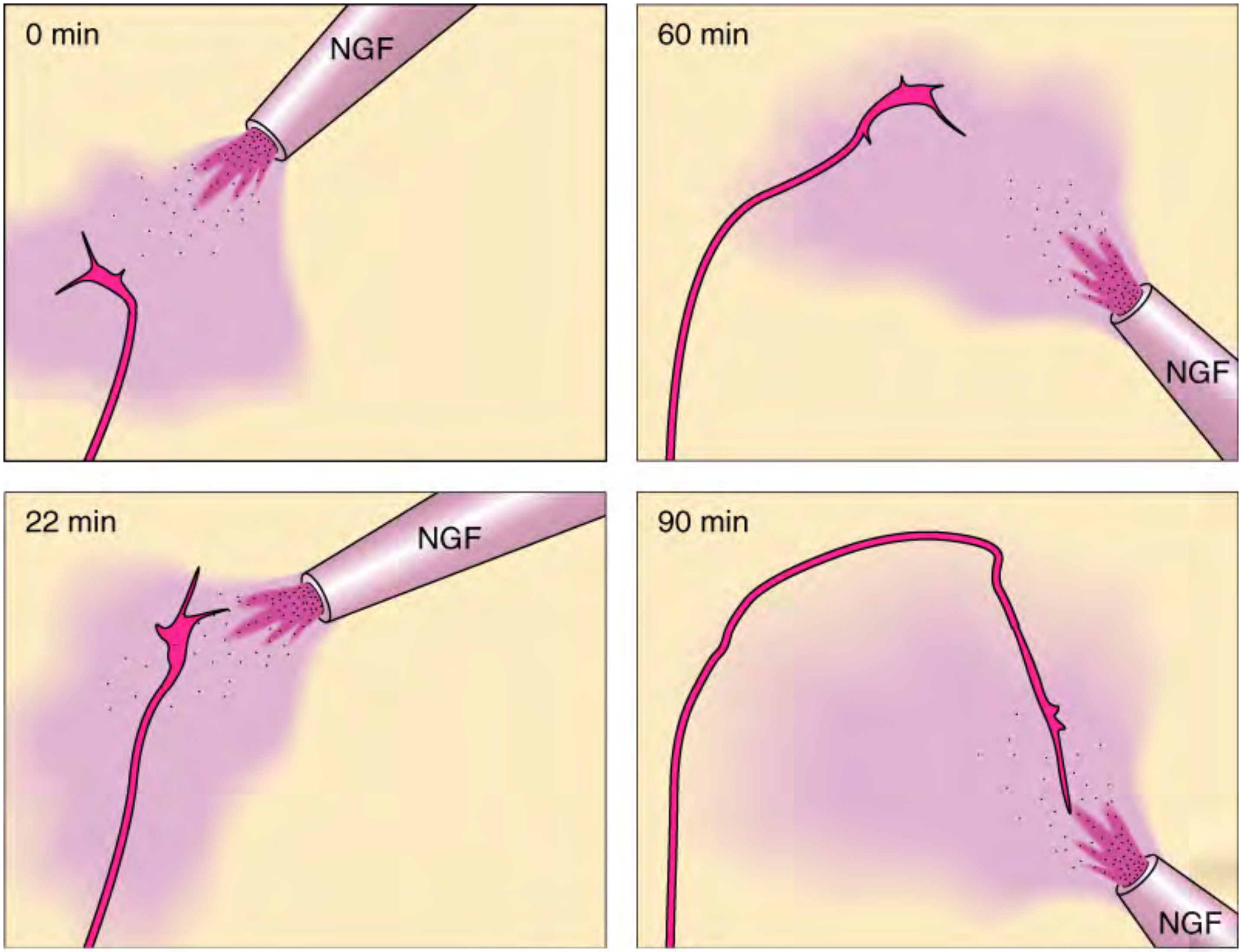
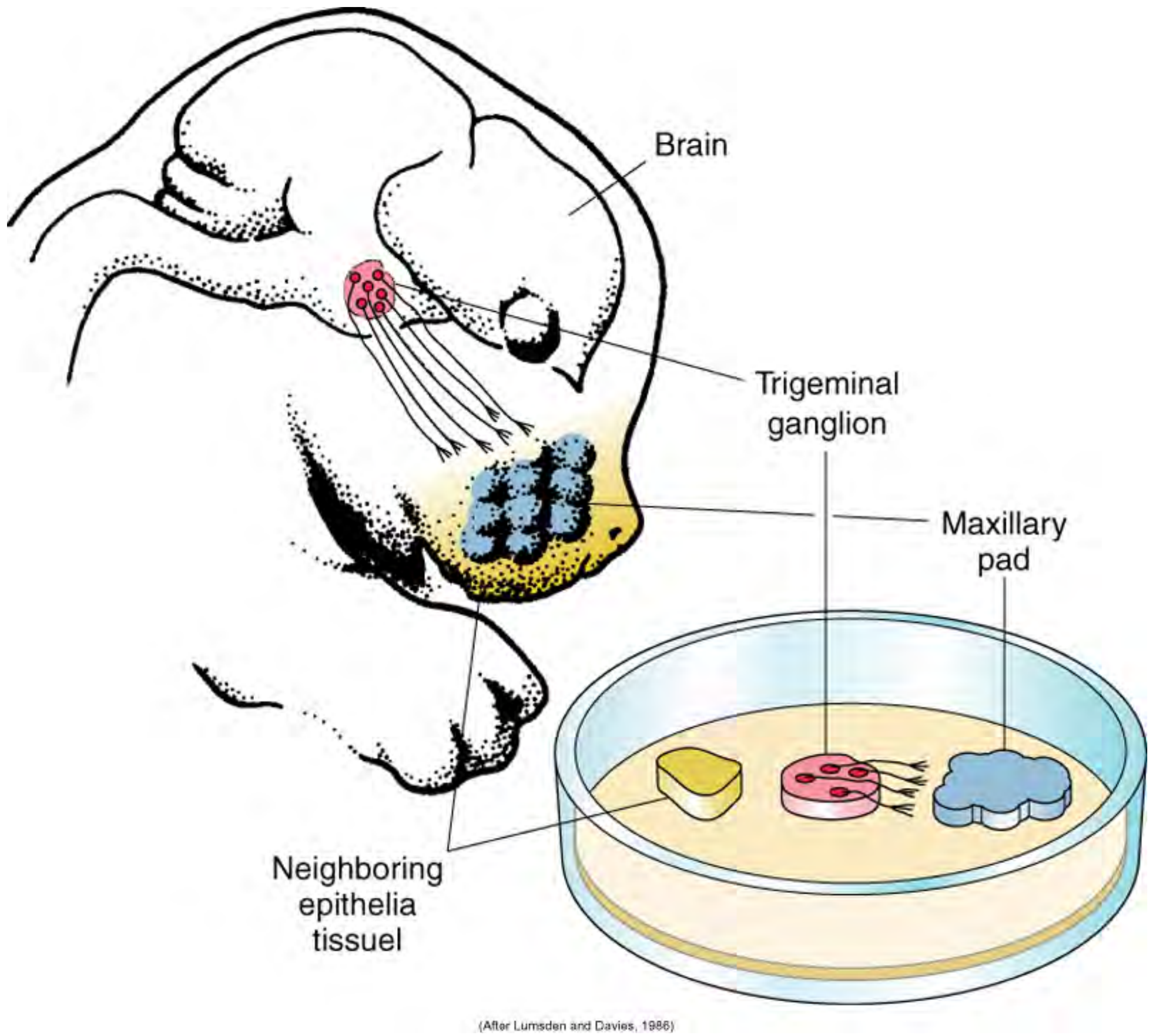
2. Guidance by Gradients of Diffusable Molecules
   chemo-attractants and chemo-repellants
   Remember semaphorin 3A and neural tube cell migration?

Growth cones can use soluble factors for "chemotaxis" (chemo-attractants and chemo-repellents)



(After Gundersen and Barrett, 1979)

Chemo-attractant(s) from target tissues

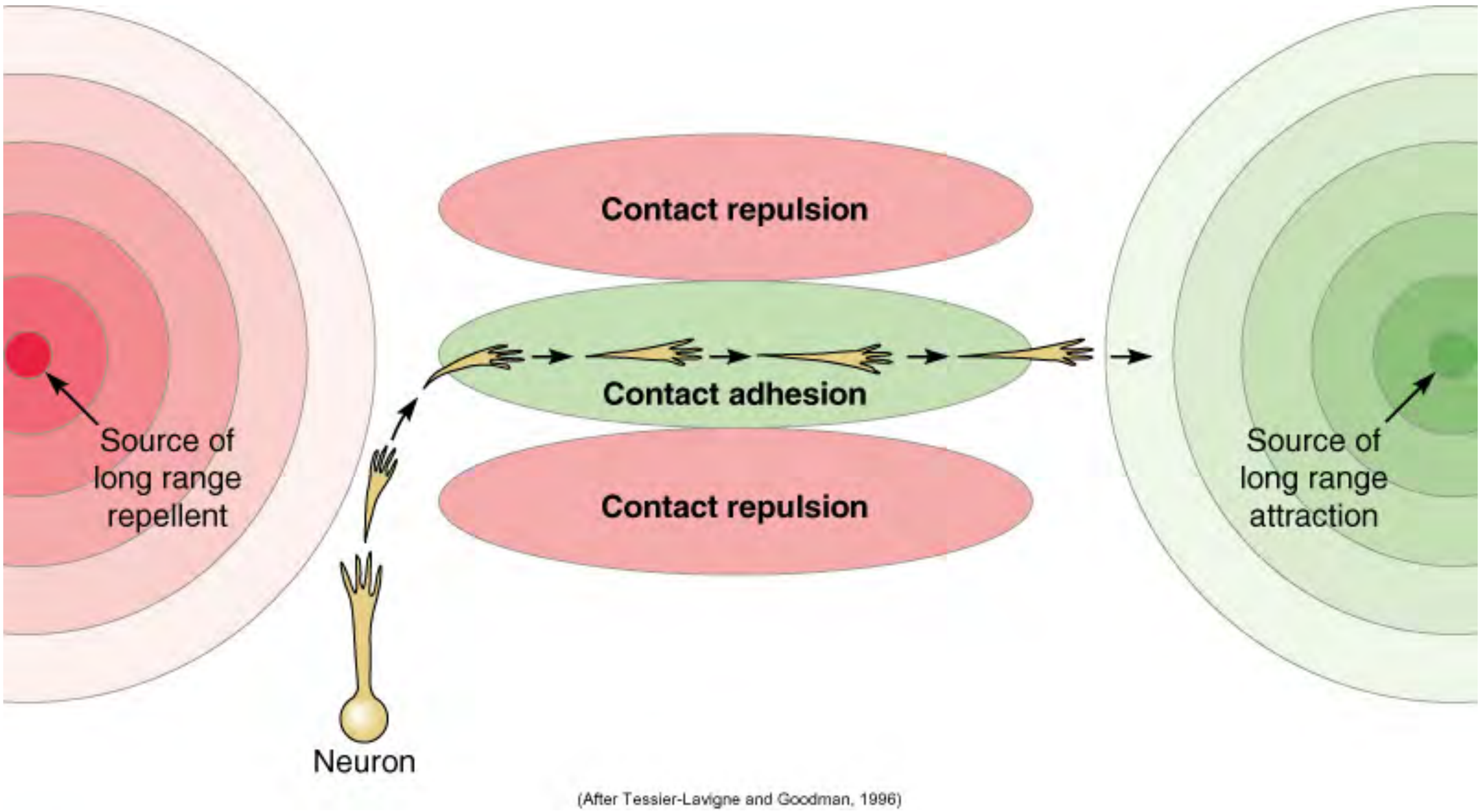


How does it all fit together?

1. Many different “molecular roadways” exist
   ECM, CAMs, diffusable gradients………
2. Different growth cones prefer different roadways;
   think about attractants and repellents;
   think molecularly about ligands and receptors
3. As development proceeds, roadways change
4. As development proceeds, growth cone preferences change

Net Result: There is an incredibly complex system of navigational cues and sensors, which is what is necessary to assemble the intricate network of connections that is the nervous system.

Short and long-range guidance cues
What are some of the real “cues”?



What is the evidence that all this exists?

Critical questions to ask:

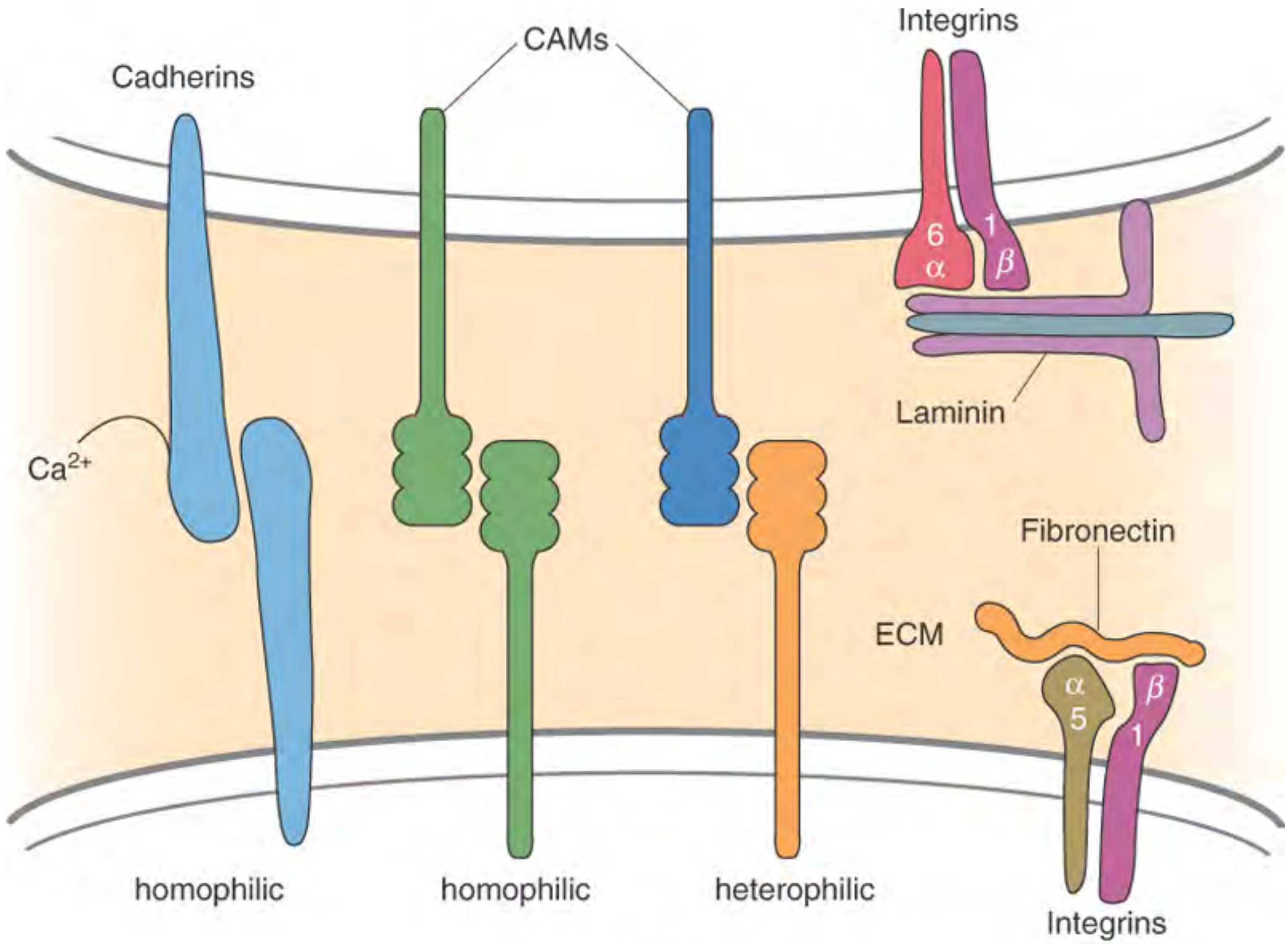
1. What are the roadways? What molecules? Do the roadways even exist?
2. How do roadways get built?
3. How do roadways get changed with time?
4. How do cells establish their preferences?
5. How do cells change their preferences?

We don't have all the answers, but we have some….

Main classes of ligands/receptors that affect axon growth & guidance

Ligands	Receptors	Primary function
Netrin	DCC/frazzled UNC5	Attraction Repulsion
Ephrin A EphA	EphA Ephrin A	Repulsion Attraction
Ephrin B EphB	EphBs Ephrin B	Repulsion Attraction
CXCL12/Sdf1	CXCR4	Attraction
Slit	Robo	Repulsion
Semaphorin	Plexin Neuropilin	Repulsion Repulsion
Wnt	Frazzled Ryk	Attraction Repulsion
Hedgehog	Bok Patched	A and R A and R
IgCAM	IgCAM	Homophilic cell adhesion
Cadherin	Cadherin	Homophilic cell adhesion
ECM SAM	Integrin	Substrate adhesion
Growth factors	GFRs	Attraction
Trophic factors	Trks	Attraction
Sphingolipids	S1P	Homophilic cell adhesion
GAGs/carbohydrates	Various receptors	Modulation

Examples of adhesion molecules expressed on growth cones (all associate intracellularly with cytoskeletal elements)



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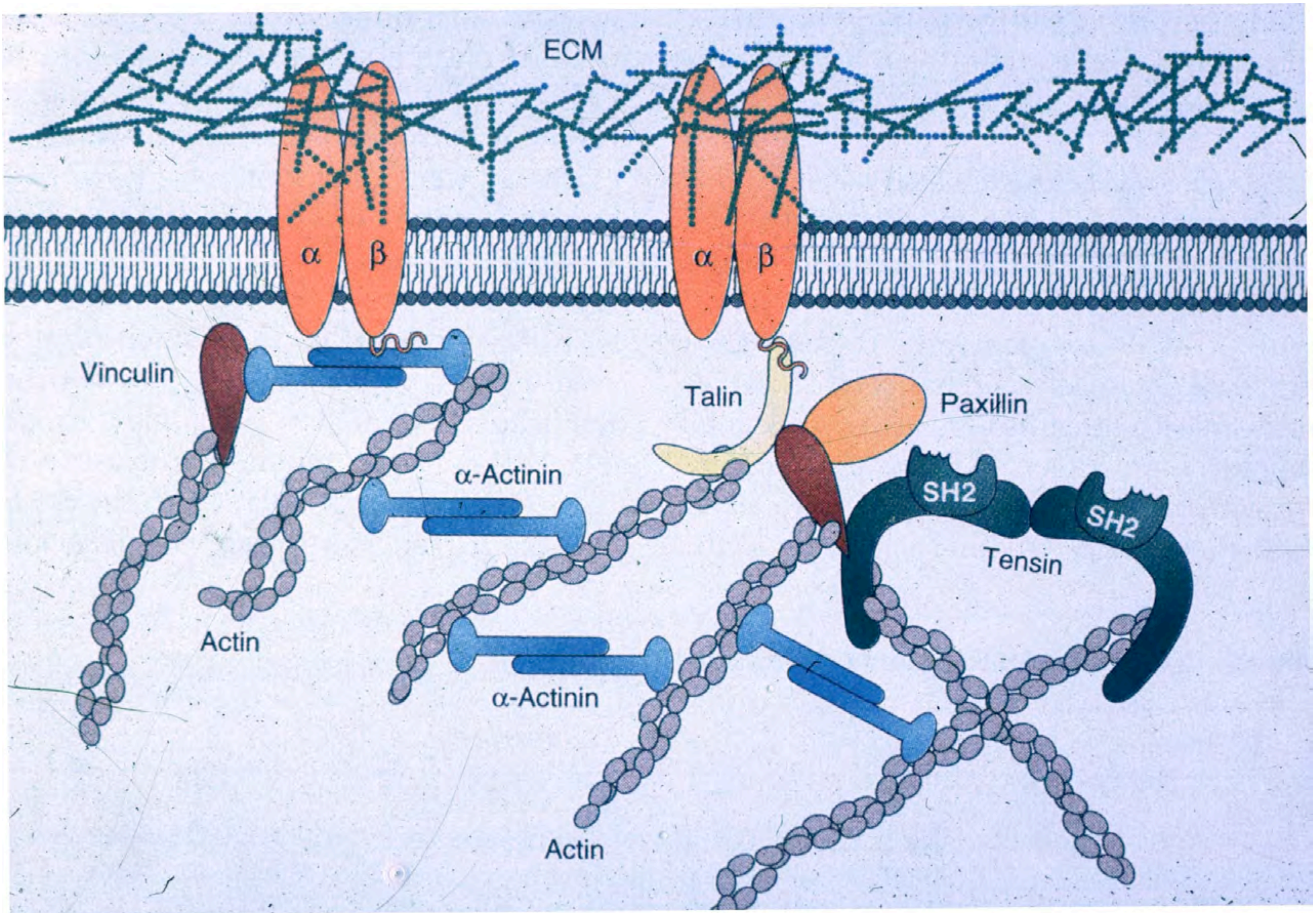
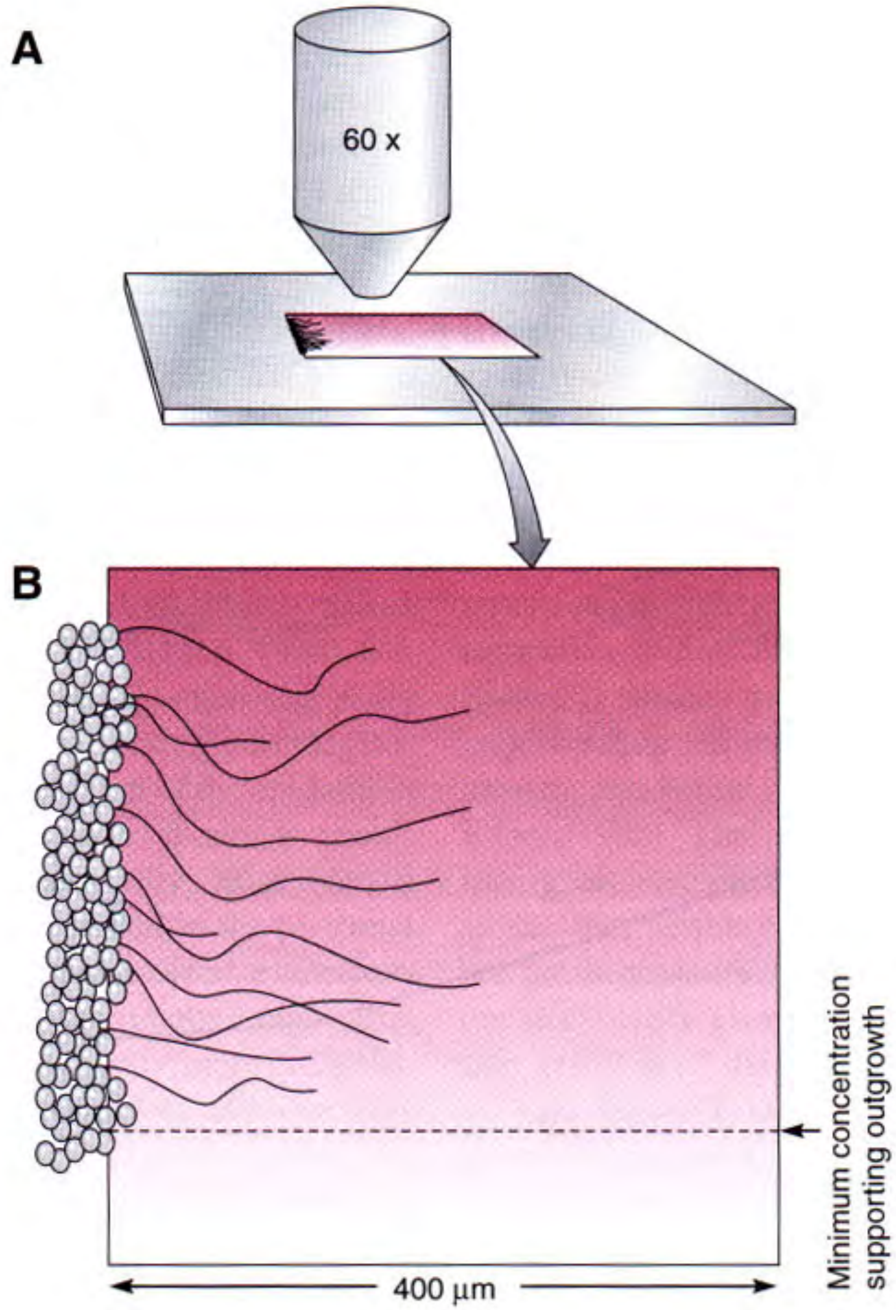
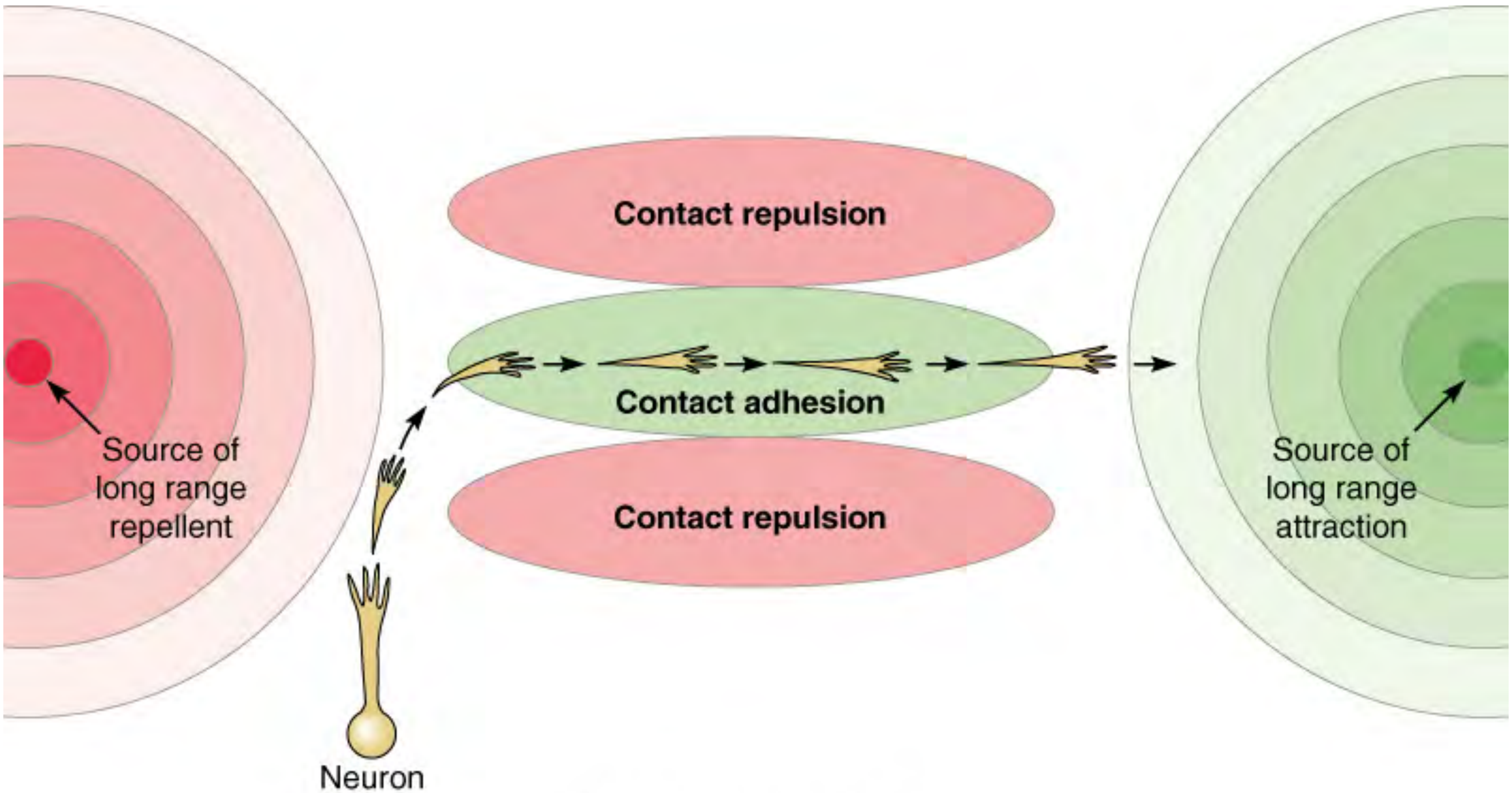
Integrins “integrate” the extracellular matrix with the cytoskeleton



Adhesive gradients do not direct growth cones (laminin gradient)



Short and long-range guidance cues



(After Tessier-Lavigne and Goodman, 1996)

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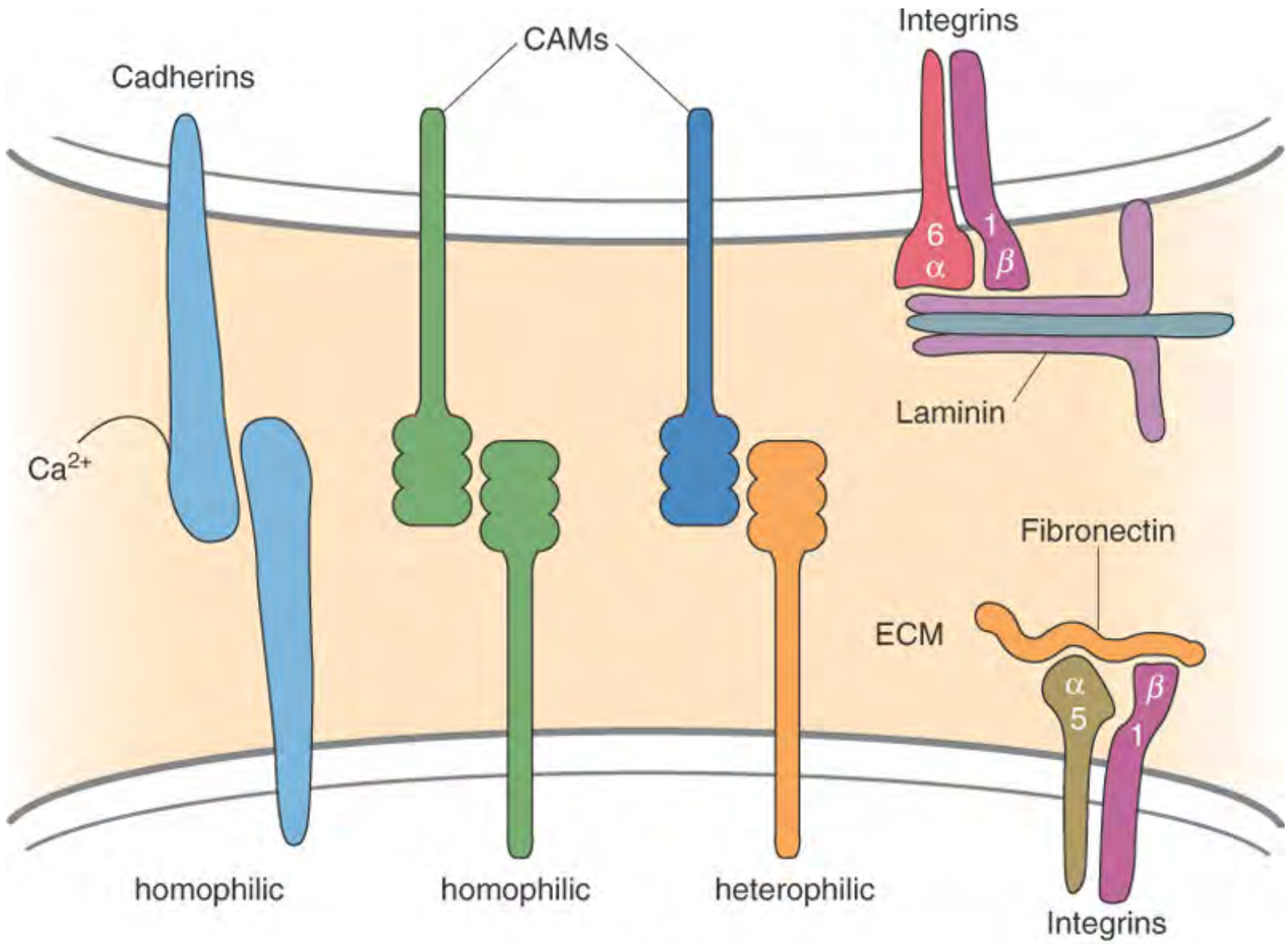

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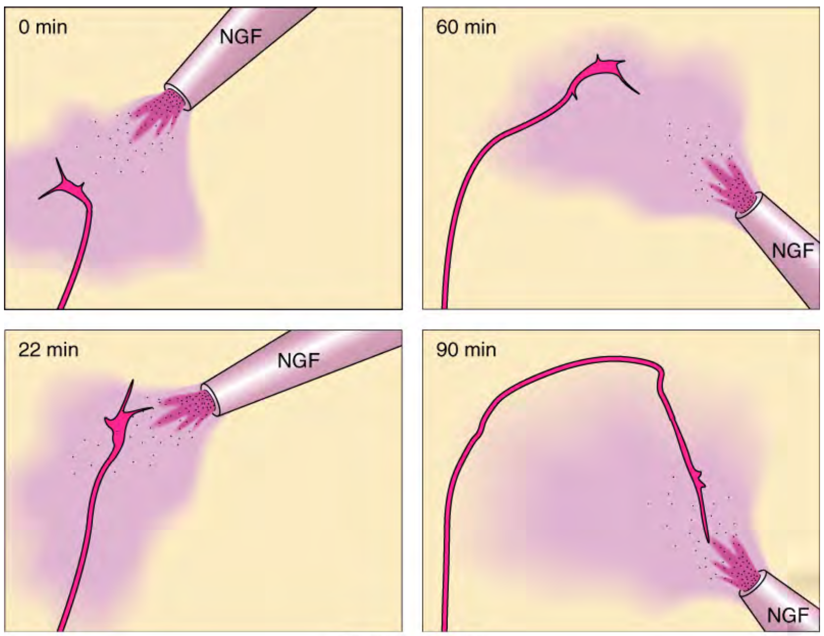
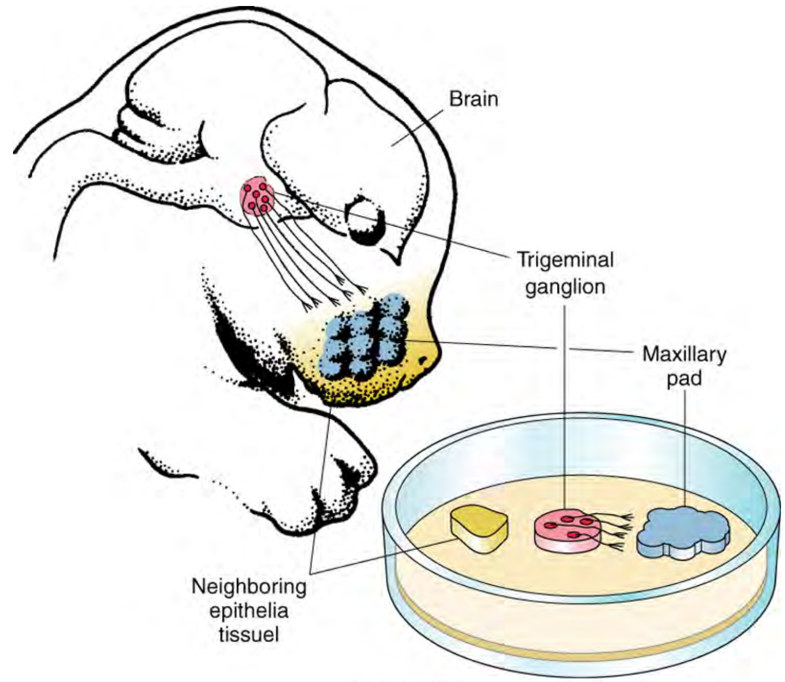
If the insoluble local cues are permissive or non-permissive but not instructive, something else must be instructive….how about soluble/diffusable gradients?

Growth cones can use soluble factors for “chemotaxis” (chemo-attractants and chemo-repellents)

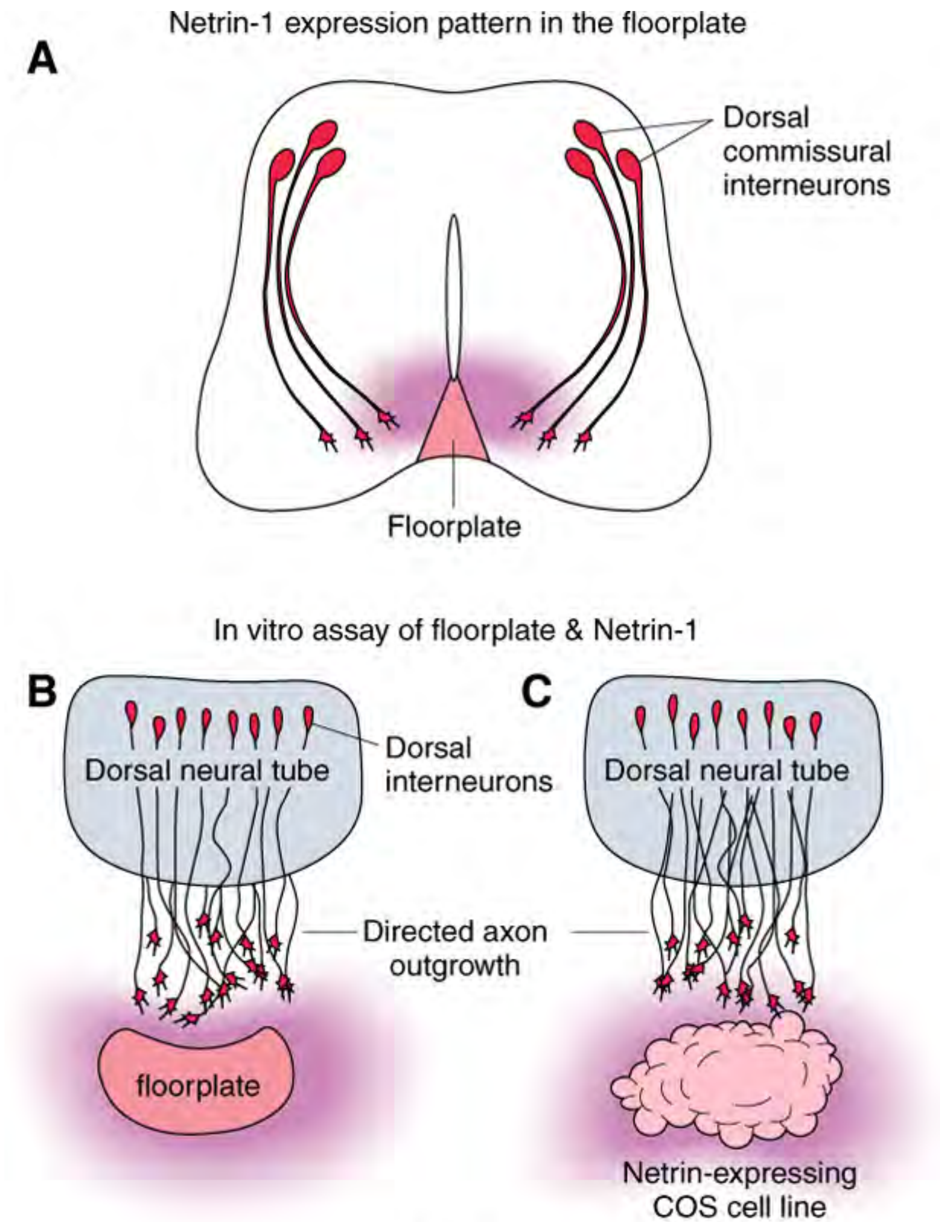


(After Gundersen and Barrett, 1979)



(After Lumsden and Davies, 1986)

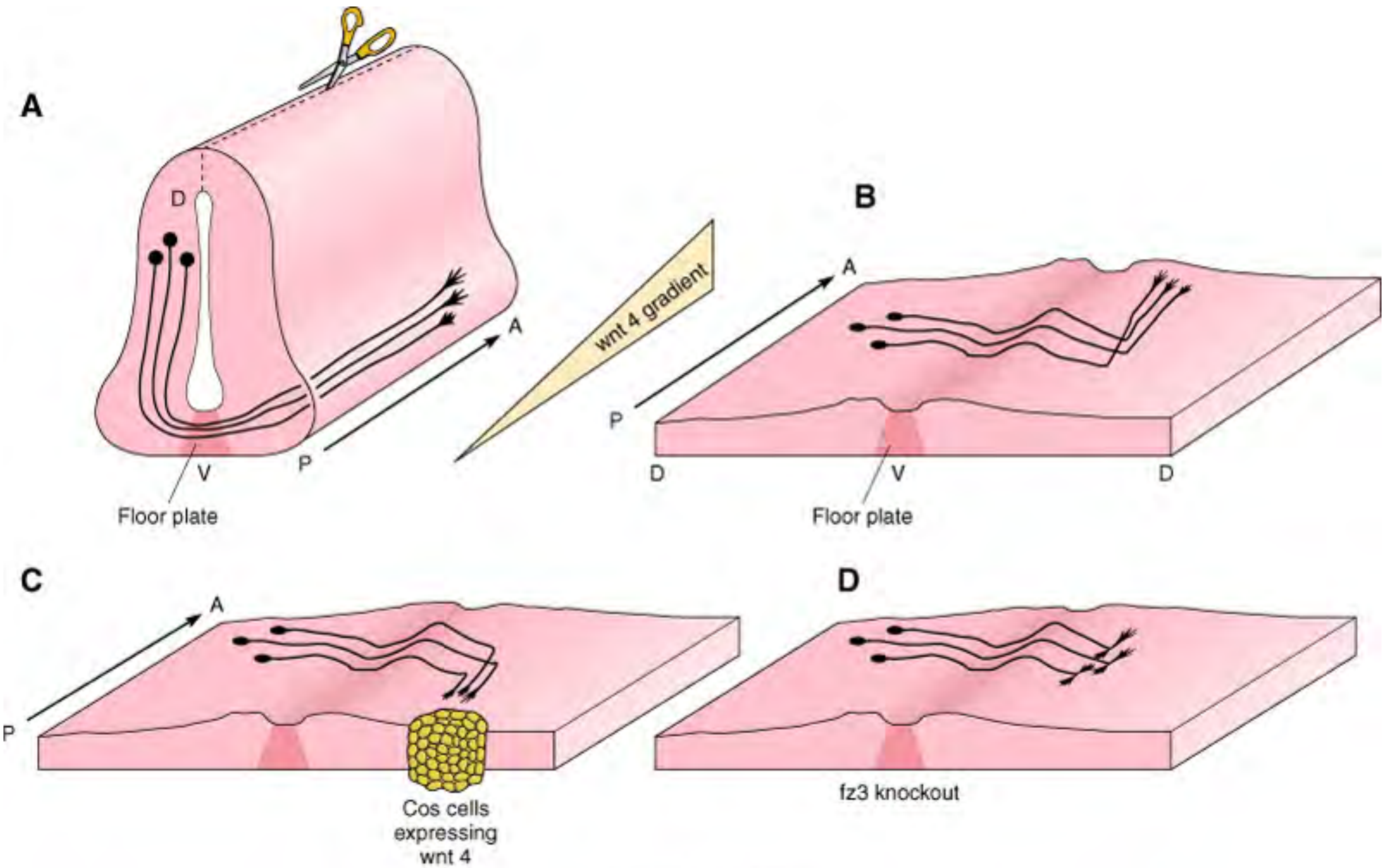
Dorsal neurons are attracted by a netrin gradient



(After Kennedy et al., 1994)

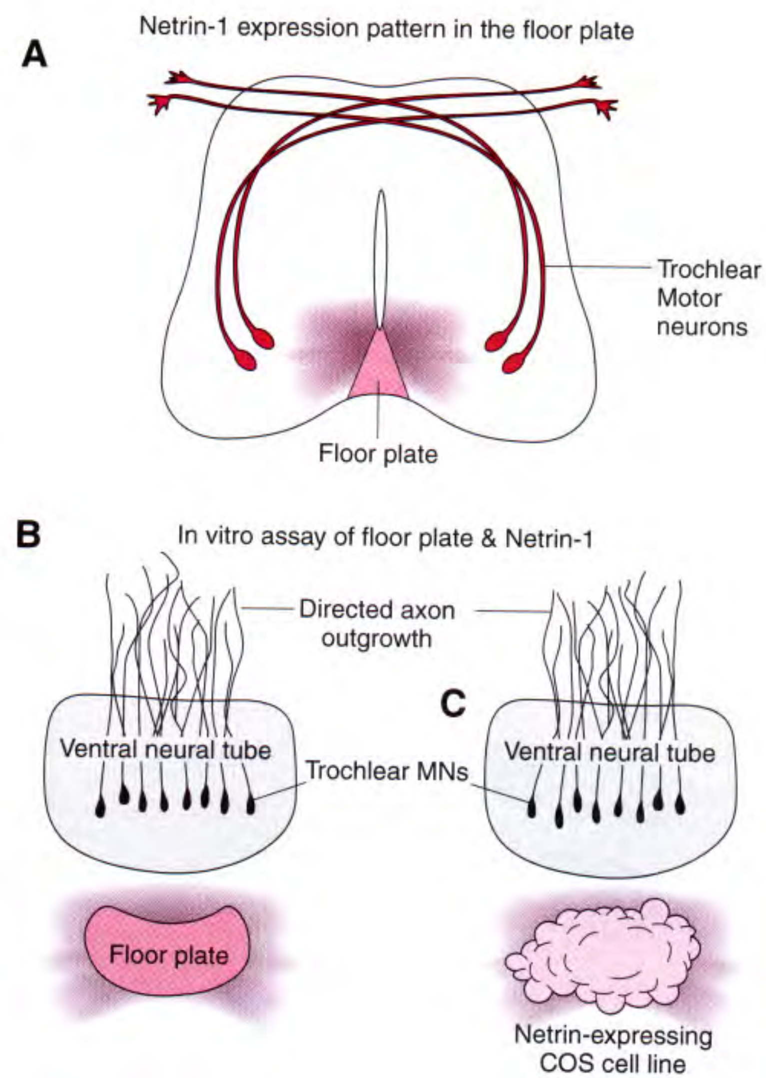
Local gradients of morphogens can orient dorsal cell axons

Netrin →Ventral…..then wnt4 →anterior

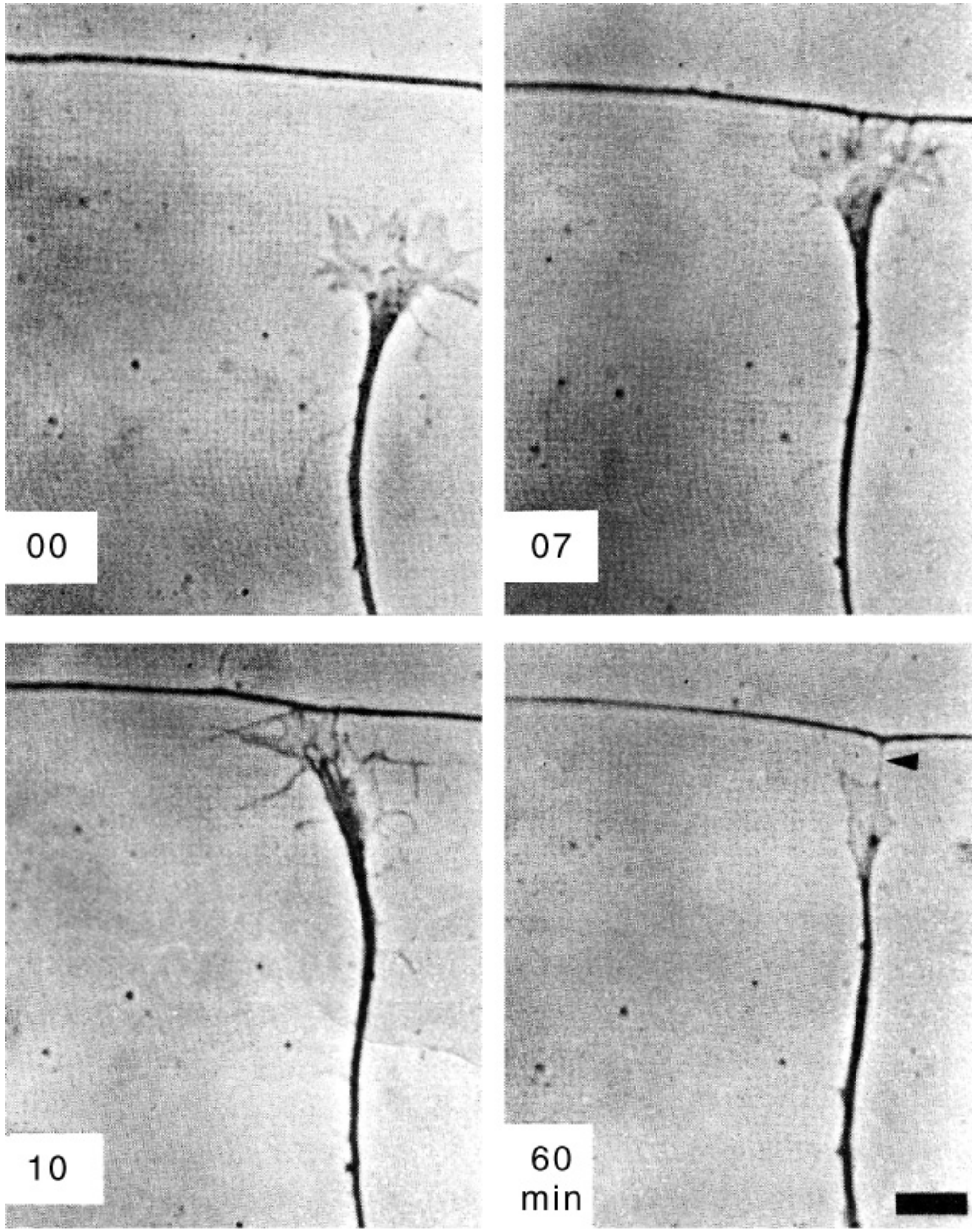


(After Lyuksyutova et al., 2003)

Ventral neurons are repelled by a netrin gradient

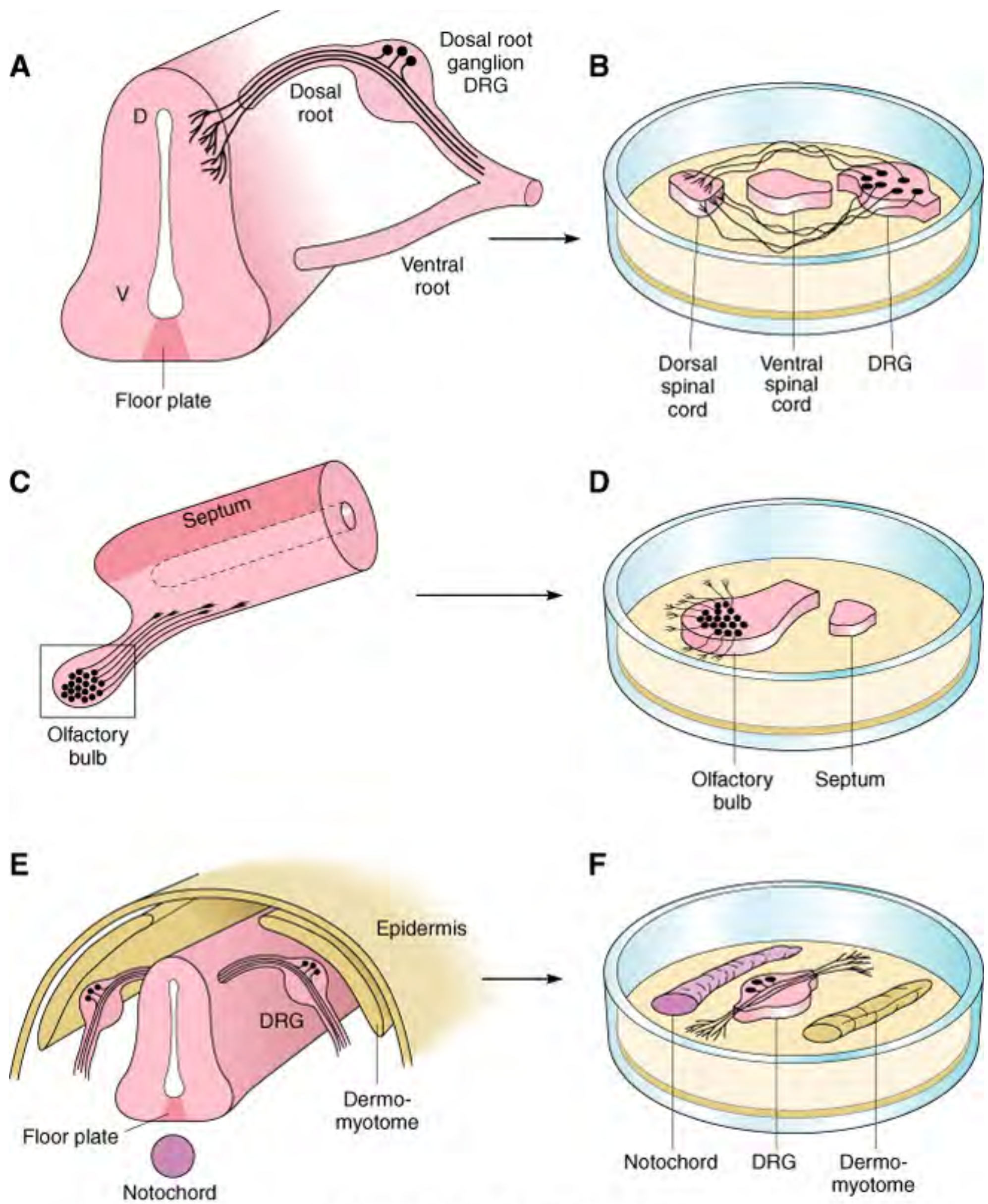


Negative Cues: Growth cone collapse



(From Kapfhammer and Raper, 1987a)

Repulsive Guidance

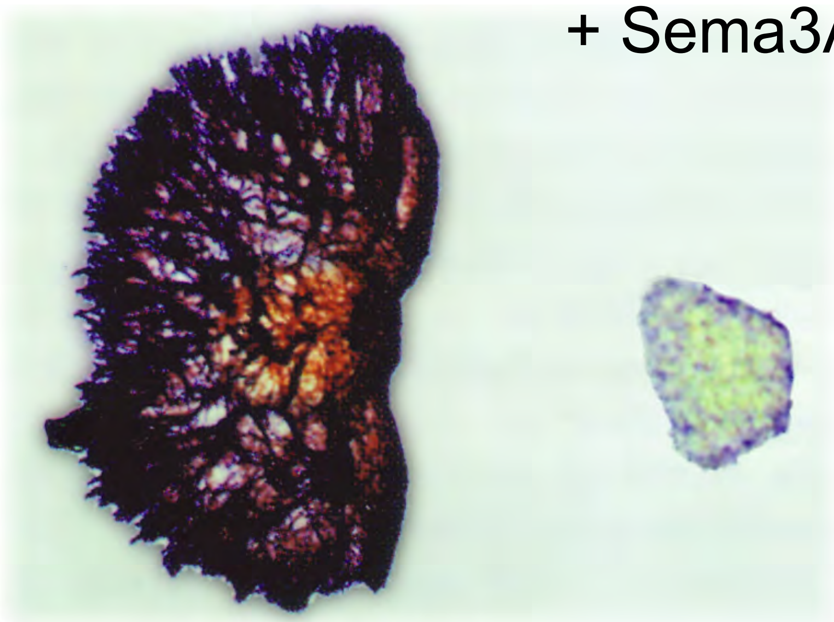


(After Peterson and Crain, 1981; Pini, 1993; Keynes et al., 1997)

Sema3A is repulsive to pain fibers of the DRG

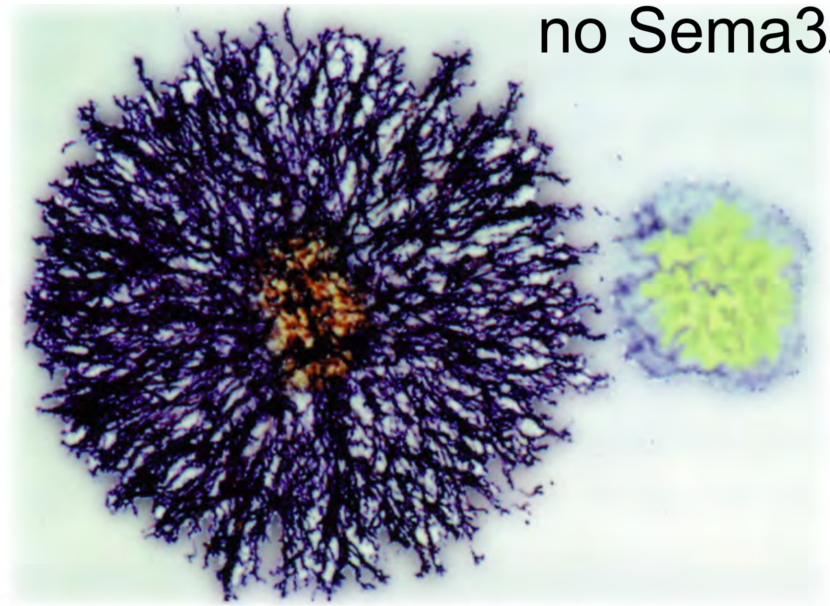
cos cells
+ Sema3A

cos cells
no Sema3A



A

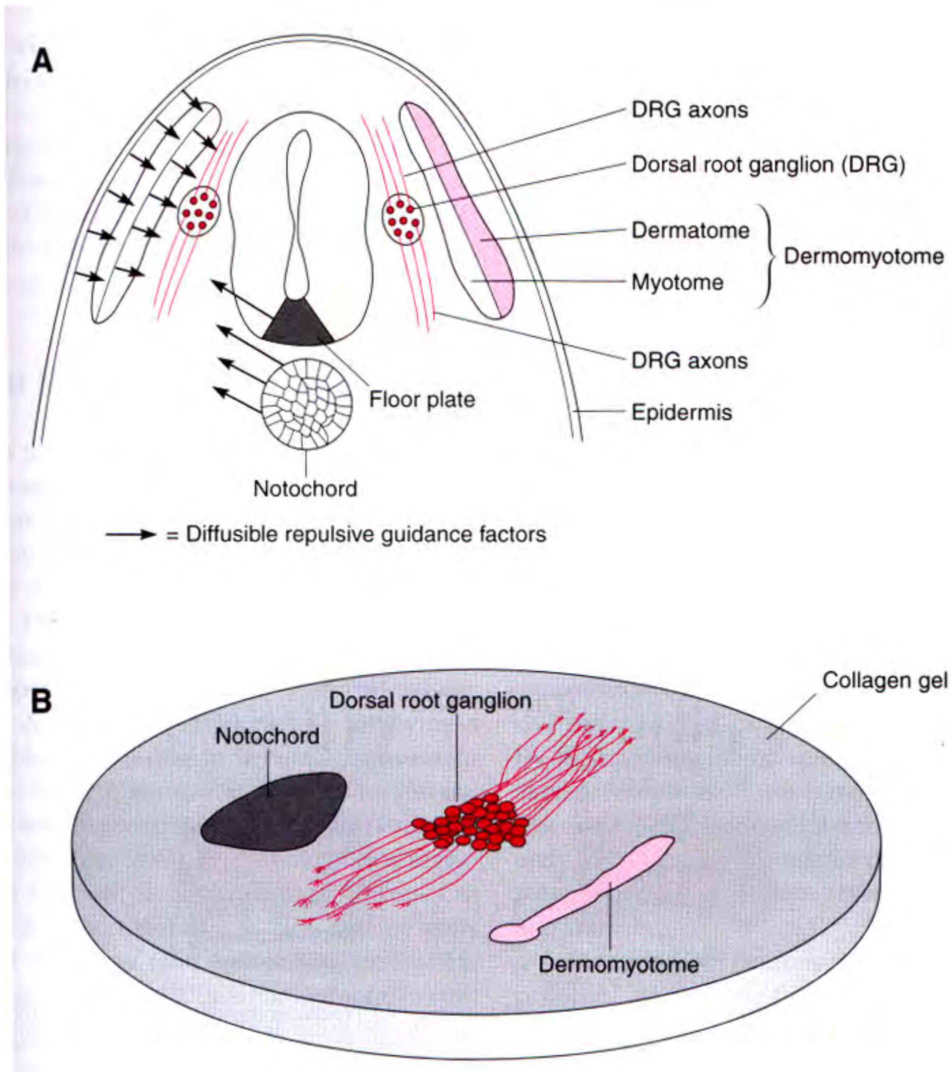
(After Messersmith et al., 1995)



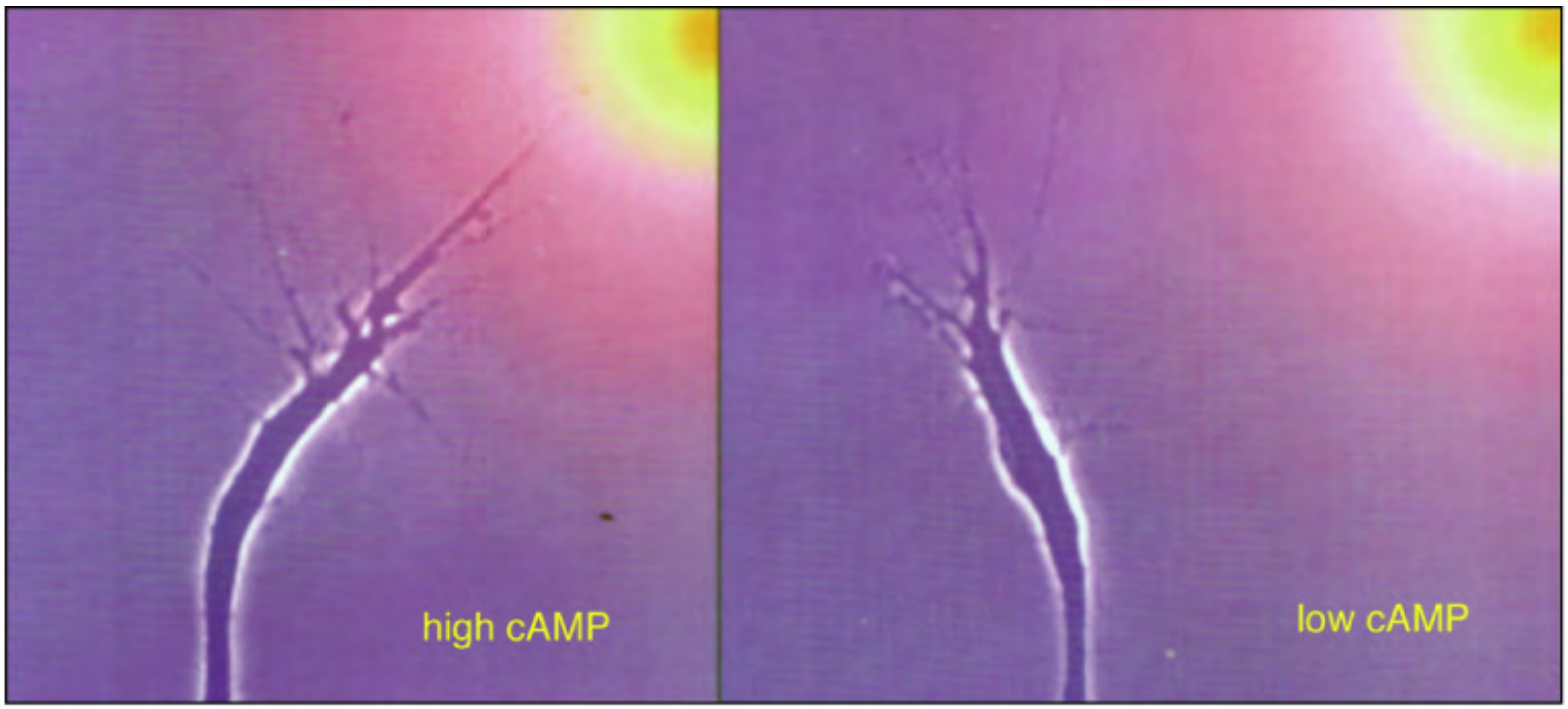
B

(After Messersmith et al., 1995)

Creating a pathway with repellents

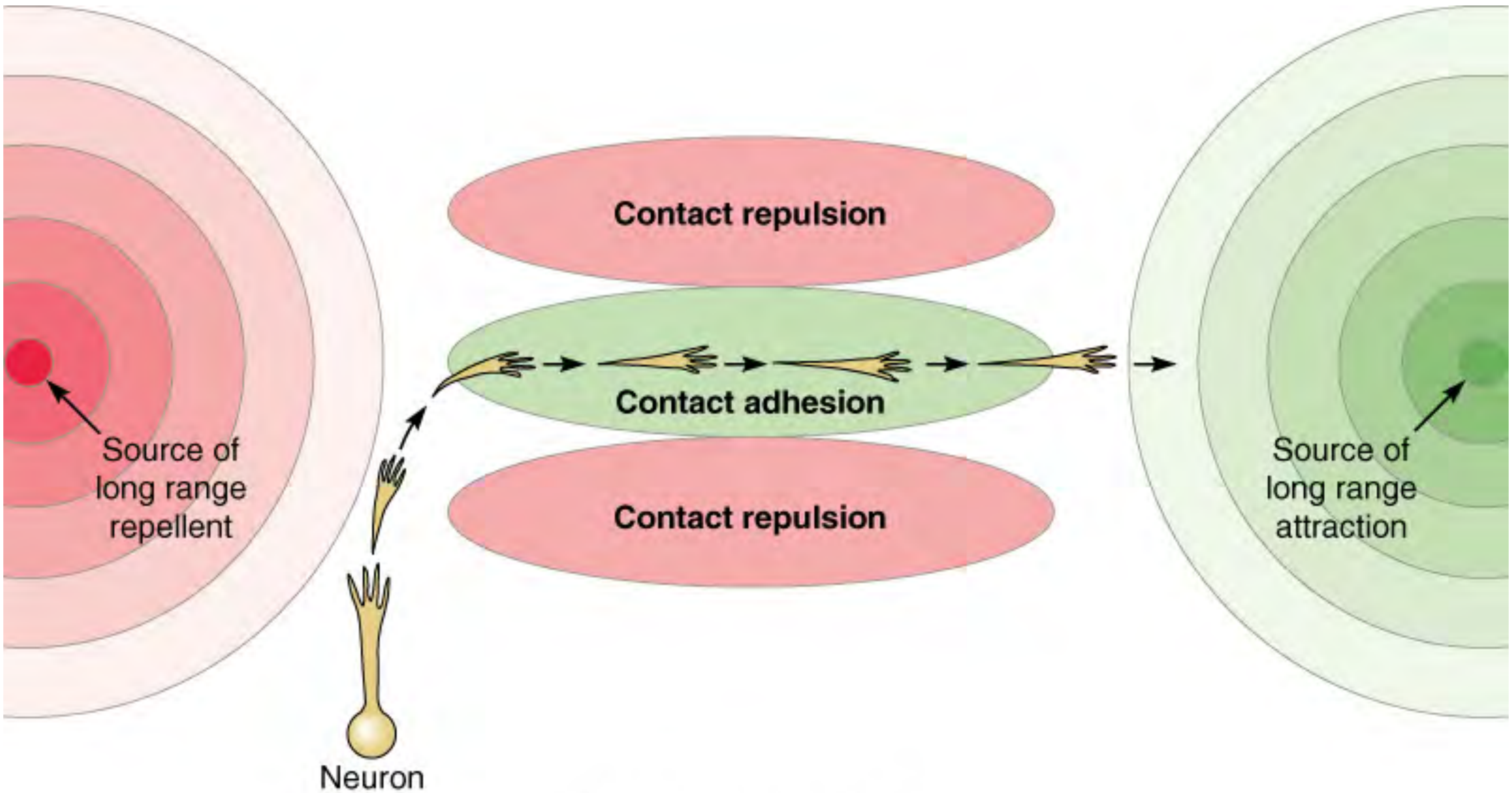


Intracellular cAMP levels regulate growth cone response to netrin - a mechanism for change in preference/response to roadways

A  B

(After Ming et al., 1997)

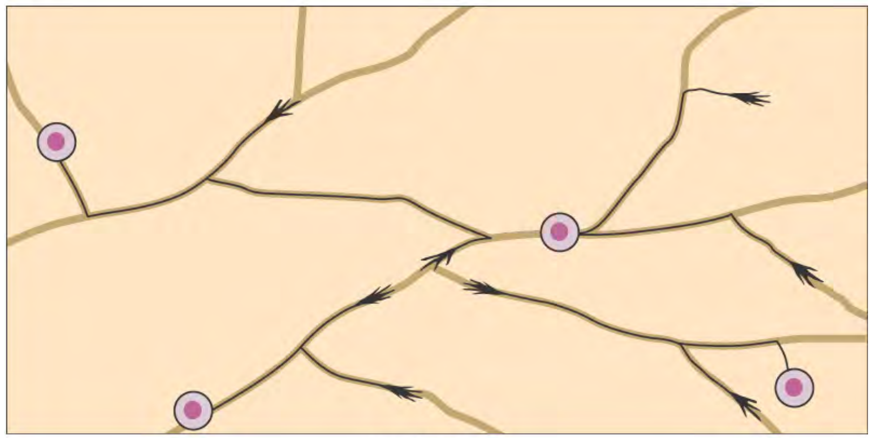
Short and long-range guidance cues



(After Tessier-Lavigne and Goodman, 1996)

Axons may follow mechanical pathways

A



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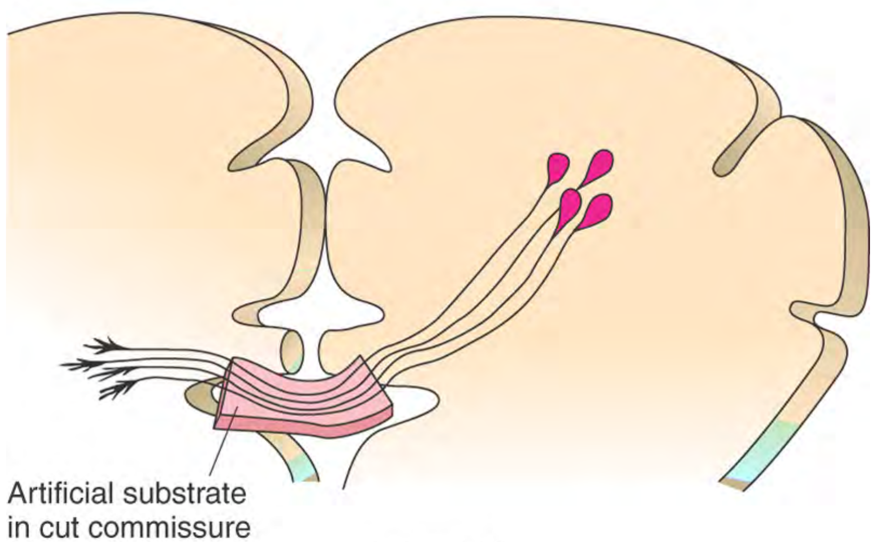
B



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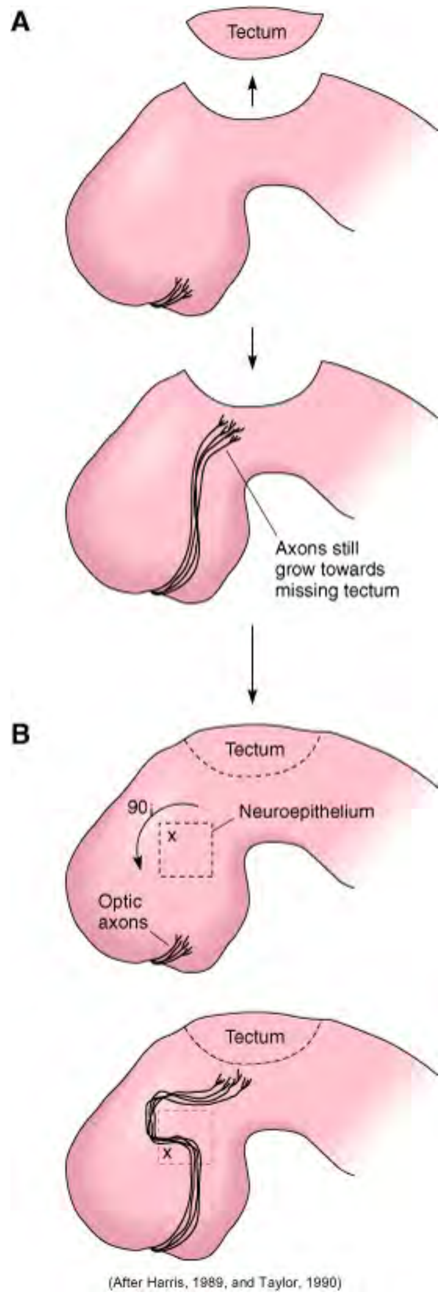
Retinal axons follow local guidance cues in the neuroepithelim



When thinking about changes in cell shape and cell movement……..think cytoskeleton!

Neuronal Cytoskeleton is composed of:

- Microtubules
- Actin Microfilaments
- Neurofilaments

Cell Movement involves reaching forward, grabbing the surface, pulling and reaching forward again

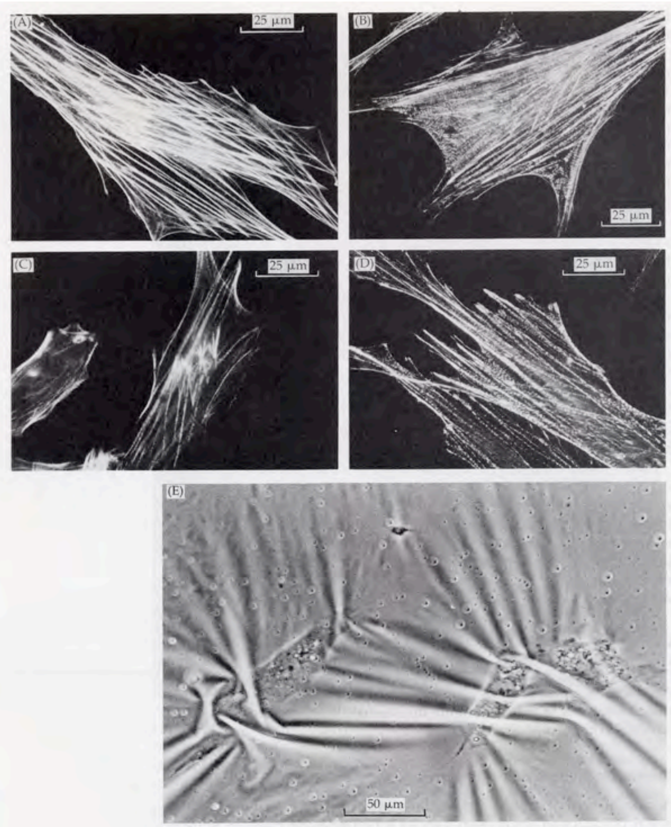
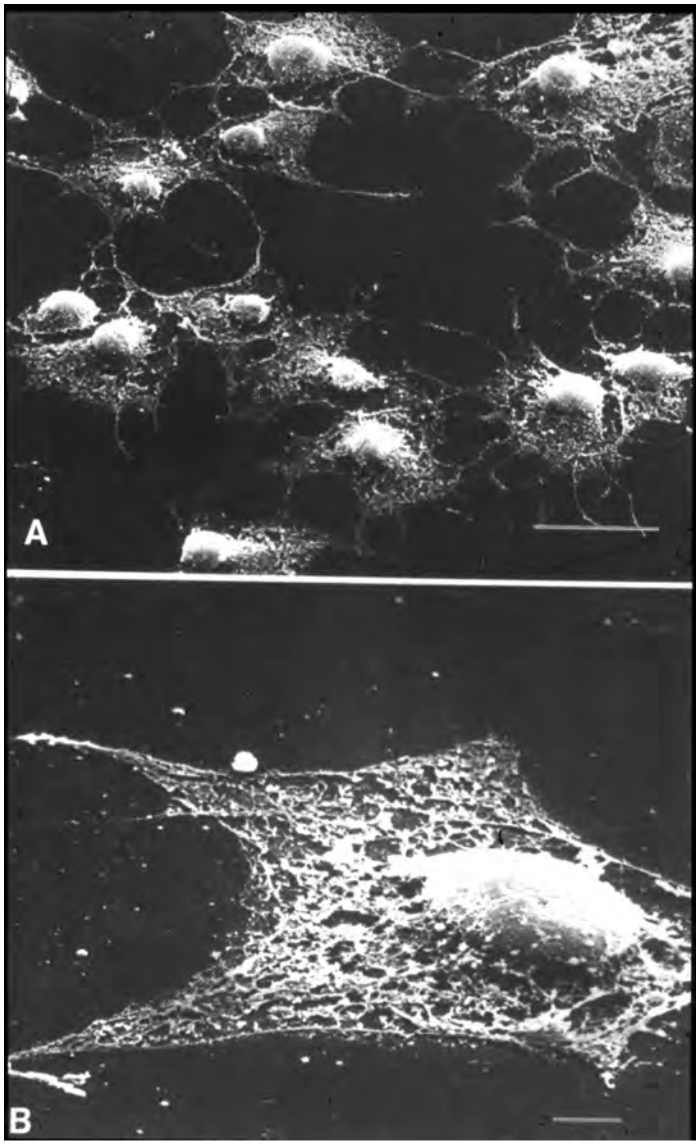
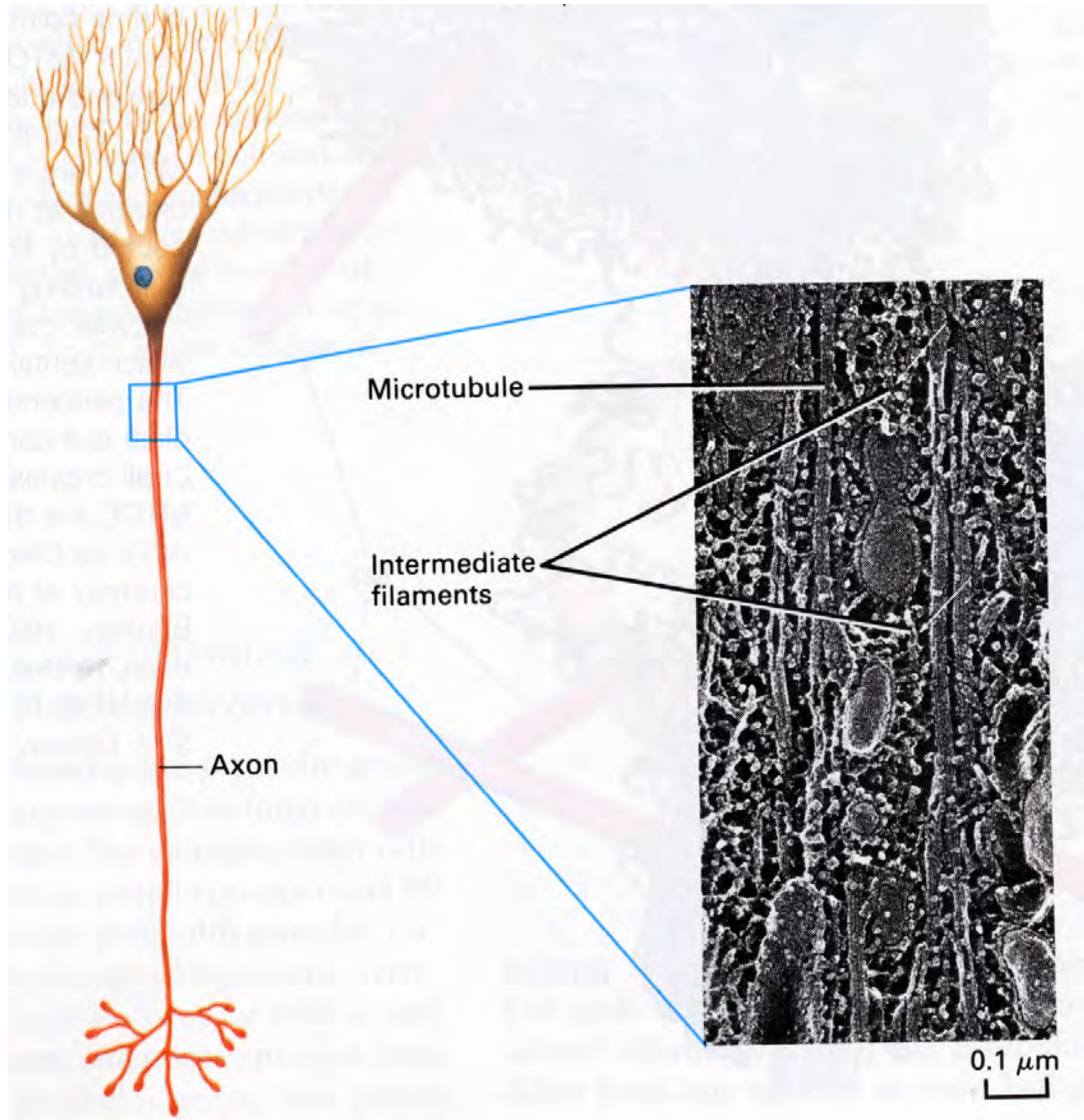


FIGURE 2. *Cultured fibroblasts stained with fluorescent antibodies to various contractile proteins. (A) Actin. (B) Myosin. (C) Tropomyosin. (D) α-Actinin. (E) Fibroblasts cultured on a thin film of silicone rubber demonstrate directly the traction generated during cell movement; note the wrinkles produced in the rubber film. (A–D from Abercrombie, 1982; courtesy of M. Osborne; E from Harris, 1982.)*

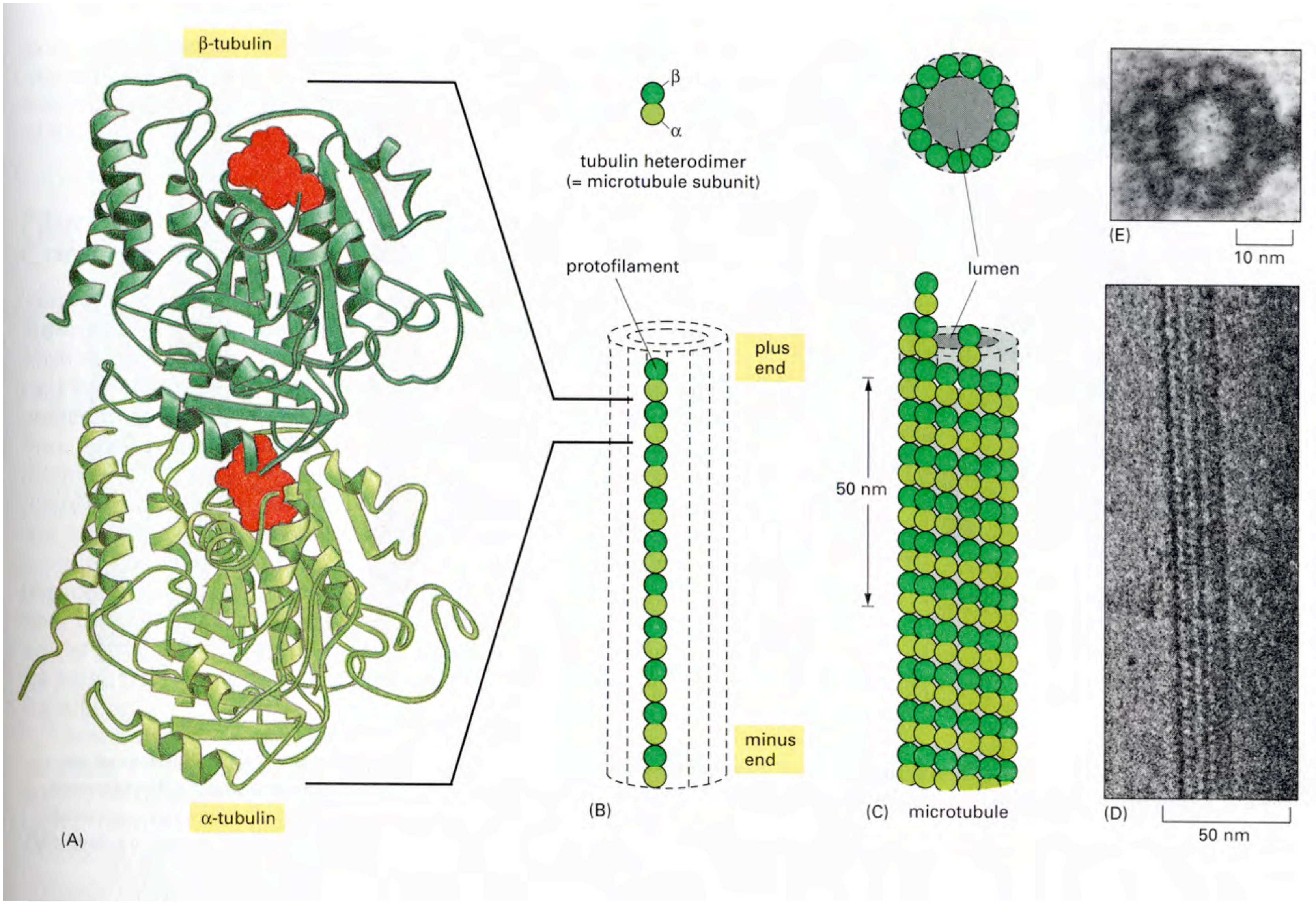
The Neuronal Cytoskeleton



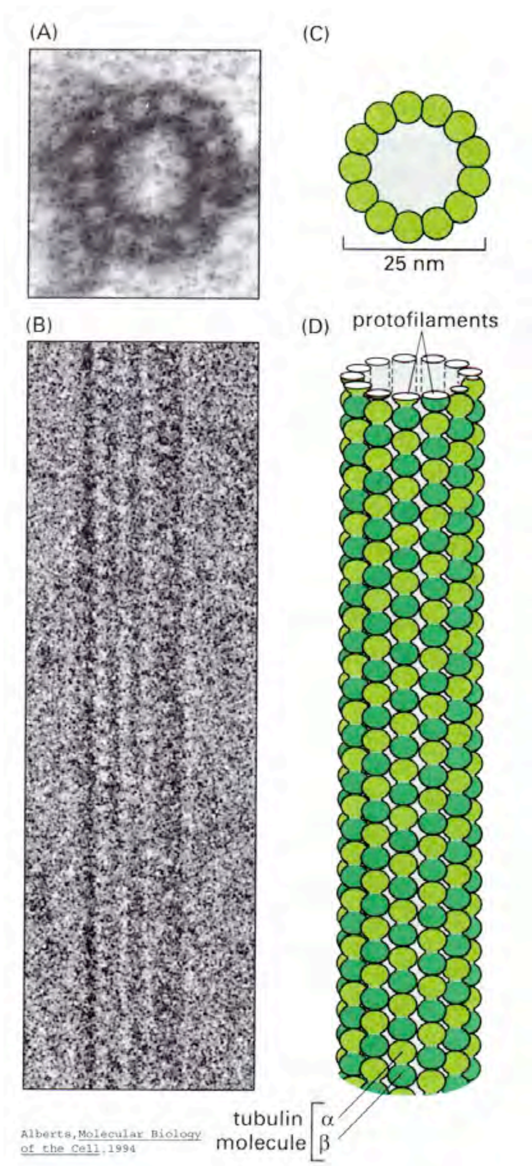


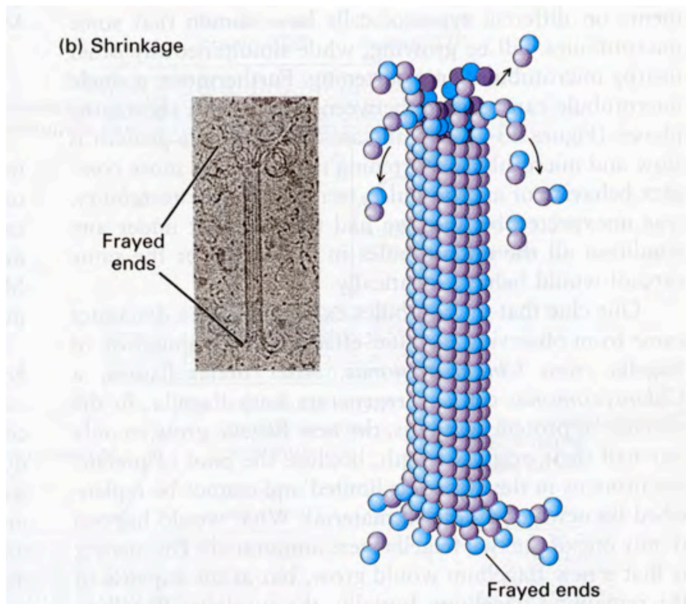
Images from M. Ignatius

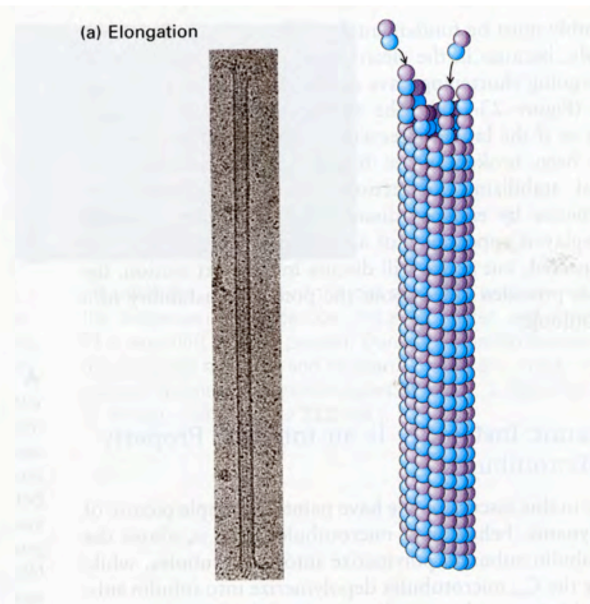
Microtubules are Non-Covalent Polymers Assembled from α and β Tubulin Subunits, plus "MAPs"



Microtubules are (i) Non-covalent, (ii) Dynamic Polymers Assembled from α and β Tubulin Subunits

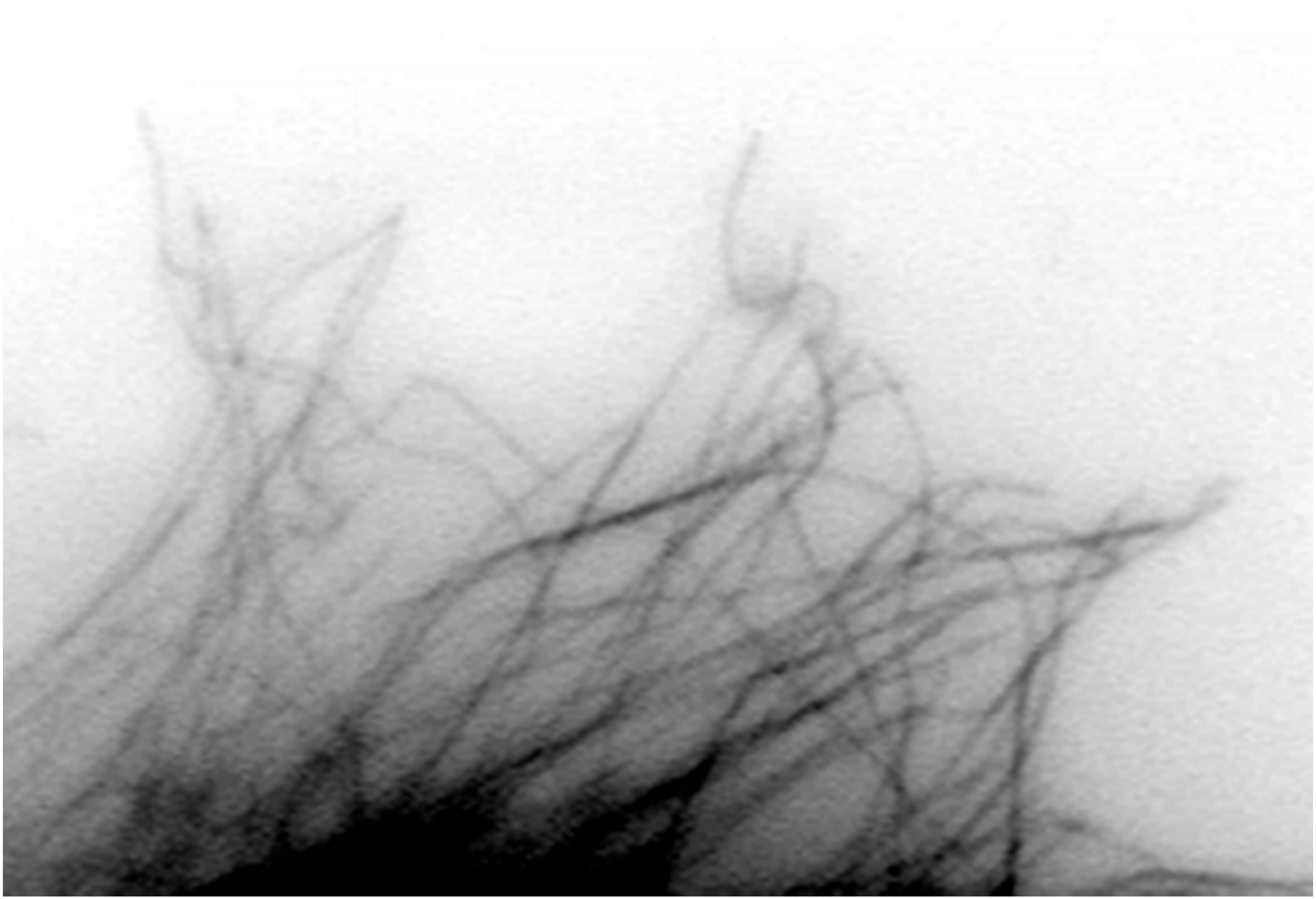






and Properly Regulated Microtubule Dynamics are Essential for Cell Function And Viability!

Microtubules are <u>Dynamic</u> Polymers



actin filaments
(also dynamic)

neurofilaments
(not dynamic)

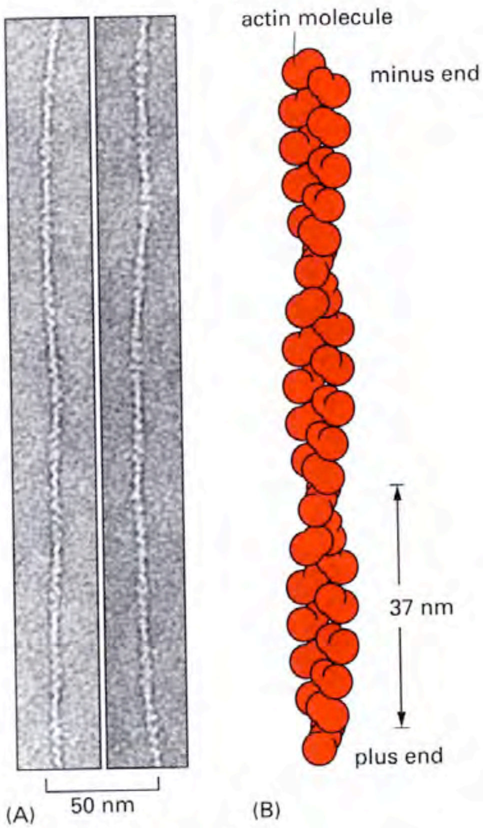
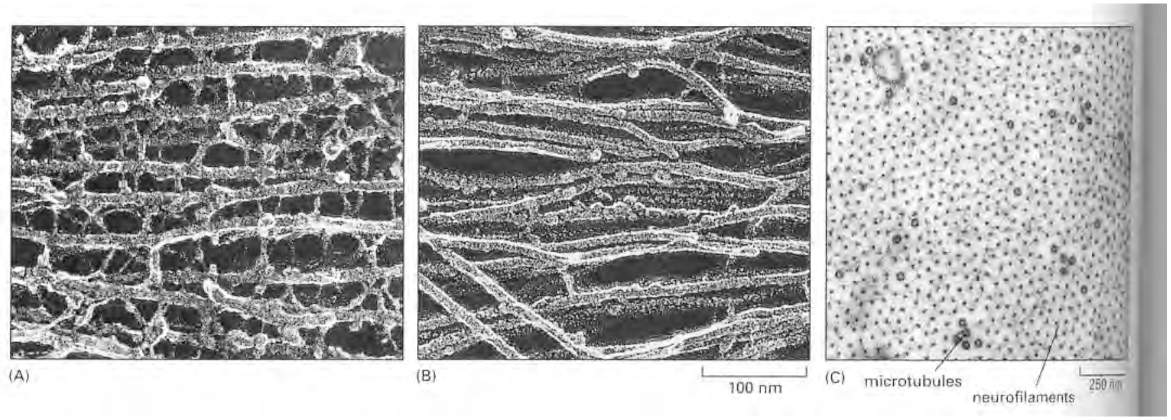
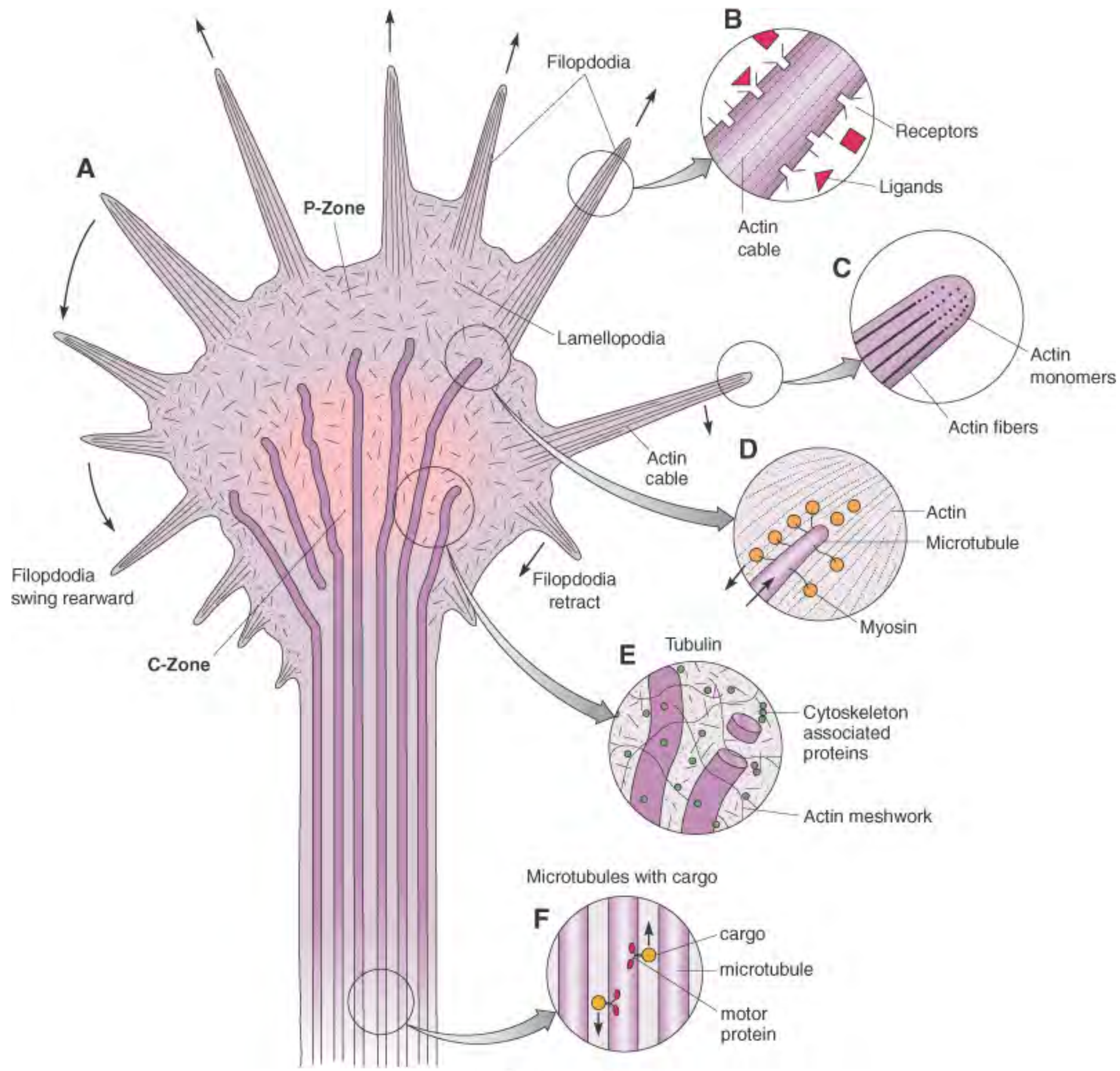


Figure 16–49 Actin filaments. (A) Electron micrographs of negatively stained actin filaments. (B) The helical arrangement of actin molecules in an actin filament. (A, courtesy of Roger Craig.)



Structure of a growth cone



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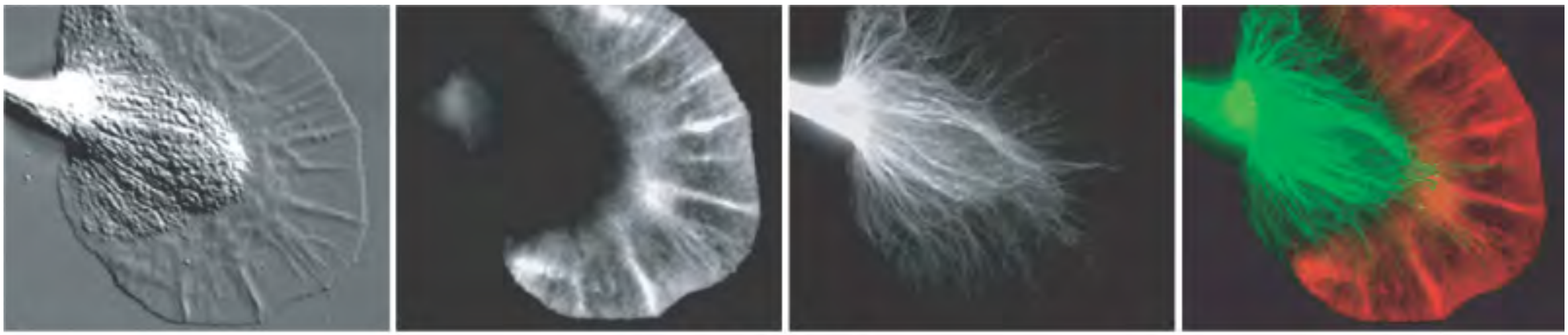
Views of Aplysia Growth Cones

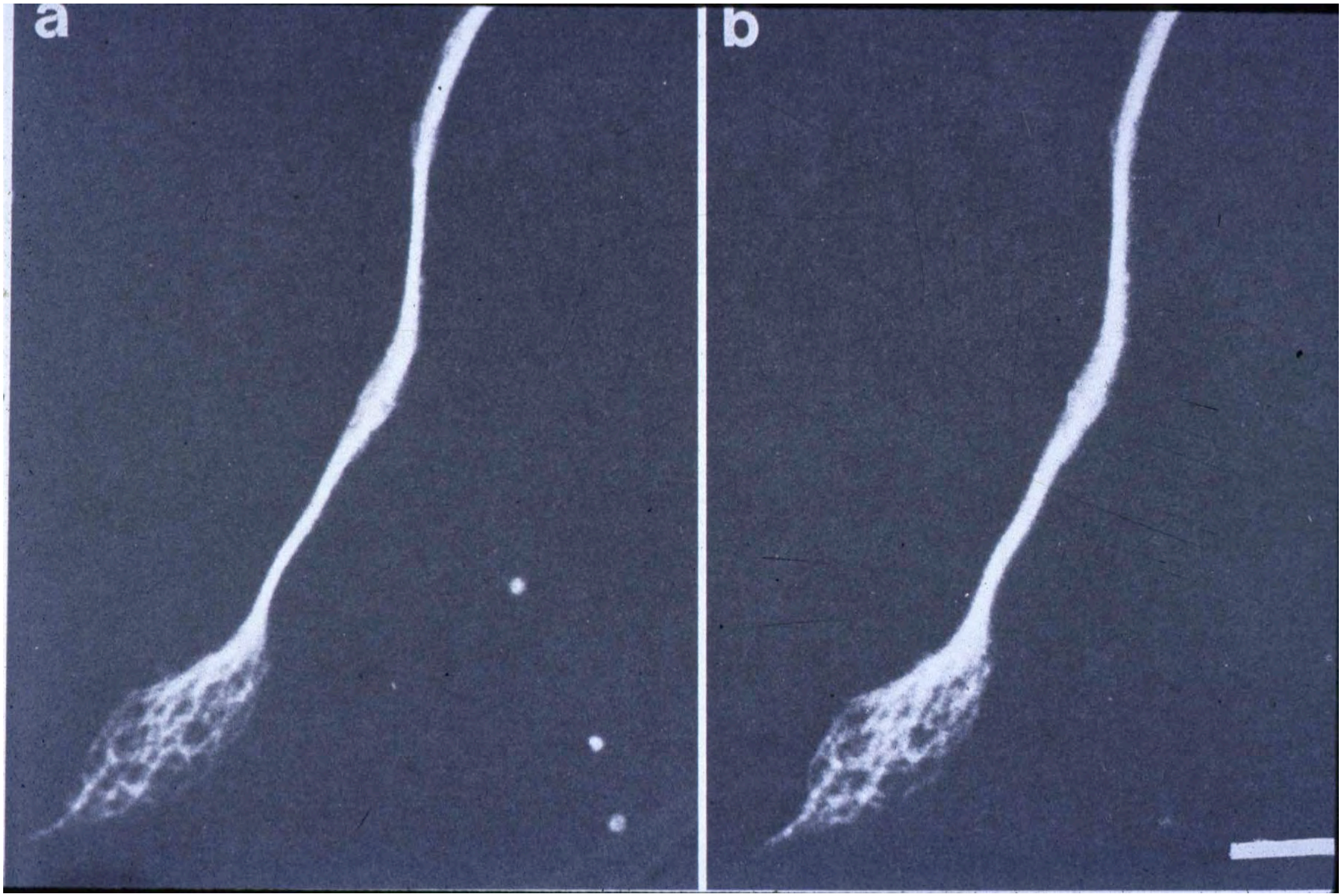


(From Paul Forscher)

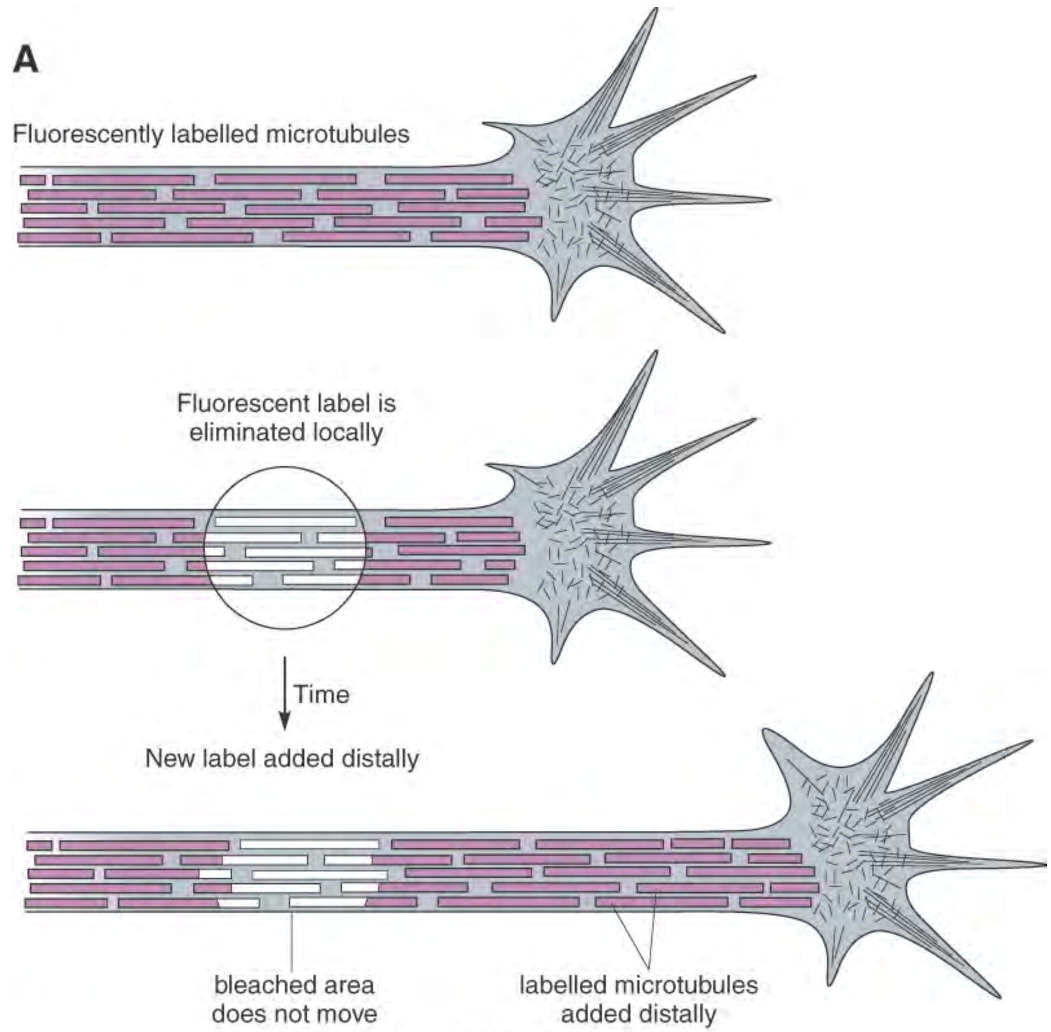
Nomarski Actin Microtubules Merge

Tubulin and Tau in a Neuronal Growth Cone

(Images by Dave Drubin)



Microtubules are added at the growing end of the axon



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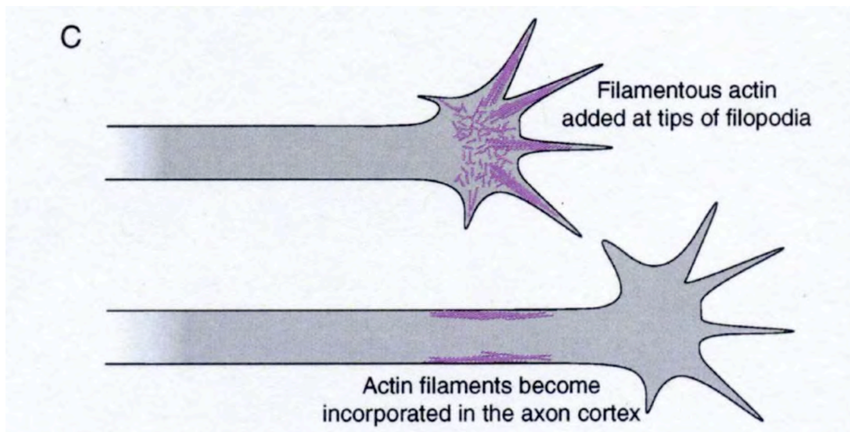
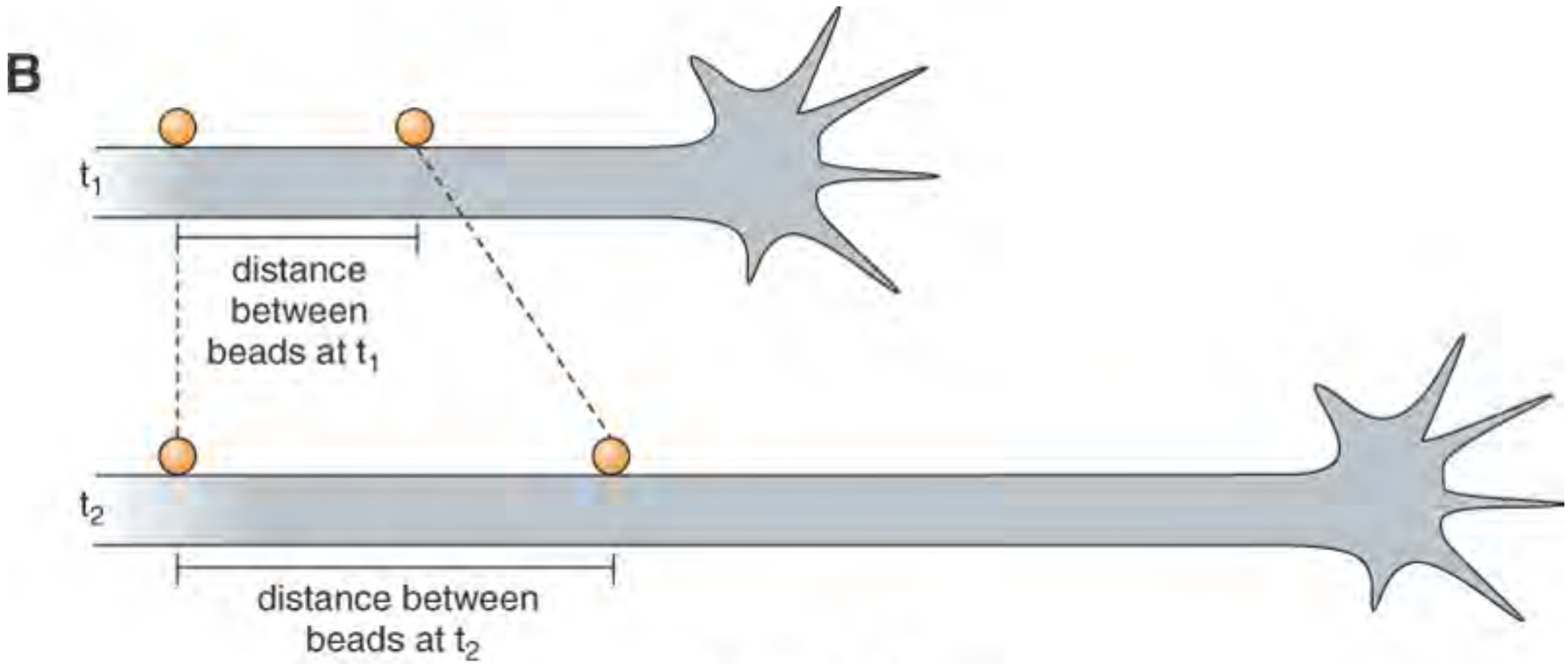
Filamentous Actin is assembled in the filopodia of the growth cone and some is left behind in the axon cortex



Membrane can be added along the length of the axon



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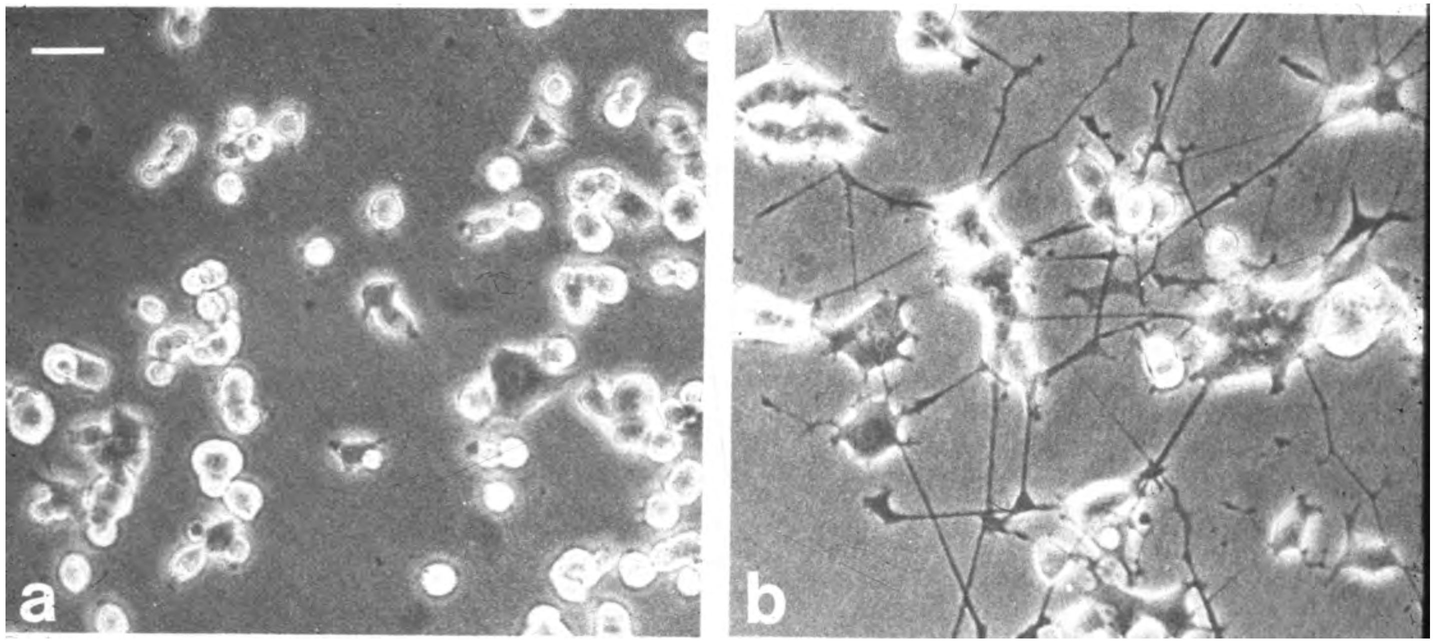
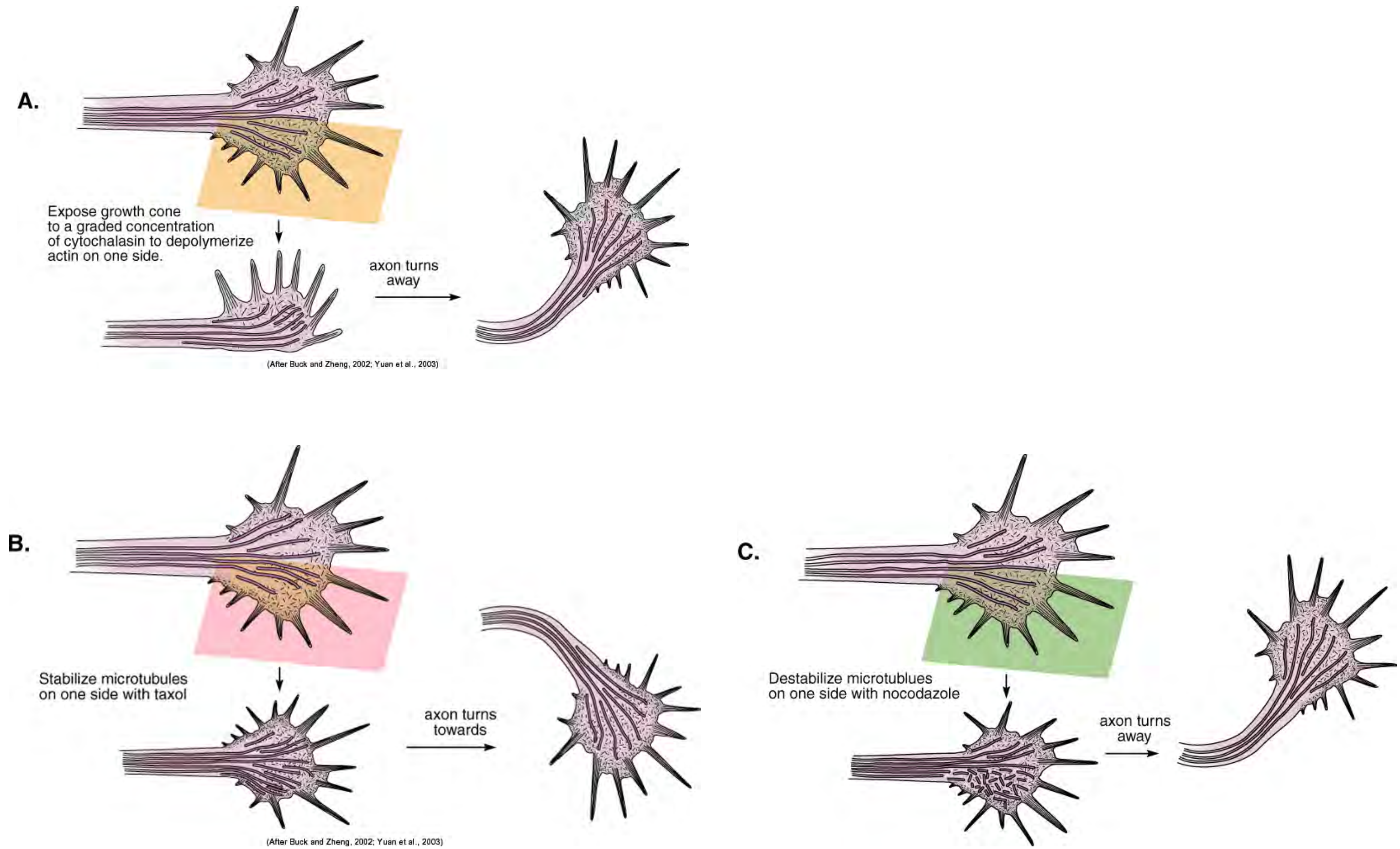
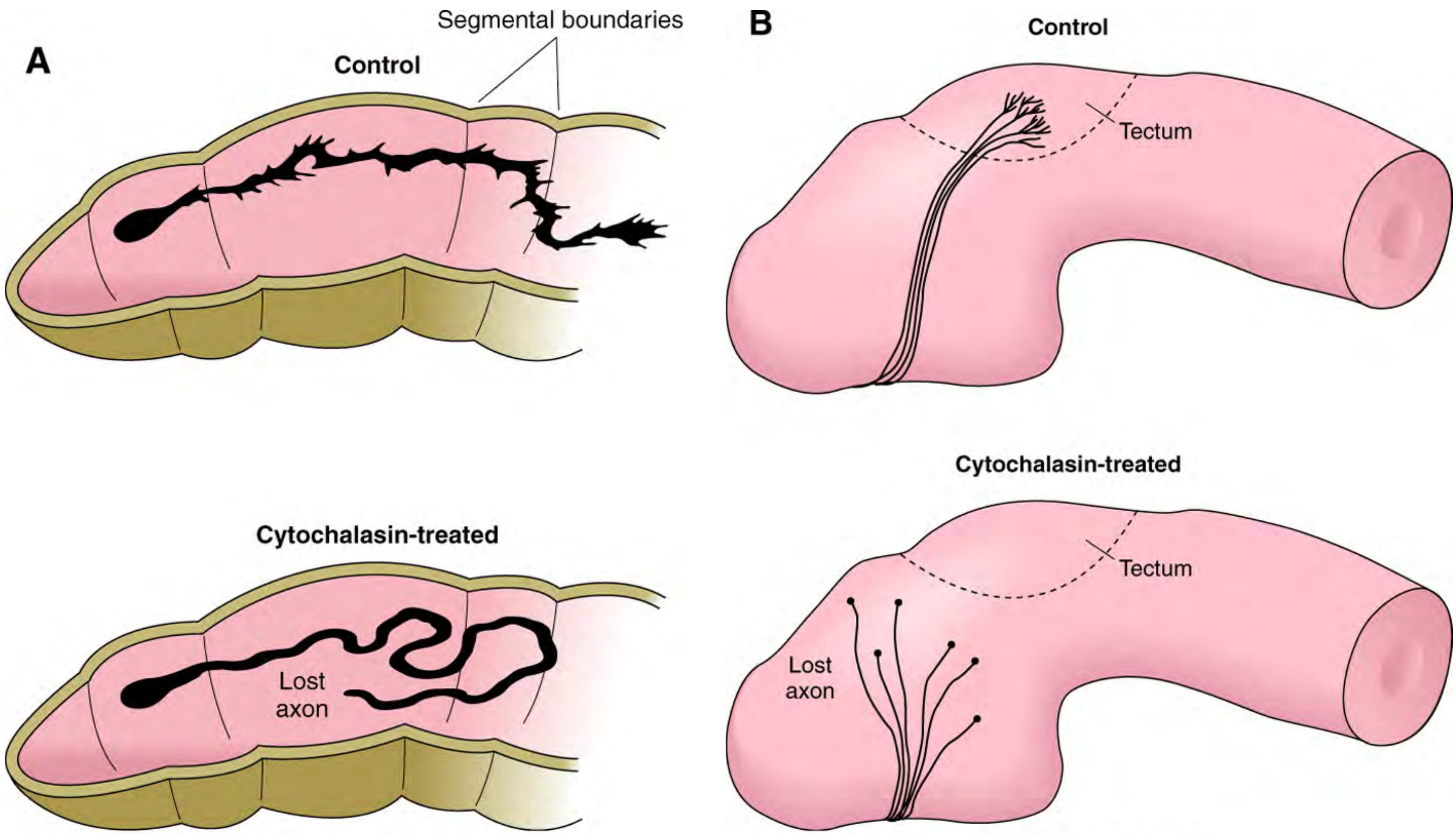

a
b


VINBLASTINE

Actin and Microtubules steer growth cones



Actin filaments are necessary to guide growth cones



(After Bentley and Toroian-Raymond, 1986; Chien et al., 1993)

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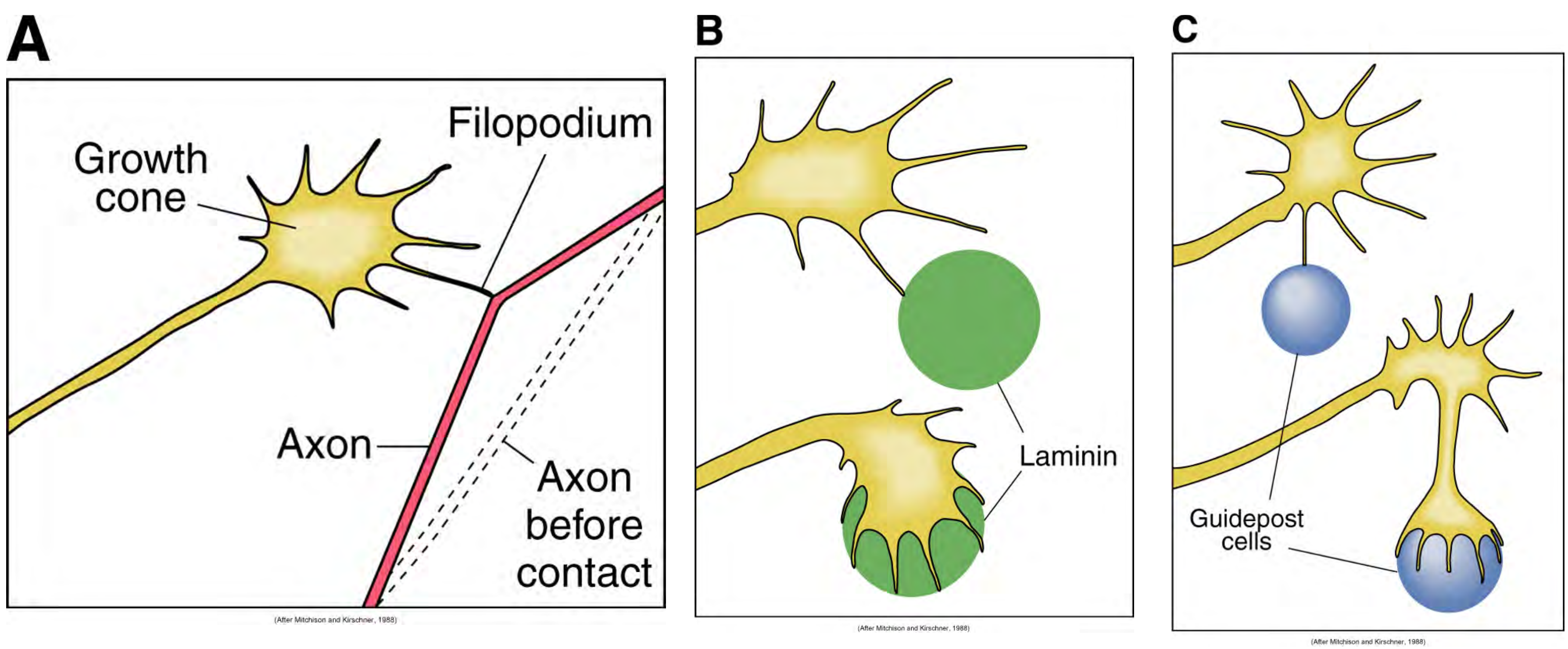
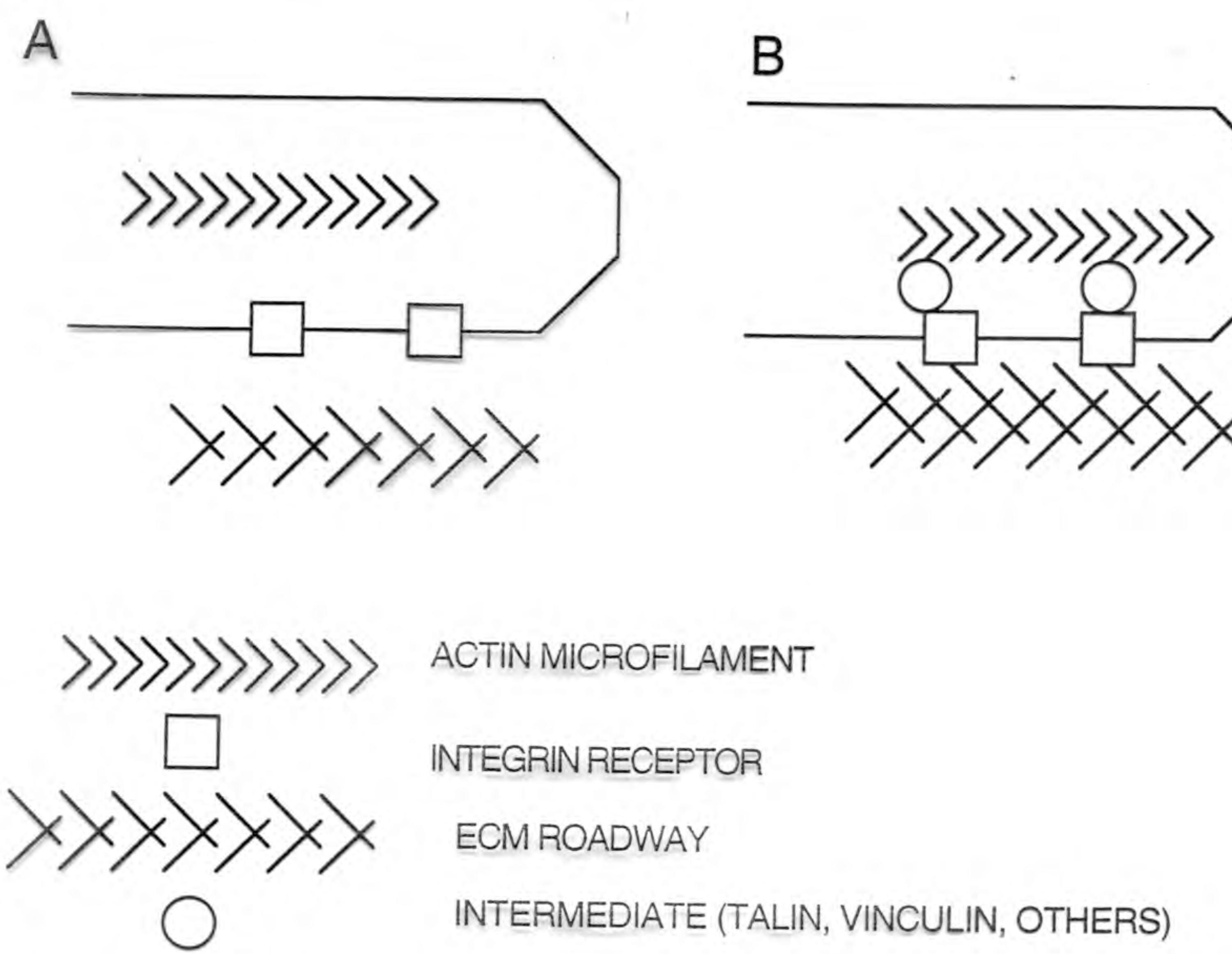
Single filopodia can direct growth cones



A "clutch" model for integrating inside and outside of growth cone



Integrins “integrate” the extracellular matrix with the cytoskeleton