A sketch of the central nervous system and its origins

G. E. Schneider 2005

Part 5: Differentiation of the brain vesicles

MIT 9.14 Class 12-13

The growth of the long extensions of neurons
and related topics

Major stages of nervous system development

- **Proliferation** of cells
- **Migration** from birth places to destinations
- **Differentiation** of neurons and cell groups
  - **Growth of extensions (axons, dendrites, spines)**
  - Sculpting by branch loss and cell death
  - Maturation
- **Plasticity**

Figure removed due to copyright reasons.
Please see:
Cajal, S. Ramón y. *Histology of the Nervous System of Man and Vertebrates*. Translated from the French by Neely Swanson and Larry W. Swanson. 2 vols. New York, NY: Oxford University Press, 1995. ISBN: 0195074017.

Nerve fiber development (Cajal)

How did he observe such developmental dynamics?

Membrane incorporation in the growing axon

- What do Purves & Lichtman say about this? Where is new membrane added, and how does it occur? *(Chapter 4b, p. 98-99)*

Developmental dynamics:

More questions from Purves & Lichtman *(chapter 4b)*

What technical advances in neuroembryology can attributed to Ross G. Harrison *(p.96)*?

Tissue culture method

How did Speidel's method *(p. 105)* differ from Harrison's?

Growth of dendrites and axons

- The growth cone
  - Motile filopodia (plural of filopodium) containing actin filaments

- Selective adhesion by filopodial tips;
  - Contraction of filopodia due to contractile proteins (mostly actin).
  - CAMs (cell adhesion molecules, like N-CAM)

Axon growth cone

scanning EM picture

Figure removed due to copyright reasons.

From Wessells and Nuttall, 1978 (reproduced in Zigmond et al., 1999)

Screenshot removed due to copyright reasons.

Growth of axons in tissue culture: NGF Assay

NEXT: Living growth cones in tissue culture; growth factors

(Video clip)

Large growth cone in tissue culture

Screenshot removed due to copyright reasons.

(Video clip)

Growing axons in culture: chick retina and DRG

Screenshot removed due to copyright reasons.

(Video clip)

Three growth cones *in vitro*

Screenshot removed due to copyright reasons.

(Video clip)

Axonal growth cone of sympathetic ganglion neuron and fibroblast *in vitro*

Screenshot removed due to copyright reasons.

Screenshot removed due to copyright reasons.

neurotrophins

a family of growth factors

Using tissue culture:

NGF Assay

First developed by

Rita Levi-Montalcini

Growth of dendrites and axons

- The growth cone
  - Motile filopodia *(plural of filopodium)*
- Selective adhesion by filopodial tips
  - CAMs (cell adhesion molecules, like N-CAM)
- Contraction of filopodia due to contractile proteins (mostly actin)

Schematic Illustration:

Growth cone

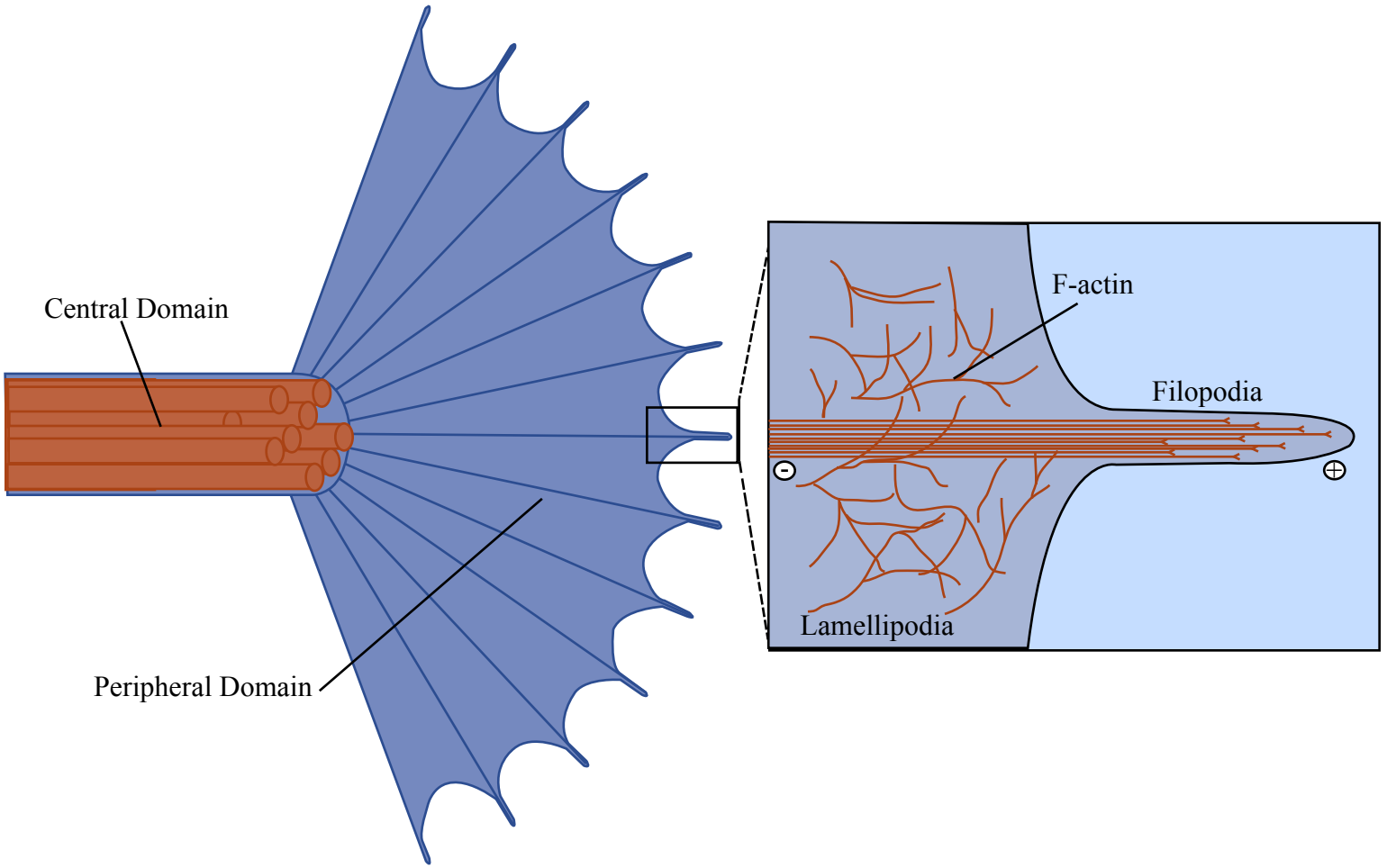


Figure by MIT OCW.

Singular terms (Latin):
Filopodium,
lamellipodium

An experiment on the growth of sensory axons in the developing grasshoppper leg

- How can the axons be observed?
- How does an axon find its way to its target ganglion?

Tracer injection, e.g. HRP.

From Purves & Lichtman ('85); Zigmond et al., '99.

*

2) Followers: selective fasciculation

1) Pioneers: "Guidepost cells"

Figure removed due to copyright reasons.

Grasshopper leg

- The role of "guidepost cells" and long filopodia of growth cones of the primary sensory neurons in the epithelium.
- This is not what happens in the mammalian optic tract, where later-growing axons do not grow along the surfaces of the earlier ones, but rather space themselves between them.

An experiment on specificity of axon growth

What is the major result in Hibbard's experiment on transplanted amphibian Mauthner cells?

(Purves & Lichtman, pp.118-119)

Hibbard's experiment on transplanted amphibian Mauthner cells

Figures removed due to copyright reasons.
Please see from p. 118 in:
Purves, Dale, and Jeff W. Lichtman.
Principles of Neural Development.
Sunderland, MA: Sinauer Associates, 1985,
pp. 3-23. ISBN: 0878937447.

Guidance Mechanisms for axon outgrowth: Four mechanisms as seen in 1985

- **Stereotropism**
- Galvanotropism
- Tropism based on **differential adhesion**
- **Chemotropism**
  - Membrane contact
  - Diffusable signals

(Purves & Lichtman pp. 119-129)

Recent studies have supplemented this picture considerably

They have distinguished four types of chemical guidance, adding new detail to the above. What are they?

(Summarized in Zigmond et al. '99)

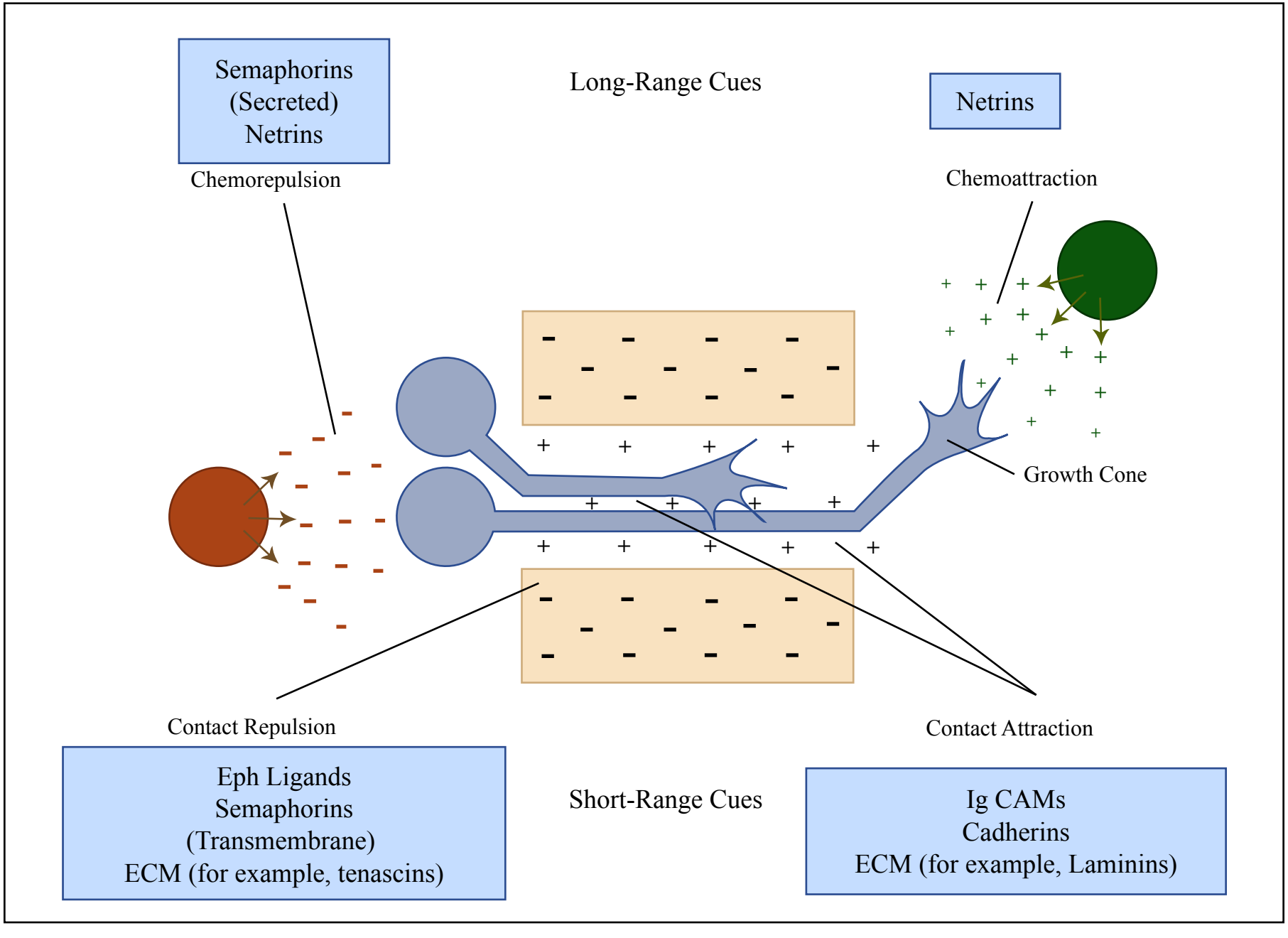


Figure by MIT OCW.

More detail from a slightly different viewpoint:

Chemical specificity 1: attraction effects

- Cell-cell adhesion (CAMs; ECM molecules like the laminins and cadherins)
- Growth factors:
  - Contact (Semaphorins)
  - Diffusable (Netrins; NGF and other neurotrophins; other families of GFs)

More about diffusable growth factors: Contrasting "trophic" and "tropic" effects of NGF

Actually, there are three distinguishable effects of NGF on certain neurons. What are they?

Tropic : effects on direction of growth

Trophic : survival promoting,

2nd trophic effect: growth promoting, increased growth vigor, with greater ability to compete.

Chemical specificity 2: barriers; inhibition of growth

- Midline barriers
  - by contact repulsion, e.g., by certain proteoglycans secreted by midline radial glia
- Oligodendrocyte factors
  - A membrane protein, *Nogo*, which inhibits axon growth
- Secreted and transmembrane proteins (Semaphorins; neurotrophins and other GFs; ephrins)

Guidance mechanisms are not fixed:

- Modulation by intrinsic metabolic factors (discoveries by Mu-ming Poo's group)

"Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides" by H.-j. Song, G.-L. Ming, Z. He, M. Lehmann, L. McKerracher, M. Tessier-Levine, M.-m. Poo. **Science**, *1988, 281, 1515-1518.*

Semaphoria III

Sema III and cGMP *(M. Poo '98)*

Screenshot removed due to copyright reasons.

Figure removed due to copyright reasons.

A study of Xenopus spinal neuron axon growth in tissue culture:

Growth cone turning in a gradient of Sema III (Song et al. 1998):

Effects of manipulating cGMP (A), cAMP (B), …

Figure removed due to copyright reasons.

{8-bromo-cGMP: a membrane permeable agonist of cGMP signalling pathways.}

An apparent role of Semaphorin III (collapsin) in the innervation of the spinal cord by dorsal root axons:

Figure removed due to copyright reasons.

A molecular sieve? Semaphorin III produced in the ventral half of the embryonic spinal cord (dark tan) may repel axons of temperature- and pain-sensory neurons while allowing in those of Ia afferent neurons that respond to muscle stretch.

From Marx, Jean (1995). Helping Neurons Find their Way. Science 268: 971-973 [a "Research News" article].

Similarly, we can describe a role of the netrin molecules in the formation of the spinothalamic tract decussations

- Discovery of the netrin molecules: Tom Jessell and co-workers at Columbia
- Netrins diffuse from floor plate region
- They have tropic effects on axons of dorsal horn cells which form the spinothalamic tract.
- *If they attract the axons growing from the dorsal horn, how can this result in a decussation?* Remember the findings just discussed (M. Poo's work)

Developing axons do much more than simply find their path to a target

- We have been considering the elongation mode of axonal growth.
- In this mode, they grow much faster, and branch much less, than during the subsequent arborization mode of growth.
- The following picture is taken from a study of optic-tract axon growth.

Two modes of axon growth

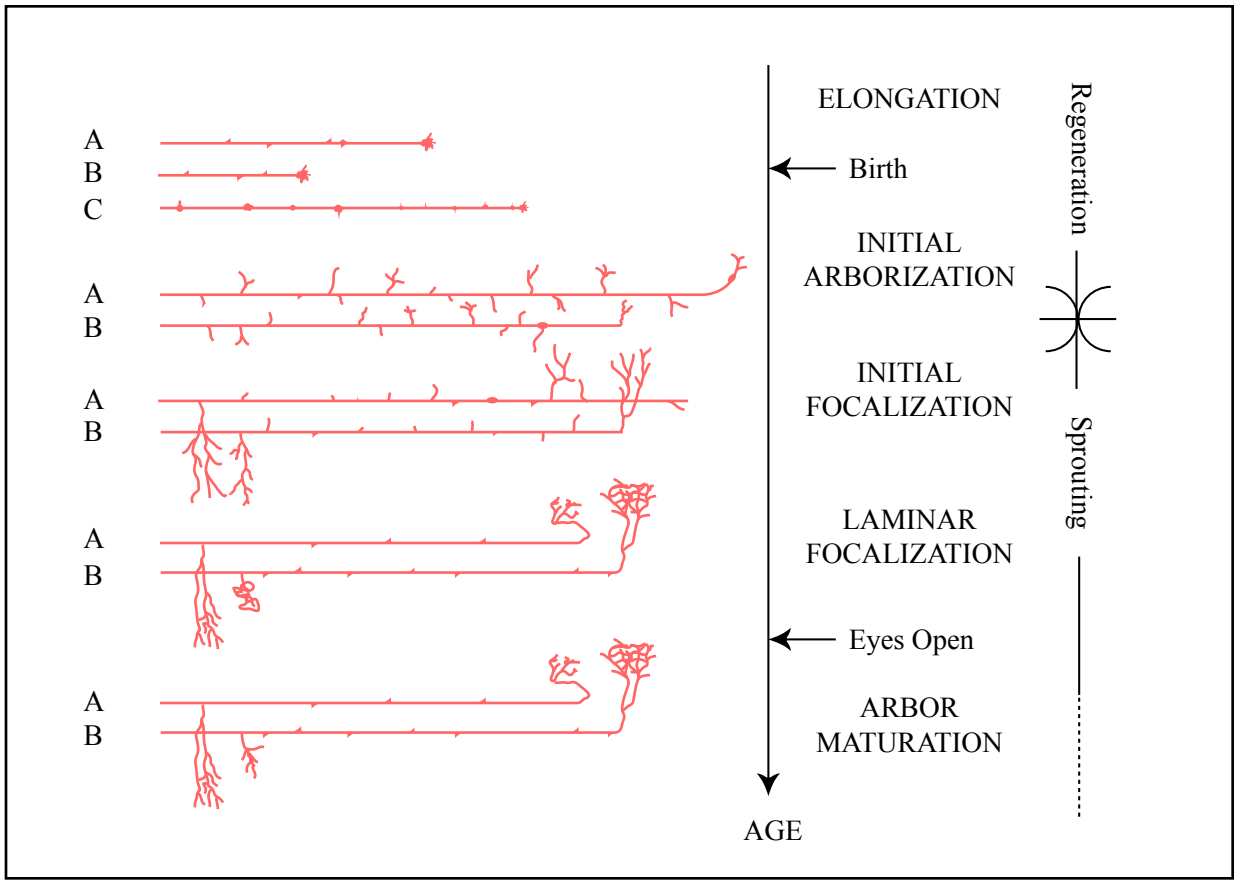


Figure by MIT OCW.

End, session 13

Topics in the study of optic-tract development & plasticity: these apply also to other axonal systems

- Embryonic formation; 2 modes of growth
  - *Optic tract;* geniculo-striate pathway; other connections
- Map formation; chemoaffinity
- Map plasticity: lesions
- Collateral sprouting; competitive interactions in axonal growth
- Roles of cell death
- Regeneration in development and adulthood

Map formation; chemoaffinity

Ephrins and Eph receptors are responsible for the naso-temporal retinal axis representation in the tectum (superior colliculus). How does it work?

Discoveries of specific mechanisms at the cell-molecular level came many years after Roger Sperry formulated his chemoaffinity hypothesis, based on his studies of regeneration in frog and goldfish.

Distribution of Eph receptors and ephrin ligands in the developing chick retinotectal system related to retinotopic projections

Figure removed due to copyright reasons.

From O'Leary, Yates & McLaughlin, 1999

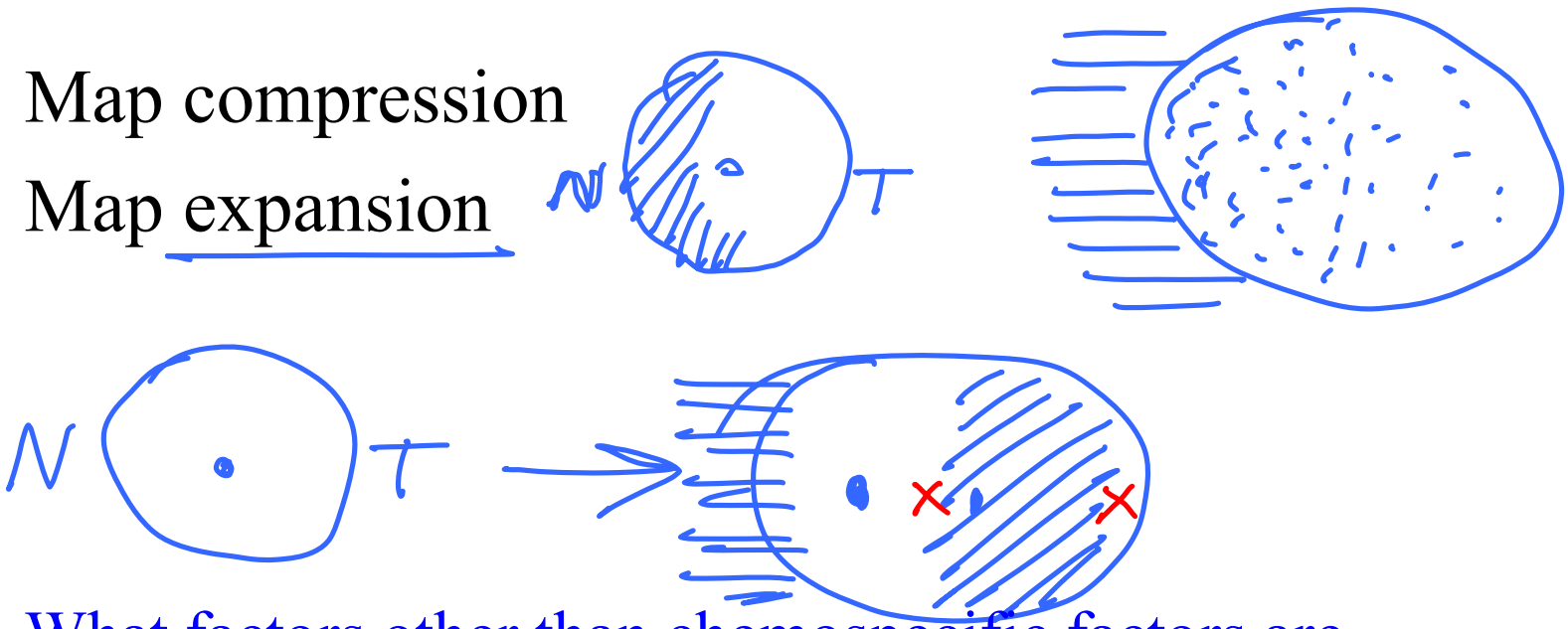
After discoveries by Bonhoeffer et al., by Flanagan et al, and others

Figure removed due to copyright reasons.

The mechanism: selective repulsion. Response of temporal and nasal RGC axons to a gradient of tectal membranes, from purely anterior to purely posterior

Such chemical specificity does not prevent plasticity of the developing maps

- Map compression
- Map expansion



- What factors other than chemospecific factors are active?
- Evidence for other factors has been obtained from studies of effects of damage during development.

“Collateral sprouting“ in development

- Can cause developing axons to violate the normal rules of regional specificity:
  - e.g., in developing hamster or ferret, the optic tract can be induced to grow into the medial geniculate body of thalamus (normally part of auditory system) or the ventrobasal nucleus (normally in receipt of somatosensory system axons from spinal cord).

Effects of early ablation of SC

Newborn hamster, studies using axonal tracing with Nauta silver stains for degenerating axons

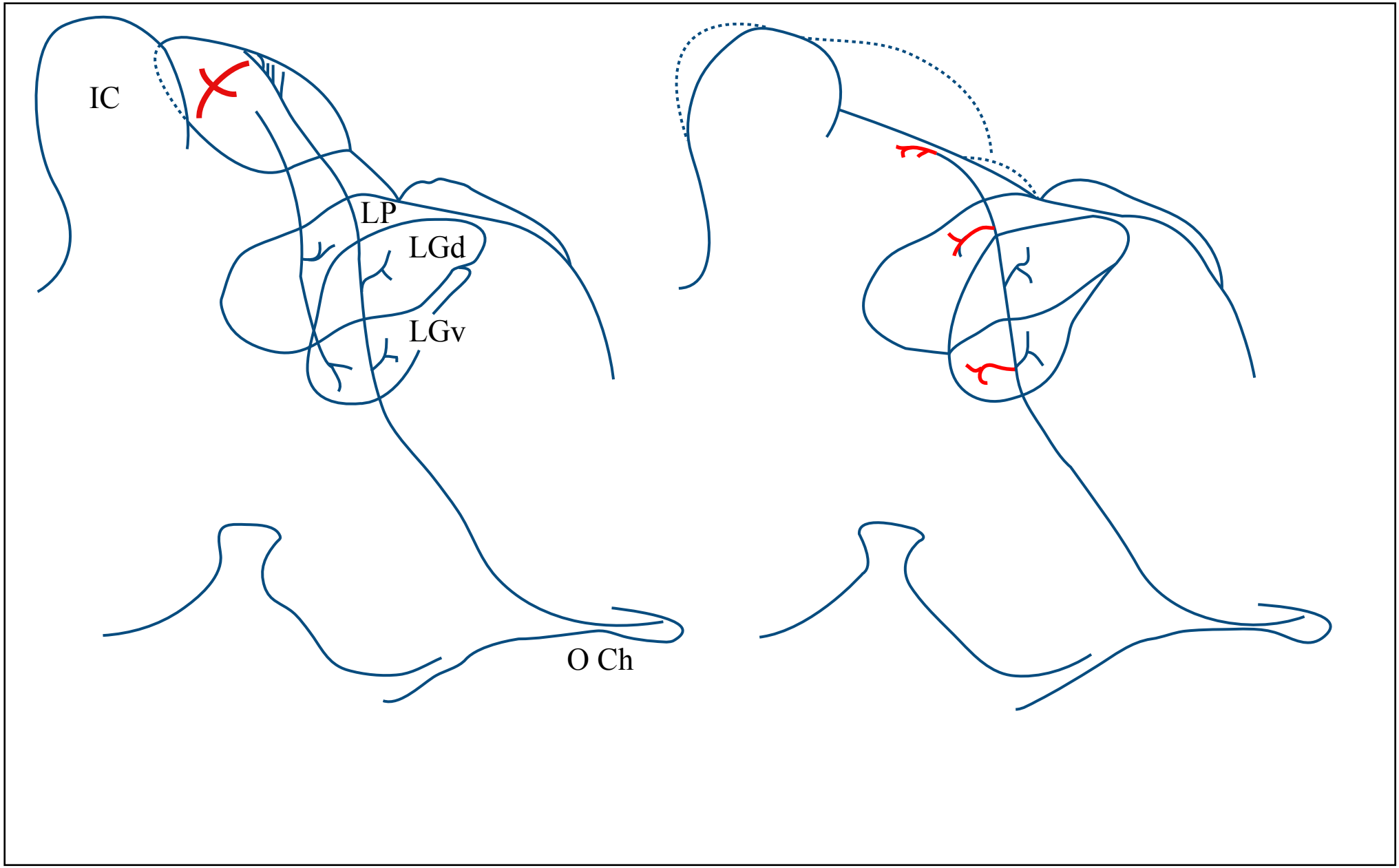


Figure by MIT OCW.

Note the sprouting in LP and LGv as well as in the remaining SC.

Effects of early ablation of SC and BIC

Newborn hamster, studies using axonal tracing with Nauta silver stains for degenerating axons

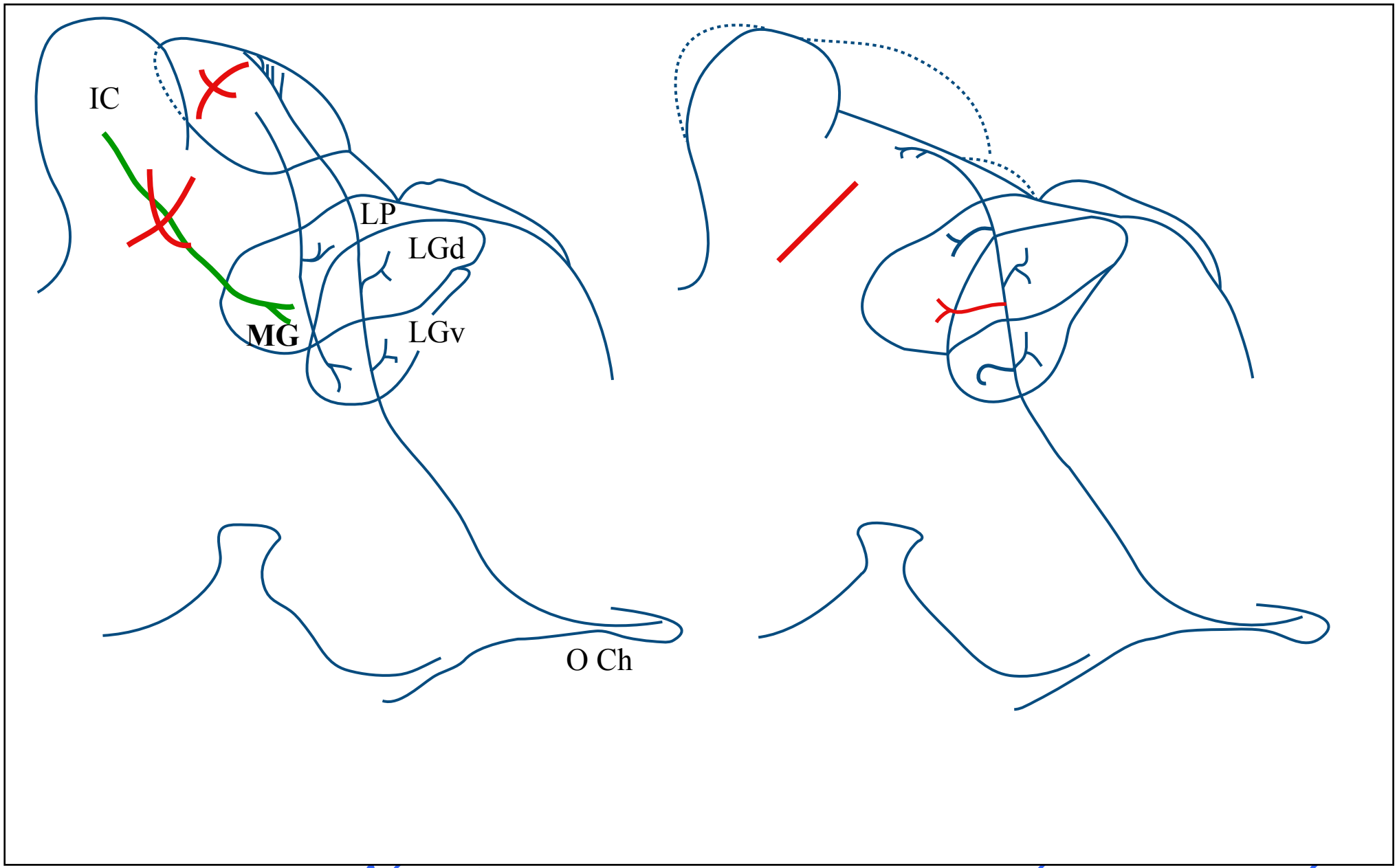


Figure by MIT OCW.

Next: 2 major reasons for sprouting other than chemical specificity

Sprouting phenomena:
axonal competition and spreading

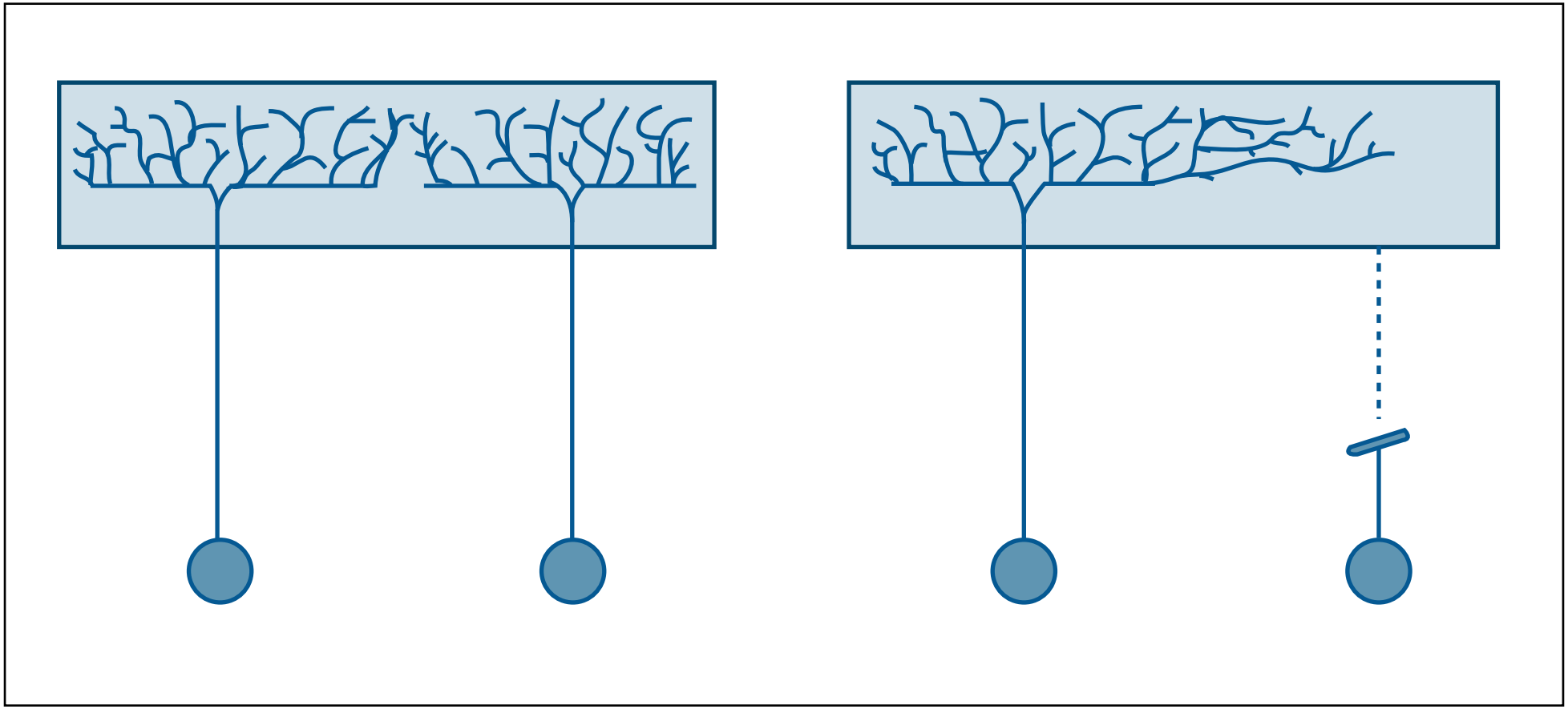


Figure by MIT OCW.

Competition among axons: What is it?

- Competition for terminal space
  - for growth factors
  - for occupancy of synaptic sites

- Axon-axon contact interactions
  - Retraction reactions; "collapsin" molecules causing a contact inhibition of extension

Modulation of "competitive growth vigor"

The more growth vigor an axon has, the more it grows and the better it competes for terminal space.

- By chemical factors: more growth with more growth factor
  - E.g., NGF (see figure).
  - There are also molecular factors intrinsic to the cells which determine growth capacity, in either elongation or arborization.
- By activity:
  - More growth by more active axons, as in formation of ocular dominance columns in visual cortex
- By "pruning":
  - Sprouting in one region due to blockage of or damage to an axon in another region (see figure)

Screenshot removed due to copyright reasons.

NGF: effects on growth vigor in DRG axons

Intrinsic, competitive vigor of axon growth

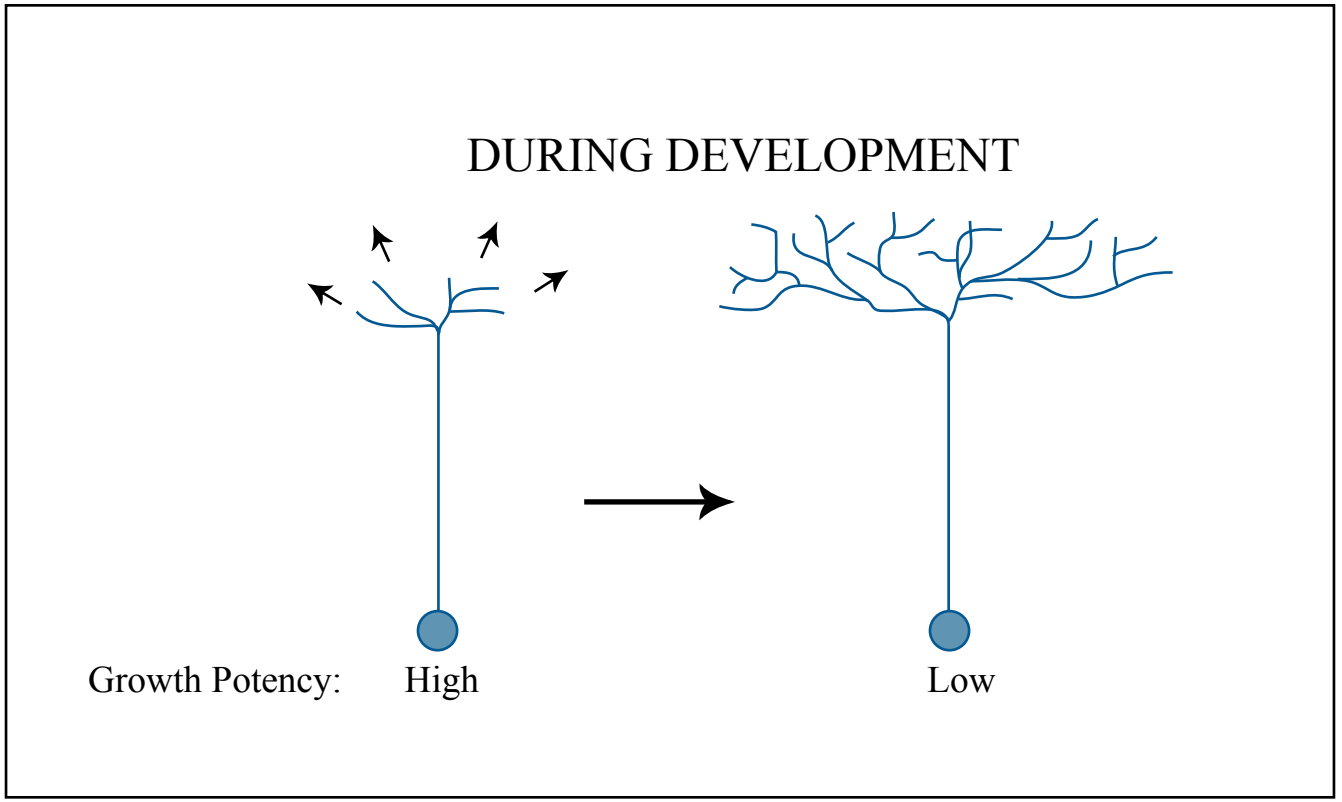


Figure by MIT OCW.

Intrinsic, competitive vigor of axon growth

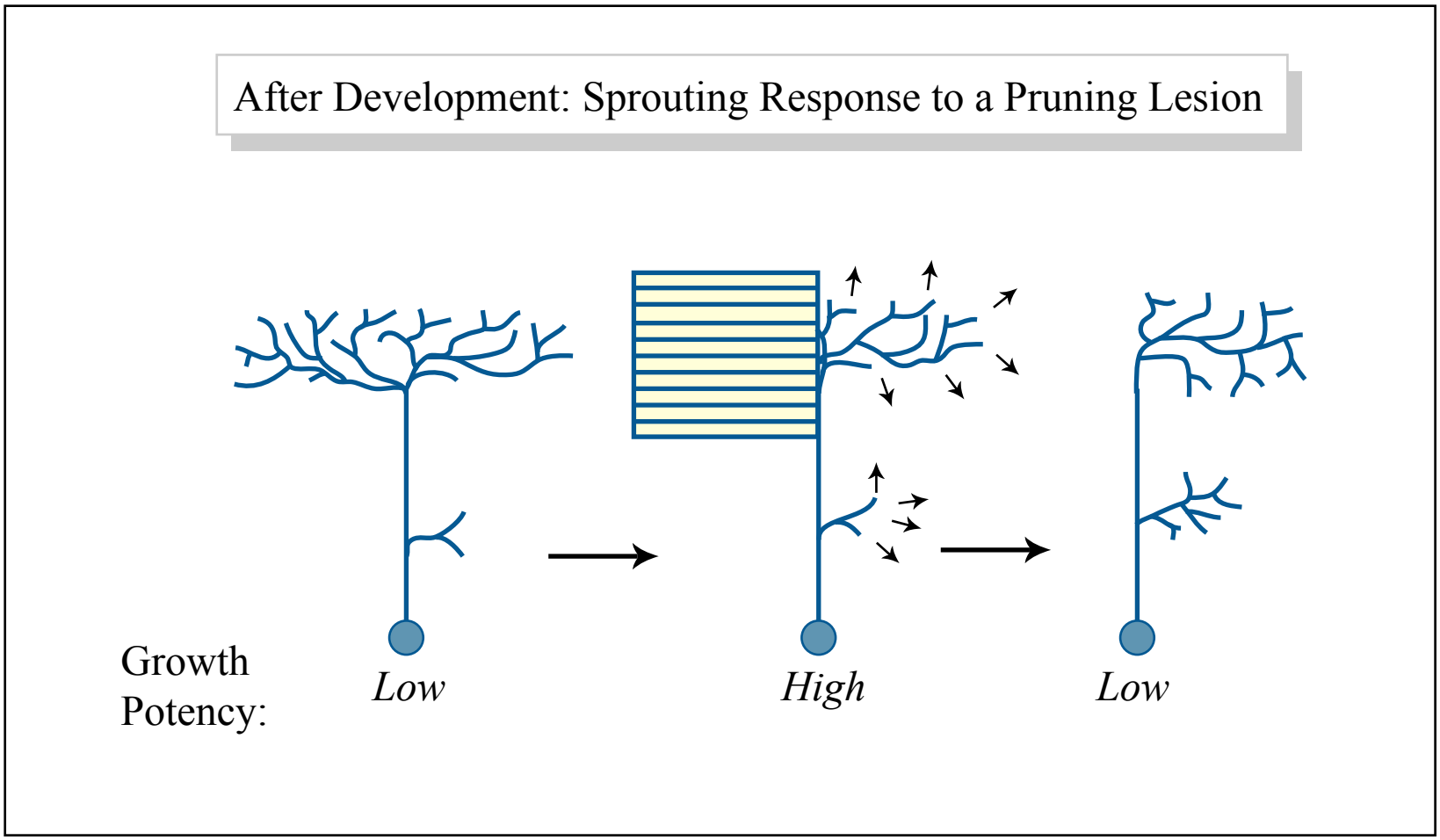


Figure by MIT OCW.

Pruning effect: Effects of pruning lesion on growth vigor. Such phenomena provide evidence for "Conservation of Terminal arbor size", discovered in studies of developing optic and olfactory tracts.

Thus, we have two types of factors that could play roles in both development and evolutionary change

1) Extrinsic factors in axon-axon competition
2) Intrinsic factors in “conservation of terminal quantity”

Major stages of nervous system development

- **Proliferation** of cells
- **Migration** from birth places to destinations
- **Differentiation** of neurons and cell groups
  - Growth of extensions (axons, dendrites, spines)
  - **Sculpting by branch loss and cell death**
  - Maturation
- Plasticity

Phenomena of neuronal death & survival; roles of neurotrophic factors and intrinsic factors

- Many neurons depend on axon target contact for survival. The target tissue gives them trophic factors.
- Without sufficient trophic factor (growth factor), they undergo apotosis (cell suicide, or "programmed cell death") unless protected by intrinsic factors.

Example:
CNS effects of limb-bud extirpation *vs.* grafting of a supernumerary limb in the embryo

- Greater than normal motor neuron death after limb-bud extirpation
- Less than normal motor neuron death after grafting of supernumerary limb in the embryo

Purves & Lichtman, ch. 6 pp 144f

Supernumerary Limb

Figures removed due to copyright reasons.Please see:
Figures from pg. 145 in Purves, Dale, and Jeff W. Lichtman. *Principles of neural Development*. Sunderland, MA: Sinauer Associates, 1985, pp. 3-23. ISBN: 0878937447.

Two major possible purposes in naturally occurring neuronal death

- Population matching
- Error correction

Purves & Lichtman, ch. 6 pp 144-149

Additional roles for neurotrophins

- Activity-induced plasticity
  - E.g., in visual cortex
- Learning
  - BDNF: associated with phosphorylation of specific subunits of the NMDA receptor.
- New neurons in adult brain (BDNF)

Axon regeneration studies:

a very brief introduction

- Mammals and birds *vs* other species; developmental changes in re-growth capacity
- Need to **Preserve** the damaged cells. (Dying cells don't re-grow axons.)
- The mature tissue environment contains many inhibitory factors that may be overcome by the right procedures to **permit** axon growth.
- **Promotion** of growth vigor may be needed after the early period of development.
- **Plasticity** of the connections can play an important role.

The 4 P's of CNS regeneration
(Ph.D. thesis by Rutledge Ellis-Behnke)

Current research:

- PN bridges, autografts
- New hydrogels promote bridge formation
- Scar tissue: inhibiting it or breaking it up
- Genetic transfections

Selected References

Slide 15: Zigmond, Michael J., et. al., eds. *Fundamental Neuroscience*. Illustrations by Robert S. Woolley. Part III. San Diego, CA: Academic Press, 1999. ISBN: 0127808701.