Instructions concerning a dislocation of a vertebra in the neck. "If you examine a man with a neck injury … and find he is without sensation in both arms and both legs, and unable to move them, and he is incontinent of urine … it is due to the breaking of the spinal cord caused by dislocation of a cervical vertebra. This is a condition which cannot be treated." Edwin Smith Surgical Papyrus, Case 31. Thebes, c. 1550 BC. Taken from Breasted, J. H. (ed.) The Edwin Smith Surgical Papyrus *© The University of Chicago Press, 1930.*

Symptoms of <u>spinal cord injury</u>:

involuntary muscle spasms
***loss* of voluntary movement**
- **" sensation, balance**
- **" control of breathing**
- **" autonomic functions (blood pressure)**
- **" bladder, sexual, bowel control**

All due to destruction of long ascending or descending spinal pathways

TO REPAIR THESE PATHWAYS,
AXONS must REGROW
SYNAPTIC CIRCUITS must be REESTABLISHED

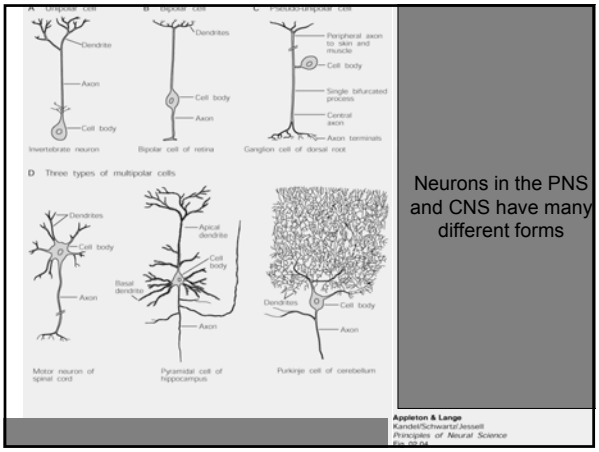
I. RESPONSE OF THE NEURON TO INJURY
All neurons react similarly

II. GLOSSARY OF GLIAL CELLS:
Normal function
Response to injury

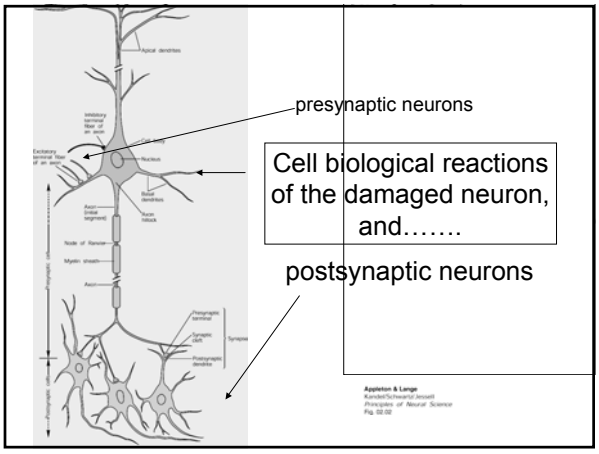
III. DEGENERATION:
Reactive changes, timecourse

IV. REGENERATION
A. Neurons in the PNS can regenerate their axons. How?
B. Neurons in the CNS have a limited capacity to regenerate axons. Why?

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: examples, recent reports



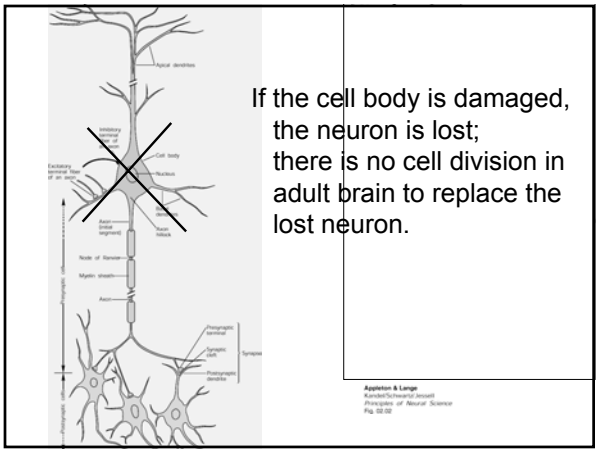
Neurons in the PNS and CNS have many different forms



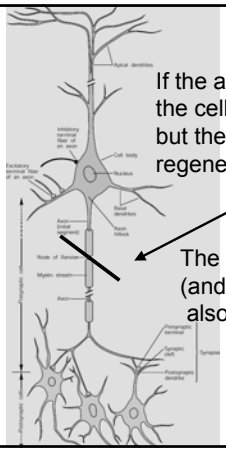
presynaptic neurons

Cell biological reactions of the damaged neuron, and…….

postsynaptic neurons



If the cell body is damaged, the neuron is lost; there is no cell division in adult brain to replace the lost neuron.



If the axon is damaged,
the cell body is lost if the cut is close,
but there is a chance that the axon will regenerate, even in the CNS.

The postsynaptic,
(and the presynaptic), neurons are also affected and may degenerate

Appleton & Lange
Kandel/Schwartz/Jessell
Principles of Neural Science
Fig. 02-02

I. RESPONSE OF THE NEURON TO INJURY (summary)

A. All neurons - despite different forms - react similarly

B. Principles

-If cell body damaged, the neuron dies, and is not replaced by cell division in mature brain.

-If the axon is damaged or severed at a distance from the soma, there is a good chance of regeneration, primarily in the PNS.

-CNS neurons have the capacity to regenerate.

I. RESPONSE OF THE NEURON TO INJURY

II. GLOSSARY OF GLIAL CELLS: Normal function, response to injury

III. DEGENERATION: Signs, Timecourse

IV. REGENERATION

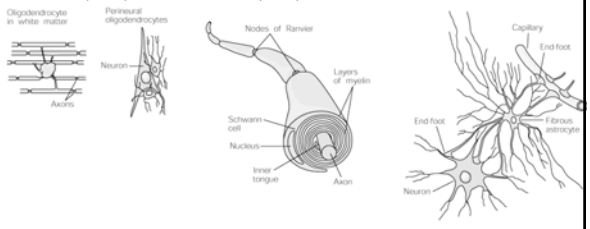
A. Neurons in the PNS can regenerate their axons. How?

B. Neurons in the CNS have a limited capacity to regenerate axons. Why?

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: Principles, examples

Types of glial cells

1. Myelin-forming:
   a. Oligodendrocytes (CNS) b. Schwann cells (PNS)
2. Astrocytes



Appleton & Lange
Kandel/Schwartz/Jessell
Principles of Neural Science
Fig. 02.01

3. Microglial cells

Related to tissue macrophages
Derive from monocytes that infiltrate brain?
Role during development?
Present in developing and mature brain
* Congregate around injured cells

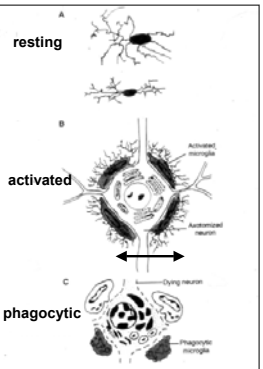


Fig. 1. Schematic illustration of microglia. (A) Resting microglia. (B) Activated microglia surrounding an axotomized neuron. (C) Phagocytic microglia surrounding a dying neuron. Modified from the original by I. Gehrmann and G. Kreutzberg.

Myelin forming cells: (myelin important for conduction).
- **oligodendroglia** in CNS
- **Schwann cells** in PNS.
  - oligodendrocytes (CNS) are inhibitory to axon regrowth in adult CNS regeneration;
  - Schwann cells (PNS) are supportive, as a growth surface and releaser of growth factors.

Astroglia -
- *development:* supports axon growth and cell migration;
- *mature:* important for ion flux, synaptic function, blood-brain barrier
- *injury:* accumulate in scar, release excess matrix; inhibit axon growth?

Microglia (resting) and macrophages (active) -
- cells of immune system, similar to monocytes.
- *injury:* help or hinder?
  - ….not well-understood

I. RESPONSE OF THE NEURON TO INJURY

II. GLOSSARY OF GLIAL CELLS: Normal function, response to injury

III. DEGENERATION: Signs, Timecourse

IV. REGENERATION

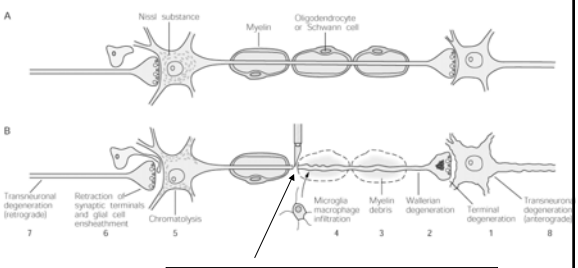
- **A. Neurons in the PNS can regenerate their axons. How?**
- **B. Neurons in the CNS have a limited capacity to regenerate axons. Why?**

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: Principles, examples

REACTIONS TO INJURY WITHIN THE NEURON:

I**mmediately** -

1. Synaptic transmission off
2. Cut ends pull apart and seal up, and swell, due to axonal transport in both directions



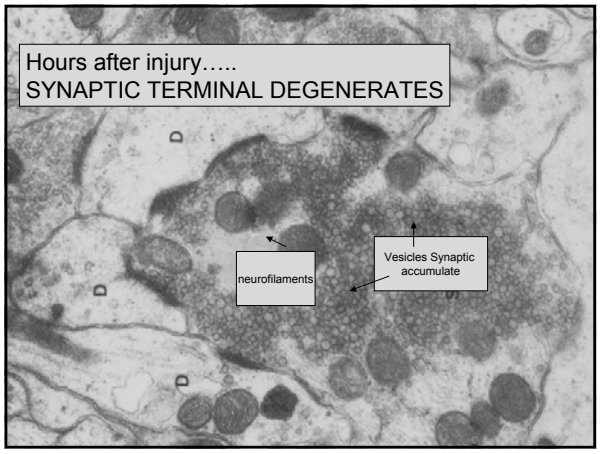
Appleton & Lange
Kandel/Schwartz/Jessell
Principles of Neural Science
Fig. 55.18

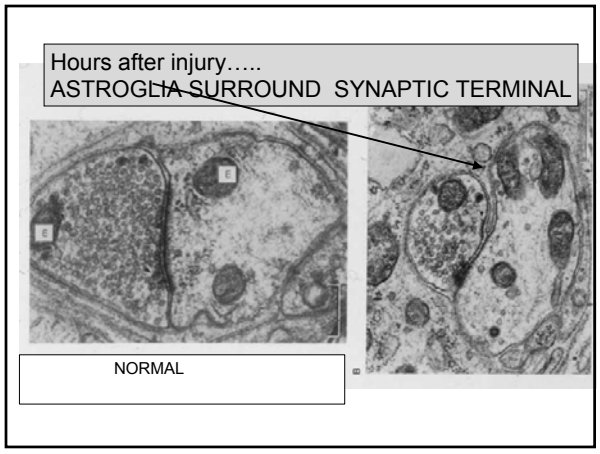
MINUTES after injury…
-synaptic transmission off
-cut ends swell

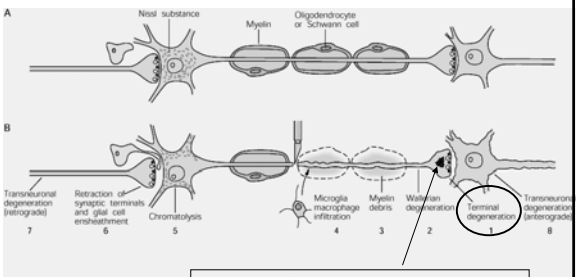
REACTIONS TO INJURY WITHIN THE NEURON:

I**mmediately** -
1. Synaptic transmission off
2. Cut ends pull apart and seal up, and swell,
   due to axonal transport in both directions

Hours later -
3. Synaptic terminal degenerates - accumulation of NF, vesicles.
4. Astroglia surround terminal normally;
   after axotomy, astroglia interpose between terminal and target
   and cause terminal to be pulled away from postsynaptic cell.







HOURS after…
synaptic terminal degenerates

REACTIONS TO INJURY WITHIN THE NEURON:

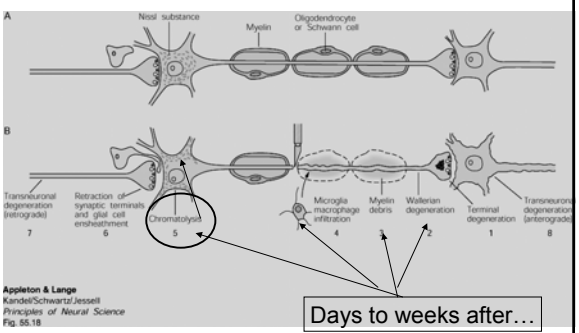
I**mmediately** -
1. Synaptic transmission off
2. Cut ends pull apart and seal up, and swell,
   due to axonal transport in both directions

Hours later -
3. Synaptic terminal degenerates - accumulation of NF, vesicles.
4. Astroglia surround terminal normally;
   after axotomy, interpose between terminal and target
   and cause terminal to be pulled away from postsynaptic cell.

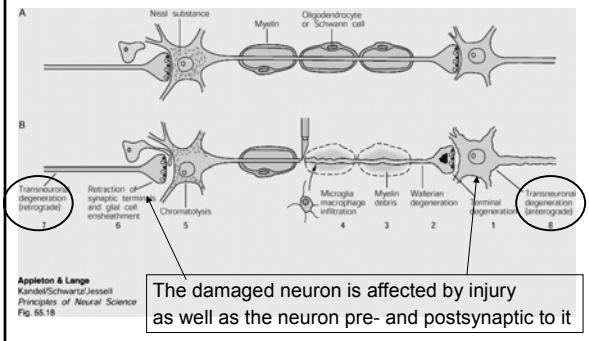
days - weeks -
5. Myelin breaks up and leaves debris (myelin hard to break down).
6. Axon undergoes **Wallerian** degeneration
7. **Chromatolysis** - cell body swells; nissl and nucleus eccentric.

**If axon cut in PNS or CNS, changes are the same.

**The damaged neuron is affected by injury,
as well as the pre- and postsynaptic neurons to it



Days to weeks after…

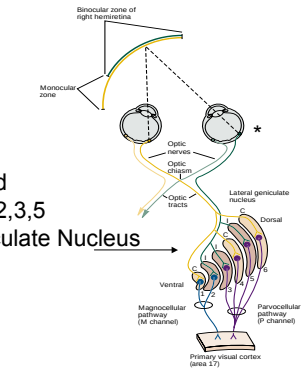


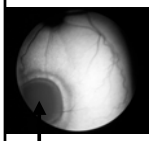
The damaged neuron is affected by injury as well as the neuron pre- and postsynaptic to it

Severing the axon causes degenerative changes in the injured neuron AND in the cells that have synaptic connections with the injured neuron.

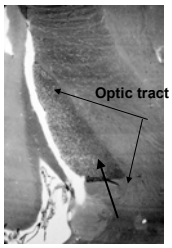
Classically, degeneration of fibers and their targets has been used to trace neuronal circuits experimentally, and still is used to understand pathology post-mortem

Fibers from the temporal retina* project laterally in the optic tract and terminate in layers 2,3,5 of the Lateral Geniculate Nucleus

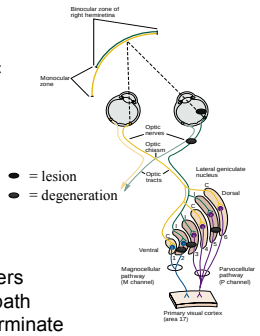




Laser lesion
(cat eye)



Degenerating axons
(myelin stain)



The localization of degenerating fibers can be used to trace where in the path the axons project, or where they terminate

Appleton & Lange
Kandel/Schwartz/Jessell
Principles of Neural Science
Fig. 27.06

I. RESPONSE OF THE NEURON TO INJURY

II. GLOSSARY OF GLIAL CELLS: Normal function, response to injury

III. DEGENERATION: Signs, Timecourse, applications of "reading" trans-synaptic degeneration

IV. REGENERATION

- **A. Neurons in the PNS can regenerate their axons. How?**
- **B. Neurons in the CNS have a limited capacity to regenerate axons. Why?**

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: Principles, examples

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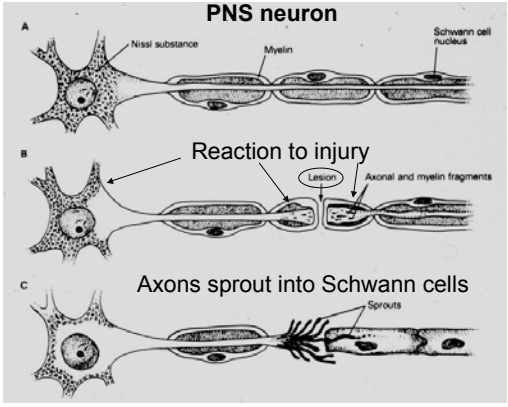
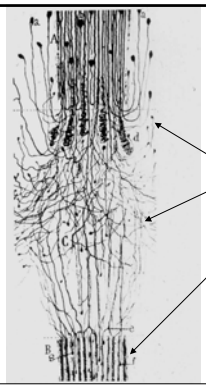
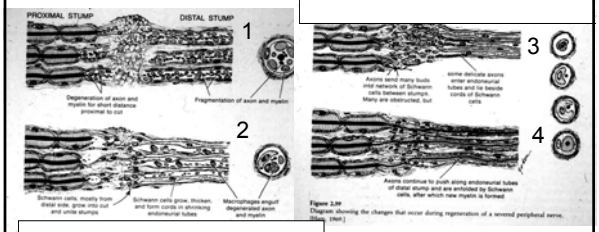


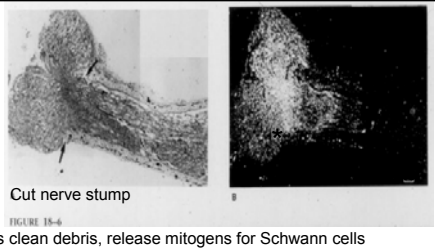


Regenerating axons form many sprouts, some of which find Schwann cell tubes

-Ramon y Cajal

Changes in the distal stump during degeneration and regeneration (PNS)





Cut nerve stump

FIGURE 18-6

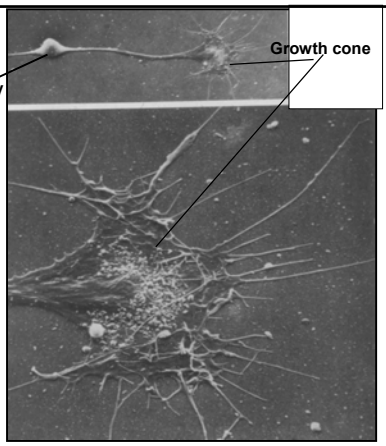
Macrophages clean debris, release mitogens for Schwann cells

New Schwann cells form tubes, a conducive environment for growth:

Schwann cells make laminin (growth-supportive extracellular matrix)

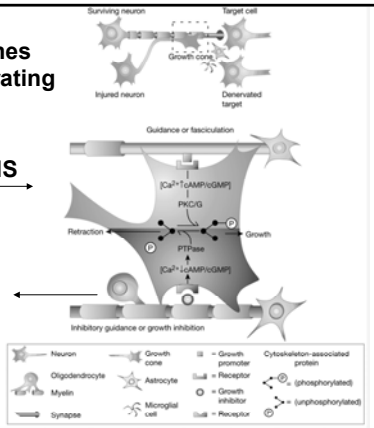
Macrophages relase interleukin; interleukin stimulates Schwann cells to make Nerve Growth Factor *

Nerve growth factor stimulates axon regeneration



growth cones on regenerating axons:

Growth in PNS



IV. Neurons in the PNS can regenerate their axons. HOW? (summary)

a. After degeneration of distal axon and myelin, macrophages clean up debris.
b. Macrophages release mitogens that induce Schwann cells to divide

c. The myelin-forming Schwann cells repopulate the nerve sheaths;
d. Schwann cells make laminin
e. Macrophages make interleukin, which induces Schwann cells to make Nerve Growth Factor.

e. Axons sprout, and some sprouts enter new Schwann cell tubes
f. Axonal growth cones successfully grow

I. RESPONSE OF THE NEURON TO INJURY

II. GLOSSARY OF GLIAL CELLS: Normal function, response to injury

III. DEGENERATION: Signs, Timecourse

IV. REGENERATION

- **A. Neurons in the PNS can regenerate their axons. How?**
- **B. Neurons in the CNS have a limited capacity to regenerate axons. Why?**

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: Principles, examples

B. Neurons in the mature CNS have a limited capacity to regenerate axons.
WHY?
CNS axons can regrow, but…

Growth is impeded by negative elements in the environment

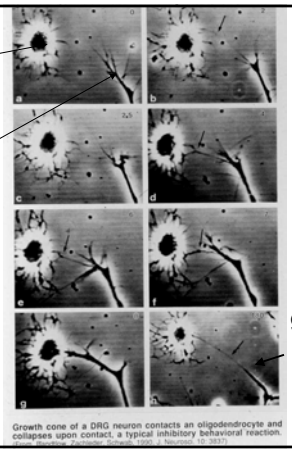
-extracellular matrix (laminin) is sparse; inhibitory proteoglycans increase

-growth factors have different distributions compared to young brain

Intracellular growth factors such as GAP-43
(important for intracellular signaling/growth cone advance) are low

oligodendrocyte
(in culture)

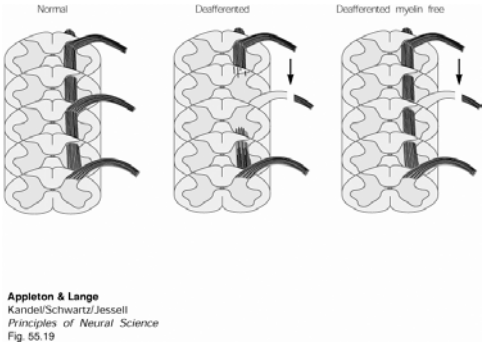
PNS (or CNS)
growth cone



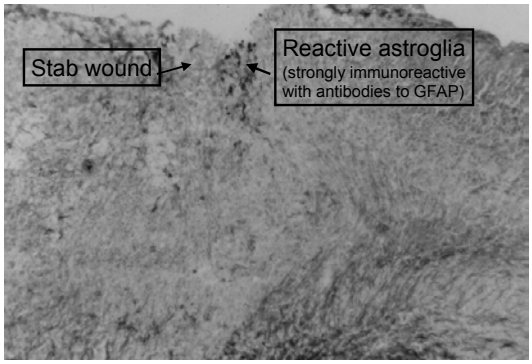
Growth cone of a DRG neuron contacts an oligodendrocyte and collapses upon contact, a typical inhibitory behavioral reaction.

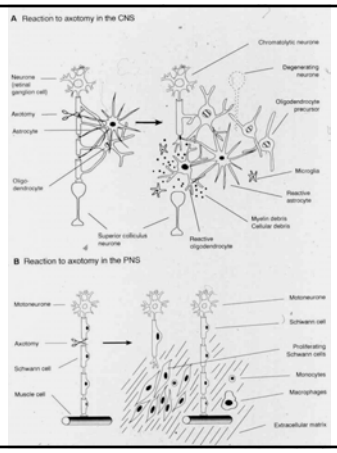
growth cone
retracts

CNS myelin, from oligodendrocytes, is inhibitory to axon growth



In the CNS, astroglia form a scar around site of injury

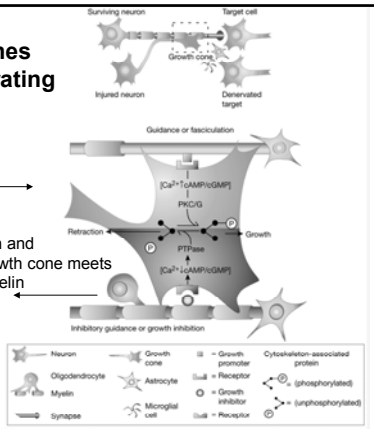




CNS

PNS

growth cones on regenerating Axons:



Growth in PNS →

CNS:
Inhibition of growth and retraction when growth cone meets oligodendrocyte/myelin ←

B. Neurons in the CNS have a limited capacity to regenerate axons. WHY?
(Summary)
CNS axons can regrow, but…

Growth is impeded by negative elements in the environment
-extracelluar matrix (laminin) is sparse; inhibitory proteoglycans increase
-growth factors have different distributions compared to young brain

Intracellular growth elements such as GAP-43
(important for intracellular signaling/growth cone advance) are low

*Glial cells inhibit growth
Oligodendrocytes (CNS myelin) are the most inhibitory
Astrocytes accumulate in the scar around injury site
Macrophages also accumulate; role of microglia unclear

I. RESPONSE OF THE NEURON TO INJURY

II. GLOSSARY OF GLIAL CELLS: Normal function, response to injury

III. DEGENERATION: Signs, Timecourse

IV. REGENERATION
A. Neurons in the PNS can regenerate their axons. How?
B. Neurons in the CNS have a limited capacity to regenerate axons. Why?

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION: principles, examples

The exciting news: CNS neurons can sprout or grow.

Challenges:

*Repopulate with neurons and “good” glia

*Overcome the “bad” glial environment:
- combat glial scars, inhibitory extracellular matrix
- add blockers of myelin

*Help axons regrow:
add neurotrophins (increase cAMP levels to prime neurons to ignore myelin-inhibitors).

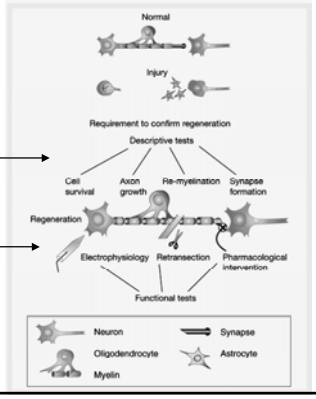
re-express ”youth" proteins - GAP-43

*Induce reformation of synapses (least understood step; how do normal synapses form?).

To determine whether regeneration has occurred….

Descriptive tests rely on microscopy.

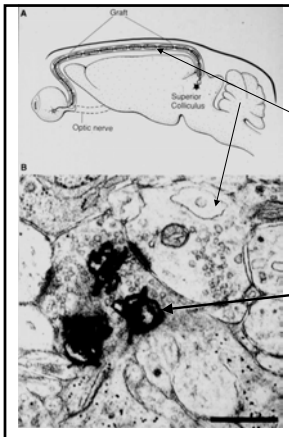
Functional tests include behavioral assays.



Therapeutic Strategies:

1. Implant
   - lengths of peripheral nerve (a natural “bridge”)

   Or
   - artificial plastic tubes lined with supportive glia



-Sciatic nerve (PNS) is cut and axons degenerate: Schwann cells repopulate nerve

-Nerve length sutured to cut optic nerve

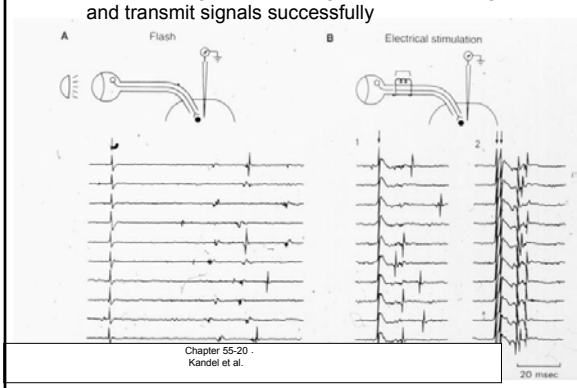
-Retinal axons regrow in grafted nerve

-Retinal axons reestablish synapses (radioactive label transported)

(work of Aguayo et al.)

Chapter 55-20
Kandel et al.

Retinal axons regenerate through the PNS nerve graft and transmit signals successfully



Chapter 55-20 .
Kandel et al.

Therapeutic Strategies:

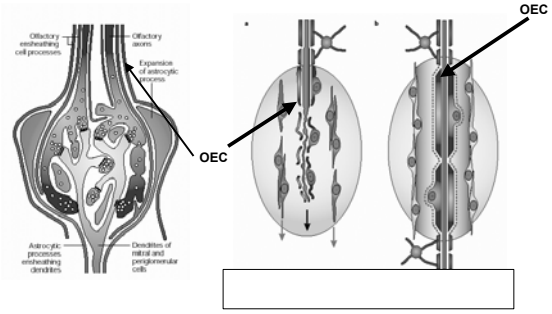
2. Transplant/ implant into or near site of injury:

-fetal tissue (containing immature neurons and glia) or stem cells, with potential of becoming either

-cell lines or normal cells transfected with a gene for
e.g., neurotrophins (positive)
antibodies (against inhibitory myelin)

-"good" glia: olfactory ensheathing glia*

Olfactory ensheathing cells, with properties of CNS and PNS glia, transplanted into transected corticospinal tract



(for Rev: Raisman, 2001, Nat. Rev. Neurosci. 2: 369)

Therapeutic Strategies:

3. Gene transfer via retroviruses,injection of RNA, anti-sense oligonucleotides);

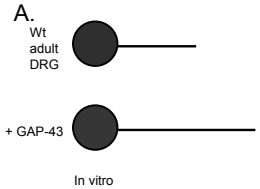
also, transgenic approach, replacing missing gene

3. Genetic Approach

Instigate events that occur during *development* by gene transfer:

GAP-43 transgenic mice:

A.



B. GAP-43 transgenic mice show a 60-fold increase in adult DRG axon regeneration into a peripheral nerve graft, in the spinal cord in vivo

Bomze et al., 2001, Nat. Neurosci., 4: 38

Therapeutic Strategies:

4. Direct delivery of growth factors to promote axon regrowth

COMBINATION OF APPROACHES:

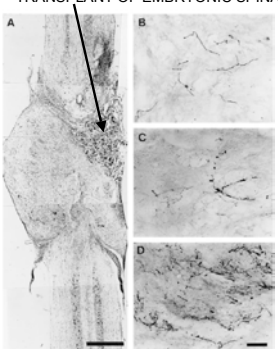
2. Cellular Transplants

Transplant piece of embryonic spinal cord

Plus….

4. Delivery of **growth factors**

TRANSPLANT OF EMBRYONIC SPINAL CORD IN LESION SITE



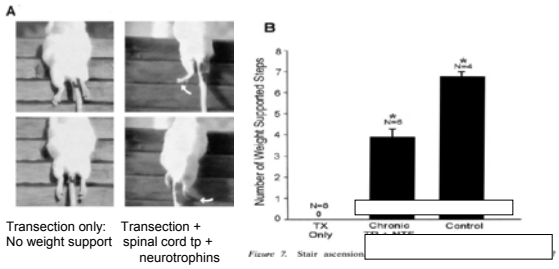
Transection +
spinal cord transplant

Transection +
spinal cord transplant
+ neurotrophins

Transection +
delayed
spinal cord transplant
+ neurotrophins
(to allow debris to be cleared)

Coumans et al., 2001, J. Neurosci. 21:9334

Embryonic spinal cord transplants plus neurotrophins lead to functional recovery after spinal cord transection



Transection only: No weight support

Transection + spinal cord tp + neurotrophins

Figure 7. Stair ascension

Therapeutic Strategies:

5. Application of "neutralizing" activity (e.g., antibodies) to combat inhibitory glia/myelin components

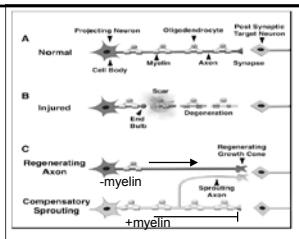


Figure 1. Damage of CNS Axons and Potential Mechanisms of Recovery

(A) Axons in CNS white matter are wrapped in myelin, produced by oligodendrocytes.

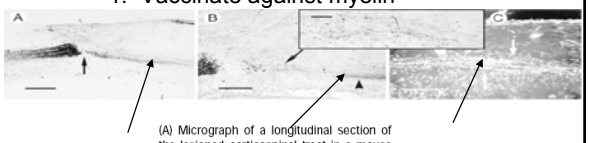
(B) Following trauma, a glial scar forms at the injury site, and distal nerve segments degenerate. CNS axons show very limited capacity to regenerate.

(C) Neutralization of inhibitors may allow improvements in function through regeneration of original axon pathways. Alternatively, sprouting of damaged axons, or other undamaged axon tracts, may form compensatory new pathways.

Axons can regenerate if myelin/oligodendrocytes are "neutralized" by antibody application (M. Schwob)

Recent advances in regeneration:

1. Vaccinate against myelin



(A) Micrograph of a longitudinal section of the lesioned corticospinal tract in a mouse immunized with spinal cord homogenate in IFA. WGA-HRP labels the tract rostral and caudal to the lesion (arrow). Many regenerated axons can be seen caudally.

Therapeutic approach: stimulate animals' own immune system by injection of spinal cord homogenate to generate polyclonal antibodies that block the inhibitory factors on myelin / adult CNS cells.

Practicalities of immunizing humans with myelin?

Huang et al., 1999, Neuron 24: 639

Mice immunized with spinal cord cells also showed functional recovery

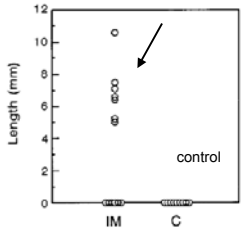


Figure 2. Maximum Length of Regenerating Axons

The maximum distance that injured corticospinal tract axons regenerated caudal to the site of lesion in mice immunized with mouse spinal cord homogenate in IFA ("IM") and controls injected with IFA alone ("C"). This distance was estimated from serial sections and by measuring the maximum distance to which WGA-HRP-labeled axons extended caudally. Each point represents one animal.

Recent advances in regeneration:

2. "prime" cells with neurotrophins, or cyclic nucleotides

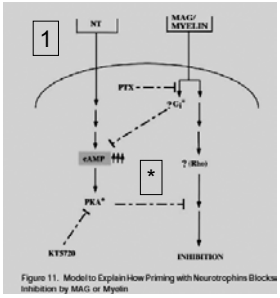
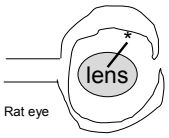


Figure 11. Model to Explain How Priming with Neurotrophins Blocks Inhibition by MAG or Myelin

If neurotrophins are presented before the neuron "sees" myelin, cAMP increases and inhibition by myelin is blocked *

M. Filbin

Recent advances in regeneration:
3. Microglial activation to help regeneration



Macrophages activated*; retinal axons regenerate

*by lens injury, or a macrophage activator

Macrophage-derived proteins < 30 kD are growth-promoting

Work of L. Benowitz

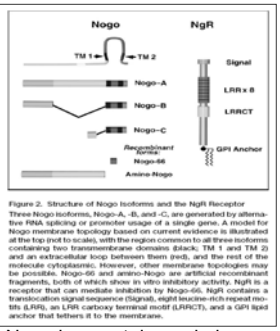


Figure 2. Structure of Nogo Isoforms and the NgR Receptor
Three Nogo isoforms, Nogo-A, -B, and -C, are generated by alternative RNA splicing or promoter usage of a single gene. A model for Nogo membrane topology based on current evidence is illustrated at the top (not to scale), with the region common to all three isoforms containing two transmembrane domains (black; TM 1 and TM 2) and an extracellular loop between them (red), and the rest of the molecule cytoplasmic. However, other membrane topologies may be possible. Nogo-66 and amino-Nogo are artificial recombinant fragments, both of which show in vitro inhibitory activity. NgR is a receptor that can mediate inhibition by Nogo-66. NgR contains a translocation signal sequence (Signal), eight leucine-rich repeat motifs (LRR), an LRR carboxy terminal motif (LRRCT), and a GPI lipid anchor that tethers it to the membrane.

Nogo is a protein made by oligodendrocytes;
NgR mediates inhibition by Nogo

Recent advances in regeneration:
4. Understanding how the Nogo receptor works

The three known myelin proteins:
MAG (myelin-associated glycoprotein)
NOGO
OMGp (Oligodendrocyte myelin glycoprotein)

interact with the Nogo Receptor (NgR), which, in turn, interacts with the P75 receptor, a known "negative" receptor, leading downstream to growth inhibition

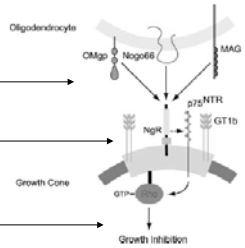
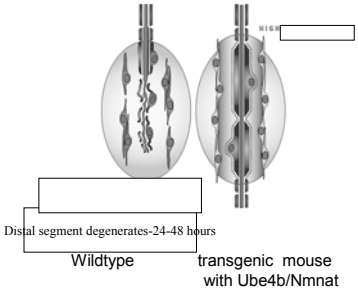


Figure 1. Schematic Diagram Showing Signaling by Myelin-Derived Growth Inhibitory Proteins

(References in outline)

3. Genetic approach

Example 1: Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat* chimeric gene



*encodes nuclear ubiquitination factor E4B fused to nicotinamide mononucleotide adenylyltransferase; leads to neuroprotection by altering pyridine nucleotide metabolism.

Mack et al., Nat. Neurosci. 4: 1199 (2001)