Symptoms of spinal cord injury:

- 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.

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

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.

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.

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Types of glial cells

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

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

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REATIONS TO INJURY WITHIN THE NEURON:
Immediately -
1. Synaptic transmission off
2. Cut ends pull apart and seal up, and swell, due to axonal transport in both directions
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4. Astroglia surround terminal normally; after axotomy, astroglia interpose between terminal and target and cause terminal to be pulled away from postsynaptic cell.
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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
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.
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

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

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

1. Cut nerve stump
2. Macrophages clean debris, release mitogens for Schwann cells
3. New Schwann cells form tubes, a conducive environment for growth:
   - Schwann cells make laminin (growth-supportive extracellular matrix)
   - Macrophages release interleukin; interleukin stimulates Schwann cells to make Nerve Growth Factor
4. Nerve growth factor stimulates axon regeneration
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.

f. Axons sprout, and some sprouts enter new Schwann cell tubes.

f. Axonal growth cones successfully grow.
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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
CNS myelin, from oligodendrocytes, is inhibitory to axon growth

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

CNS

PNS
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
- extracellular 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

1. RESPONSE OF THE NEURON TO INJURY
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5. 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?).

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To determine whether regeneration has occurred....

Descriptive tests rely on microscopy.

Functional tests include behavioral assays.

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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.)

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

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

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. Wt adult DRG

+ GAP-43

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

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

TRANPLANT 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)

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

Therapeutic Strategies:

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

Axons can regenerate if myelin/oligodendrocytes are "neutralized" by antibody application
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?

Recent advances in regeneration:

1. Vaccinate against myelin

Mice immunized with spinal cord cells also showed functional recovery

![Graph](image)

Figure 2: Maximum Length of Regenerating Axons.

The maximum distance that spared corticospinal tract axons regenerated caudally to the site of lesion in mice immunized with myelin homogenate in IFA + Ad5 and those injected with IFA alone (Fig. 3). This distance was calculated 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

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

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.

References in outline
3. Genetic approach

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

Wildtype transgenic mouse with Ube4b/Nmnat

Sheaf segment degenerates 24-48 hours

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

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