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 in the damaged neuron, presynaptic neurons 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 axon is severed close to the cell body, 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.

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

III. DEGENERATION: Signs, Timecourse

IV. REGENERATION
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Types of glial cells

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

2. Astrocytes

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

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.

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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.

**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.**

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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.

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

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*Fibers from the temporal retina*
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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### 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
   - Nerve growth factor stimulates axon regeneration

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

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### 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.
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

g. Axonal growth cones successfully grow

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**

**Stab wound**

**Reactive astroglia** (strongly immunoreactive with antibodies to GFAP)

**growth cones on regenerating Axons:**

**Growth in PNS**

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

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**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
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.

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**Therapeutic Strategies:**

1. **Implant**
   - lengths of peripheral nerve
     (a natural "bridge")
   Or
   - artificial plastic tubes lined with supportive glia

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

- 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.)

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**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

And recovery of function occurs after transplantation (caveat: some axons must be “spared”...)


Therapeutic Strategies:

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

Example of Gene transfer 1:

Instigate events that occur during development by gene transfer genetically:

GAP-43 transgenic mice:

A. Wt adult DRG

+ GAP-43

In vivo

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

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 (M. Schwob)
COMBINATION OF APPROACHES:

#2. Cellular Transplants
Transplant piece of embryonic spinal cord

Plus....

#4. Delivery of growth factors

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

Molecular mechanisms underlying regeneration:
1. Vaccination to combat myelin
2. Prime cells with neurotrophins
3. Identification of a gene underlying Wallerian degeneration
4. Increase (good) microglia in eye by stabbing lens
5. Signals that travel from injury site back to nucleus
6. Molecules that increase, decrease during inflammation, degeneration, regeneration
7. Molecular identification of 3 myelin-associated factors, their common receptor and co-receptor

Mice immunized with spinal cord cells show functional recovery

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; See also work of M. Schwartz
If neurotrophins are presented before the neuron "sees" myelin, cAMP increases and inhibition by myelin is blocked.

Molecular mechanisms underlying regeneration
2. Prime cells with neurotrophins

Filbin, 2003, Nat. Rev. Neurosci. 4: 1

Molecular mechanisms underlying regeneration: 3. Identification of a gene underlying Wallerian degeneration
Wildtype transgenic mouse

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

*encodes nuclear ubiquitination factor E4B; leads to neuroprotection by altering pyridine nucleotide metabolism or by changing ubiquitination.

Wlds* (Natural mutant)

Macrophage-derived proteins < 30 kD are growth-promoting

Macrophages activated: retinal axons regenerate

Yin...and Benowitz, 2003, J. Neurosci. 15: 2284

Molecular mechanisms underlying regeneration
5. Signals that travel from injury site back to nucleus

Importinβ increases after injury and binds to a nuclear localization signal (nls); the entire complex travels retrogradely to modulate the regenerative response

Hanz and...Fainzilber, 2003, Neuron 40:1095; See also work of R. Ambron, Columbia

Molecular mechanisms underlying regeneration
6. Molecules that increase, decrease during inflammation, degeneration, regeneration

**Information from microarrays...**

Bareyre and Schwab, 2003, TINS 26: 555
Molecular mechanisms underlying regeneration:

6. (cont.) Molecules that increase, decrease during inflammation, degeneration, regeneration

**Information from microarrays...**

Brainstem lesion

-antibody to myelin proteins

+antibody to myelin proteins

Bareyre and Schwab, 2003, TINS 26: 555

Molecular mechanisms underlying regeneration:

7. Molecular identification of 3 myelin-associated factors, their common receptor and co-receptor

All 3 myelin proteins (Nogo, Mag, Omgp) interact with the Nogo Receptor (NgR)

McGee and Strittmatter, 2003, TINS 26: 193

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

McGee and Strittmatter, 2003, TINS 26: 193

P75 receptor also counteracts neurotrophin-Trk interactions

McGee and Strittmatter, 2003, TINS 26: 193
The bottom line... what treatments work in humans with spinal cord injury??

The case of Christopher Reeves...

Mice, cats, rats and humans that have been completely spinalized can regain greater locomotor performance if they are trained to perform that task, by robotics...


(Measures of recovery: