GLIAL CELLS AND NERVE INJURY
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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 RE-ESTABLISHED

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

II. GLIAL CELLS:
- Normal function
- Response to injury

III. DEGENERATION:
- Reactive changes, timecourse

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

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

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

Cell biological reactions in the damaged neuron,
presynaptic and postsynaptic neurons

Neurons in the PNS and CNS have many different forms
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 morphologies, react similarly.
   - 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
   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

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

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Hours after injury.....
SYNAPTIC TERMINAL DEGENERATES

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NORMAL

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ASTROGLIA SURROUND SYNAPTIC TERMINAL

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Vesicles Synaptic accumulates

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neurofilaments
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.
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
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   Principles, examples

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

- 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 release interleukin; interleukin stimulates Schwann cells to make Nerve Growth Factor
  - Nerve growth factor stimulates axon regeneration

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

Laser lesion (cat eye)

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

PNS neuron

Axons sprout into Schwann cells

Ramon y Cajal

Recovery and regeneration

Retina

Primary visual cortex (area 17)

Optic tract

Optic nerves

Optic tracts

Lateral geniculate nucleus

Dorsal Parvocellular pathway (P channel)

Magnocellular pathway (M channel)

C I C I I C

Degenerating axons (myelin stain)

Radioactive nerve growth factor

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 release interleukin; interleukin stimulates Schwann cells to make Nerve Growth Factor

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

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

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
  - myelin proteins (NOGO, MAG, Omgp) increase
  - inhibitory proteoglycans increase

- Intracellular growth factors such as GAP-43
  (important for intracellular signaling/growth cone advance) are low
- -growth factors have different distributions compared to young brain
- -normally growth-supporting extracellular matrix (laminin) is sparse

V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION:
Principles, examples
CNS myelin, from oligodendrocytes, is inhibitory to axon growth.

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

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

(Commentary)

CNS axons can grow, 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

**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?
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**V. EXPERIMENTAL STRATEGIES TO PROMOTE REPAIR AND RECOVERY OF FUNCTION:**

principles, examples
The exciting news: CNS neurons can sprout or grow.

Challenges:
* Overcome the "bad" glial environment:
  - combat glial scars, inhibitory extracellular matrix
  - add blockers of myelin
  - repopulate with neurons and "good" glia

* 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?

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

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*

To determine whether axons have regenerated....

Descriptive tests based on microscopy.

Functional tests, including behavioral assays.

- 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.
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 might 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
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. Schwab)
#2. Cellular Transplants
Transplant embryonic spinal cord

#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
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

Molecular mechanisms underlying regeneration
2. (cont.) Prime cells with neurotrophins, or increase cAMP (or CREB) directly

Molecular mechanisms underlying regeneration
3. Identification of a gene underlying Wallerian degeneration

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

Barreyre and Schwab, 2003, TINS 26: 545

Molecular mechanisms underlying regeneration:

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

Work of S. Strittmatter (Yale);
Also Tae-Wan Kim, Joseph Gogos (Columbia - P&S)

McGee and Strittmatter, 2003, TINS 26: 193

Molecular mechanisms underlying regeneration:

7. (cont.) 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

Molecular mechanisms underlying regeneration:

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

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:

Molecular mechanisms underlying regeneration:
1. Vaccination to combat myelin

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 26: 629; See also work of M. Schwartz