Hotwiring the Hardwired

CNS Injury and Repair

“Gentlemen, we can rebuild him. We have the technology.”

SHP – Neurobiology of Development and Disease
Spinal Injury

- There are an estimated 10,000 to 12,000 spinal cord injuries every year in the United States.
- A quarter of a million Americans are currently living with spinal cord injuries.
- The cost of managing the care of spinal cord injury patients approaches $4 billion each year.
- 38.5 percent of all spinal cord injuries happen during car accidents. Almost a quarter, 24.5 percent, are the result of injuries relating to violent encounters, often involving guns and knives. The rest are due to sporting accidents, falls, and work-related accidents.
- 55 percent of spinal cord injury victims are between 16 and 30 years old.
- More than 80 percent of spinal cord injury patients are men.

National Spinal Cord Injury Statistical Center
Progression of CNS injury
(Spinal cord as a model)

- Local swelling at the site of injury which pinches off blood perfusion → ischemia
- Excessive release of glutamate and excitotoxicity of neurons and oligodendrocytes at the site of injury
- Infiltration by immune cells (microglia, neutrophils)
- Free radical toxicity
- Apoptosis/necrosis
Restructuring in Response to Damage

- Astrocytes begin production and secretion of cytokines, which “reactivates” their proliferation. They infiltrate lesion and form a scar.
- Astrocytes express a complex milieu of proteoglycans (chondroitin sulfate proteoglycans) at the scar boundary.
- Damage to axons in the central nervous system results in retraction of resealed growth cone where it stalls indefinitely.
- Axons are demyelinated and degenerate or remain “fixed” in place for years.
CNS injury

- Conversely, regeneration axons and functional recovery following peripheral nervous system damage does occur.
- This CNS-specific “hostile environment” has been attributed to two entities within the CNS: 1) reactive astrocytes and 2) oligodendrocyte myelin-associated inhibitors (such as Nogo, MAG, OMgp, chondroitin sulfate proteoglycans).
Early Highlights in Neural Regeneration

• Egyptian papyrus from ~1700 bce details two cases of fracture or dislocation of vertebrae in the neck and suggest that they were “an ailment not to be treated”
• Over the next few centuries Greek, Hindu, Arab and Chinese physicians develop traction methods to treat spinal fracture without paralysis.
• Roman physician Galen (200 ce) pioneers the introduces the concept that the spinal cord is an extension of the brain that carries sensation to the limbs and back.
• Paulus of Aegina (7th century ce) recommended surgery for spinal fractures where broken vertebral fragments are to be removed.

National Institute of Neurological Disorders and Stroke
Later Highlights in Neural Regeneration

- Development of X-ray imaging technology in the 1920s allowed visualization of spinal injuries for the first time and more accurate prognosis of the outcome.
- By middle of the 20th century, standard methods were present to stabilize injuries, fix them in place, and rehabilitate disabilities with exercise.
- In the 1990s, it was found that the anti-inflammatory steroid methylprednisalone could be employed to minimize cell death and tissue damage if administered early enough after injury.
CNS Damage, What Can We Do About It?

Fix what we have
- Prevent cell death
- Promote axon regrowth
- Remove blockades

Build around it
- Brian-machine interfaces that can interpret neural codes and output activity to periphery (organic or machine)
There have been many focuses to induce regeneration

- Inhibiting the axon regeneration blockers in CNS myelin
- Removing barriers formed by glial scars.
- Stimulating regrowth by signaling pathways
- Replacement of neurons damaged during injury with embryonic stem cells
- Engineering brain-machine interfaces to produce enhanced sensory feedback prosthetics (bionic/cybernetics)
History of Neural Regeneration

- **1830s** Anatomist Theodor Schwann finds first evidence of regeneration of severed sciatic nerve in rabbits.
- **1890s** Santiago Ramon y Cajal reports that CNS nerves appear to attempt to regenerate but cannot. Introduces that “hostile CNS environment” concept.
- **1969** Utilizing EM imaging Geoffrey Raisman demonstrates that neurons can establish new synapses and reorganize networks after injury.
- **1982** Albert Aguayo shows that rat axons could growth through the CNS in the presence of a peripheral nerve graft but stall when they reach the boundary of the CNS.
PNS vs CNS Environment

- Peripheral nerve is crushing (to cause degeneration and test regeneration) and labeled with an enzymatic marker.
- These nerve explants were transferred into rat brain and tracing of regenerated axons is seen at later stages.
- These axons can re-extend for the length of the explant but stall before entering the CNS tissue.

This signifies very clearly that the CNS environment is not conducive to regeneration of axons.
Is Regeneration Deficit Due to Lack of Trophic Support or Inhibitory Influence?

- They cultured sympathetic neurons with a “bridge” into adjacent chambers composed of either optic nerve (CNS) or sciatic nerve (PNS)
- Neurons grow axons preferentially through the peripheral nerve into the chamber.
- Axons can enter the chamber of the optic nerve only if they grow around it.

This is taken as evidence that CNS tissue (and not PNS) possesses an active property of specifically and actively inhibiting regrowth of axons through them.

Schwab & Thoenen, 1985
Immunization with Myelin Improves Regeneration from Spinal cord lesion

IFA

People begin to think of the differences in myelination between CNS and PNS. (oligodendrocyte vs Schwann cell)

Liver homogenate

Immunization of rats against myelin promotes regeneration of axons through spinal cord lesion.

Spinal cord homogenate

This provides further evidence for the role of myelin as a regeneration inhibitor.
Identifying Myelin Inhibitor Proteins

- Martin Schwab in the 1980s develops a variety of antibodies against myelin. One, IN-1 becomes favored in later studies.
- Adding these antibodies to culture allows axon outgrowth on myelin.
- These antibodies were eventually used on protein fractions of myelin to identify the first myelin associated inhibitor, Nogo
Inactivation of Myelin Antigen Leads to Functional Recovery

- Addition of IN-1 anti-myelin antibody promotes axon regeneration past the lesion in the spinal cord.
- IN-1 treatment after injury also results in functional recovery of motor coordination in feeding test.
Nogo

- Reticulon family protein.
- Three isoforms Nogo-A, Nogo-B, and Nogo-C, expressed from differential splicing (A/B) and alternate promoter usage (Nogo-C).
- C-terminus contains two hydrophobic regions thought to be transmembrane sequences and are required for ER membrane localization in other reticulons.
- Localized to the plasma membrane, endoplasmic reticulum, and neuronal synapses.
- Nogo-A is expressed in CNS, Nogo-B in the CNS/lung/liver, and Nogo-C in the skeletal muscle.
Nogo KOs exhibit high variable phenotypes

Simonen et al, 2003 (Schwab)

Kim et al, 2003 (Strittmatter)

Zheng et al, 2003 (Tessier-Lavigne)
Strittmatter Model

GrandPre’ et al, 2000
Amino-Nogo

Amino-Nogo (Nogo-A sequence up to common exon) inhibits neurite outgrowth of CGN cells in a dose-dependent manner.

This is likely another extracellular domain of Nogo that can interact specifically with a receptor.

Prinjha et al, 2000
Researcher Subsequently Purify Two Additional Myelin-Associated Inhibitors

• Myelin-Associated Glycoprotein (MAG): transmembrane protein with 5 immunoglobulin-like repeats in the extracellular domain.

• Oligodendrocyte Myelin Glycoprotein (OMgp): GPI-linked containing leucine-rich repeat (LRR) domains with serine/threonine repeats.
Nogo Receptor (NGR)

- Receptor for Nogo, MAG, and OMgp
- GPI-linked protein with LRR repeats
- Since it has no transmembrane motifs, it requires a coreceptor to transmit its signal across the membrane.
- p75 neurotrophin receptor acts as this coreceptor
- Since NGR is the convergent target of all myelin-associated inhibitors, there is much research to specifically inactivate this receptor
Myelin Associated Protein
Inhibitors aren’t the Whole Story

• Chondroitin sulfate proteoglycans (CSPGs) are upregulated in the glial scar following spinal cord lesion.

• Treatment with chondroitinase (which cleaves CSPGs from the surface of the cell) enhances regrowth through the lesion.

Davies et al, 1999
Bradbury et al, 2002

Bradbury et al, 2002
Myelin Associated Protein Inhibitors aren’t the Whole Story

- By stimulating the cortex and measuring electrical activity at various points in the spinal cord, they show that severed neurons reestablish their connectivity.

- Animals treated with ChABC do not have recovery of fine motor behaviors (tape removal) but have dramatic improvement in their ability to walk.

Bradbury et al, 2002
cAMP Pathway Regulates MAG Signaling

Growth Cone Turning Assay

MAG

Song et al, 1998

Forkolin or db-cAMP

Cai et al, 2001

cAMP levels drop in CNS during development

anti-cAMP

Cai et al, 2001

### A

**E18 RG neurons on MAG cells**

![Graph A](image1)

**control**  
**MAG**  
**+KT**  
**+Rp**  
**PKG Inh.**

Cai et al, 2001

### B

**P5 RG neurons on MAG cells**

![Graph B](image2)

**control**  
**MAG**  
**+dbcAMP**  
**+Sp**  
**+dbcGMP**

Cai et al, 2001
Activation of cAMP Signaling Enhances Regeneration

Cai et al, 2001

Neumann et al, 2002
Stem Cells Approaches for CNS Repair

- Transplantation of oligodendrocyte progenitor cells to treat myelination disorders
- Transplantation of phenotypically restricted (unipotent) neuronal progenitors to treat neurodegeneration
- Implantation of mixed progenitor pools or multipotent stem cells to reconstruct disorders with losses of several lineages
- Mobilization of endogenous neural precursors to replace neuronal loss in disease
Brain-Machine Interface:

- **neural code problem**: how neural activity is built into networks and how those networks produce specific activities and interact with other networks.
- When these neural codes are understood, artificial devices can be built to mimic physiological neural circuit activity.
- These breakthroughs can restore function of lost neural networks and replace missing systems (ie eyes, relay past a damaged spinal cord etc)
General Design of a Neuroprosthetic Device

- Chronic intracranial recording → large-scale brain activity from motor areas in the cortex
- Data is processed by real-time mathematical models for extracting motor commands from raw electrical brain activity
- Model is used to output specific motor commands to a peripheral prosthetic device or artificial actuator
Approaches to BMI

- Scullcaps fitted with electrodes on the head have been shown to work.
  **Pro:** These measure brain wave patterns (EEG) and small differences can be modulated by the individual as an output for control of a device.
  **Con:** It takes considerable training to learn how to use this interface. It is very slow and the triggers used to control devices are limited.

- Electrodes fitted into the brain (either deep brain electrodes or chips sitting on the surface).
  **Pro:** These devices are more intuitive (depending how they are set up), much faster, and they can provide a lot of options for control of output devices.
  **Con:** They require surgery to install. Their use comes with all the normal concerns of implanting foreign objects. The neural activity measured by these devices, and therefore their sensitivity, seems to diminish over time.
How To Train a Computer to Talk Brain

- Monkeys are trained in a gripping and reaching task on a computer simulation.
- "pole control" neurons are recorded while the monkey controls a robotic arm with a joystick. This builds the motor control model.
- "brain control" one the model converges, monkeys realize that they don’t have to move the joystick anymore. Joystick is removed and monkey can control the actuator by thought alone.
BMI in humans
• A: superimposed action potentials from individual electrodes.
• B: field potentials from during neural cursor control.
• C: three traces of electrical recordings following a “go” cue, instructing the cursor to move.
• Matt was able to control a cursor with his mind with a strong degree of accuracy, even when compared to someone controlling it with their hands (a and b).

• He was also able to move around a cursor in a field while avoiding obstacles (orange squares).
Alternatives

- Xerograms are an additional focus with similar intent.
- They implant stimulators that can pass current through wires in the limbs to activate muscles and restore motor movement.
Overall Architecture of the Eye
Regeneration in the Eye

- They crush the optic nerve. Normally the axons distal to the eye (to the right) will complete denervate the nerve. The axons proximal to the site crush injury will shrink back to their cell bodies on the retinal ganglion cells (RGC) shortly thereafter.
- The retinal ganglion cells often die shortly after that (possibly from loss of neurotrophin from their postsynaptic targets).
- Larry Benowitz’s group discovered that a small puncture injury to the eye lense increases the RGC survival by 8-fold and reinnervation of the crush site by 100-fold.
Activation of Monocytes Promotes Regeneration

- They then went further and showed that adding a monocyte activating factor, Zymosan (basically an irritant) to the eye dramatically upregulated the reinnervation of the optic nerve.
- They implanted a peripheral nerve graft and asked whether the addition of Zymosan to the lens would induce regrowth through that as well. They saw a dramatic (2x) increase in innervation of the peripheral explant, which was even more striking (3x) if Zymosan is added 3 days after the injury.
Oncomodulin is the Monocyte Regenerating Factor

- The same group finally purified the factor secreted by the macrophages, called oncomodulin, that induces the regeneration of these axons.
- Implanting beads soaked in oncomodulin induces regrowth of axons effectively through the crush site of an optic nerve.
- Oncomodulin also enhances the axon outgrowth from cultured neurons. It promotes the growth of axons grown on CSPG, and outgrowth is very exuberant when the CSPGs are removed.
Bionic Vision