

REGENERATION AND REPAIR OF THE CENTRAL NERVOUS SYSTEM

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PURPOSES OF THIS LECTURE

Consider:

- the effects of injury on the function of the CNS
- the mechanisms responsible for those effects
- methods that may promote CNS healing and repair
- unintended consequences of spinal cord repair

Although numerous vascular and traumatic events can affect both the brain and the spinal cord, we will use spinal cord injury as our model in this lecture.

FACTS:

- Axons in the peripheral nervous system (PNS) regenerate.
- Axons in the CNS axons do not regenerate.
- Axons of PNS neurons will not regenerate into the CNS

TENTATIVE CONCLUSION:

- Failure to regenerate is not a property of axons but, instead, a property of the site of injury.

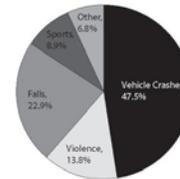
Between 250,000 and 400,000 Americans live with spinal cord injury.

79.6% of new SCI patients are male.

48.2% are married at the time of injury.

The average age at injury is 37.6 years.

Causes of SCI Since 2000



Average Yearly Expenses

Severity of Injury	First Year	Each Subsequent Year
High Quadriplegia (C1-C4)	\$710,275	\$127,227
Low Quadriplegia (C5-C8)	\$458,666	\$52,114
Paraplegia	\$259,531	\$26,410
Incomplete Motor Function at any level	\$209,324	\$14,670

- Length of initial hospitalization following injury in acute care units: 19 days
- Average stay in rehabilitation unit: 45 days
- 88.1% of all spinal cord injured individuals are discharged from hospitals to a private home, 5.3% are discharged to nursing homes.

Estimated Lifetime Costs by Age of Injury

Severity of Injury	25 Years Old	50 Years Old
High Quadriplegia (C1-C4)	\$2,801,642	\$1,649,342
Low Quadriplegia (C5-C8)	\$1,584,132	\$1,003,192
Paraplegia	\$936,088	\$638,472
Incomplete Motor Function at any level	\$624,441	\$452,545

The effects of injury on the function of the CNS:

- Loss of movement caudal to the lesion (including respiratory movement after high cervical lesions).
- Loss of sensation (cutaneous, proprioceptive) **OR** persistent pain caudal to the lesion!
- Loss of urinary bladder function. ***
- Compromised cardiovascular regulation.
- Loss of temperature regulatory sweating caudal to the lesion.

*** Of these, the loss of bladder function is the most dangerous, WHY?

A VERY INSTRUCTIVE PHENOMENON EXPERIENCED BY ABOUT 80% OF SPINALLY INJURY PATIENTS IS AUTONOMIC DYSREFLEXIA, LEADING TO HYPERTENSIVE CRISES.

What follows will give you a good idea of what it is like to be trapped in a wheelchair when your blood pressure goes to 200+ mmHg and your heart rate drops to 30 bpm. The quote is from a quadriplegic spinal cord researcher.

“Something’s wrong.
I’m hot.
No, wait, now I’m cold.
But why am I sweating? I never sweat.....
Why does my scalp itch?
On no, the headache is starting.
I’m getting dysreflexic.
What is causing it?
My catheter seems okay.
I don’t feel any kinks.
But why isn’t there any urine in the leg bag?
Oh no, is the catheter clogged up?
The headache is getting worse.
It feels like a nail is being hammered into my head.
What am I going to do? It’s going to get worse. I know it.
I know what’s going to happen.....”

Kim Anderson, Ph.D., 2003

What is happening here?

Dr. Anderson’s bladder catheter is plugged or kinked in her urethra. Her bladder fills with urine, finally reaching a high pressure.

Of course, because of her injury, she doesn’t feel a thing. However, the afferents from the bladder still send their messages into the spinal cord.

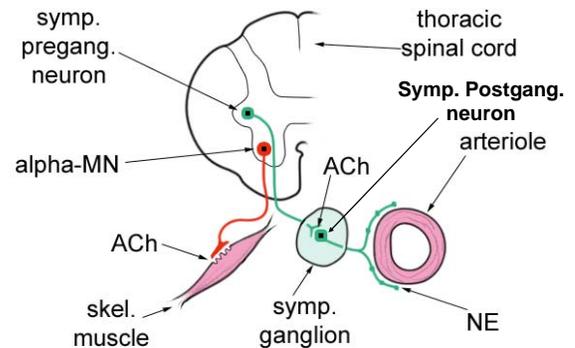
Normally, we would act on those messages, but even if we did not descending inhibitory pathways from the brain (destroyed in Dr. Anderson) would normally prevent messages from the bladder from reaching the sympathetic nervous system.

Without this descending inhibition, bladder information cause almost seizure-like increases in activity to the sympathetic axons that innervate vascular smooth muscle, causing a large increase in vascular resistance and decrease in venous compliance.

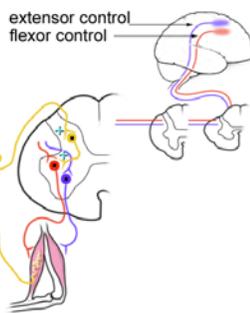
Arterial pressure rises, but the baroreceptor reflex only has its vagal limb to compensate. So, heart rate plunges. This produces very large pulse pressures, resulting in extreme headaches and, sometimes, stroke.

This process is illustrated on the next slide.

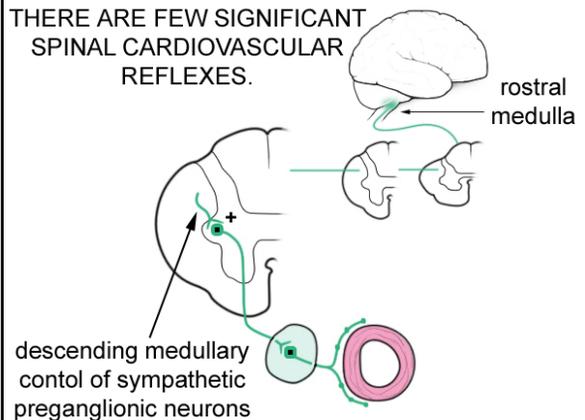
SOME BASIC ANATOMY

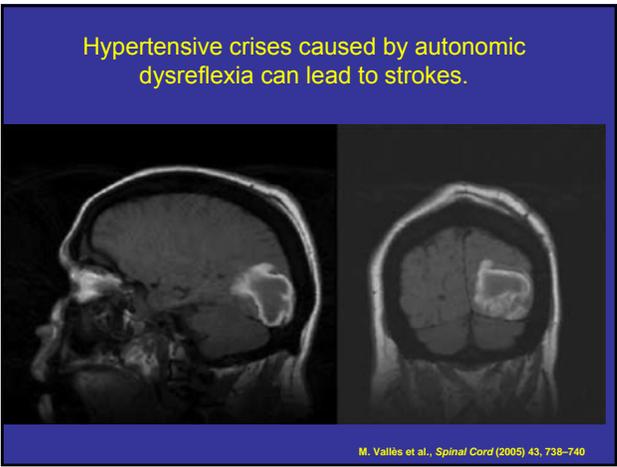
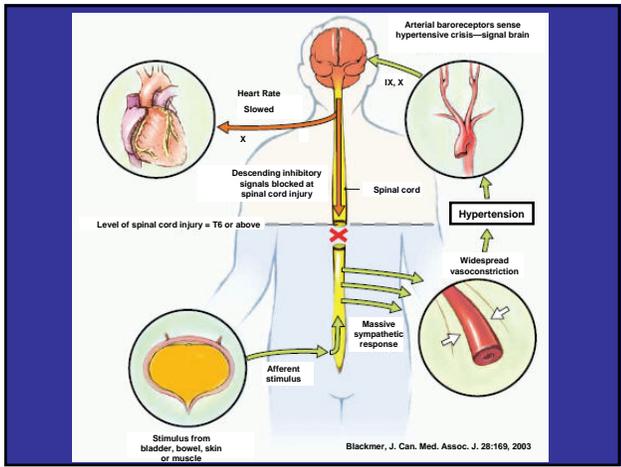
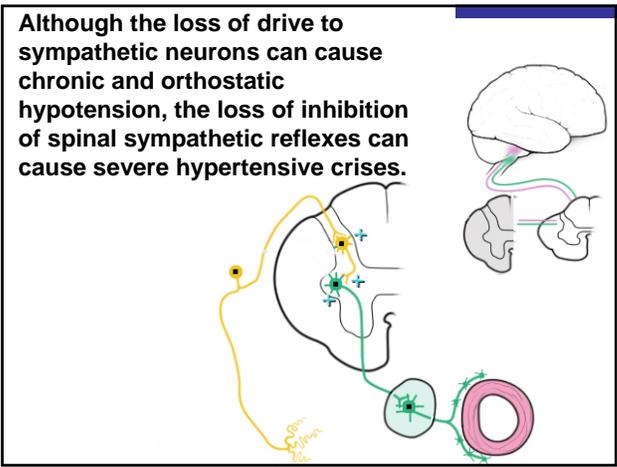
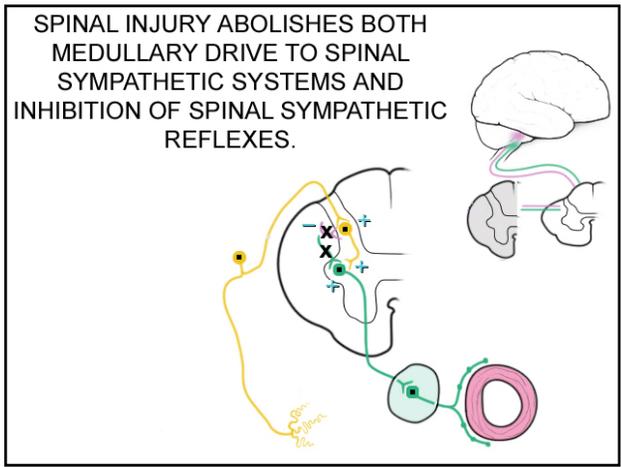
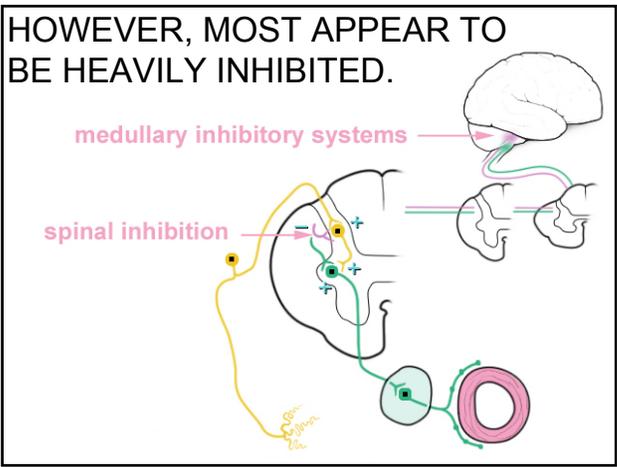
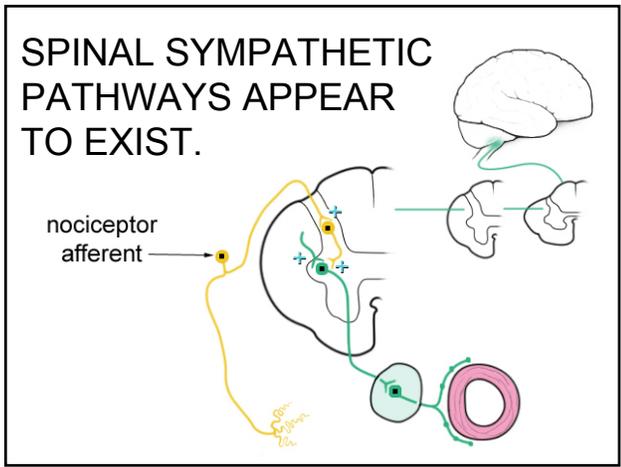


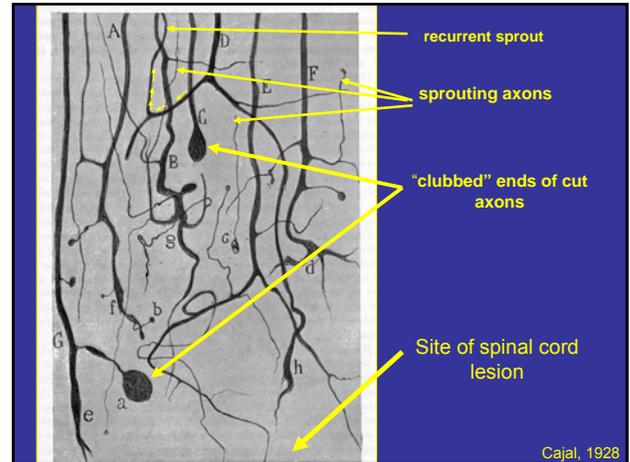
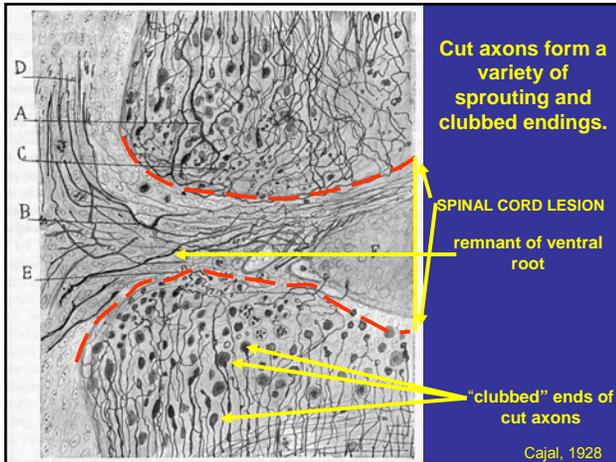
SOMATIC CONTROL INVOLVES IMPORTANT SPINAL REFLEXES



THERE ARE FEW SIGNIFICANT SPINAL CARDIOVASCULAR REFLEXES.







CNS axons fail to regenerate for 2 reasons:

- Release of inhibitors of axon regeneration by degenerating myelin
- Formation of a scar by astrocytes

One interpretation of the presence of inhibitors of axon regeneration in myelin is that the CNS is "not supposed to regenerate" after injury. What does that say about our attempts to cause regeneration?

Normal: Projecting Neuron, Cell Body, Myelin, Axon, Synapse, Post Synaptic Target Neuron, Oligodendrocyte

Injured: End Bulb, Astroglial Scar & Myelin Debris, Degeneration

Brittis, Neuron, 2001

What are these "inhibitors of axon regeneration?"

To date, 3 regeneration-inhibiting proteins and one proteoglycan have been identified, either in degenerating myelin or in the astrocyte-generated glial scar.

- Nogo-A
- MAG, Myelin associated glycoprotein
- OMgp, Oligodendrocyte myelin glycoprotein
- Chondroitin sulfate proteoglycan (CSPG)

Until recently, the only receptor for any of the first 3 of these molecules was that for Nogo-A. It is called NgR.

CSPGs do not act *via* receptors.

A few years ago, the astonishing discovery was made that all 3 inhibitors of axon regeneration were bound by NgR (see red arrows)!

Oligodendrocyte: MAG, OMgp, Nogo-A

Neuron: NgR, Unknown transducer/coreceptor, Rho GTPase, Rho kinase, PKA, CAMK, Rho G

GO: Growth cone collapse

Woolf, Science, 2002

Were it only that simple, simply blocking NgR would permit regeneration! However, the figure shows (red arrows) that both MAG and OMgp have other binding sites. These may explain why knocking out the gene for NgR does not permit regeneration.

Oligodendrocyte: MAG, OMgp, Nogo-A

Neuron: NgR, Unknown transducer/coreceptor, Rho GTPase, Rho kinase, PKA, CAMK, Rho G

GO: Growth cone collapse

Woolf, Science, 2002

The inhibitory system that has been most aggressively targeted is the Nogo-A/NgR system. Numerous methods have been used, and two will be described:

Preventing the binding of the “active” portion of Nogo-A (called Nogo-66, see figure), to NgR.

NEP1-40 is a peptide that binds to NgR and completely blocks binding of Nogo-66. In some preparations, it has improved regeneration moderately.

Passive immunization against Nogo-A with IN-1, an antibody that recognizes Nogo-A.

Treatment with IN-1 appears to improve regeneration. However, it also causes reorganization of intact axons, suggesting that Nogo-A normally stabilizes CNS axons.

STRATEGIES FOR PERMITTING AND ENCOURAGING REGENERATION

BLOCKING EFFECTS OF REGENERATION INHIBITORS

- antibodies
- enzymes

BRIDGES AND SCAFFOLDS

- peripheral nerves as bridges
- spinal roots as bridges
- artificial materials as bridges and scaffolds
- combined therapies

ANTIBODIES AND ENZYMES

We have already discussed an example of the use of antibodies above (IN-1).

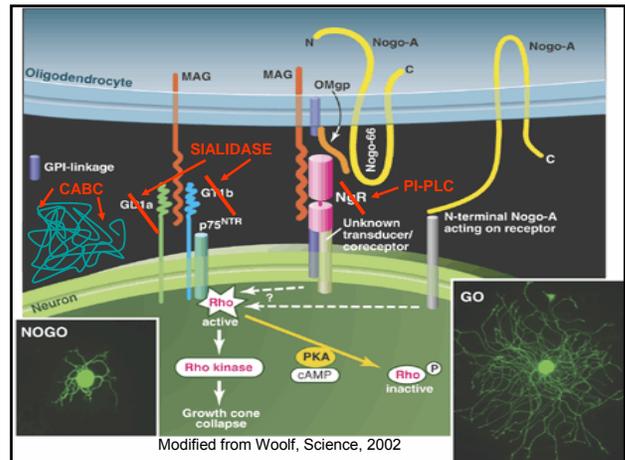
ENZYMES:

Chondroitinase ABC (CABC)—“digests” the CSPGs in the glial scar produced by activated astrocytes.

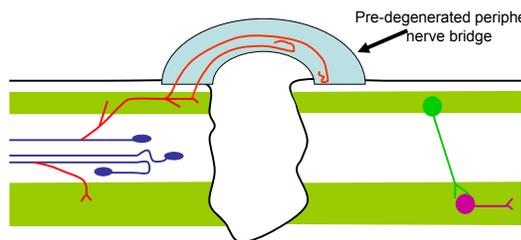
Phosphatidylinositol-specific phospholipase C (PI-PLC) removes NgR from axons.

Sialidase destroys the alternate sialoglycan receptors for MAG on axons.

These sites of action are shown on the next slide.

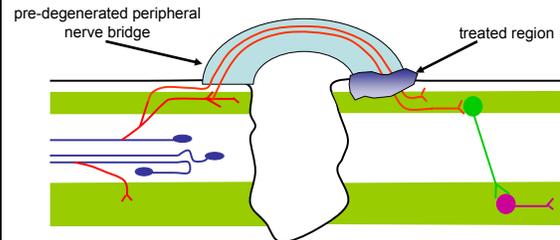


Axons will avidly grow **into** pre-degenerated peripheral nerve bridges, but without “help” few (if any) axons will grow **out**.



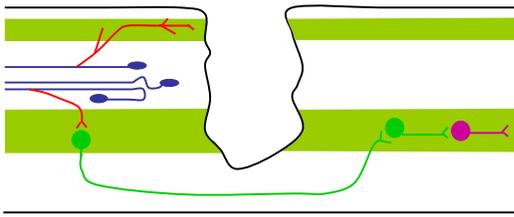
- Transsected CNS Tract
- Newly formed collaterals from CNS Tract
- Existing Propriospinal Interneurons
- Motorneuron

Treatments with enzymes or growth factors permit axons to leave the bridge.



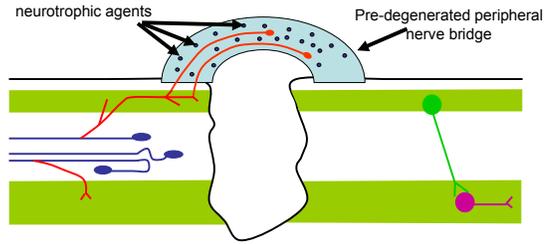
- Transsected CNS Tract
- Newly formed collaterals from CNS Tract
- Existing Propriospinal Interneurons
- Motorneuron

Collateralization: Redefining Outcomes after SCI



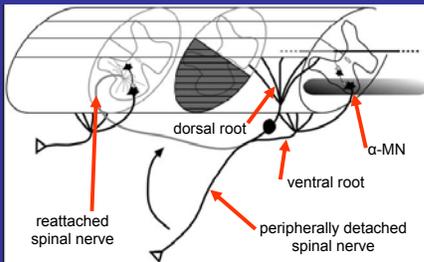
- Transected CST Tract
- Newly formed collaterals from CST
- Existing Propriospinal Interneurons
- Motoneuron

Incorporation of neurotrophic agents in artificial scaffolds promotes growth into, but not out of bridges.



- Transected CNS Tract
- Newly formed collaterals from CNS Tract
- Existing Propriospinal Interneurons
- Motoneuron

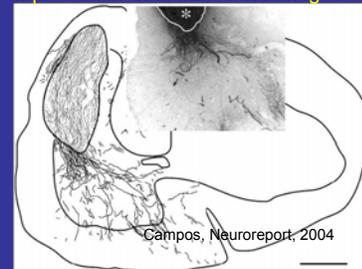
An interesting method uses the peripheral end of a spinal nerve to bridge a spinal cord lesion.



Campos, Neuroreport, 2004

Remarkably, the axons of α -motoneurons grow into the spinal cord caudal to the lesion and appear to make synaptic contacts on neurons. A significant improvement in function occurs.

What experiment could you do to show that the improvement was due to the bridge?



Campos, Neuroreport, 2004

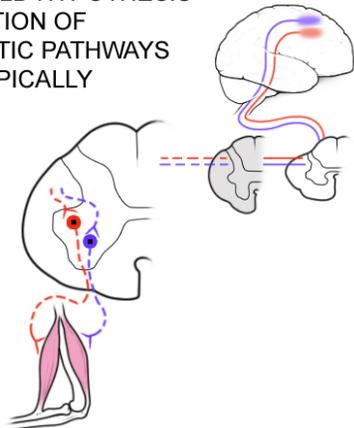
How is improvement after a treatment quantified? Below is a modified version of the "BBB" score that is most commonly used. See any problems?

Score	Description
0	No observable hindlimb (HL) movement
1	Slight movement of one or two joints, usually the hip and/or knee
2	Extensive movement of one or two joints or Extensive movement of one joint <i>and</i> slight movement of one other joint or Slight movement of all three joints
3	Slight movement of two joints <i>and</i> extensive movement of the third
4	Extensive movement of two joints <i>and</i> slight movement of the third
5	Extensive movement of all three joints of the HL
6	Sweeping with no weight support or Plantar placement of the paw with no weight support
7	Plantar placement of the paw with weight support in stance only (i.e., when stationary) or occasional, frequent, or consistent weight supported dorsal stepping and no plantar stepping
8	Occasional weight supported plantar steps, no forelimb (FL)-HL coordination
9	Frequent to consistent weight supported plantar steps <i>and</i> no FL-HL coordination
10	Frequent to consistent weight supported plantar steps <i>and</i> occasional FL-HL coordination
11	Frequent to consistent weight supported plantar steps <i>and</i> frequent FL-HL coordination
12	Consistent weight supported plantar steps, consistent FL-HL coordination or frequent plantar stepping, consistent FL-HL coordination, and occasional dorsal stepping

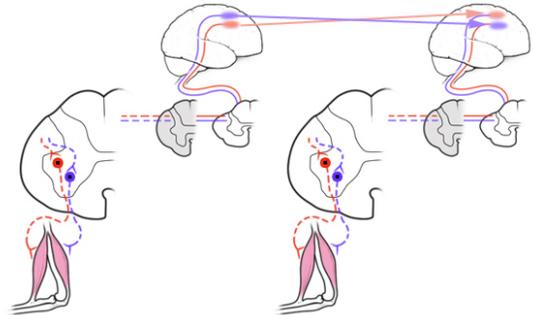
WILL WE BE ANY BETTER OFF AFTER SPINAL CORD REGENERATION?

HOW COULD SPINAL CORD REGENERATION OR SPROUTING AFTER SPINAL CORD INJURY LEAD TO SERIOUS PATHOLOGY?

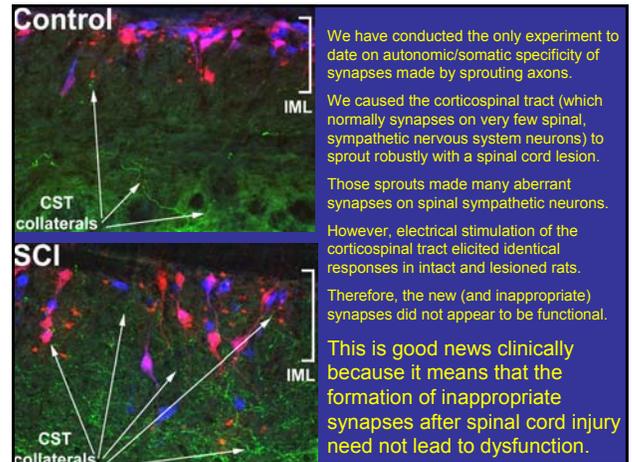
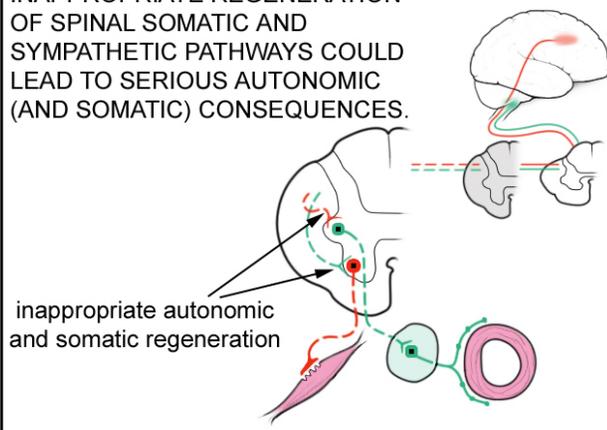
THE CURRENTLY HELD HYPOTHESIS IS THAT REGENERATION OF DESCENDING SOMATIC PATHWAYS WILL BE SOMATOTOPICALLY CORRECT.



CORTICAL PLASTICITY VERY LIKELY COULD COMPENSATE FOR INCORRECT REGENERATION OF DESCENDING SOMATIC NEURONS.



INAPPROPRIATE REGENERATION OF SPINAL SOMATIC AND SYMPATHETIC PATHWAYS COULD LEAD TO SERIOUS AUTONOMIC (AND SOMATIC) CONSEQUENCES.



We have conducted the only experiment to date on autonomic/somatic specificity of synapses made by sprouting axons.

We caused the corticospinal tract (which normally synapses on very few spinal, sympathetic nervous system neurons) to sprout robustly with a spinal cord lesion.

Those sprouts made many aberrant synapses on spinal sympathetic neurons.

However, electrical stimulation of the corticospinal tract elicited identical responses in intact and lesioned rats.

Therefore, the new (and inappropriate) synapses did not appear to be functional.

This is good news clinically because it means that the formation of inappropriate synapses after spinal cord injury need not lead to dysfunction.

SUMMARY

Care of the spinally injured is a major human and financial burden.

The injured CNS does not repair itself, and this is a property of the injury environment.

Spinal injury causes a loss of both somatic and autonomic function.

The loss of autonomic function is usually the more dangerous.

Hypertensive crises are the most dangerous cardiovascular effect of spinal injury.

CNS axons are prevented from regenerating by a glial scar and release of inhibitors.

You should know what these inhibitors are and their mechanisms of action.

An aggressive search is on for inhibitors of regeneration inhibitors.

Both antibodies and enzymes have had some success. Know what they are.

A variety of synthetic and natural bridges have been used to bypass spinal lesions.

The rule has been that axons will readily grow into these bridges, but not out of them.

Recent treatments have improved the function of these bridges.

Quantification of the effects of spinal injury and its treatments needs to be improved.

Spinal cord regeneration can lead to the formation of aberrant synapses.

The one study to date indicates that these synapses may not be functional.