

Stem Cell Transplantation for Spinal Cord Injury

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Back to School
Managing Your Injury to Independence
CPA and Thomson Rogers
Toronto
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Historical Perspective: It Has Been Extremely Difficult to Develop Effective Strategies to Improve Recovery after SCI because.....

- There are many primary and secondary injury mechanisms that need to be treated
- The loss of tissue is usually extensive, and thus tissue regeneration will be required
- The regeneration of axons will have to extend for long distances to make reconnections
- The axons will have to reach precise targets
- There are serious inhibitory factors eg scar tissue and myelin based inhibitors

Major Questions Requiring Answers-WHICH CELLS????

Neural or Non-Neural Cells which are better for transplantation?

Endogenous vs. Exogenous (transplanted) Neural Stem Cells? - which source is better?

Developmental Age of the Transplanted Cells - Embryonic, Fetal, Neonatal, or Adult?-which is better?

If adult cells, what is best source of cells? Spinal Cord or Brain?

Answer: We do not know, and we need to continue to examine many types of cells in experimental studies and clinical trials.

We prefer ADULT SPINAL CORD DERIVED TRANSPLANTED NEURAL STEM/PROGENITOR CELLS (NSPC)

ADULT MAMMALIAN SPINAL CORD CONTAINS NEURAL STEM CELLS

Weiss et al • J. Neurosci. 16:7599, 1996

Shihabuddin et al • Exp. Neurol. 148:577, 1997

Johansson et al • Cell, 96:25, 1999

**Namiki and Tator • J. Neuropath. Exp. Neurol.
58:489, 1999.**

Neural Stem Cells are present in the Brain and Spinal Cord

- can self-renew and are multipotential for neurons and glia
- In the brain and spinal cord, multipotential stem/progenitor cells reside in the ependymal region around the ventricles and central canal of the spinal cord,
- In amphibians, these cells proliferate and differentiate into neurons and glia to regenerate the injured cord
- In mammals, these cells proliferate in response to injury but have limited regenerative ability

SEE THIS
REVIEW PAPER
FOR DETAILS

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REVIEW OF TREATMENT TRIALS IN HUMAN SPINAL CORD INJURY: ISSUES, DIFFICULTIES, AND RECOMMENDATIONS

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OBJECTIVE: To provide a comprehensive review of the treatment trials in the field of spinal cord injury, emphasizing what has been learned about the effectiveness of the agents and strategies tested and the quality of the methodology. The review aims to provide useful information for the improvement of future trials. The review audience includes practitioners, researchers, and consumers.

METHODS: All publications describing organized trials since the 1960s were analyzed in detail, emphasizing randomized, prospective controlled trials and published Phase I and II trials. Trials were categorized into neuroprotection, surgery, regeneration, and rehabilitation trials. Special attention was paid to design, outcome measures, and case selection.

RESULTS: There are 10 randomized prospective control trials in the acute phase that have provided much useful information. Current neurological grading systems are greatly improved, but still have significant shortcomings, and independent, trained, and blinded examiners are mandatory. Other trial designs should be considered, especially those using adaptive randomization. Only methylprednisolone and thyrotropin-releasing hormone have been shown to be effective, but the results of the former are controversial, and studies involving the latter involved too few patients. None of the surgical trials has proven effectiveness. Currently, a multitude of cell-based Phase I

TWO Categories of Strategies

#1 Endogenous Neural Stem Cells.

Stimulate them with growth factors or other agents to regenerate the damaged spinal cord

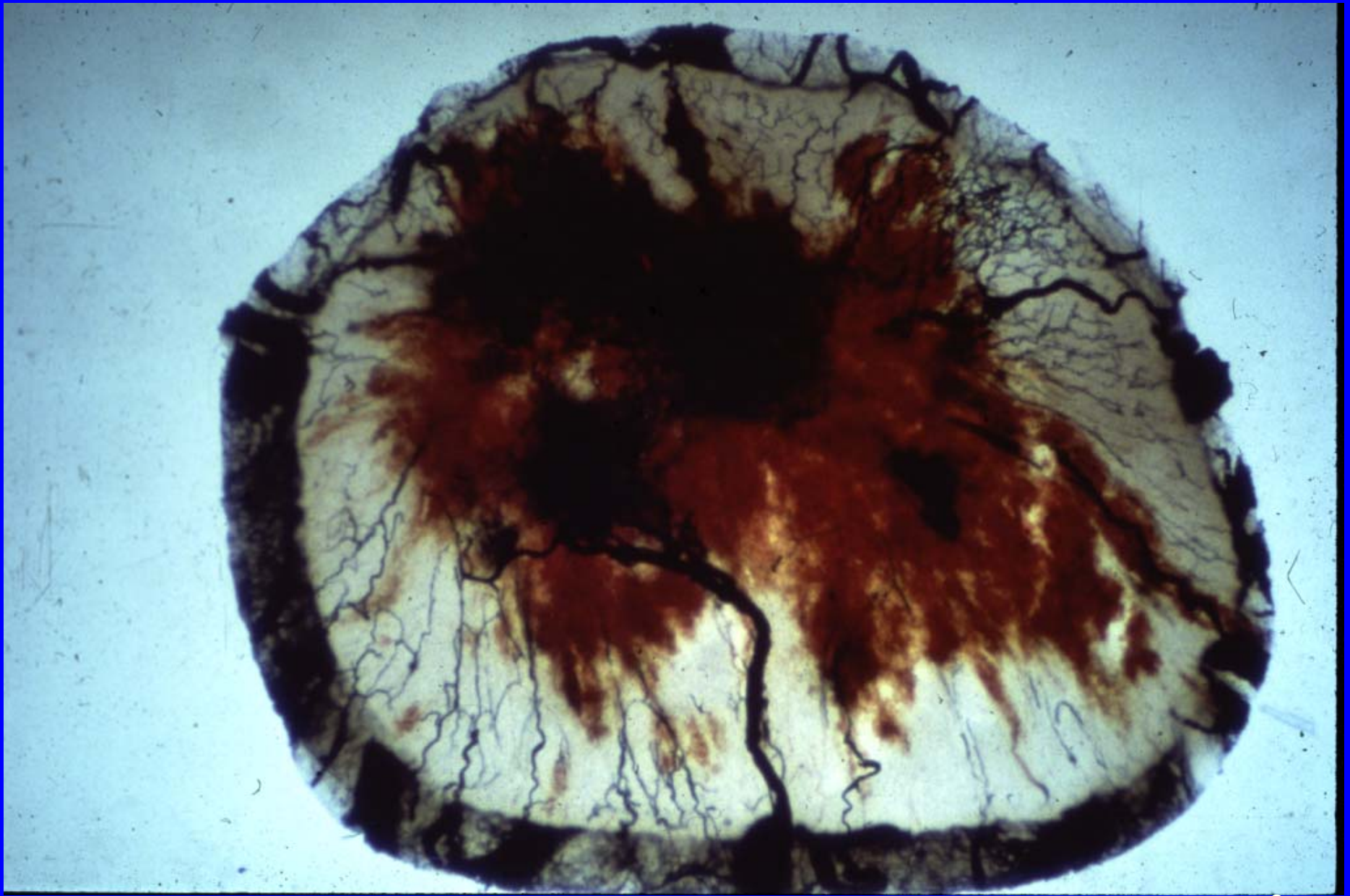
#2 Transplanted Neural Stem Cells.

Harvest them, grow them in culture and then transplant them into the injured cord

Two Potential Strategies Involving Neural Stem/Progenitor Cells for Repair of the Injured Spinal Cord in Patients

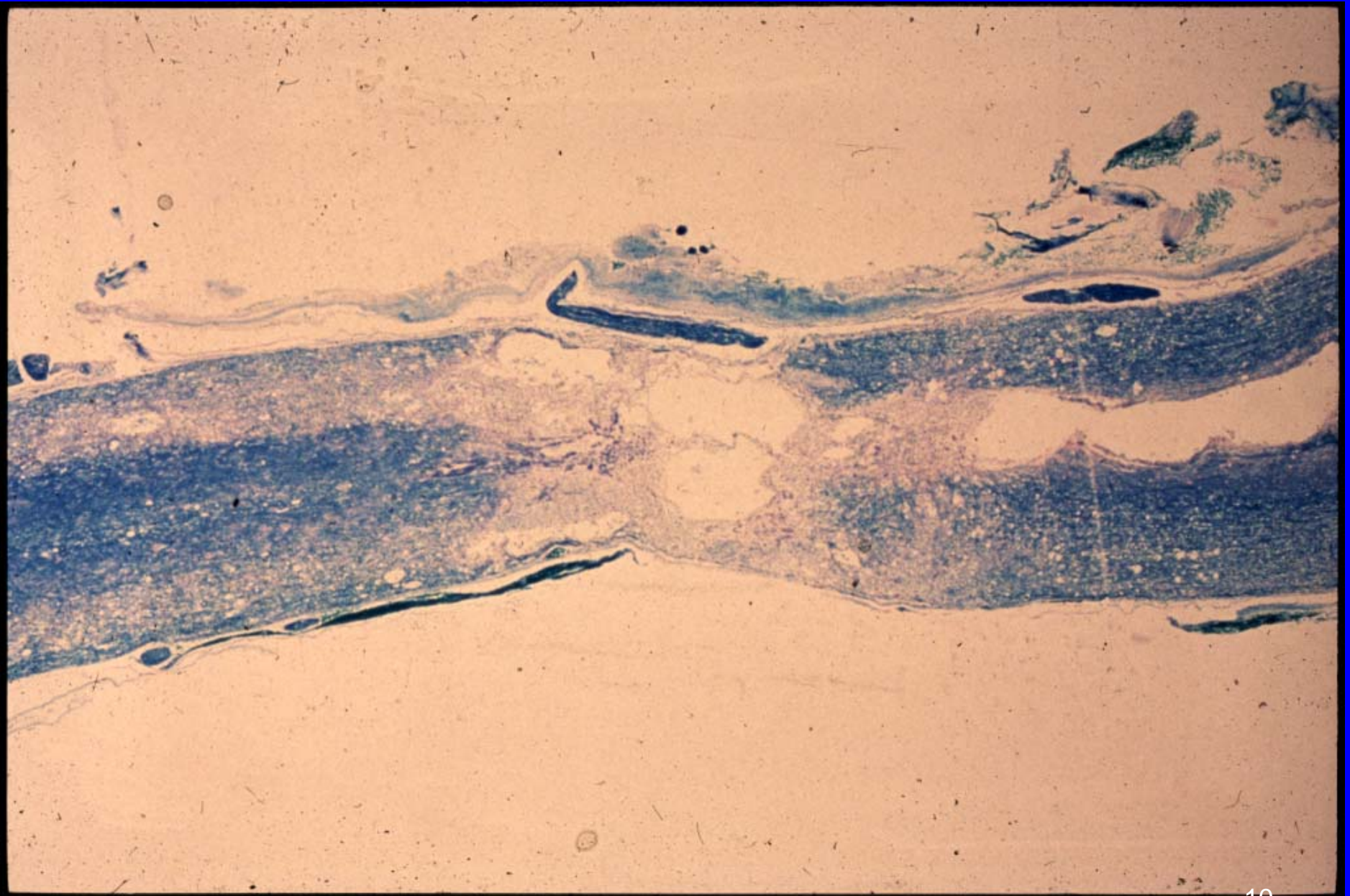
1. Manipulation of Endogenous stem cells .
That is those already present in the spinal cord
2. Transplantation of Exogenous stem cells
harvested from somewhere else in the body or
from another person or species

Rat Clip Compression Injury - 1 Hour



Several Rat Models Produce Injuries Similar to Humans

Rat Clip Compression Injury at 3 Months

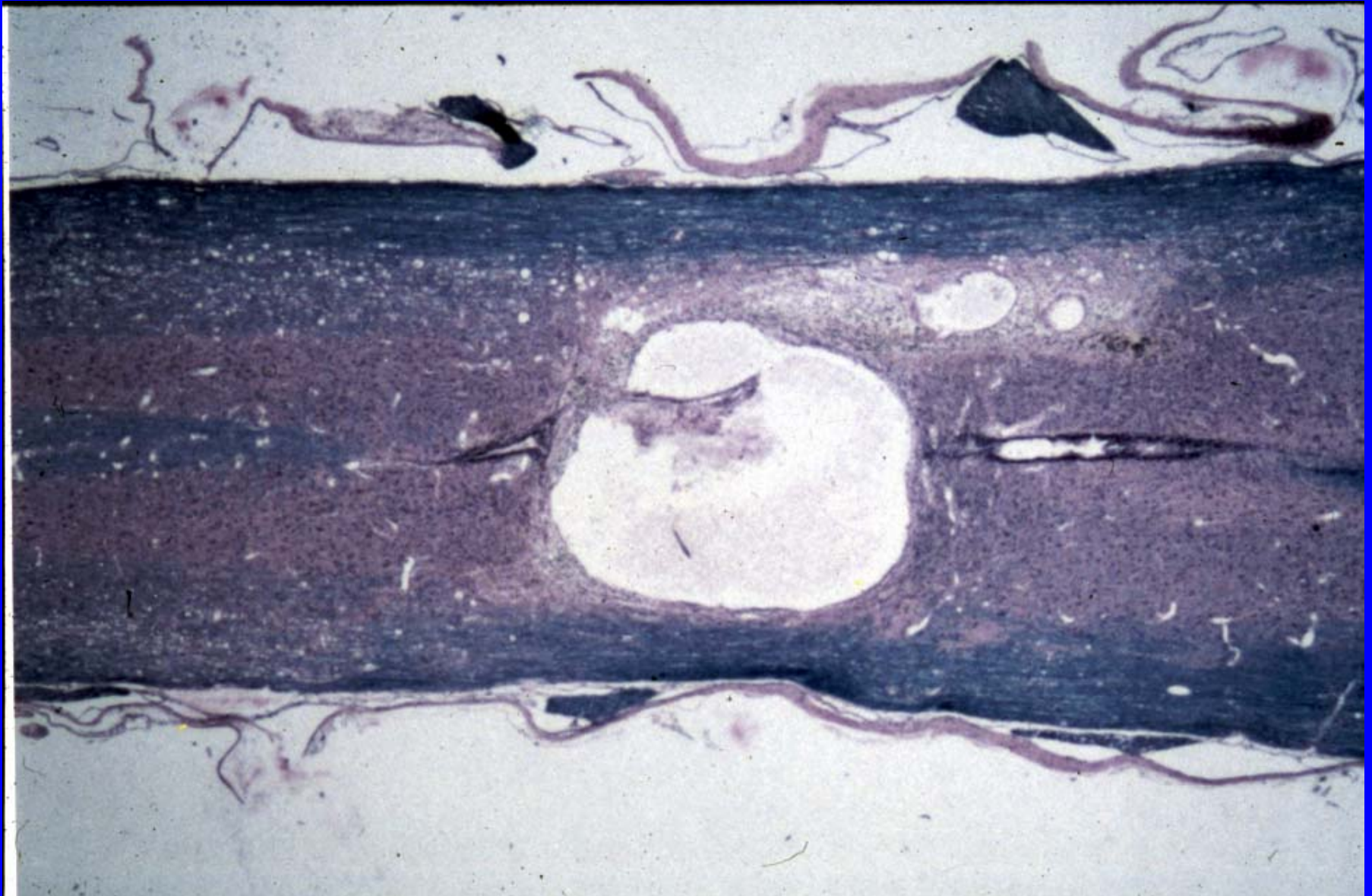


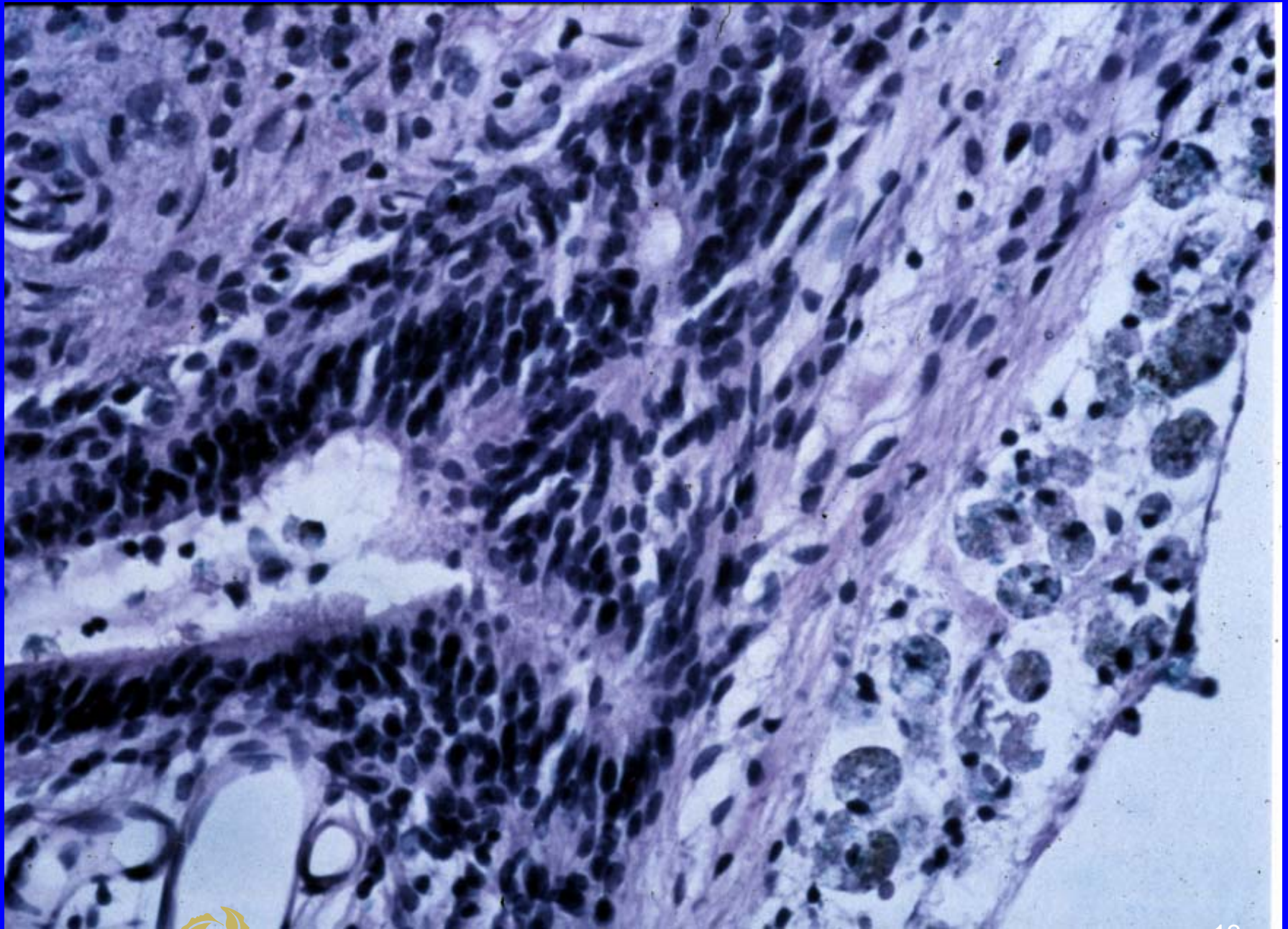
Identical to Human Injury- Cavitation, Syringomyelia, Ependymal Proliferation¹⁰

Stem/Progenitor Cells in the Brain and Spinal Cord

- Neural stem/progenitor cells can self-renew and are multipotential for neurons and glia
- In the spinal cord, stem/progenitor cells reside in the ependymal region around the central canal
- In amphibians, these cells proliferate and differentiate into neurons and glia to regenerate the injured cord
- In mammals, these cells proliferate in response to injury but have limited regenerative ability
- *Would increasing the number of stem cells by stimulation or transplantation improve regeneration?*





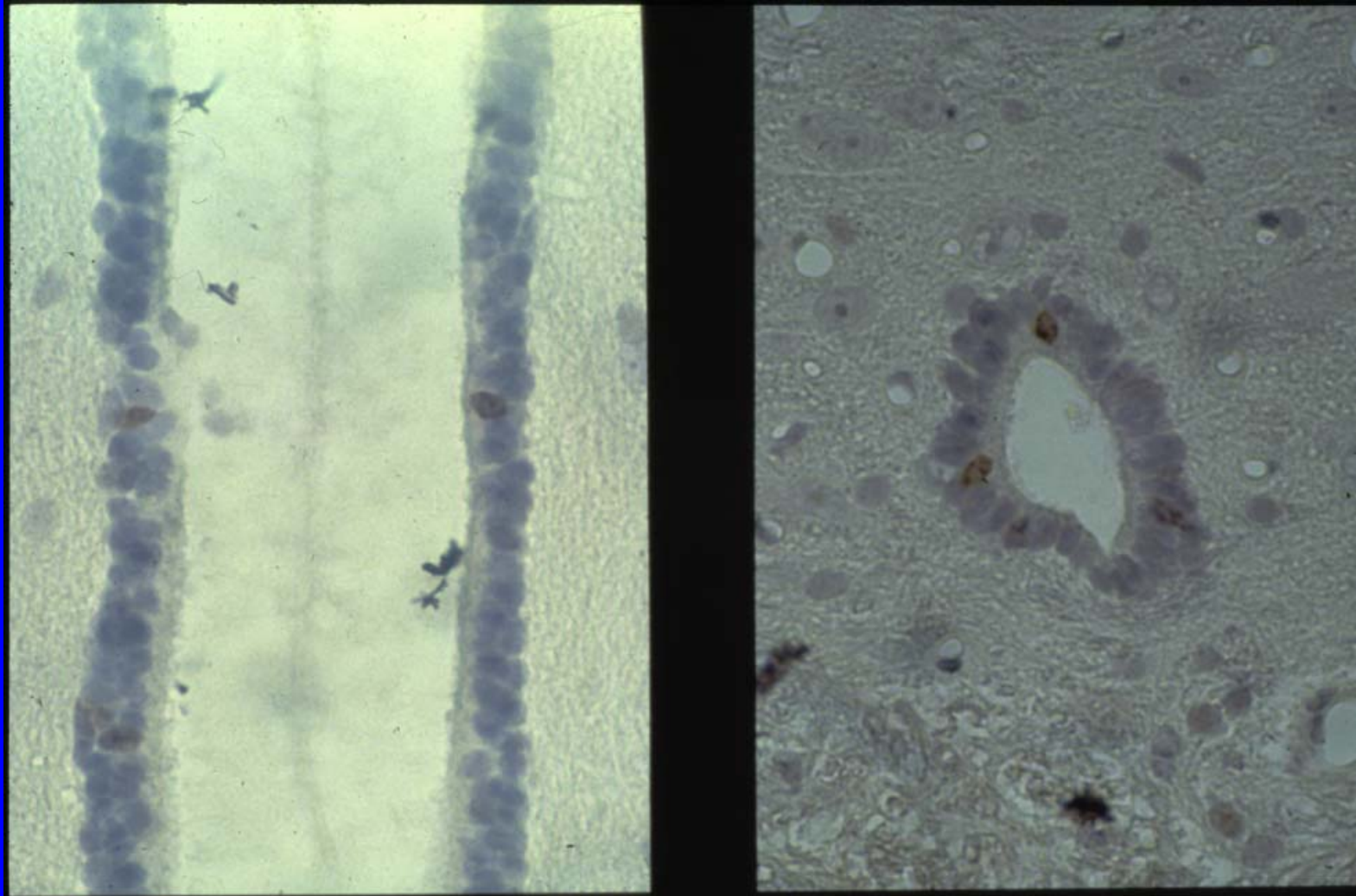


Ependymal Cells in the Adult Mammalian Spinal Cord

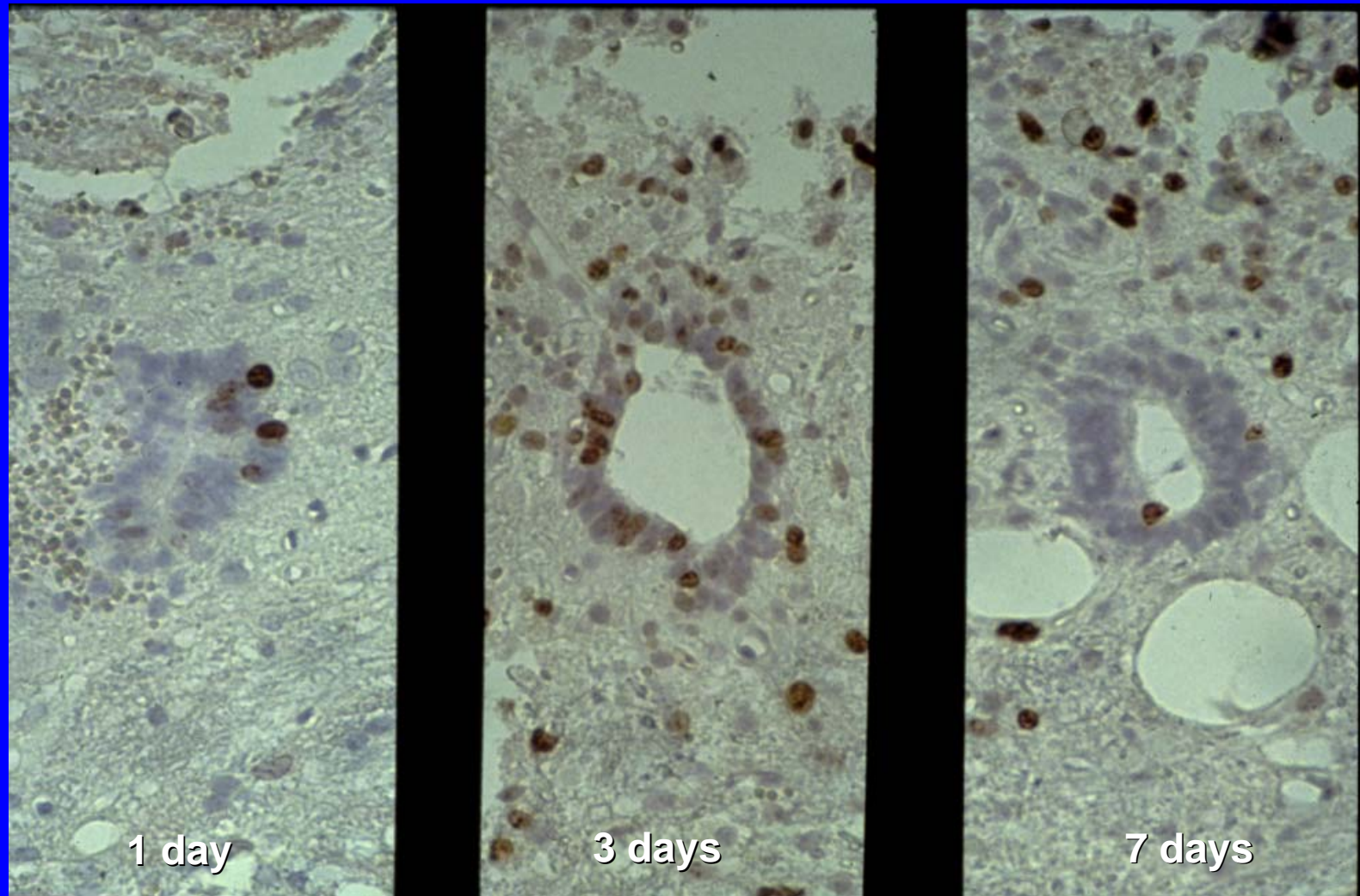
- 1. Normal ependymal cells - proliferate with a labelling index of 1-2%.**
- 2. After injury ependymal cells proliferate, migrate, and act as stem cells.**



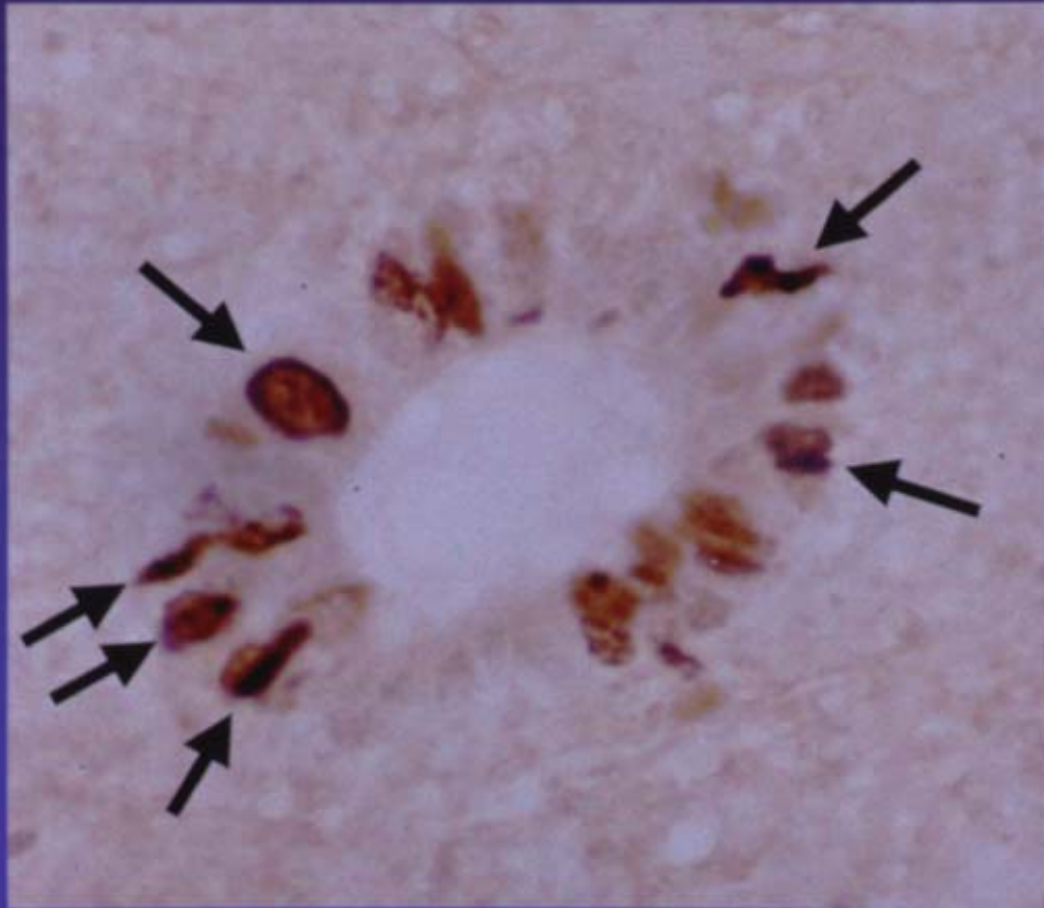
Normal Adult Spinal Cord.
BRDU Administration.
Anti-BRDU Immunohistochemistry for dividing cells 1-2%



SCI Causes Marked Proliferation of Stem/Progenitor Cells



Double Label (BrdU/Nestin)



Stem/Progenitor Expansion



Transplanted Stem Cells are better than Endogenous Stem Cells

- We spent 6 years, from 1998-2004 trying to encourage endogenous NSPCs to repair the spinal cord, but achieved minimal success. To do this we had to massively stimulate the injured spinal cord with mitogens and growth factors (EGF and FGF2).
- We got minimal functional recovery and tissue repair.
- We were concerned about the growth factors causing tumor formation

TRANSPLANTATION TRIALS and “EXPERIMENTS” IN HUMANS with SCI

- Many recent trials in patients, mostly Phase 1 uncontrolled, small number of patients, many without published reports
- Many non-trial “Experiments” in humans
- “Stem Cell Tourism”
- **Many types of cells have been transplanted into humans with SCI in several countries**

Transplantation Trials in Humans with SCI – Recent All Phase 1 and many not reported

Strategy	Name of Study, Authors and Reference	Countries	Year of Published Report if any	No. of Patients	Neurologic Result	Other Results and Comments
<u>Fetal Porcine Stem Cells – into cord</u>	Diacrin Study	USA		28		No further recruitment
<u>Autologous Activated Macrophages – into cord</u>	Procord Study Knoller et al	Israel	2005	6	Improvement in some	Phase 2 Trial in Progress in 2006
	Schwartz and Yoles Lammertse	Israel, Belgium USA and Canada	2005 ??2009	14 ?????	Improvement in 5 patients	
<u>Peripheral Nerve Transplants</u>				4		
<u>1..Peripheral Nerve Grafts – cord to cord</u>	Cheng	Taiwan	2004	1		Continuing to Recruit
<u>2. Peripheral Nerve Grafts – cord to cord</u>	Barros	Brazil	2003	8	No improvement	20
<u>3. Peripheral Nerve Grafts – cord to nerve</u>	Brunelli	Italy	2003, 2009	2	Improvement	

Transplantation Trials in Humans with SCI – Cont'd

Strategy	Name of Study, Authors and Reference	Countries	Year of Published Report if any	No. of Patients	Neurologic Result	Other Results and Comments
<u>4. Avulsed Root Inserted Into Cord-brachial plexus injury</u>	Carlstedt et al (26)	Sweden	2000	10	Improvement	
<u>Human Olfactory Ensheathing Glia (fetal and adult) - into cord</u>	Huang et al	China	2003	171	Improvement	Continuing to recruit
	Feron et al	Australia	2005	3		
	Rabinovich et al(124)	Russia	2003	15	Improvement	Continuing to recruit
	Lima	Portugal	2006	??		
<u>Blood Derived Stem Cells - ALS- intrathecal</u> <u>- SCI - intrathecal</u>	Janson et al	USA Brazil Russia	2001			
<u>Schwann Cells</u>	Zhu et al	China		47	Improvement	Continuing to recruit
	Feng et al	China		9	Improvement	Continuing to recruit

Transplantation Trials in Humans with SCI – Cont'd

Strategy	Name of Study, Authors and Reference	Countries	Year of Published Report if any	No. of Patients	Neurologic Result	Other Results and Comments
<u>Human Fetal Spinal Cord - syringomyelia</u>	Falci et al	Sweden	1997	1		Syrinx smaller
	Wirth et al	USA	2001	8	No further deterioration	Syrinx smaller
<u>(Rho Antagonist)</u>	Cethrin Study / Bio-Axone	USA, Canada	2008		Some good neurological	Completed- some Patients recovered
<u>(Anti-Nogo-A Antibody)</u>	Novartis Study	Europe				Planning stage
<u>Human Bone Marrow Stromal or Hematopoietic Cells</u>	Park et al)	South Korea	2005	5	Improvement	
	Zhang et al	China		90		
	Bryukhovetskiy	Egypt				
	Neuronyx	Russia Czech Republic USA				
<u>NSPC-Human Embryonic Stem Cells</u>	GERON (Keirstead, Wirth)	USA				Began 2009
<u>NSPC-Human Fetal Brain</u>	STEM CELLS INC.	USA				Planning Stage
<u>(Electrical Stimulation)</u>	Shapiro et al	USA	2005	10	Some improvement	Continuing to recruit 22
	Xu, Liu	China		>100	Some improvement	Continuing to recruit

Human Neural Stem Cells: Two Recent/Current Trials in Humans

Human Embryonic Stem Cells for SCI- in USA and Canada

- Embryonic and therefore fear of producing tumors .
- Geron, Inc. used human embryonic stem cells (hESC), differentiated toward oligo lineage by Hans Keirstead, and in Jan. 2009 the FDA gave permission to start a trial in human SCI in US centres. Trial began in 2010.
- Transplanted in five **ACUTE** patients up to 14 days after SCI
- 2011 trial discontinued-ran out of money!

Human Fetal Stem Cells for SCI- in Switzerland

- Fetal source was challenged because of ethical concerns (still).
- StemCells Inc. completed a transplant trial of human fetal brain stem cells programmed to differentiate into oligos in 6 children with Batten's Disease.,
- Trial started in 2011 in Zurich in SUBACUTE and CHRONIC CASES 3-12 months after injury. So far 3 thoracic ASIA A patients have received transplants.

My Choice for Spinal Cord Repair- Neural Stem/Progenitor Cells (NSPC) from the Adult Spinal Cord

- Multipotential and have inherent ability to divide
- Transplanted rather than endogenous
- Rodent now, humans-pending
- Adult cells- no ethical concerns and do not cause cancer (versus embryonic)
- From spinal cord because they differentiate preferentially toward oligos without needing extra growth factors
- With differentiating factors such as C-AMP we can generate neuronally enriched NSPC

Why Spinal Cord Source of Cells Rather than Brain?

- After several years, we and several other labs have not established that one is superior to the other, and both have shown good results.
- **Examples:** Karimi et al 2008 in the Fehlings lab and other labs have shown good results with **brain-derived neural stem cells**.
- The Tator lab and others have shown good results with **spinal cord-derived neural stem cells** - see Parr et al 2008.

Highlights from Our Recent Studies with Transplanted Adult Rat Spinal Cord-Derived NSPCs for Spinal Cord Repair in the Rat-to show the potential of these cells

1. Subacute spinal cord compression model- **Ann Parr et al** in: Neuroscience 155:760-770, 2008
2. Demyelination and dysmyelination models– **Andrea Mothe et al** in: Experimental Neurology 213:176-190, 2008

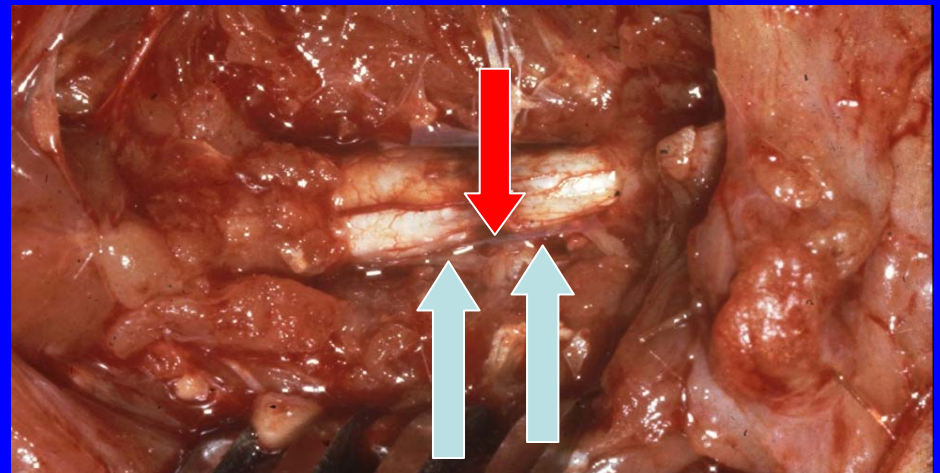
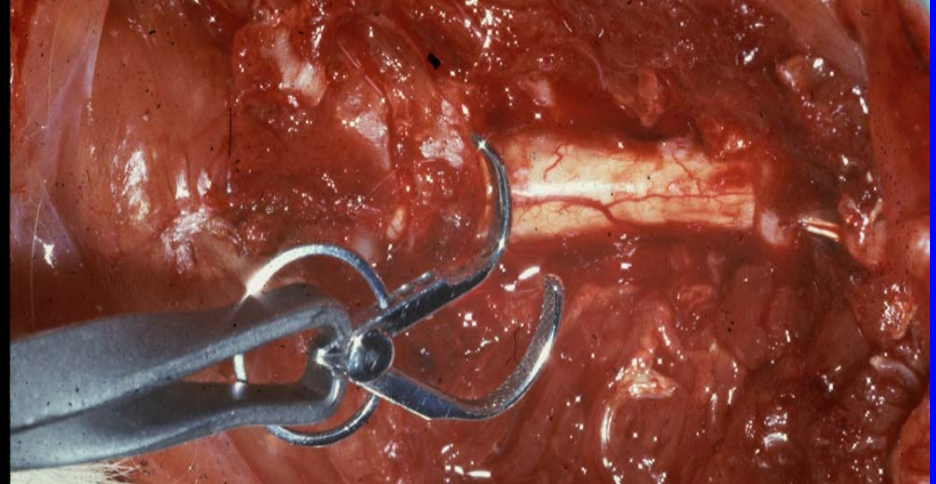
Transplanted Adult Spinal Cord-Derived NSPCs Promote Functional Recovery After Rat Spinal Cord Compression Injury.

Parr AM, Kulbatski I, Zahir T, Wang X,
Yue C, Keating A, Tator CH.

Neuroscience. 26;155(3):760-70, 2008.

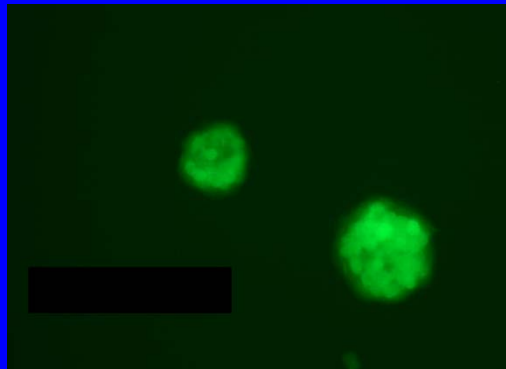
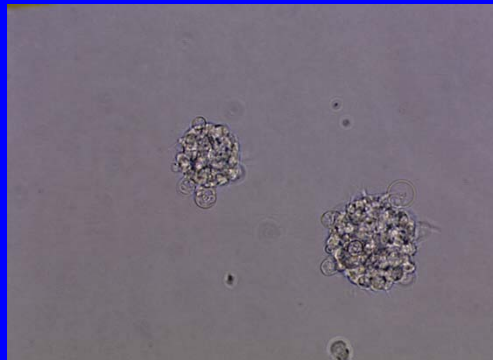
Methods - Bone Marrow Stem Cells versus Neural Stem Cells

- Acute extradural clip compression of spinal cord
 - T8
 - 27g injury- a moderate injury
- Bone Marrow Stem cells (transpl day 0), Neural Stem Cells(transpl day 9) were injected 1mm rostral and caudal to the injury site (200,000 cells). Control-culture medium
- 12 week survival
- Daily cyclosporine to prevent rejection



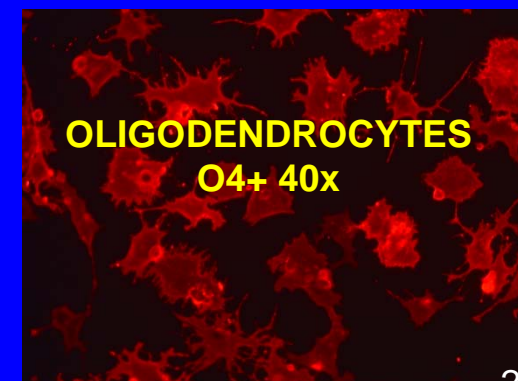
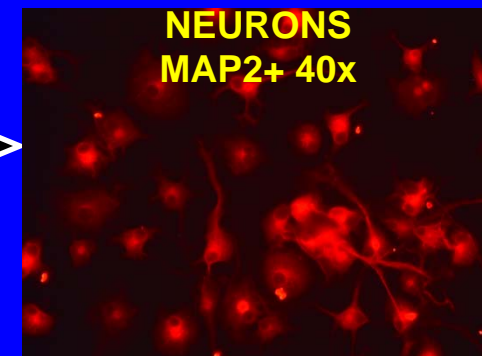
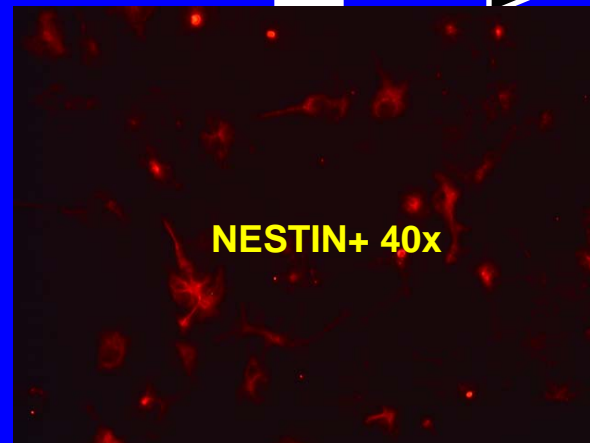
Adult Rat Spinal Cord Stem Cells

GFP adult rat spinal
cord neurospheres
Day 7 in culture P3
Phase 40x



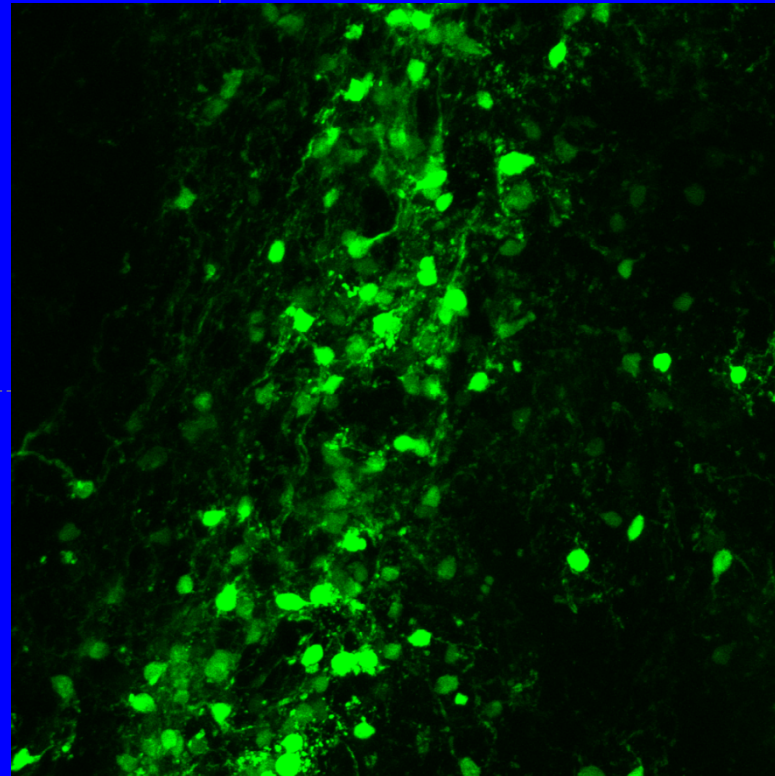
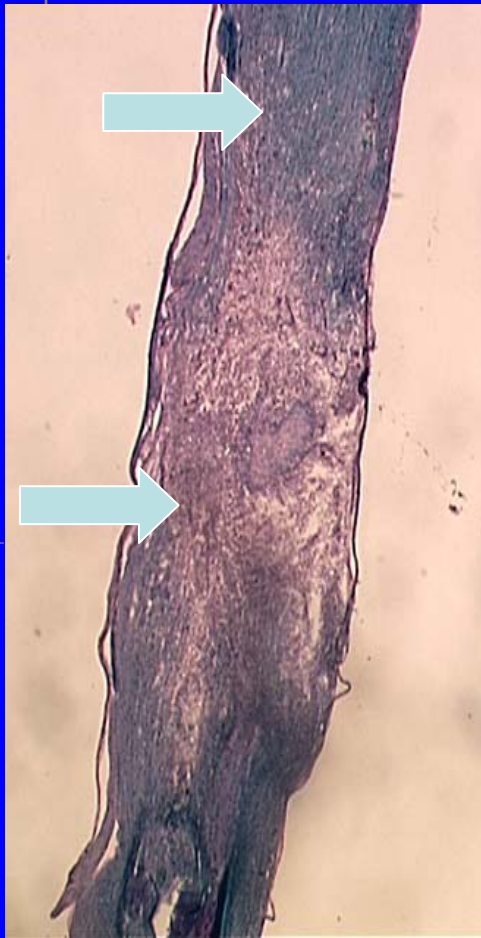
DIFFERENTIATION

After plating
on Matrigel,
removing growth
factors, and
adding 1% FBS



Transplantation of Stem Cells

U W T V

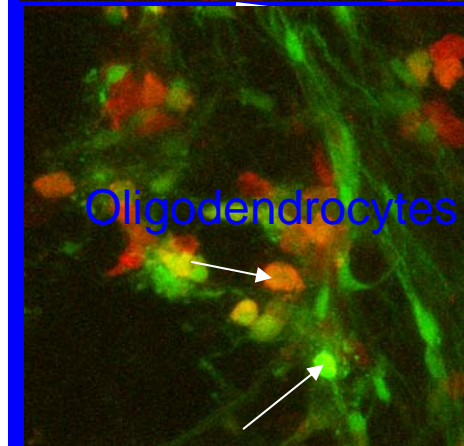
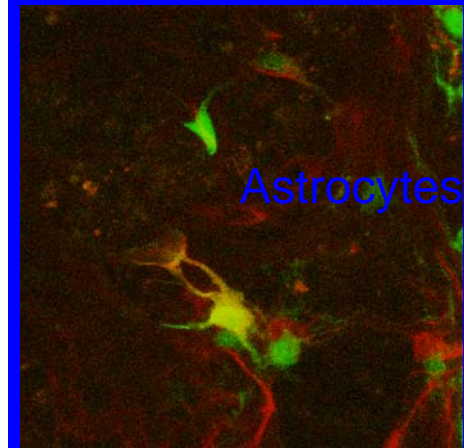
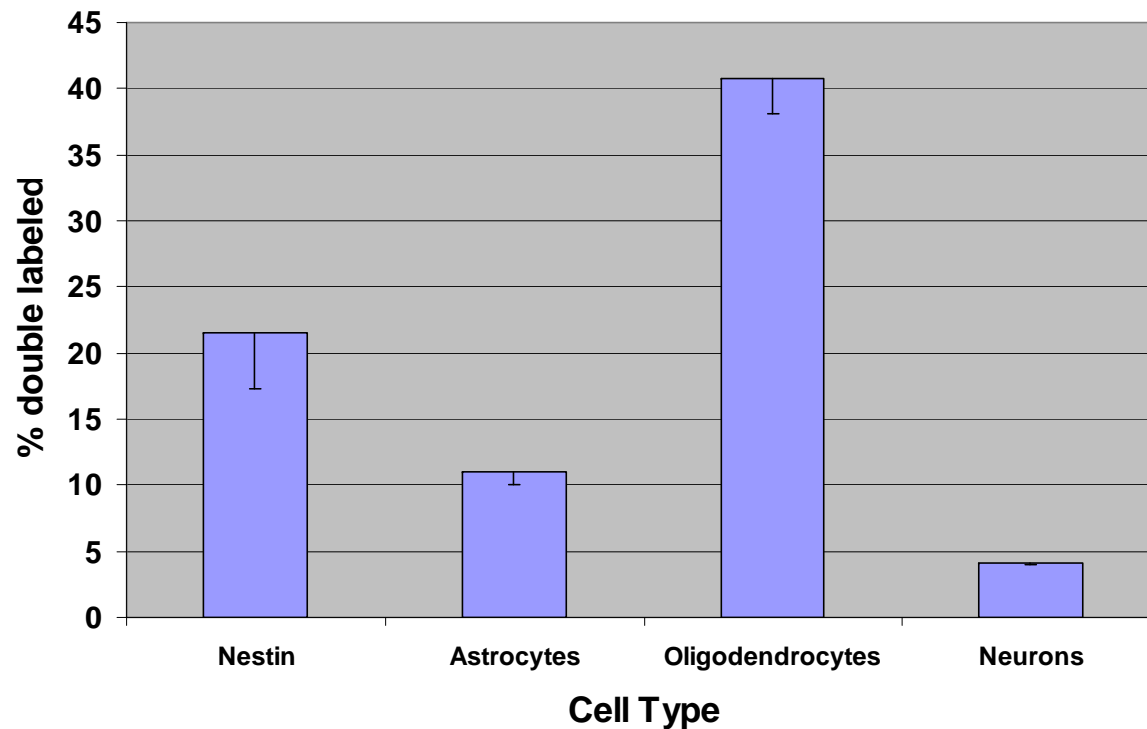


- Transplantation of eGFP adult rat spinal cord
- stem/precursor cells at 7 days, rostral and caudal to SCI site

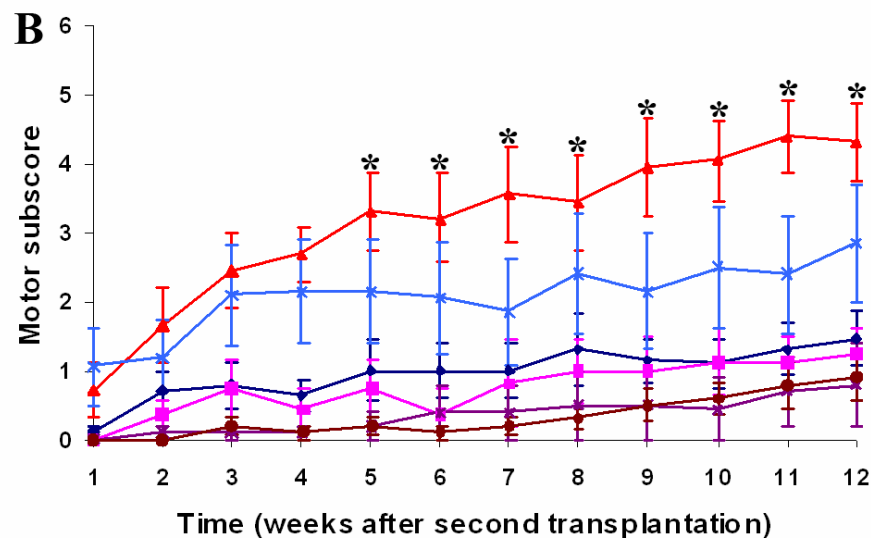
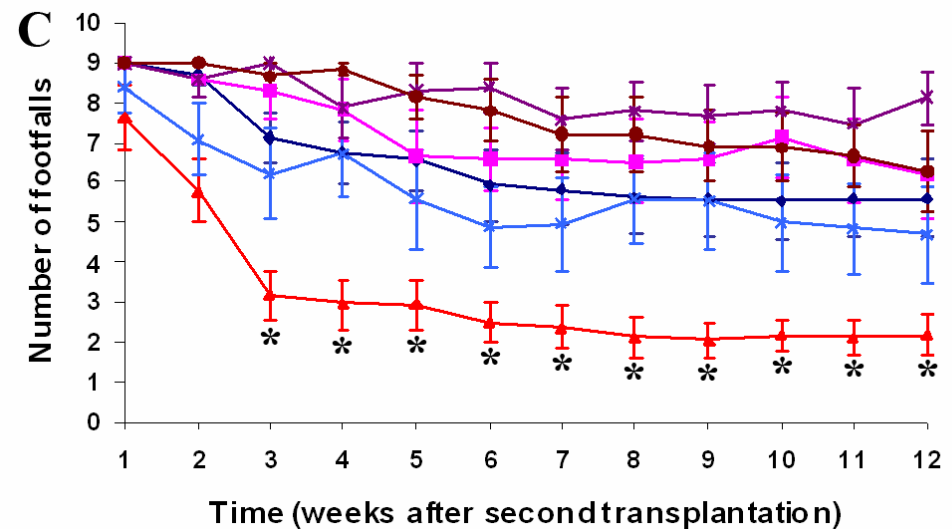
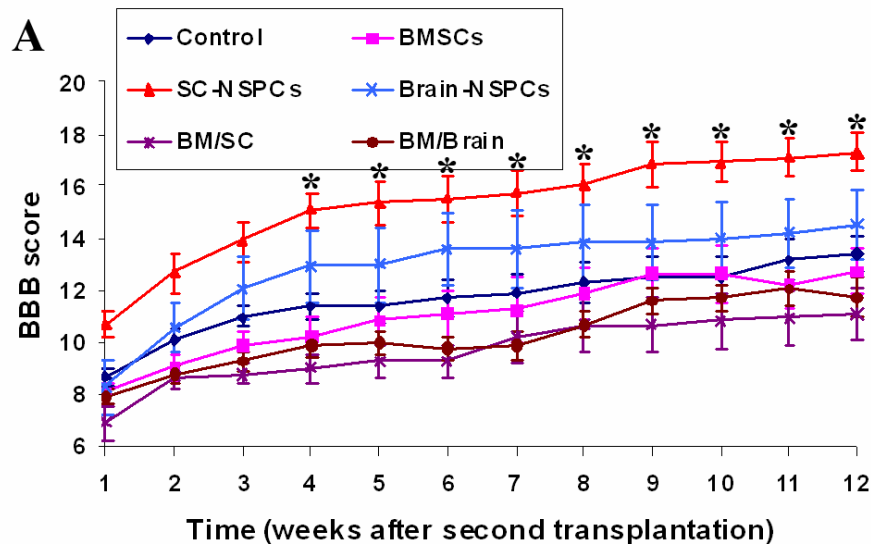


NSPCs Differentiated into Oligodendrocytes and Astrocytes

Cell Fate of SC-NSPCs



Transplantation of Neural Stem Cells Resulted in Functional Improvement (red line)



(A) Locomotor performance

(B) Motor subscore

(C) Ladderwalk

significant improvement in rats receiving Neural Stem Cells from the Spinal Cord

Conclusions of this Experiment— Cell Survival and Differentiation

- Spinal Cord derived neural stem cells had better survival and produced better recovery :
 - when rats received rostral and caudal injections
 - when cells were injected at 9 days after injury
- Neural stem cells differentiated mainly into oligodendrocytes
- Bone Marrow stem cells - better survival and reduced cavitation
 - filled the cavity with collagen and fibronectin
 - BUT did not express neural markers

Conclusions-Functional Analysis

- Spinal cord stem cells produced early functional improvement
- After 27g injury there was some preserved tissue and therefore, allowed neuroprotective effect to be demonstrated
- Bone Marrow stem cells caused a trend towards improved cell survival of the neural stem cells when transplanted as a scaffold, but did not produce functional improvement

Beneficial Mechanisms of Transplanted Stem/Progenitor Cells in CNS Injury

- **Replacement** of damaged neuronal or glial cells promote recovery through regeneration, such as axonal regeneration **YES!!!**
- **Remyelination** by transplanted cells or host cells
 - by oligodendrocytes, or Schwann cells **YES!!!!**
- **Neuroprotection** – increased host cell/axon survival, reduction of demyelination **YES!!!!**
- **Creation of a favorable environment** – proliferation of endogenous cells
 - creation of cellular bridges and guidance for regeneration
 - counteract glial scar or other inhibitors
 - expression of growth factors or cytokines for neuroprotection or axonal regeneration
- **Vascular effects** – restoration of blood flow by angiogenesis
 - repair of blood brain barrier, reduction of edema

Future Strategies to Enhance Effectiveness

A. Strategies to enhance stem cell survival:

1. Fibrin Scaffold to hold the stem cells
2. Pre-differentiation of stem cells in vitro
3. Guidance channels for the stem cells

Future Strategies

B. Strategies to enhance axonal regeneration:

1. Anti-Nogo-A
2. Chondroitinase-abc
3. Cyclic-AMP to enhance production of neurons from the stem cells

More Work is needed on the Stem Cell Strategies

- Endogenous versus transplanted
- Source and viability of cells
- More Pre-Clinical trials
- More Well-Organized Scientifically Sound Clinical Trials

Thank You