CIRM regenerative medicine consortium roundtable Neurological diseases and injury: First in human cell Therapy trials

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MILLER
SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

Panel

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James Guest, M.D., Ph.D. University of Miami
Stephen Huhn, M.D. Stem Cells Inc.
Ann Tsukamoto, Ph.D. Stem Cells Inc.

Cell therapy is of potential interest for several neurological diseases

Stroke
Brain trauma
Progressive Degenerative diseases such as ALS, Parkinson's disease
Spinal cord injury
Multiple sclerosis

Clinical Targets for cell therapy after spinal cord injury

1) Restoration of function
2) Reduction of complications

Spinal cord injury

* Frequently young men
* Mainly cervical injuries
* Degree of life-long dependence/independence is linked to residual function
* System is redundant and recovery of modest numbers of axons can provide substantial increased function

Restoration of Function

* Breathing
* Hand and arm function
* Bowel, bladder, and sexual
* Sensation
* Walking ability

Reduction of complications

* Renal and urological dysfunction
* Neuropathic pain
* Unwanted spasticity
* Skin breakdown
* Severe bone mass loss

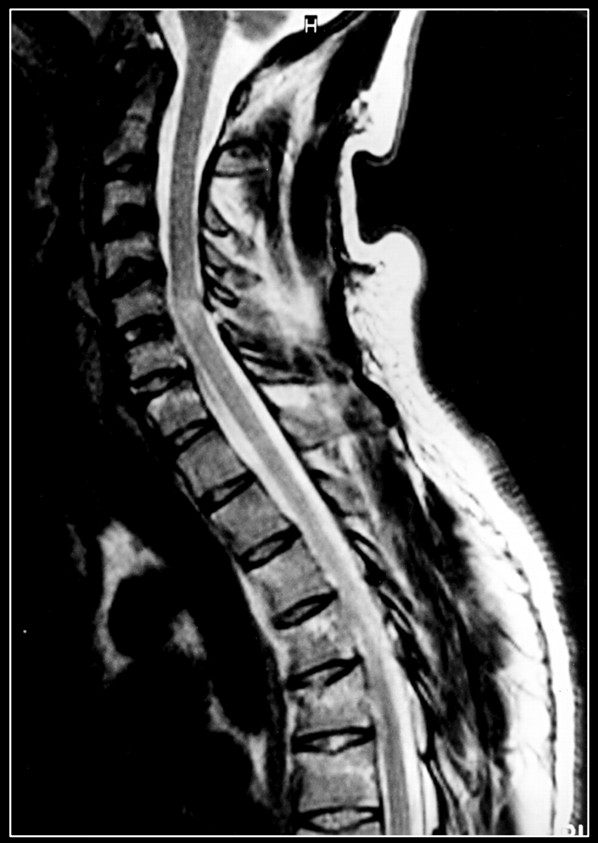
Key points

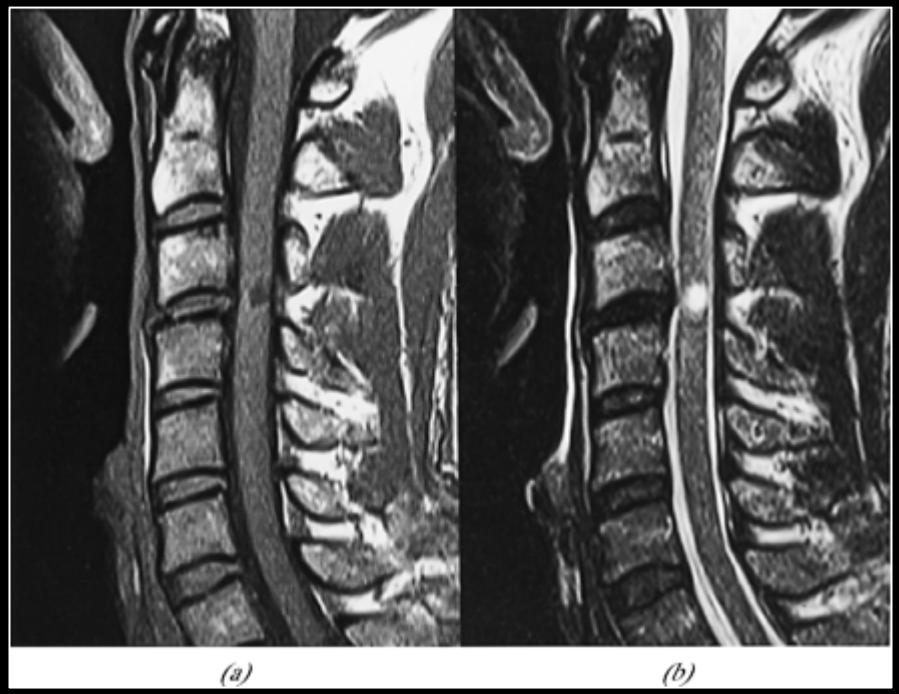
* Patient population (acute trauma in spinal cord (or TBI) versus subacute versus chronic disease); stage of disease for neurodegenerative disorders (e.g., early vs. advanced ALS, Parkinson's, Alzheimer's, Huntington's)
* Anatomic location
* Cell fate
* If it doesn't work, how do you figure out why?
* End points – e.g., is it walking or bladder/control of bodily functions for SCI
* Immune response and immunosuppression
* Dose & delivery
* Mechanism of action

Cell therapy trials for SCI

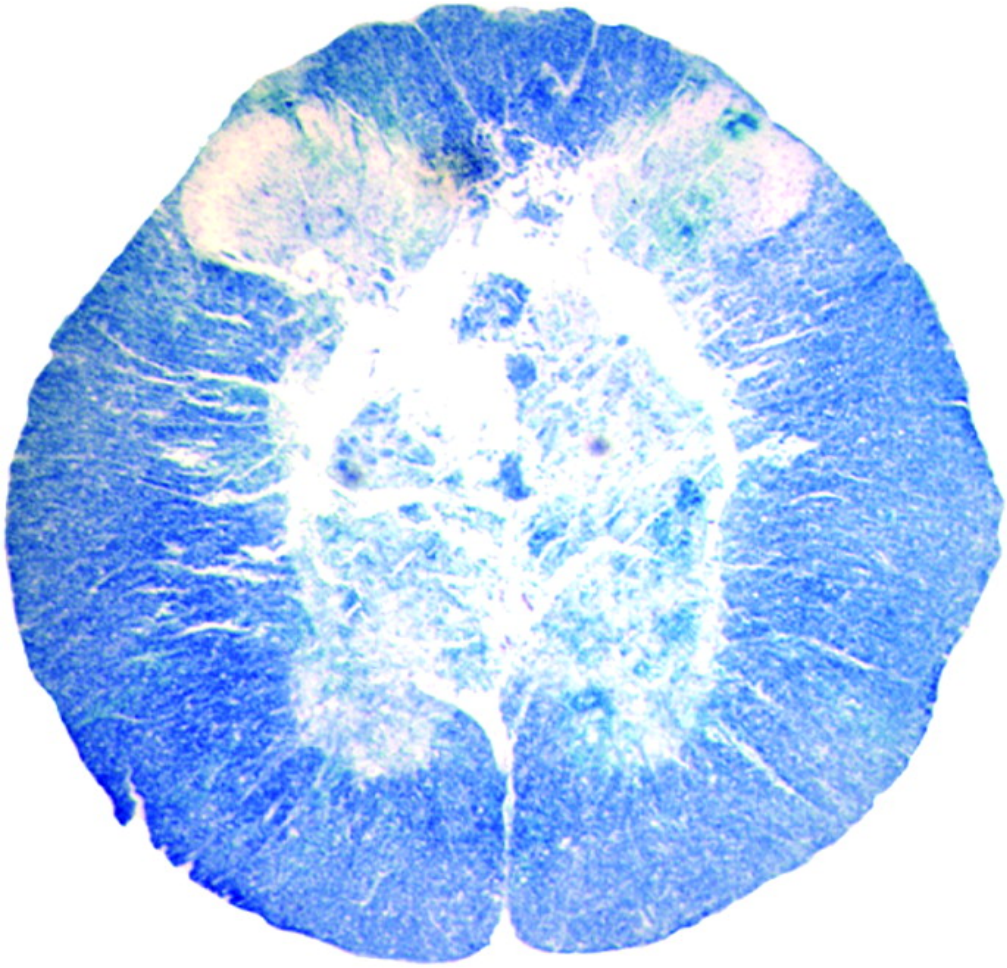
* Proneuron- “activated macrophages” Phase 1/2
* Geron- partialy differentiated ES-oligodendroglial precursors Phase 1
* Stem Cells Inc.- Neural stem cells- Early Phase
* Neuralstem-ALS- Neural stem cells- Early Phase
* Numerous foreign studies

Most spinal cord injuries evolve to form cavitation

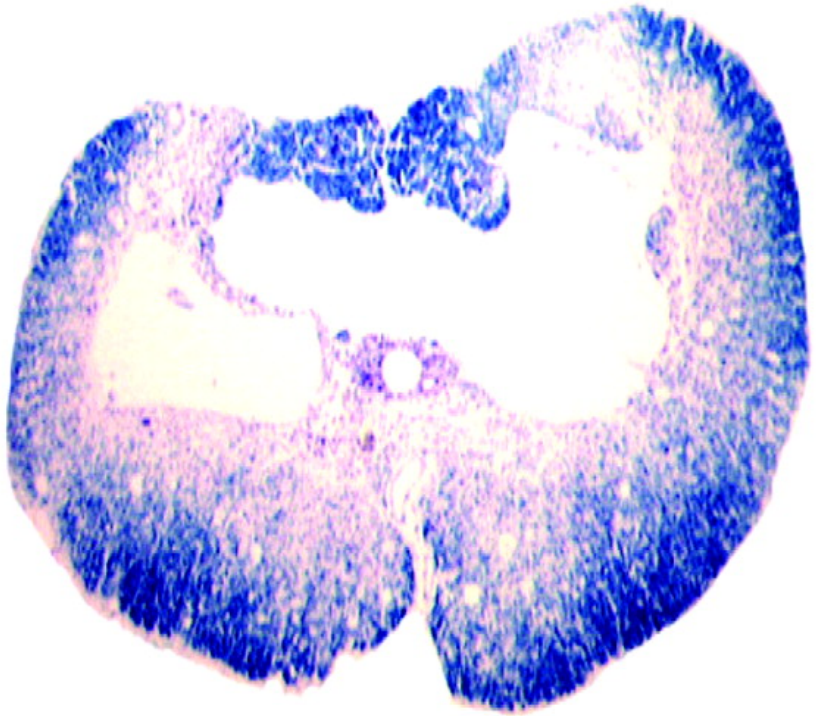




Tissue Loss after Spinal Cord Injury

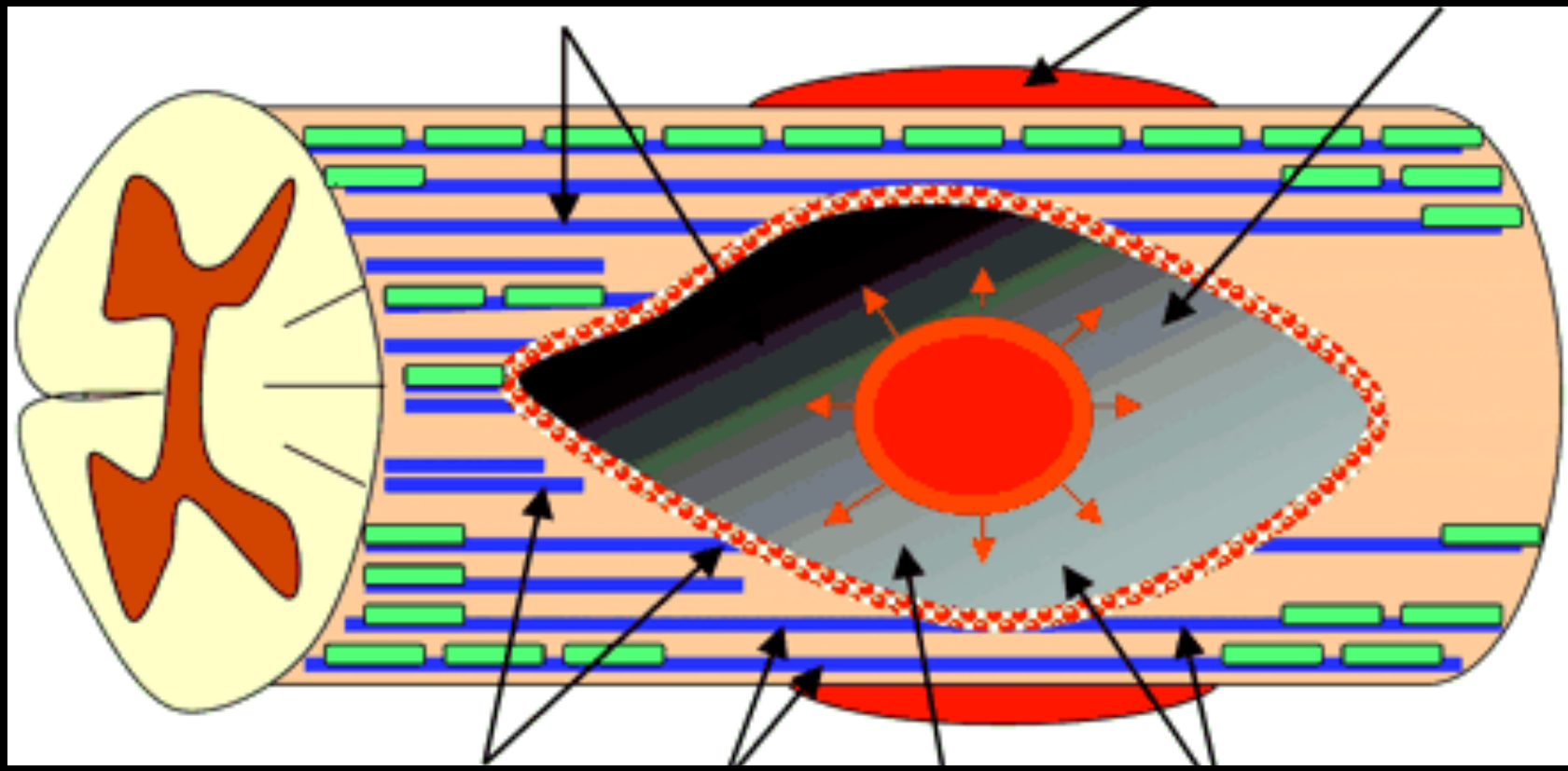


1 Hour Post Injury

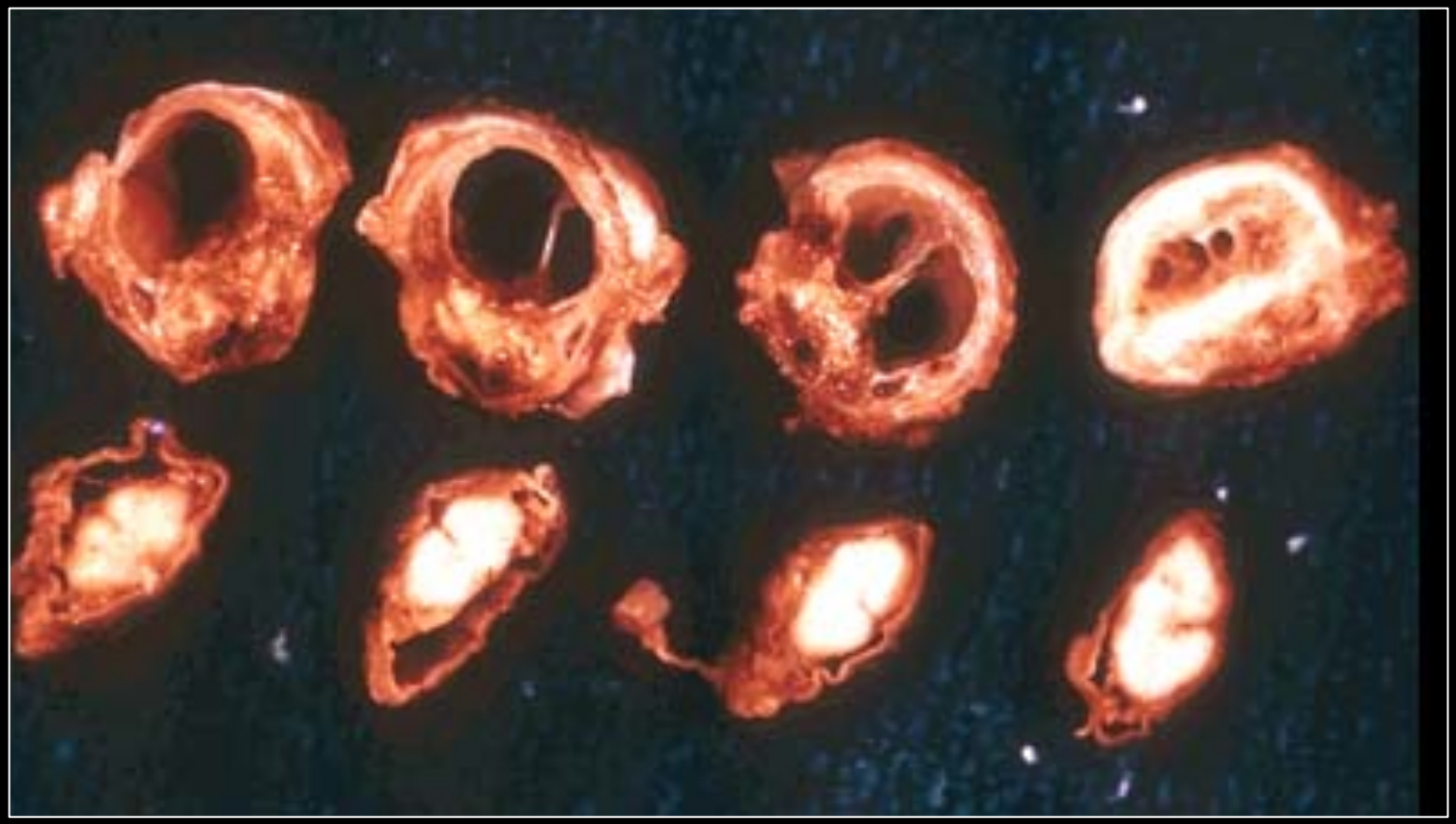


60 Days Post Injury

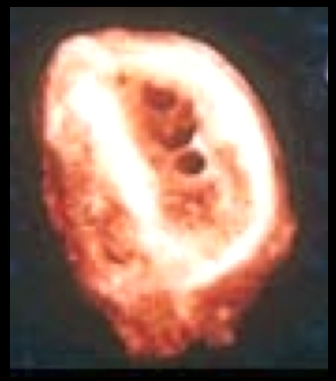
Cavity formation after SCI

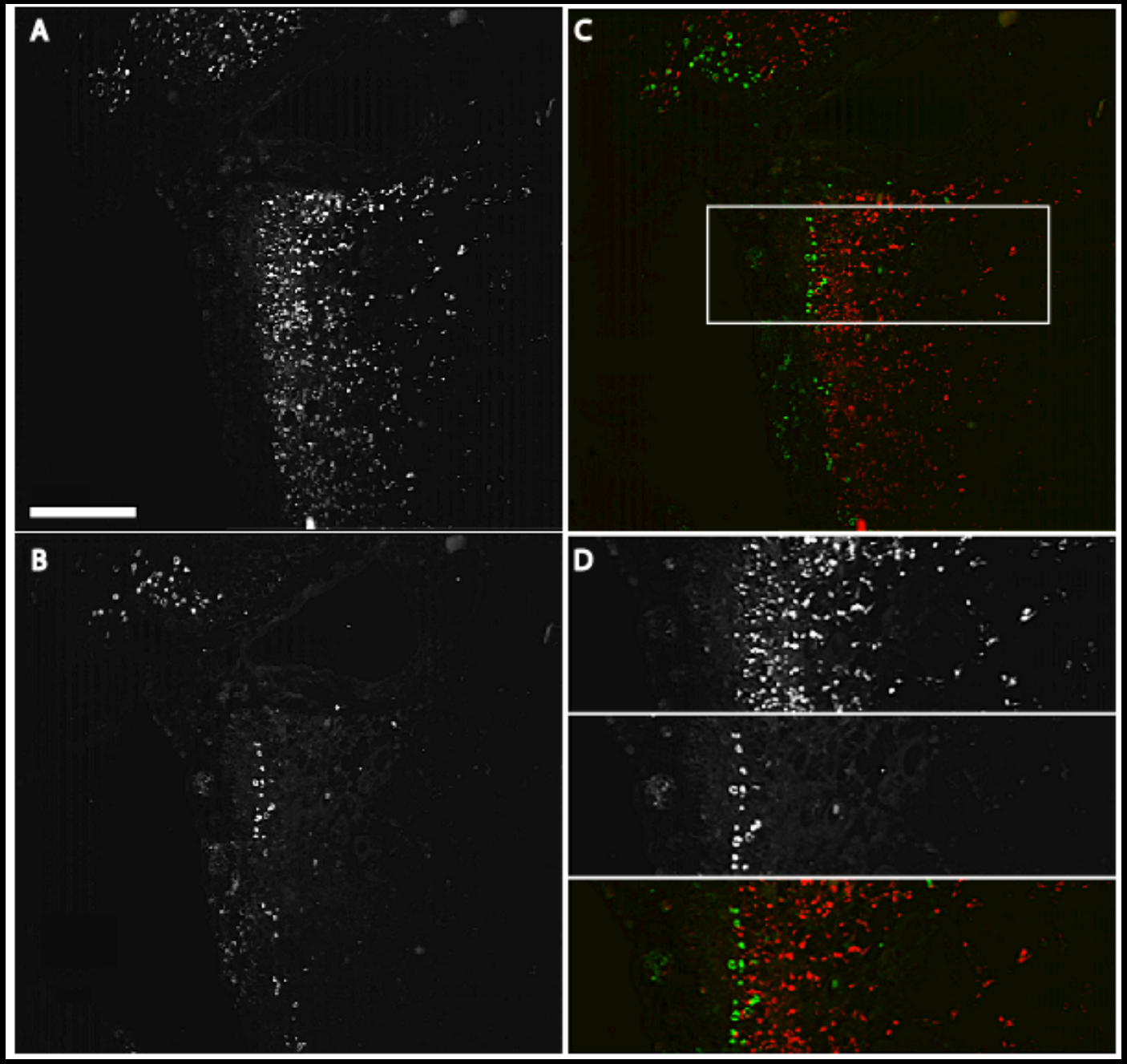


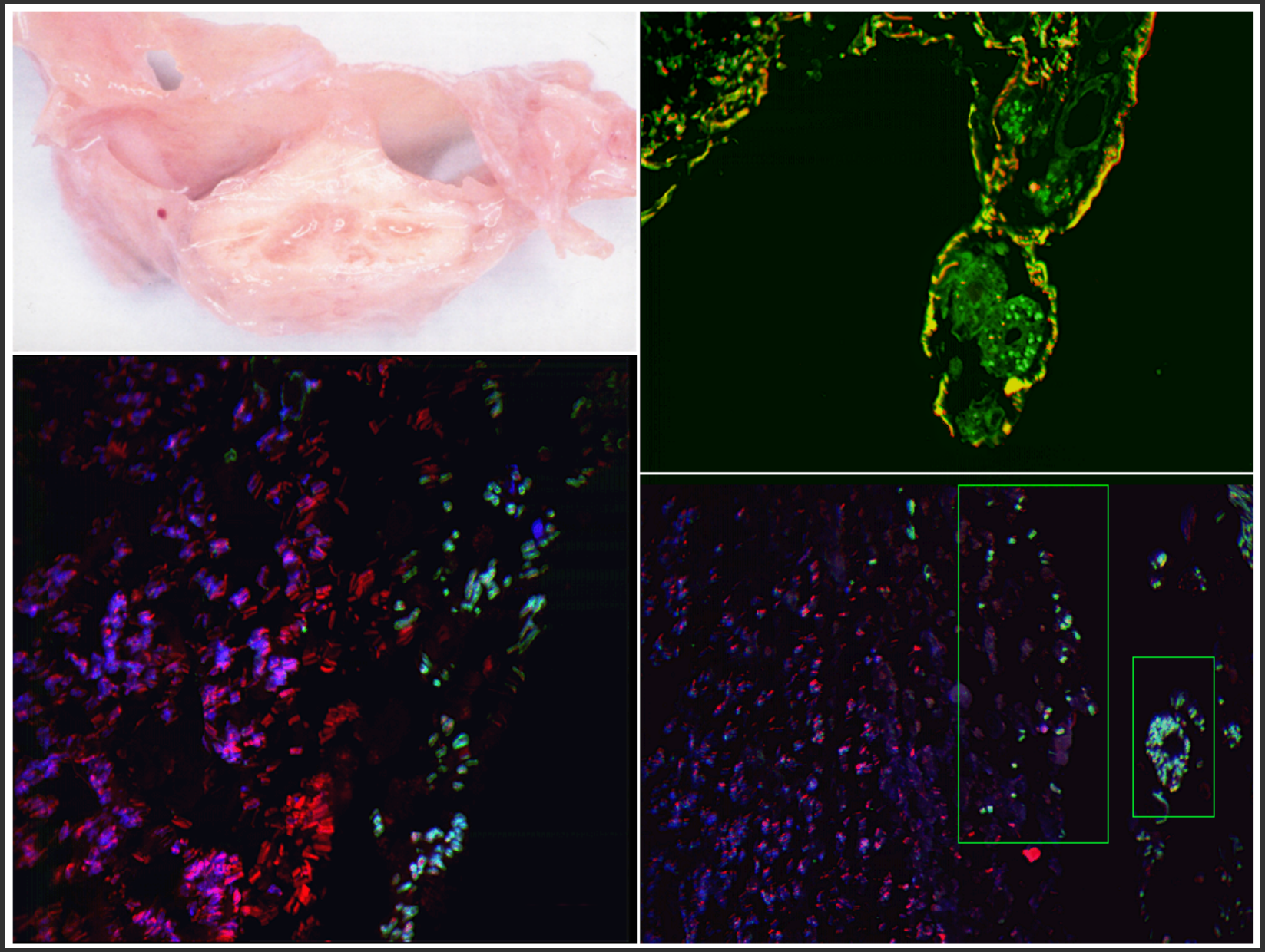
Chronic spinal cord cavitation



Axonal demyelination
After chronic SCI
Case 28, one
year post SCI
C2/3 level.

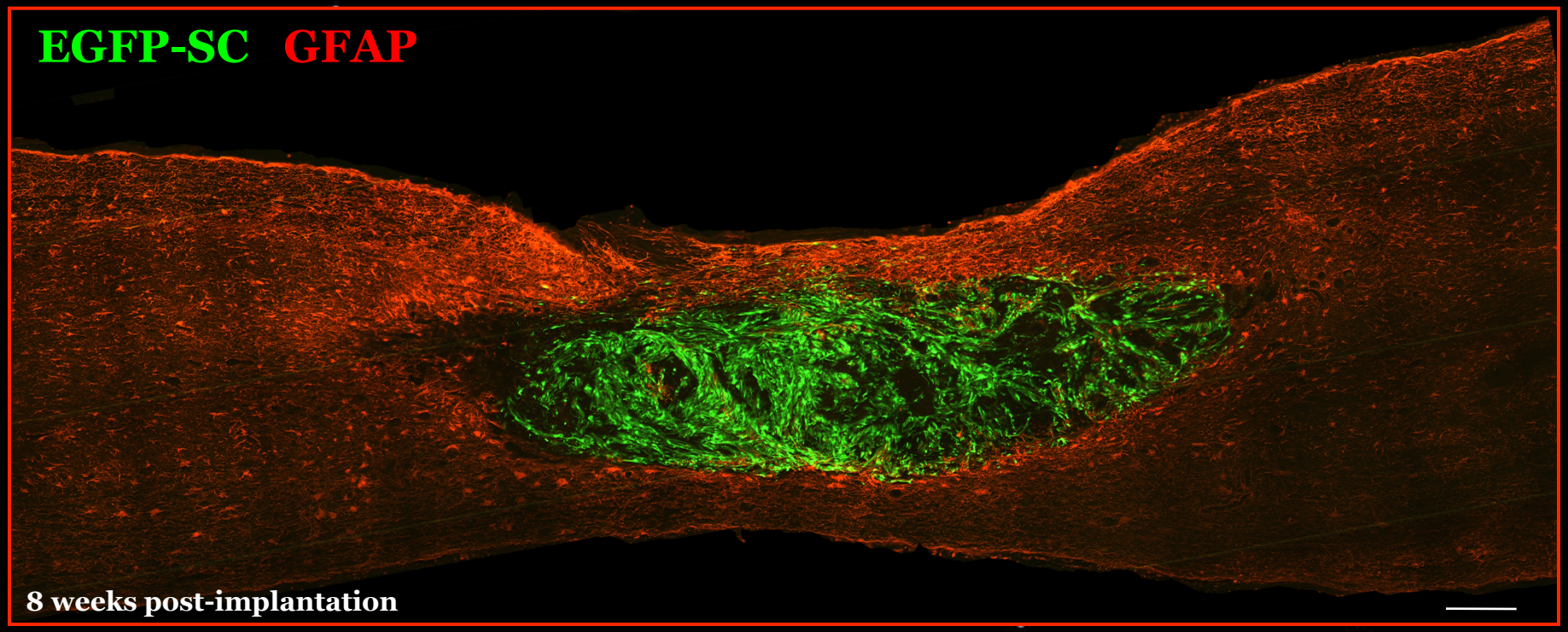






Guest, J. D., Hiester, E. D., and Bunge, R. P., 2005. Demyelination and Schwann cell responses adjacent to injury epicenter cavities following chronic human spinal cord injury. Exp Neurol 192**, 384-393.**

IDENTIFICATION OF IMPLANTED SCs



EGFP-labeled SCs, implanted at 1 week post-injury, form a substantive bridge across the injured cord

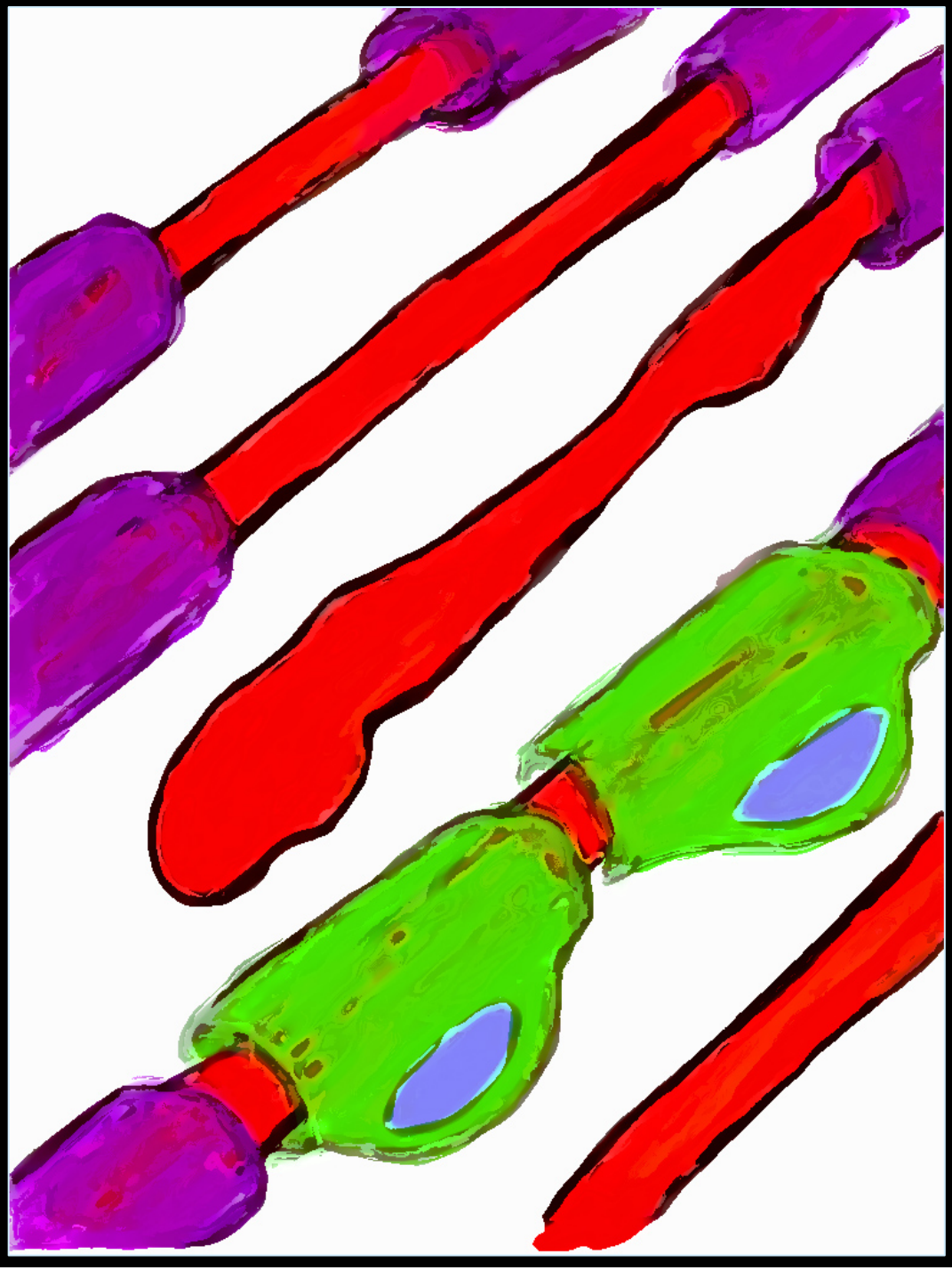
Courtesy of Dr. Damian Pearse

Advantages of Schwann cells/ SCI

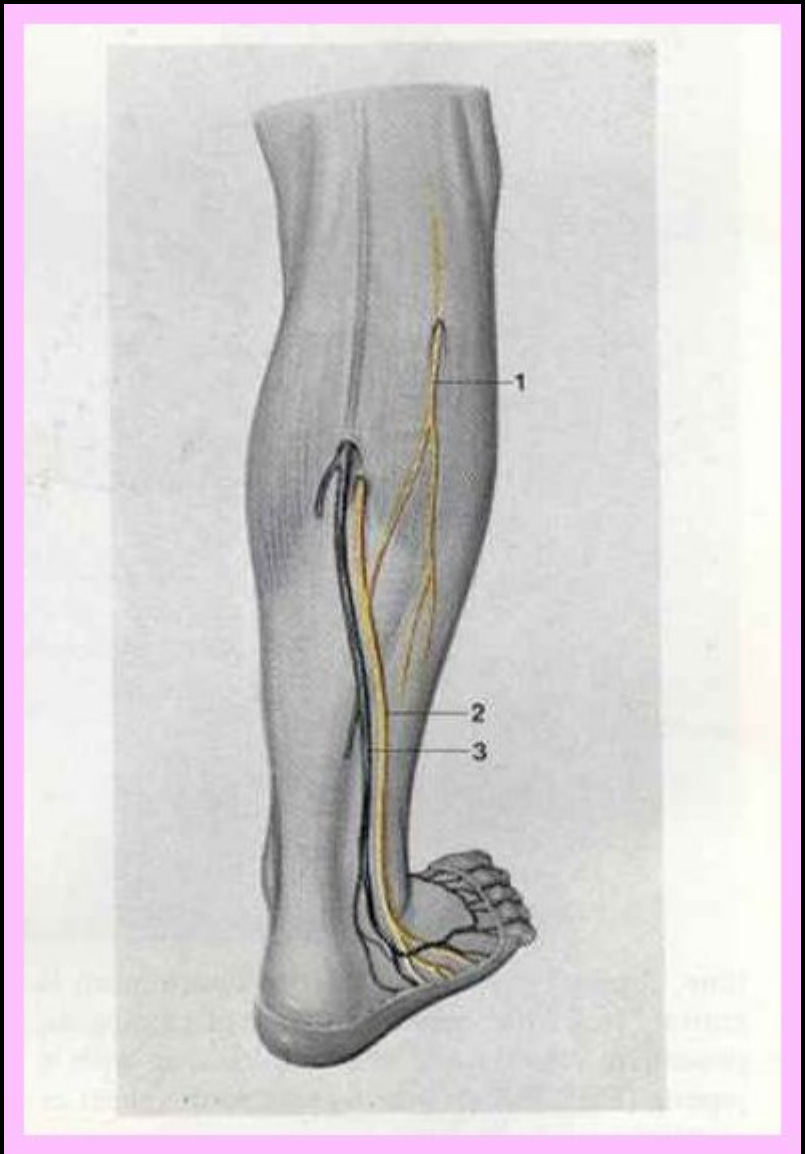
1. Promote regeneration of axons in the PNS
2. Part of the normal endogenous injury response in human SCI
3. SC are neuroprotective, secrete trophic factors
4. Produce ECM components such as laminin
5. Must myelinate or ensheath axons in CNS to survive
6. Primary nerve tissue is accessible via minor biopsy
7. Robust culture methods
8. Can be expanded to large numbers with 3 passages
9. Can be genetically engineered
10. Can be transplanted autologously obviating the need for immune suppression.

Possible Disadvantages of Schwann cells/SCI

* Do not truly integrate in CNS
* Do not directly support CST axon growth.
* Post-transplant survival is modest- <20%
* May promote excessive sensory plasticity
* Minimal migration
* Post-transplant cell death is associated with additional inflammation.

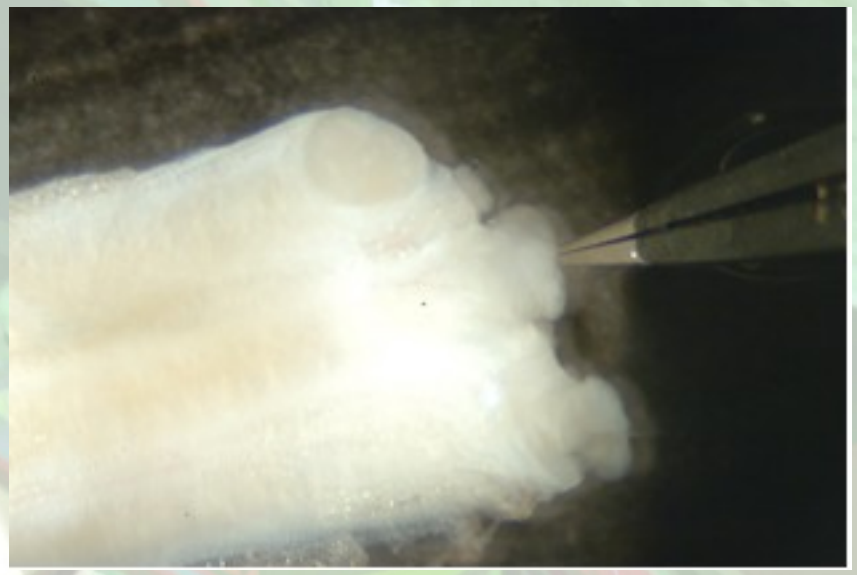


Sural nerve




0
1
2
3
4
5
6

Dissection of Fascicles from Nerve



Courtesy of Dr. Pat Wood

Dissociate nerve fasicles using collagenase and dispase , 16 -18 h

Sterility & purity check

Wash out dissociation enzymes

Plate 1 million cells per plate on laminin coated tissue culture dishes in Schwann cell growth medium with antibiotics

Culture for about 1 wk-10 days . Resuspend and replate at 500 ,000 cells per dish on laminin coated tissue culture dishes in Schwann cell growth medium without antibiotics

Sterility & purity check

Cryo-preserve excess cells

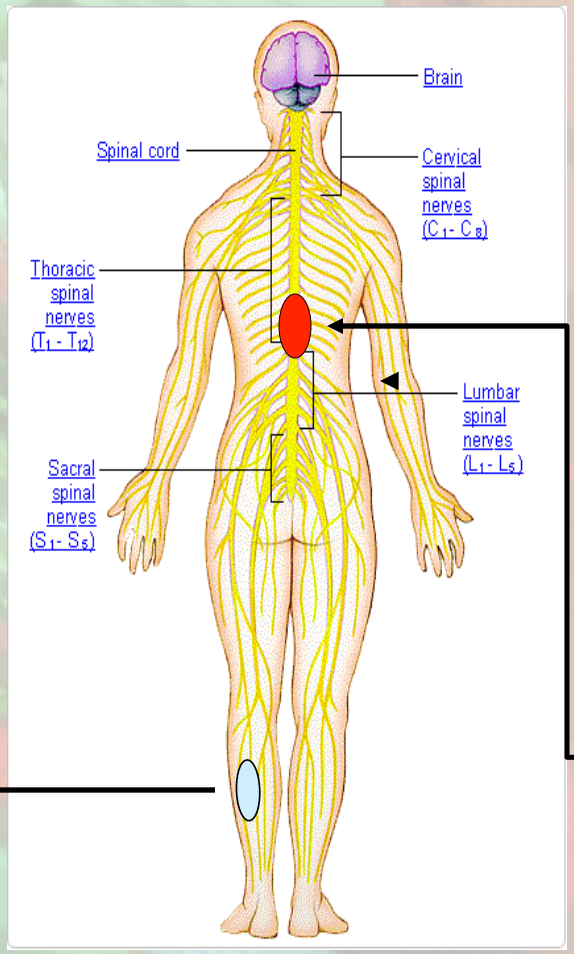
Culture nerve fasicles in Schwann cell growth medium with antibiotics , 1 week

Sterility check

Manual separation of nerve fasicles

Sterility check

Sural nerve biopsy obtained from patient



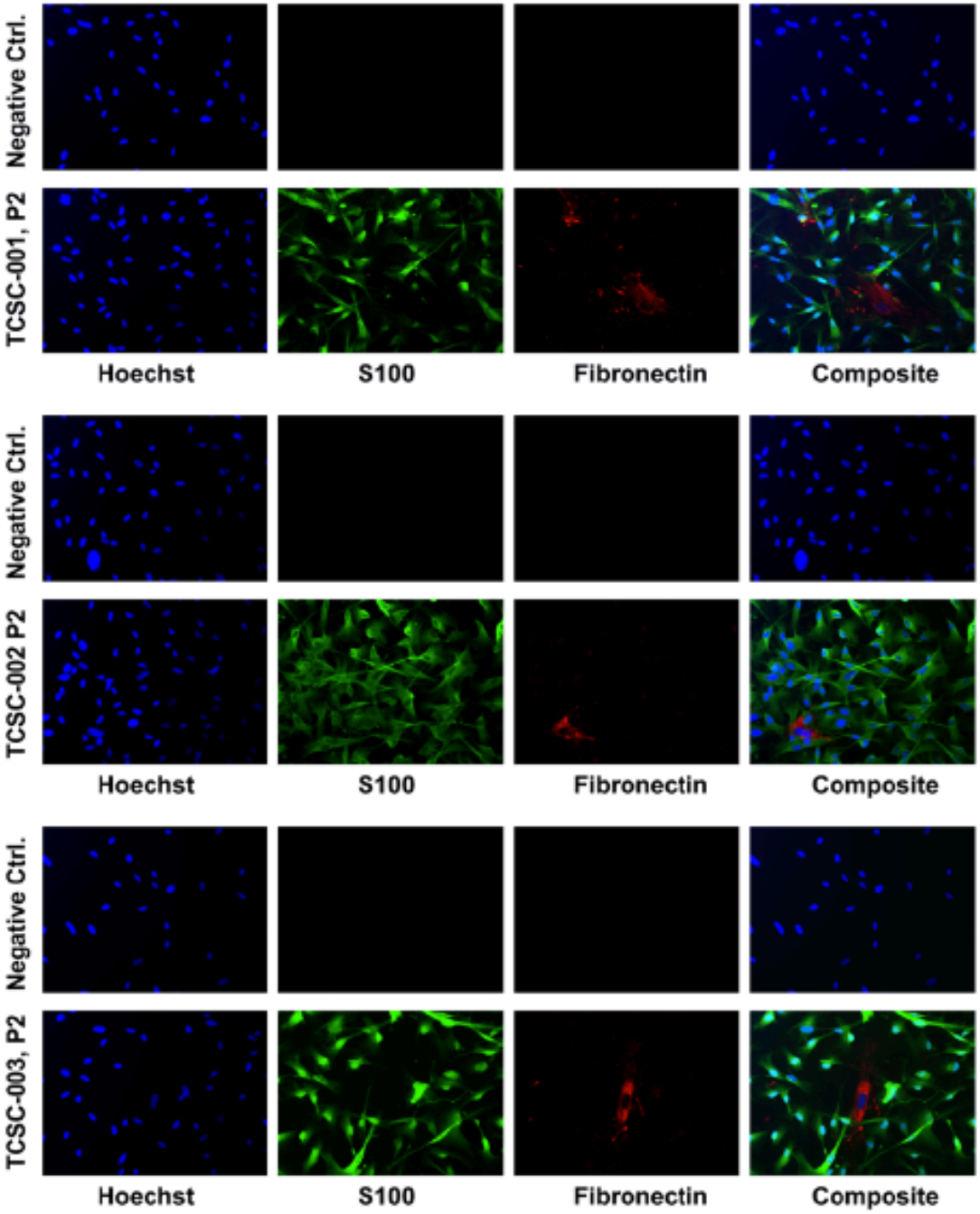
Culture for about 1 wk-10 days . Resuspend cells and prepare for transplantation

Sterility, purity & endotoxin check

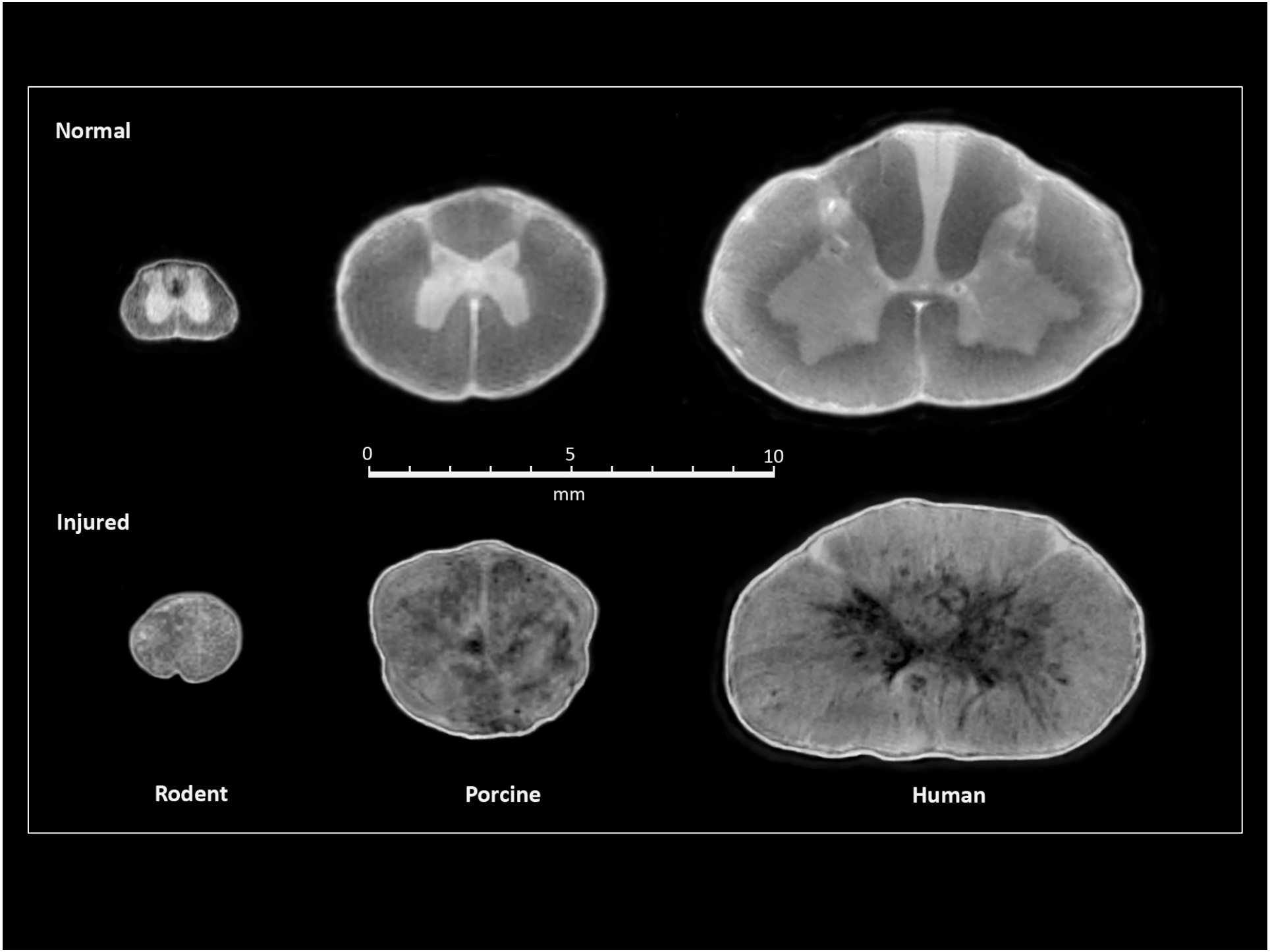
Inject into the spinal cord injury site of the same patient (autologous transplantation)

Courtesy of Dr. Pat Wood

Figure 3: An area of a well from each donor that was stained for S100 and fibronectin was selected that showed positive staining for both Schwann cells and fibroblasts.



Utility of large animal studies


Normal
0
5
10
mm
Injured
Rodent
Porcine
Human


7.5 cm
57 cm

Value of large animals for translation

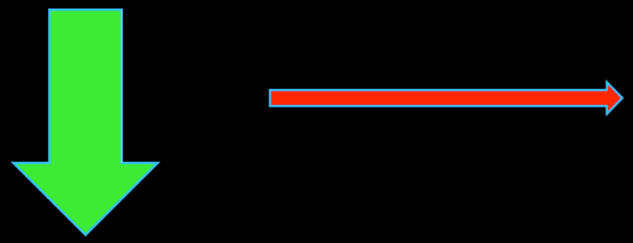
- Typical cavitation, scarring to emulate clinical conditions for therapeutic application
- Can emulate actual proposed clinical procedures
- Human-like CSF and dura
- Biodistribution much more meaningful than rodents
- Assists scale-up for cell and biomolecules near- human doses may be tested

RODENT STUDIES
Large group size with ability to show an effect **statistically**.
10 years

PRIMATE STUDIES
Allow full **autologous** protocol to be tested.
Are suitable for **chronic** injury studies. Human-like outcomes can be measured
5 years

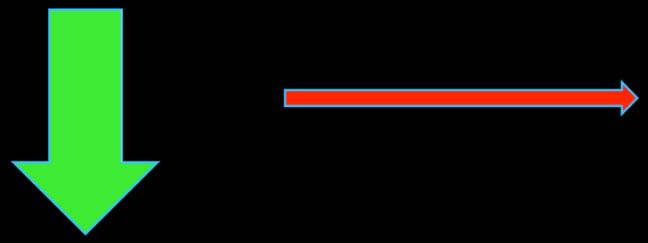
PORCINE STUDIES
Human like anatomic Spinal column and cord
Size allow **delivery methods** to be emulated exactly as per human. MRI imaging allows detection of delivery related injury.
2 years

Porcine experiment



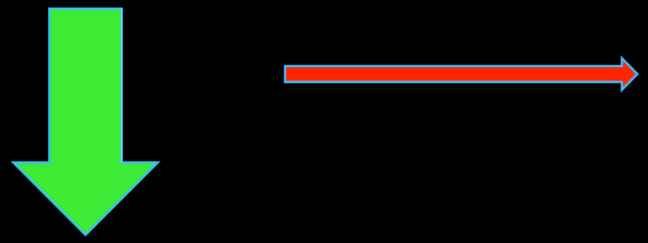
Pre-injury baseline walking scale,
Pre-contusion SSEP,MEP.

T9 contusion injury and nerve harvest



Post-injury SSEP, MEP, surface cord
Stimulation and recording, MRI,
walking scale.

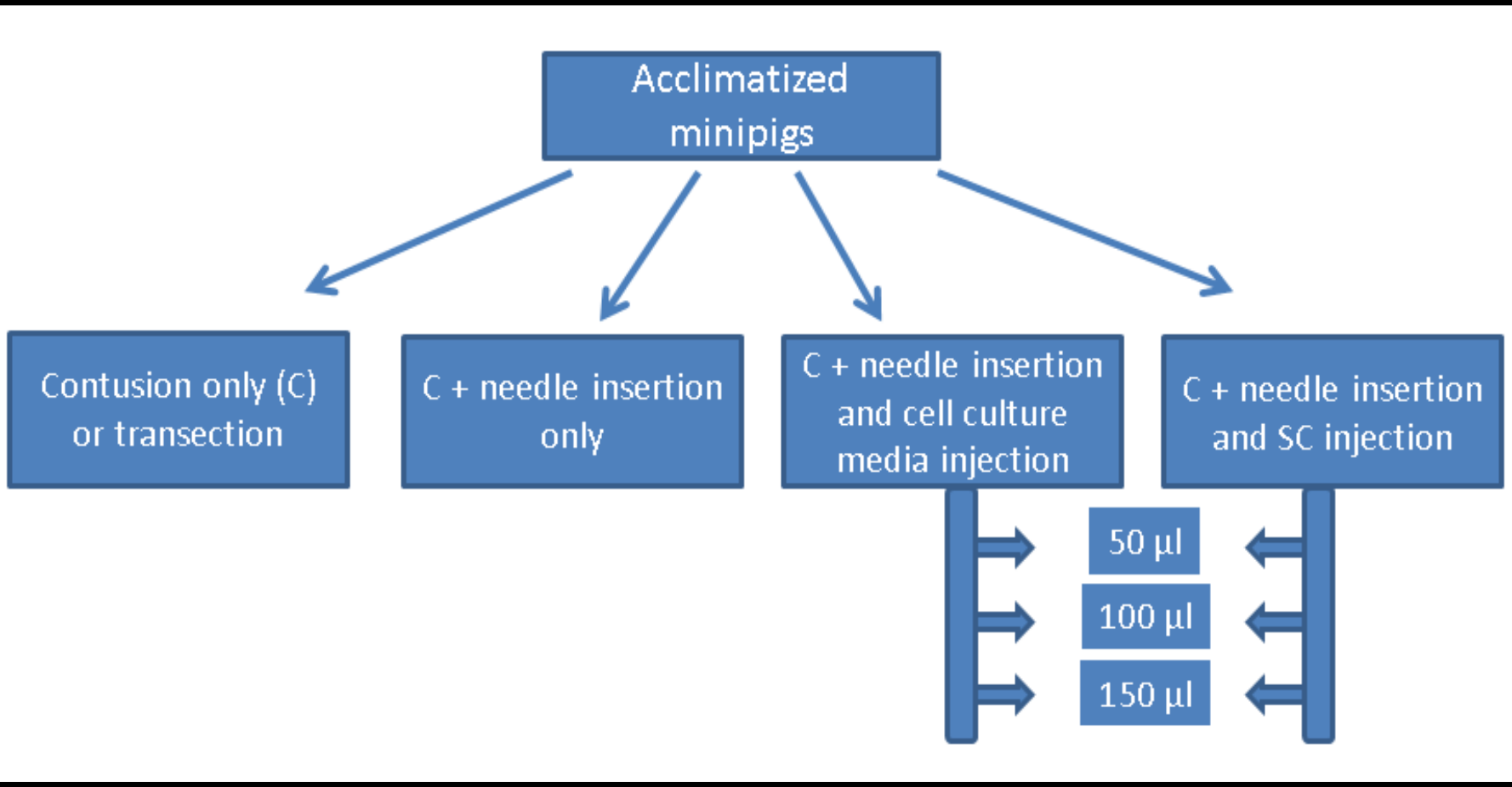
Autologous Schwann Cell injection procedure – 21-24d

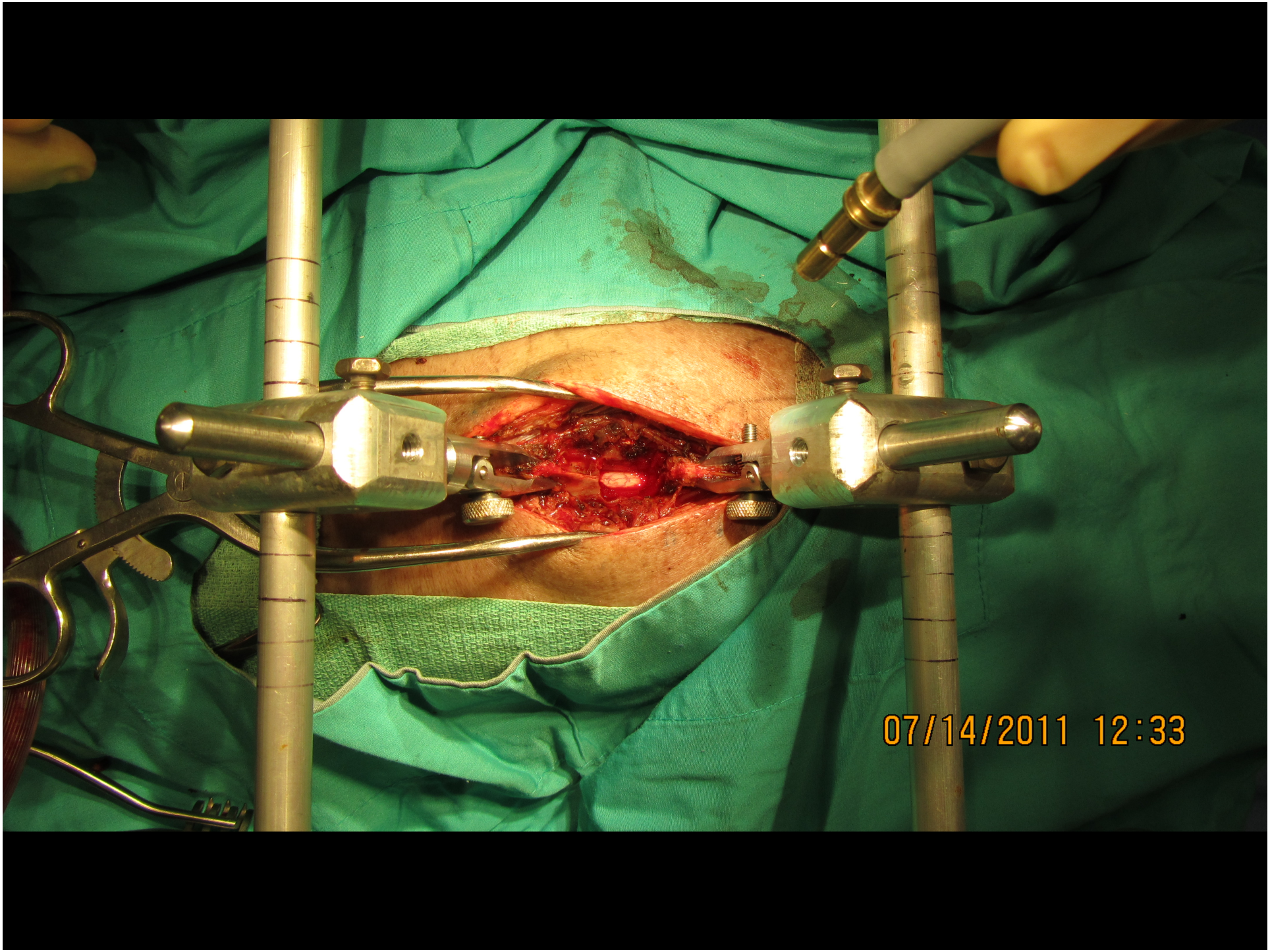


Peri- transplant SSEP, MEP,
Peri-lesional cord stimulation+
Recording. Post-transplant MRI,
walking scale.

Perfusion

3d-24 weeks. Histology


Acclimatized minipigs
Contusion only (C) or transection
C + needle insertion only
C + needle insertion and cell culture media injection
C + needle insertion and SC injection
50 µl
100 µl
150 µl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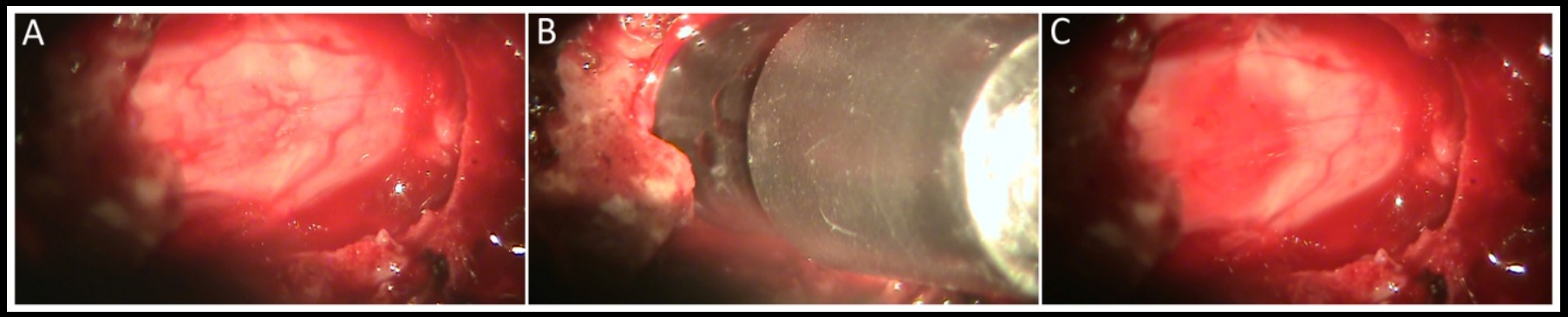
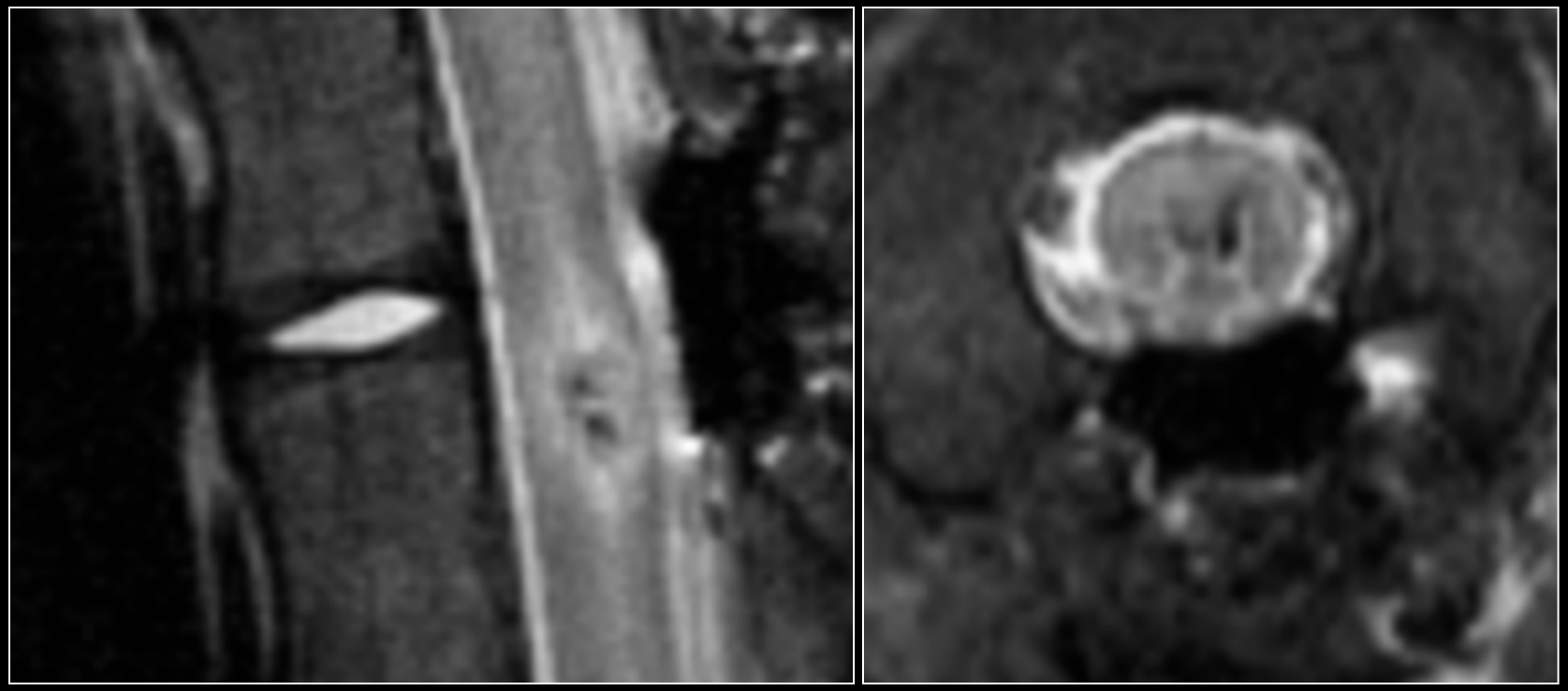
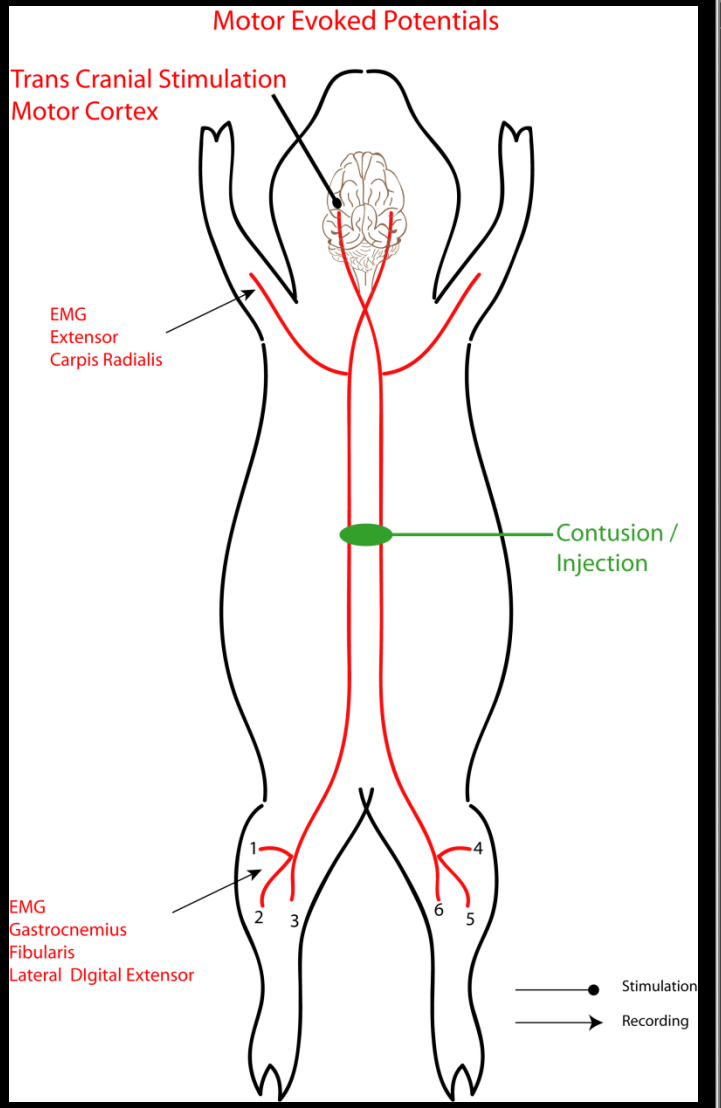
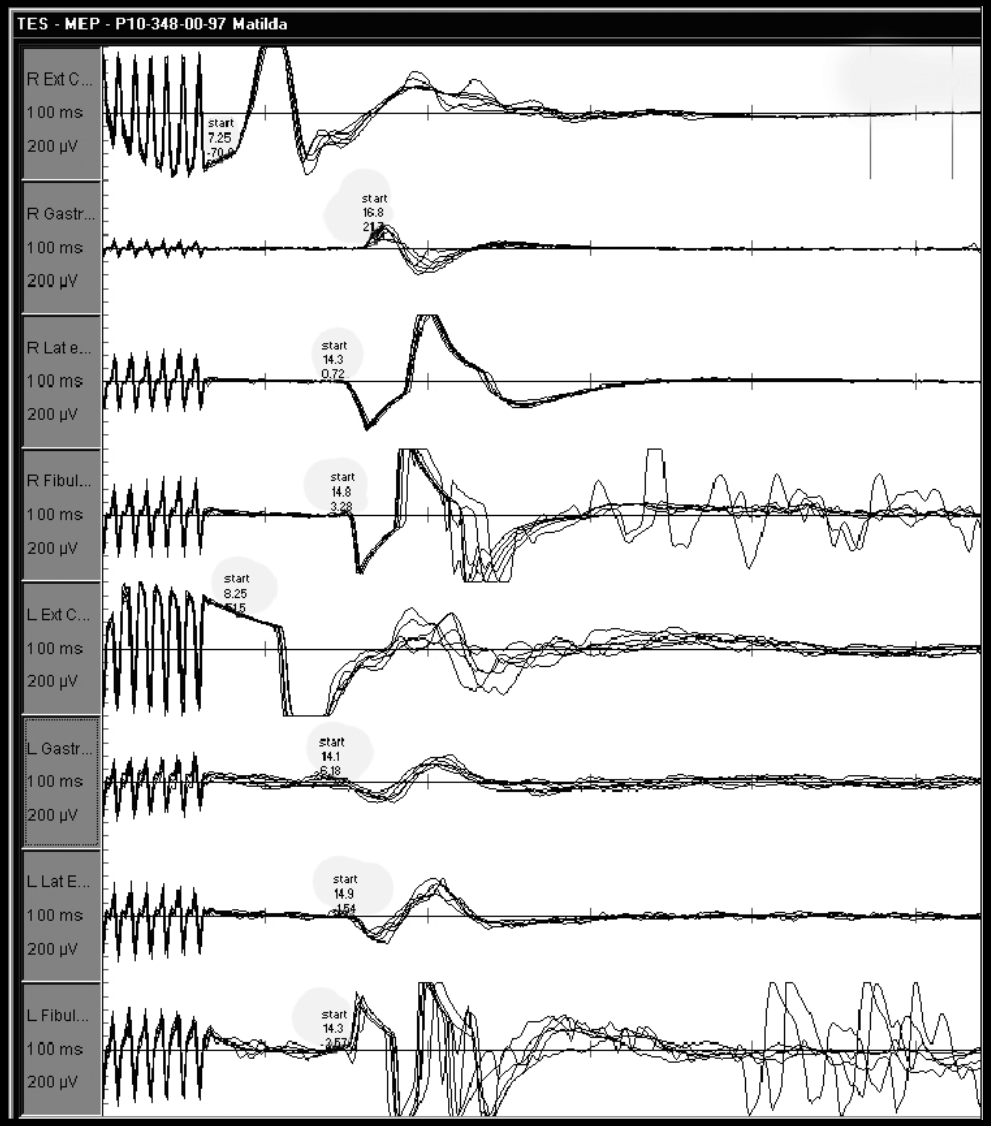

A
B
C

T9 Porcine injury, one hour post-contusion



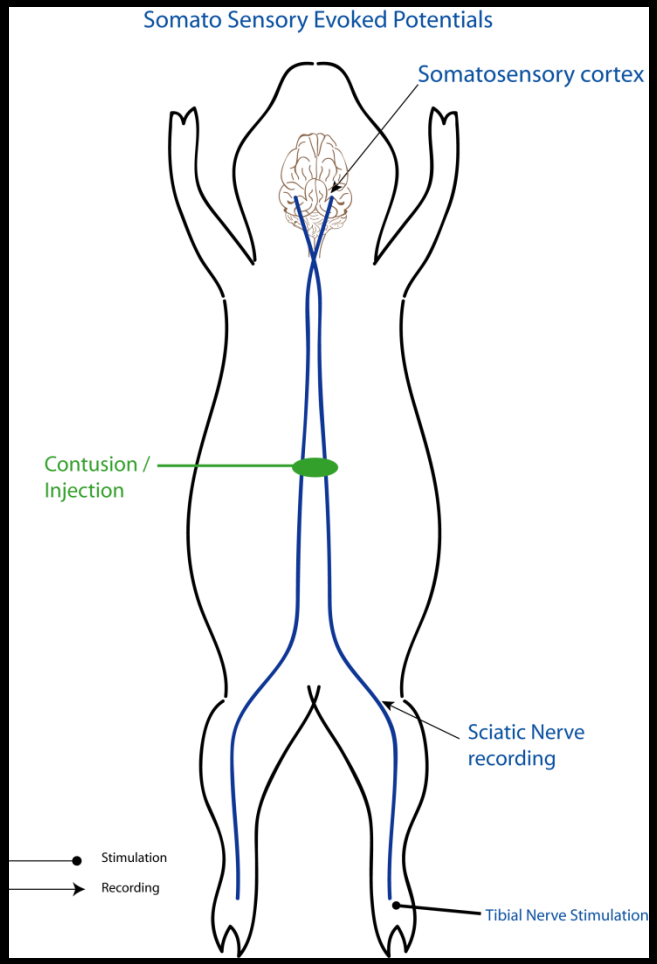
Baseline MEPs Pig 31

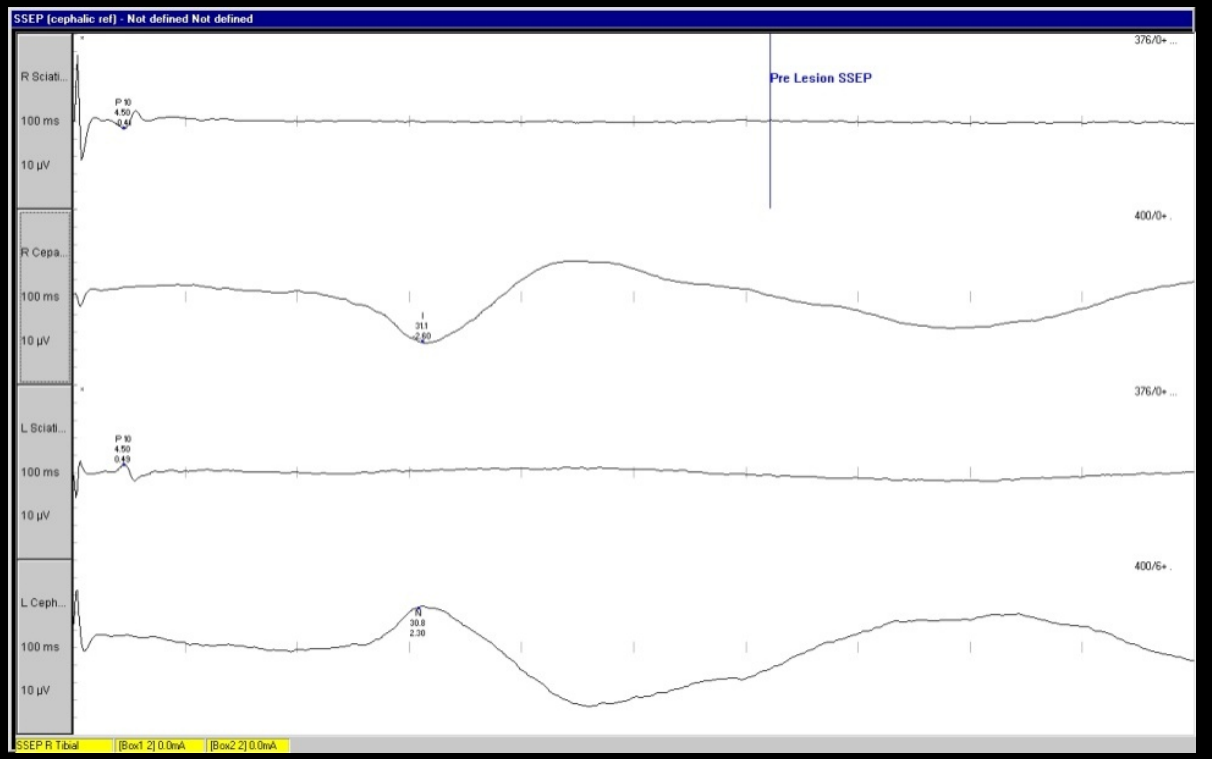




TRANSCRANIAL STIMULUS 400V (6 repetitions)

Baseline SSEPs Pig 22

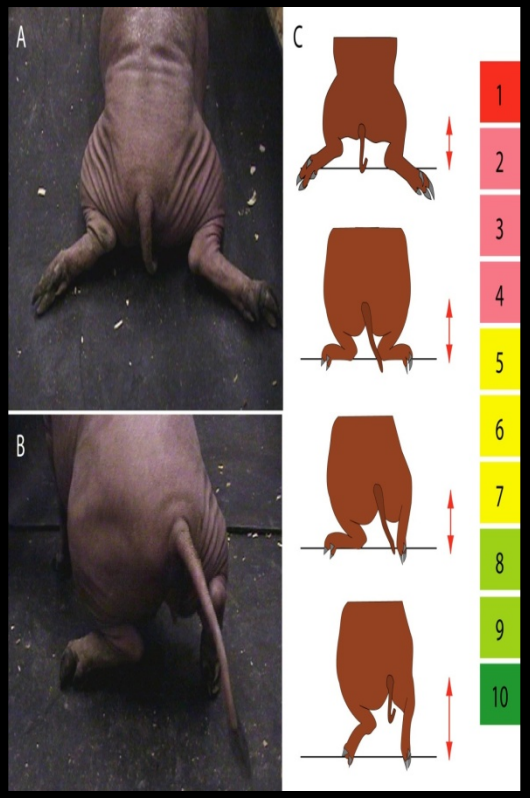


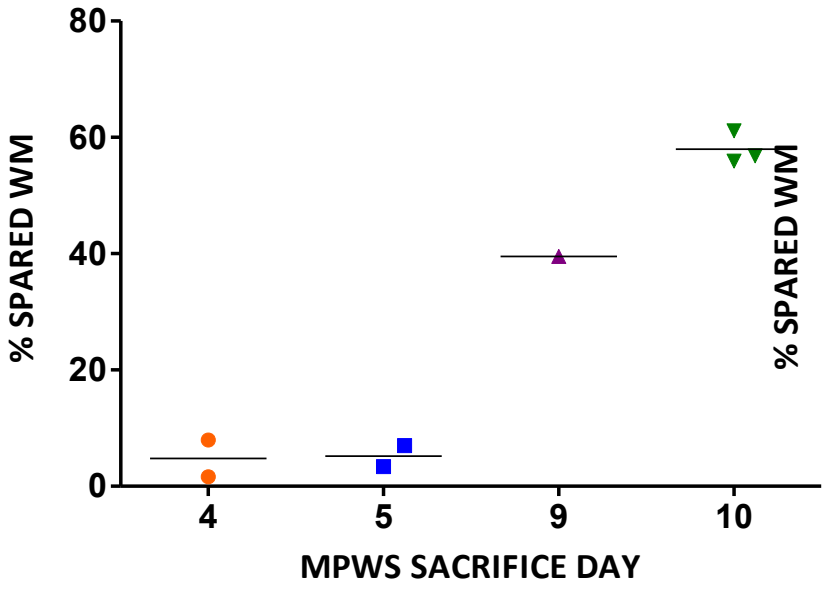


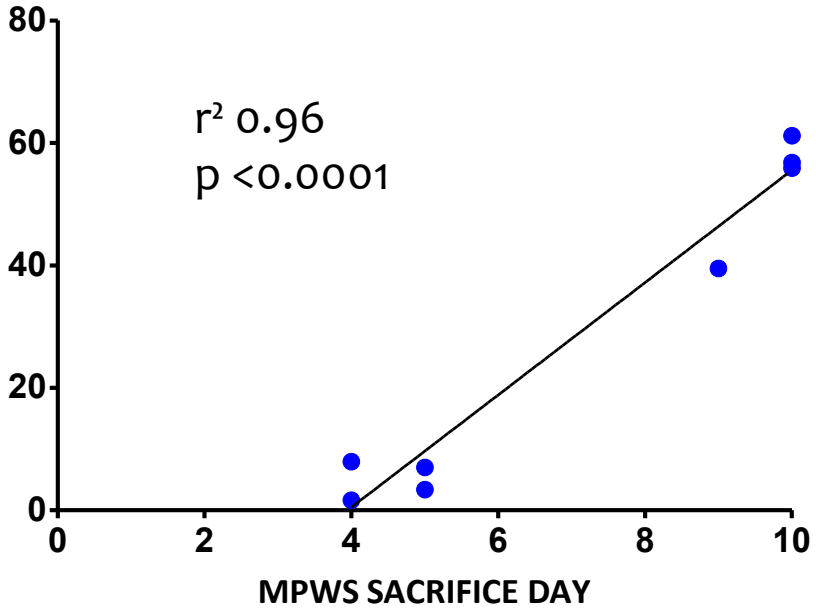
Tibial nerve Stimulus 50mA (400 repetitions)

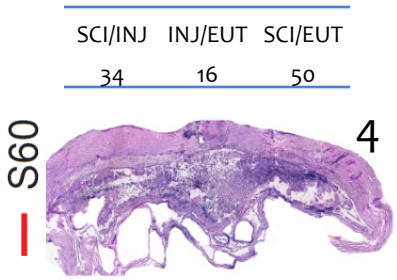
Miami Porcine Walking Scale

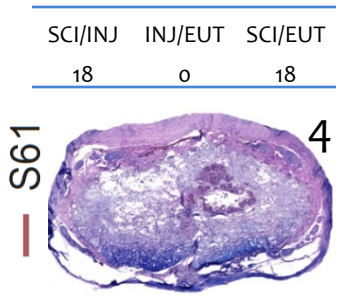
Score	Description
1	No Movement
2	Movement of hips only
3	Movement of hips and knees
4	Alternating rythmic flexion/extension movement of all joints, no weight support
5	Occasional clearance of buttock cheek from floor surface
6	Occasional sequences of dorsal steps
7	Stands with full sustained weight-bearing
8	Inconsistent sequences of consecutive plantar steps
9	Walks consistently with imperfect coordination and balance
10	No apparent deficit

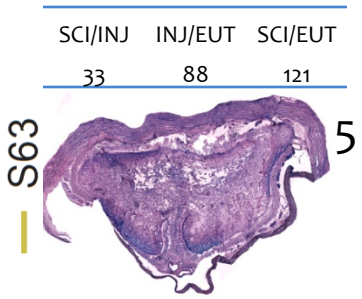



% SPARED WM
80
60
40
20
0
4
5
9
10
MPWS SACRIFICE DAY


r² 0.96
p <0.0001
% SPARED WM
80
60
40
20
0
0
2
4
6
8
10
MPWS SACRIFICE DAY


SCI/INJ INJ/EUT SCI/EUT
34 16 50
S60
4


SCI/INJ INJ/EUT SCI/EUT
18 0 18
S61
4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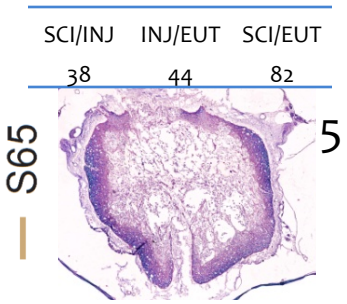
S63
5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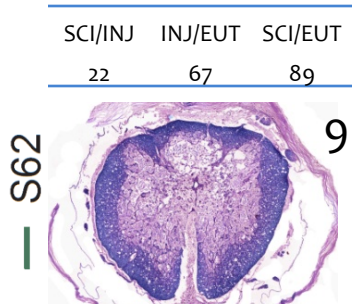
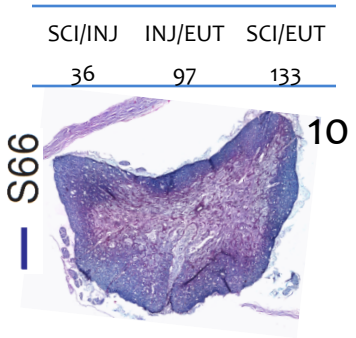
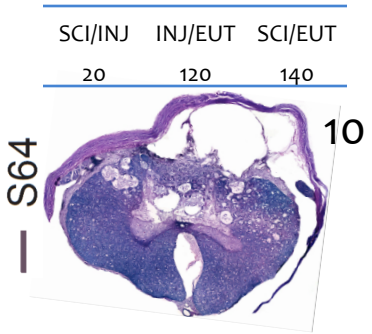
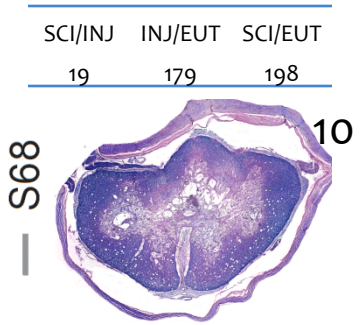
38 44 82
S65
5

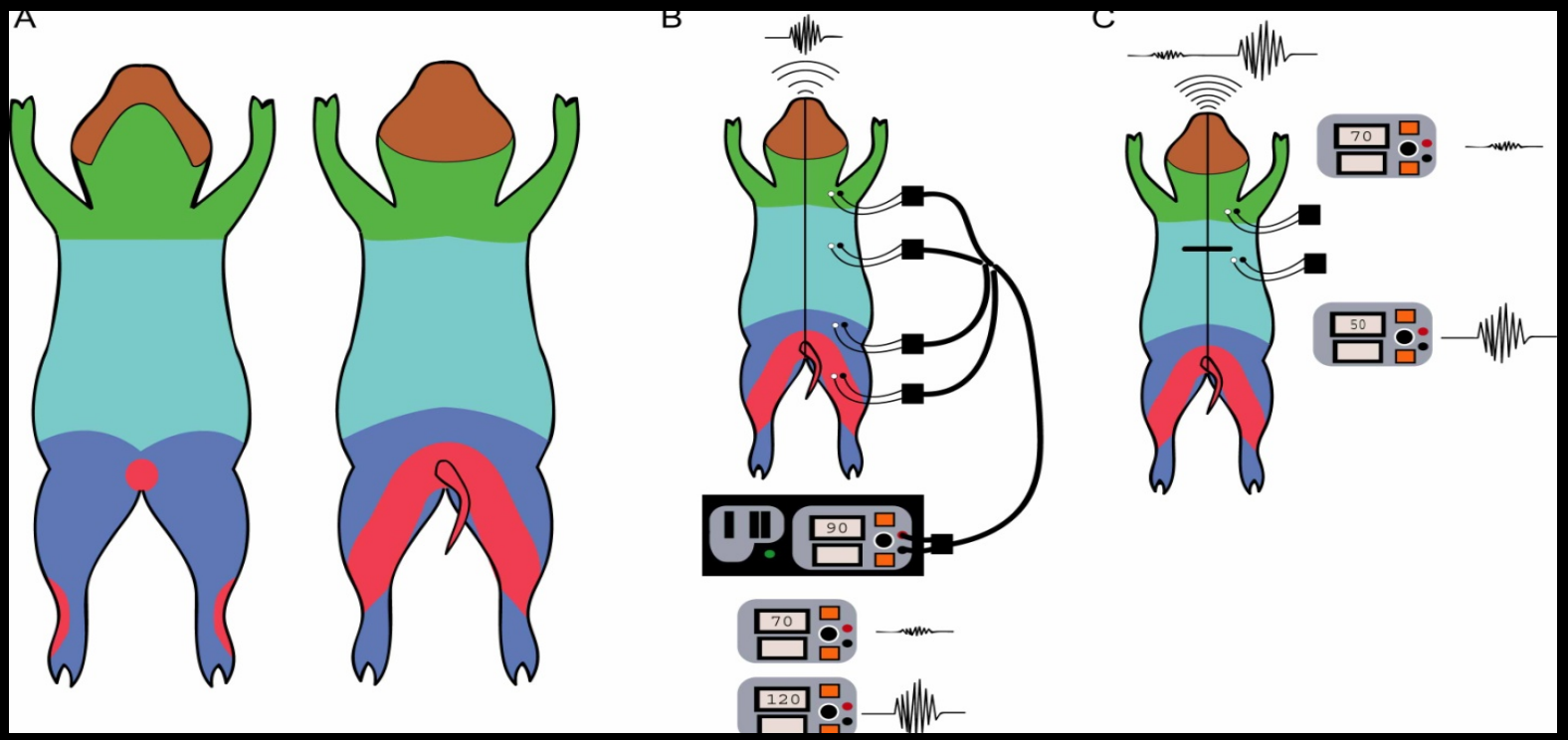

SCI/INJ INJ/EUT SCI/EUT
22 67 89
S62
9


SCI/INJ INJ/EUT SCI/EUT
36 97 133
S66
10


SCI/INJ INJ/EUT SCI/EUT
20 120 140
S64
10

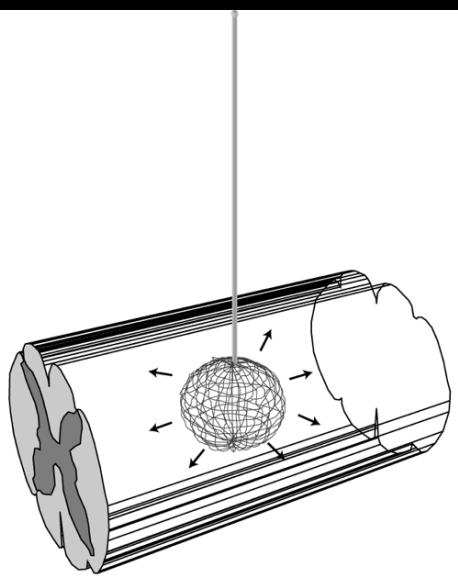

SCI/INJ INJ/EUT SCI/EUT
19 179 198
S68
10

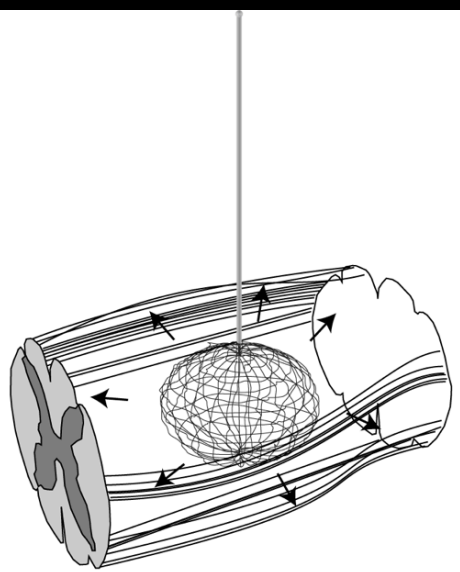
Miami Porcine Sensory Testing

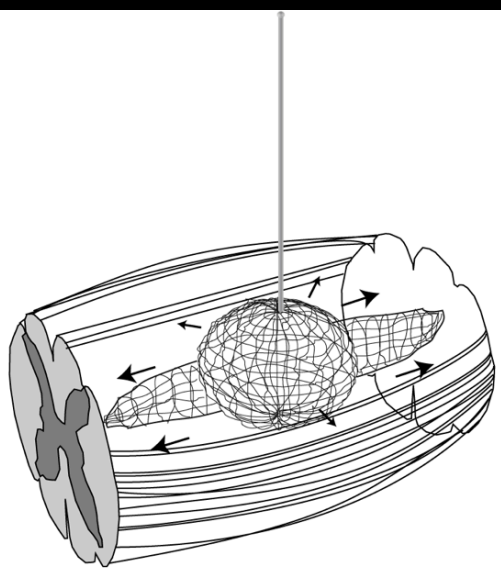


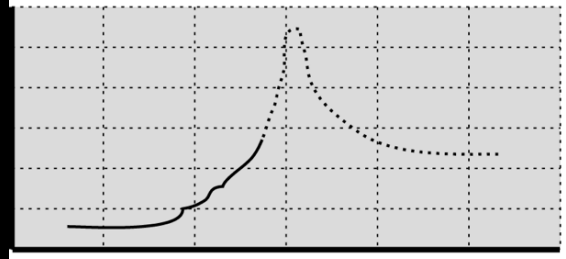
Risks of spinal cord injections

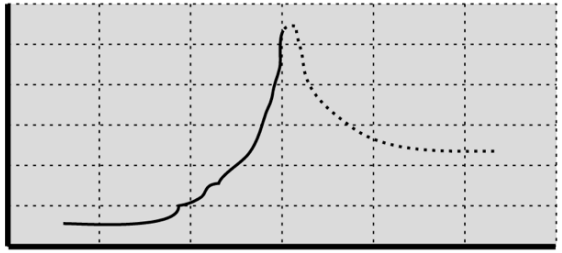
* Surface pial penetration is necessary and needle passage through the dorsal spinal cord may damage some fibers.
* Spinal cord motion against a fixed needle may cause damage.
* Depending on time point after injury, the injection site may contain active inflammation, fragile neovasculature, endogenous tissue responses and reparative structures.
* Because the pia mater is relatively inelastic, large injection volumes may cause high intra-cord pressures leading to tissue dissection and ischemia.

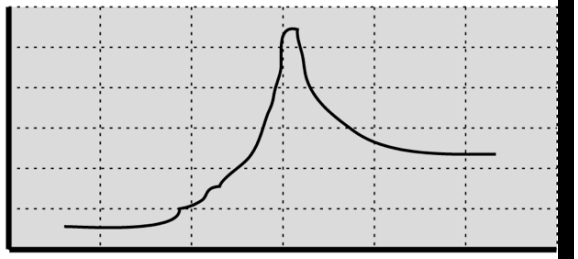




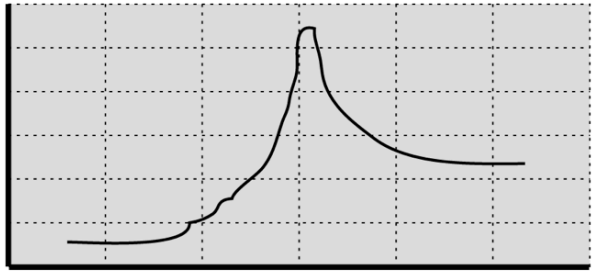




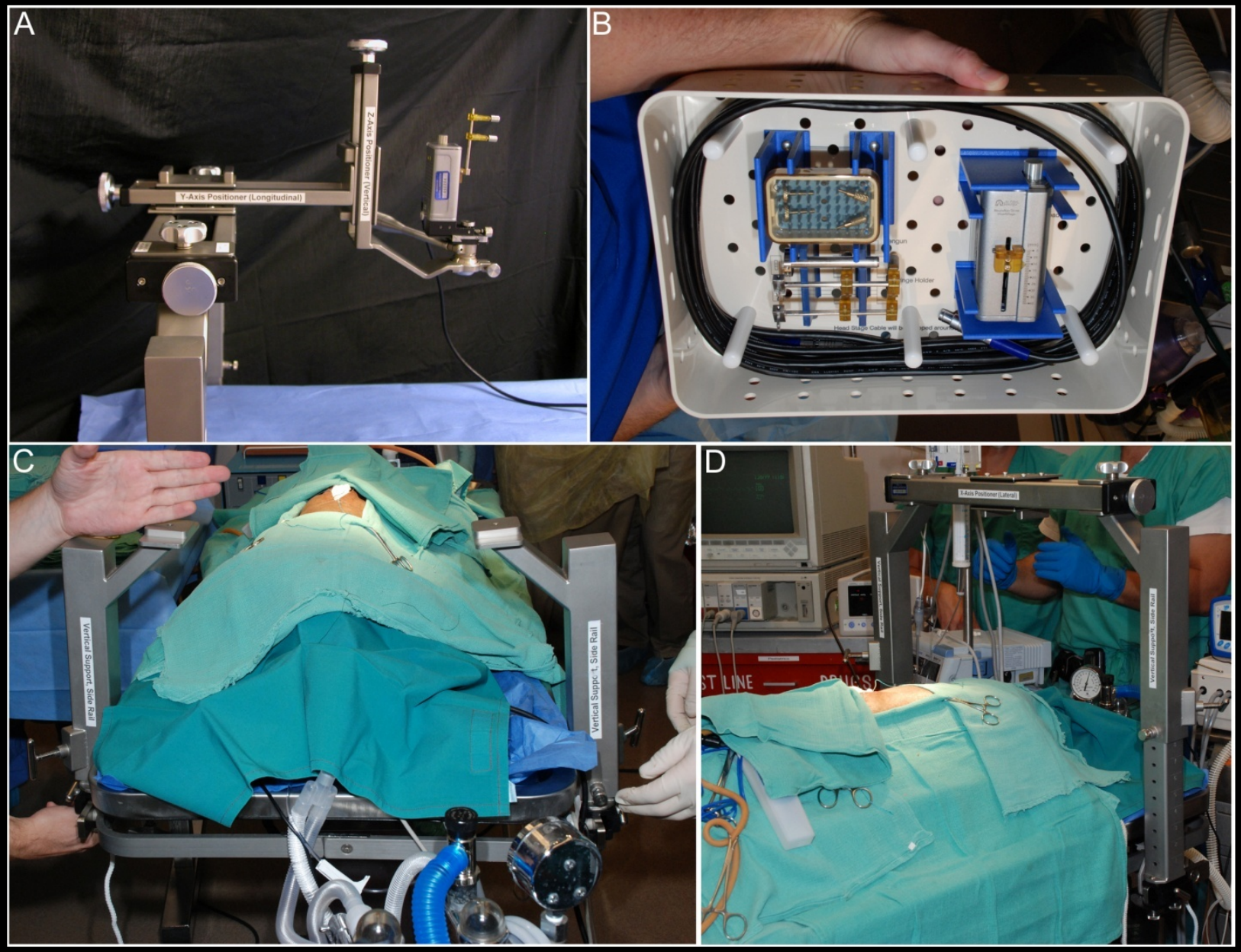


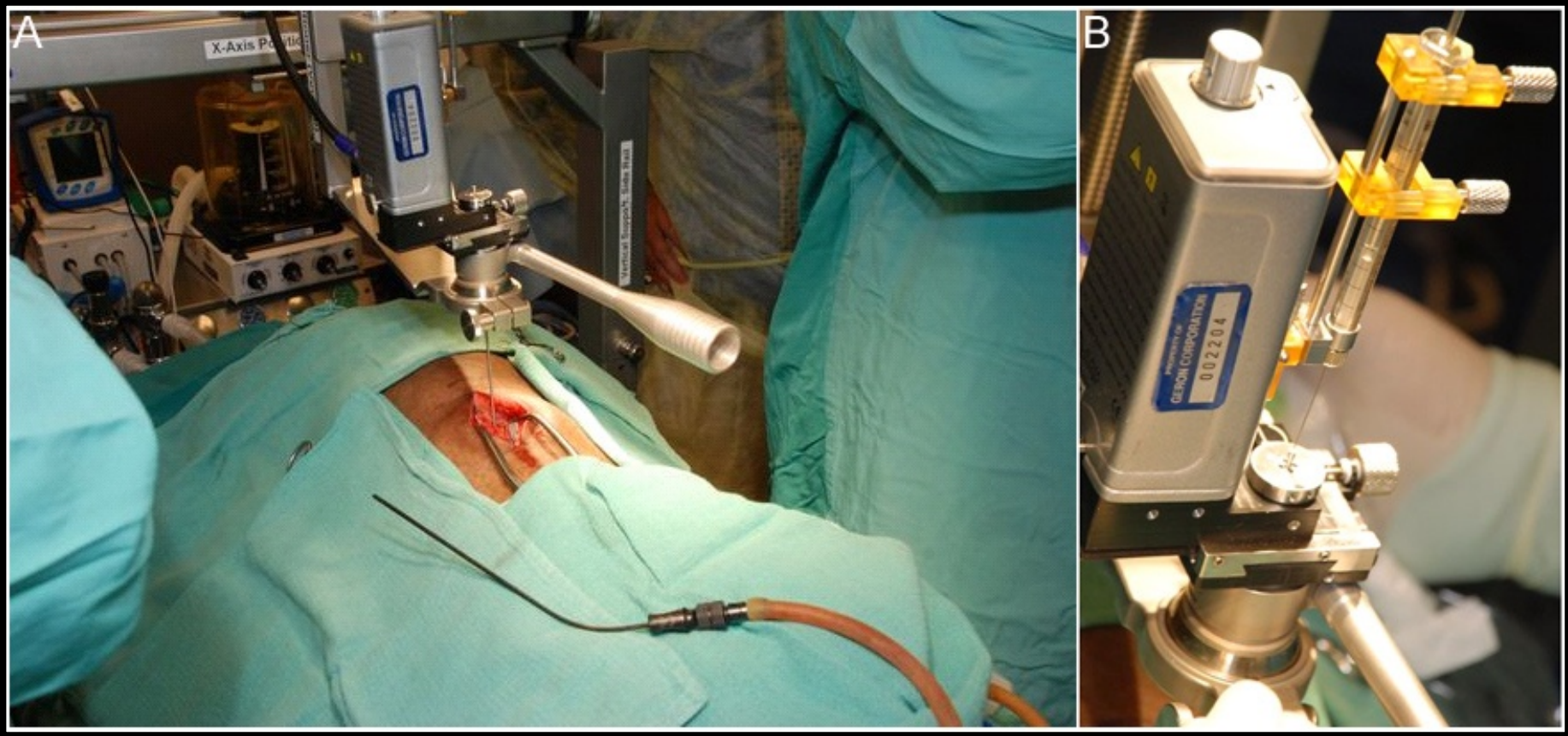


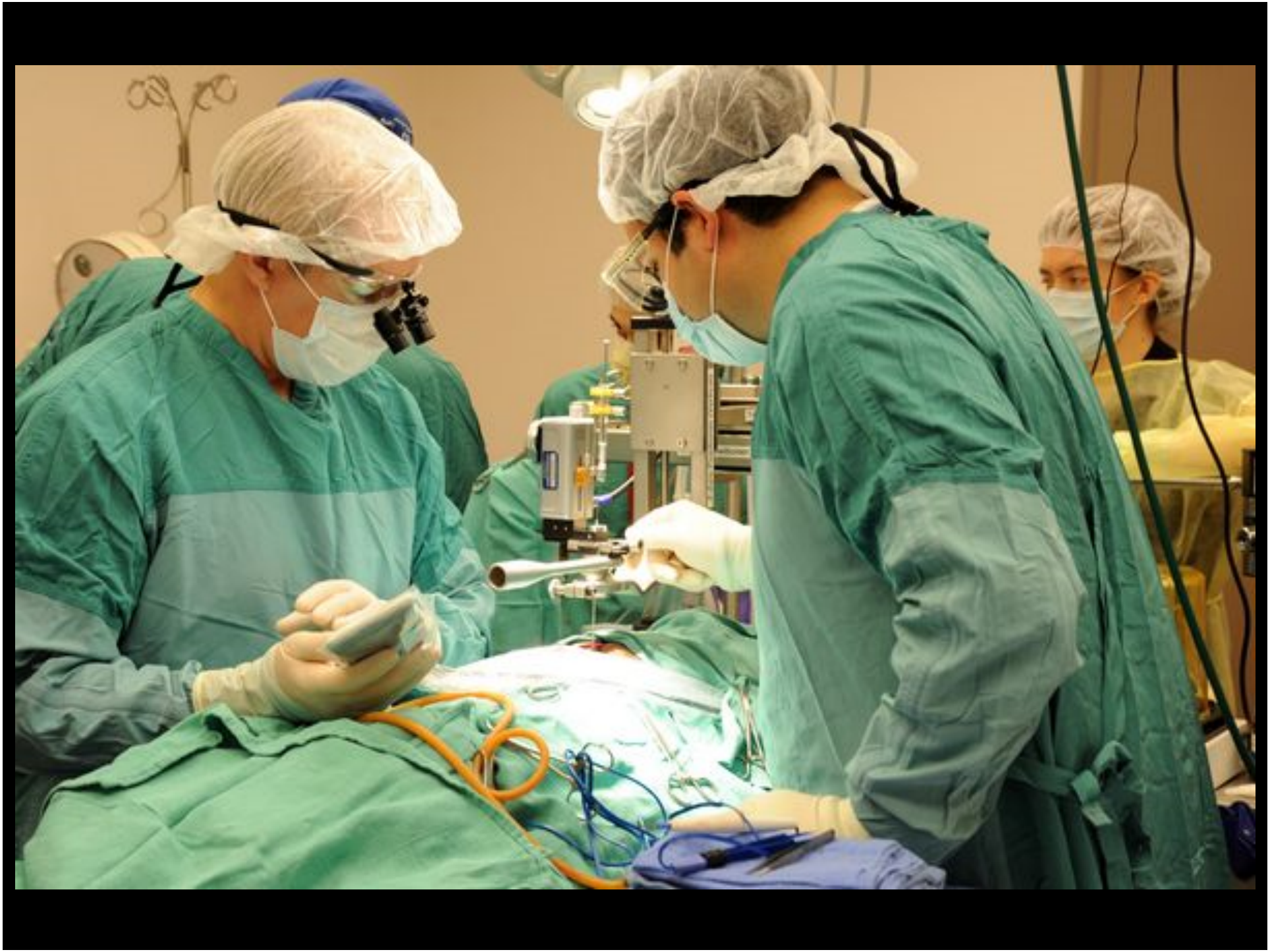
Intraparenchymal
Pressure Gradient



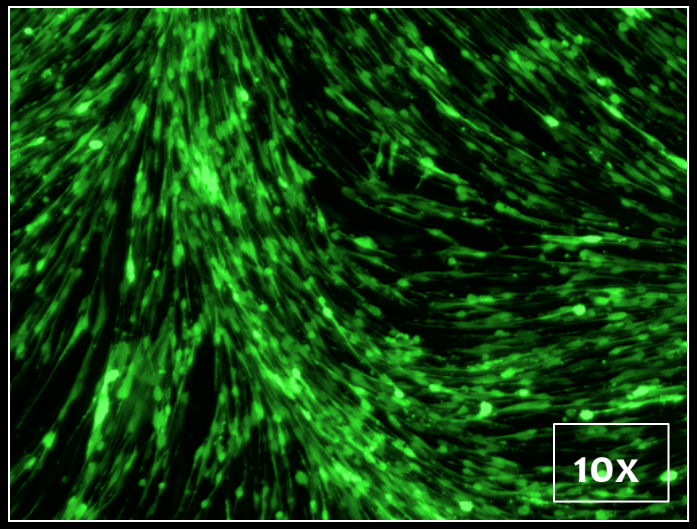
Total volume Injected

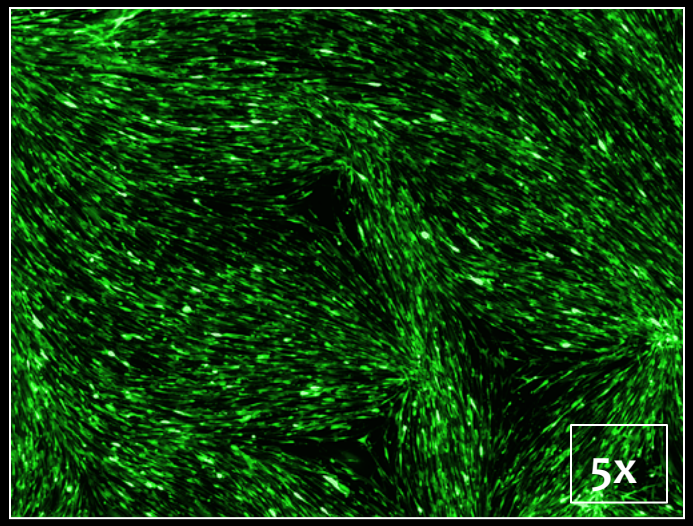

A
Y-Axis Positioner (Longitudinal)
Z-Axis Positioner (Vertical)
B
C
Vertical Support, Side Rail
Vertical Suppo t, Side Rail
D
X-Axis Positioner (Lateral)
Vertical Support, Side Rail

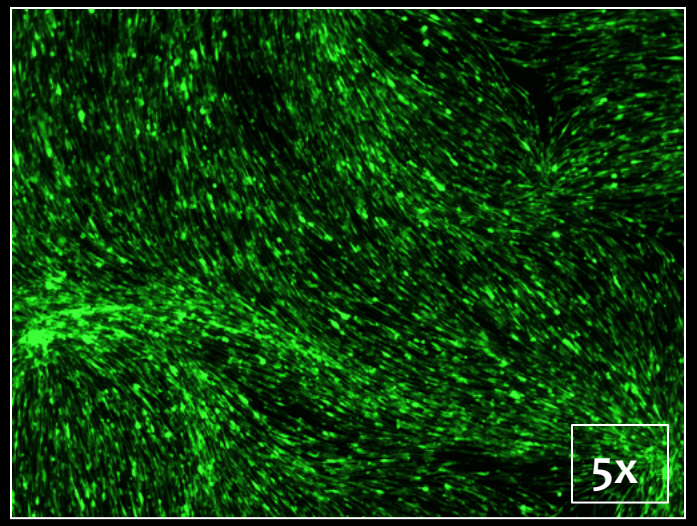

A
B
002204

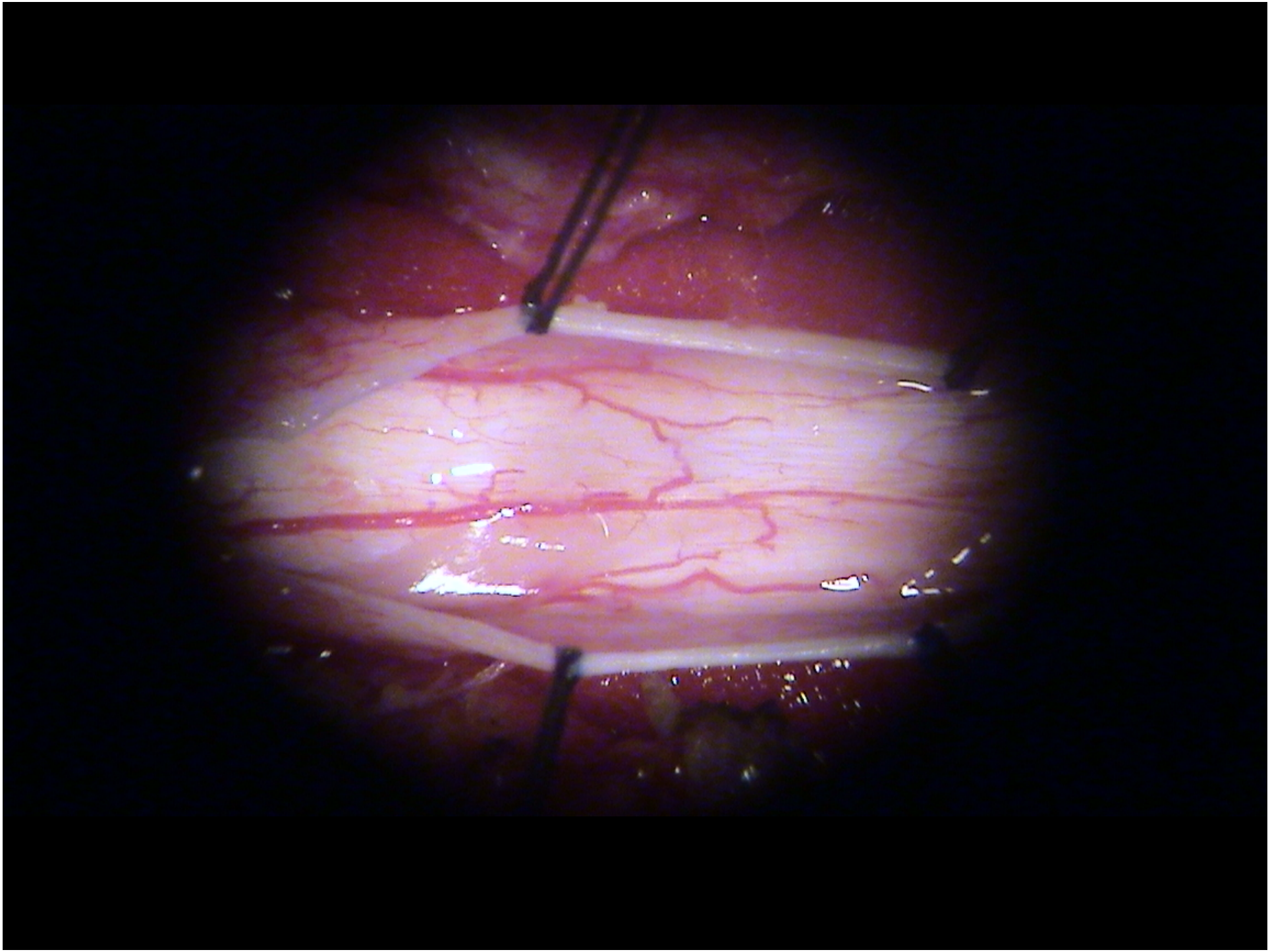


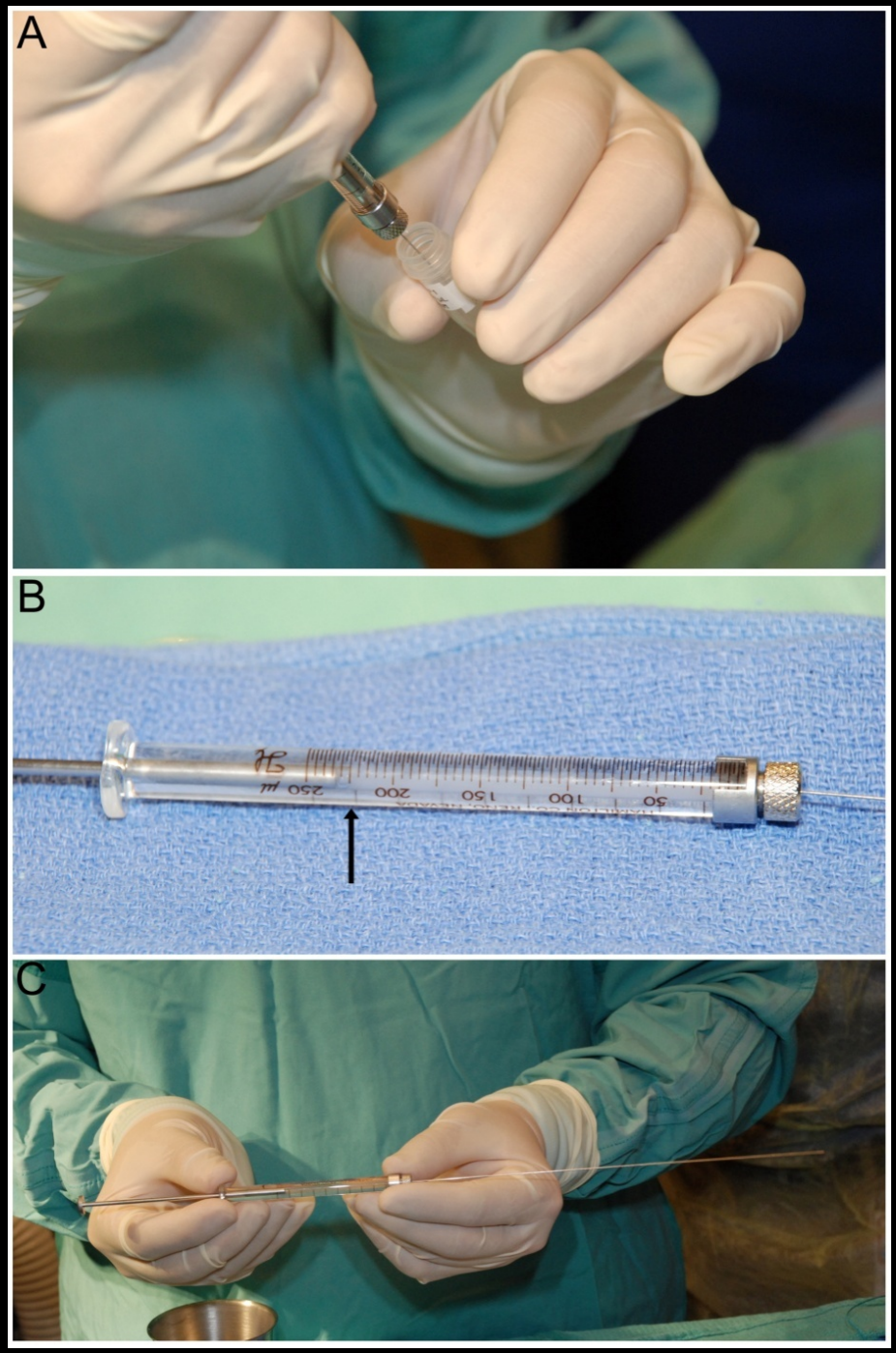
L-GFP PSC- Dr. Gagani Athauda. P1 cryopreserved Porcine SC.

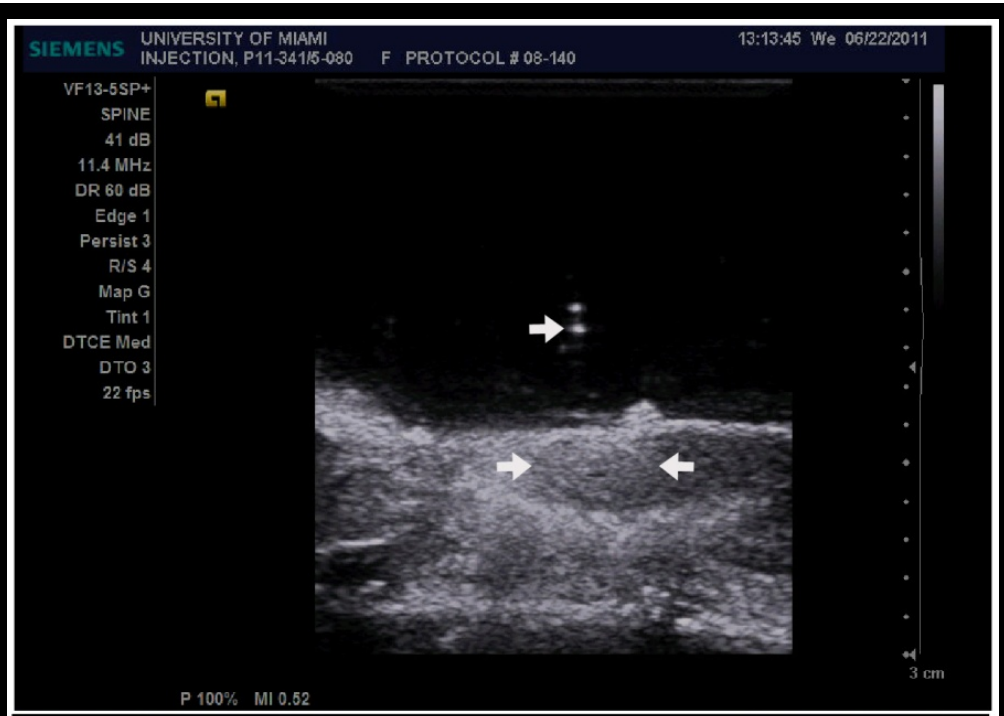


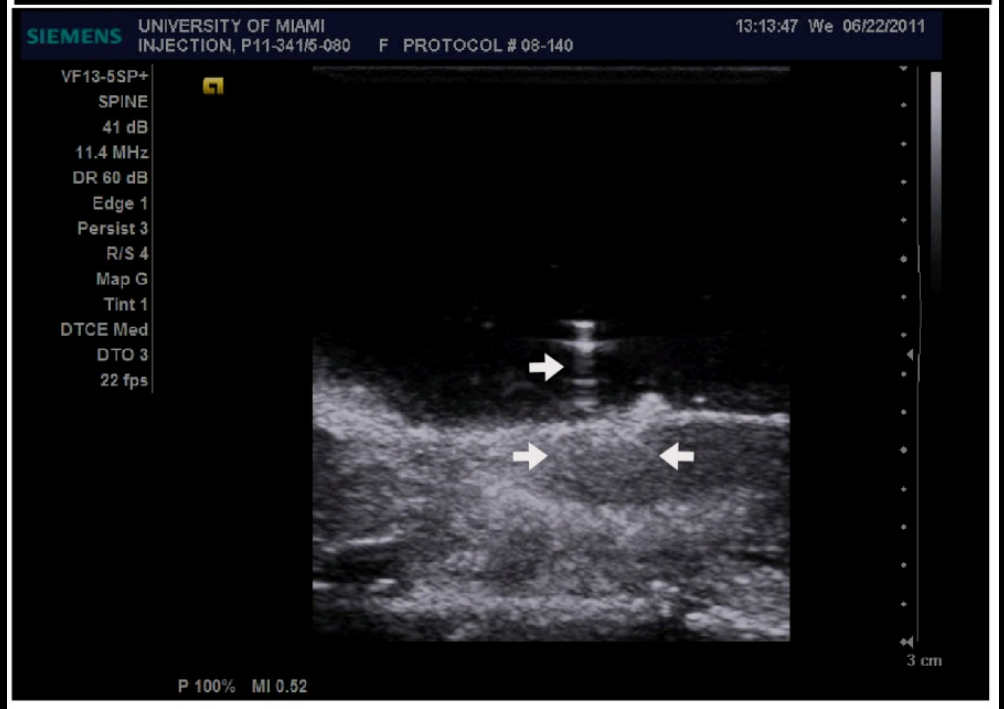


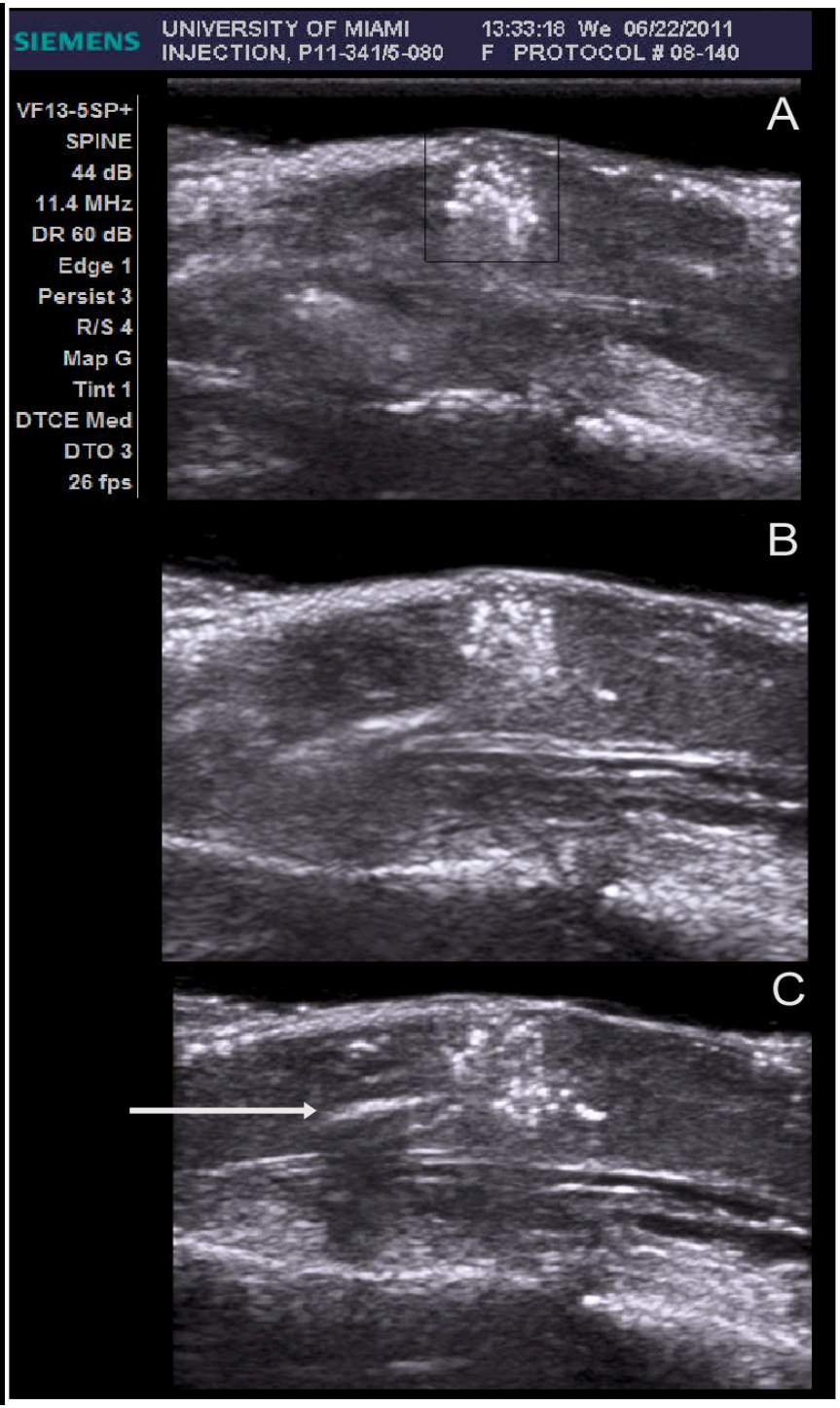


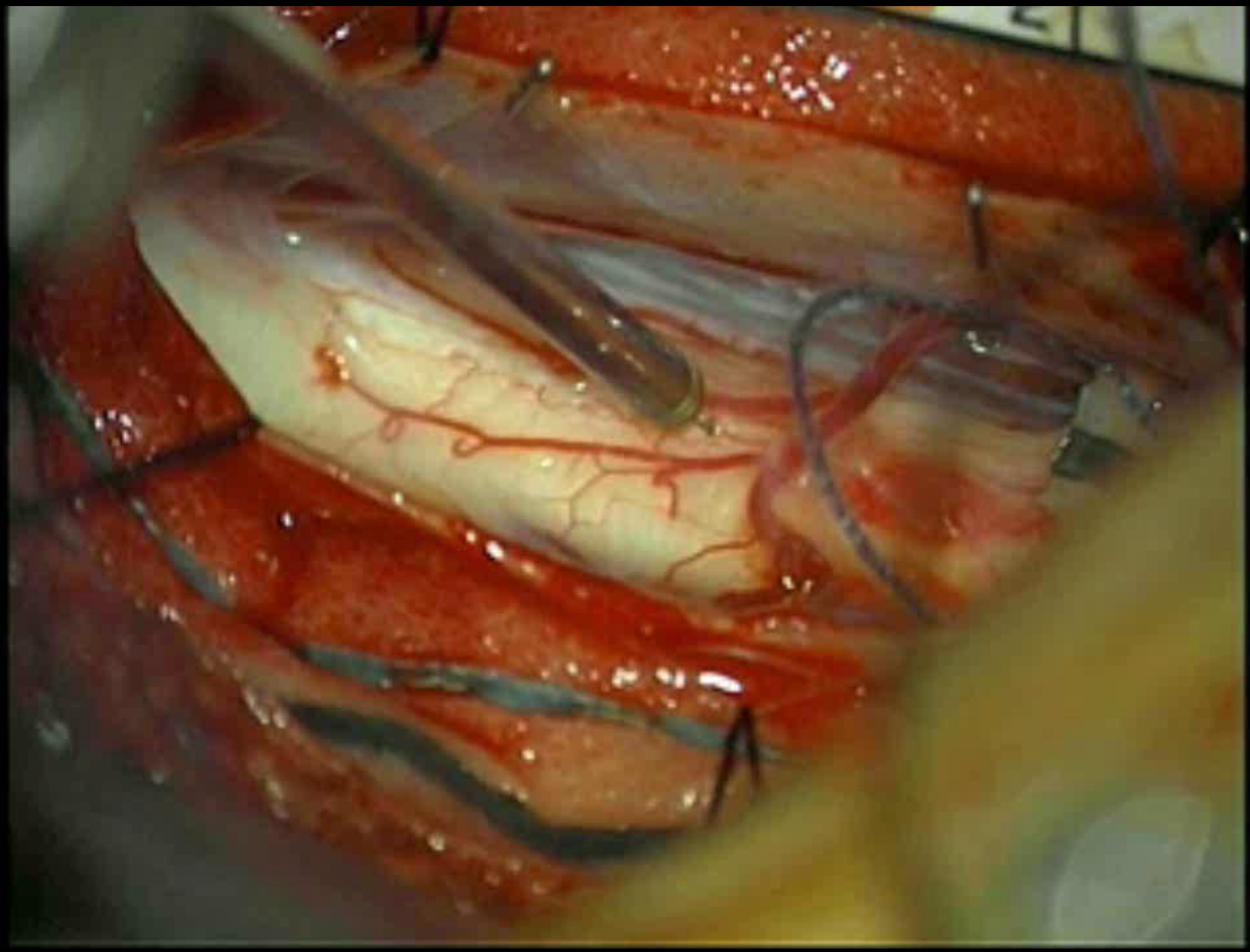


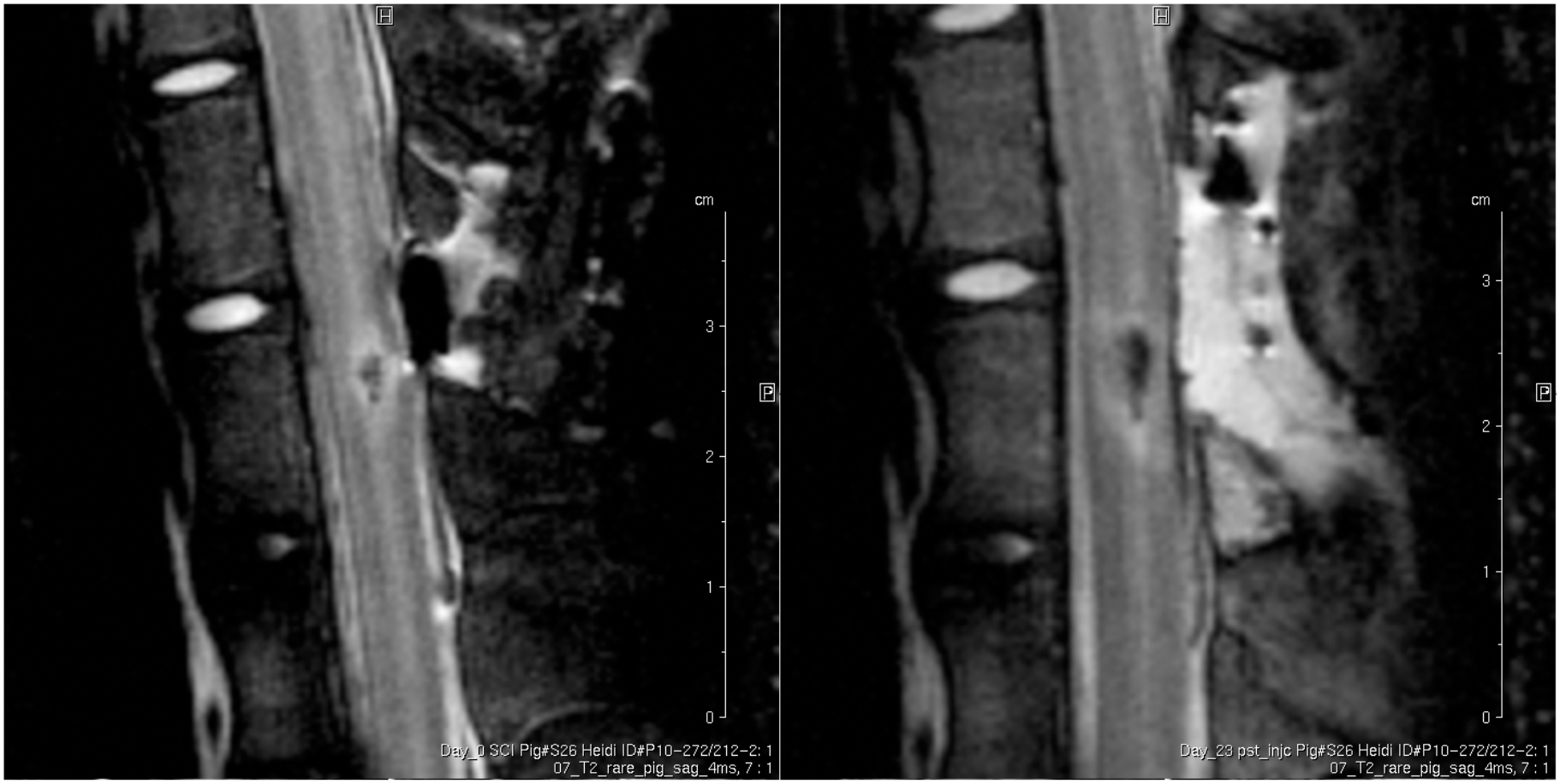

A
B
250
200
150
100
50
C


SIEMENS
UNIVERSITY OF MIAMI
INJECTION, P11-341/5-080 F PROTOCOL # 08-140
13:13:45 We 06/22/2011
VF13-5SP+
SPINE
41 dB
11.4 MHz
DR 60 dB
Edge 1
Persist 3
R/S 4
Map G
Tint 1
DTCE Med
DTO 3
22 fps
3 cm
P 100% MI 0.52


SIEMENS
UNIVERSITY OF MIAMI
INJECTION, P11-341/5-080 F PROTOCOL # 08-140
13:13:47 We 06/22/2011
VF13-5SP+
SPINE
41 dB
11.4 MHz
DR 60 dB
Edge 1
Persist 3
R/S 4
Map G
Tint 1
DTCE Med
DTO 3
22 fps
3 cm
P 100% MI 0.52


SIEMENS
UNIVERSITY OF MIAMI
INJECTION, P11-341/5-080
13:33:18 We 06/22/2011
F PROTOCOL # 08-140
VF13-5SP+
SPINE
44 dB
11.4 MHz
DR 60 dB
Edge 1
Persist 3
R/S 4
Map G
Tint 1
DTCE Med
DTO 3
26 fps
A
B
C

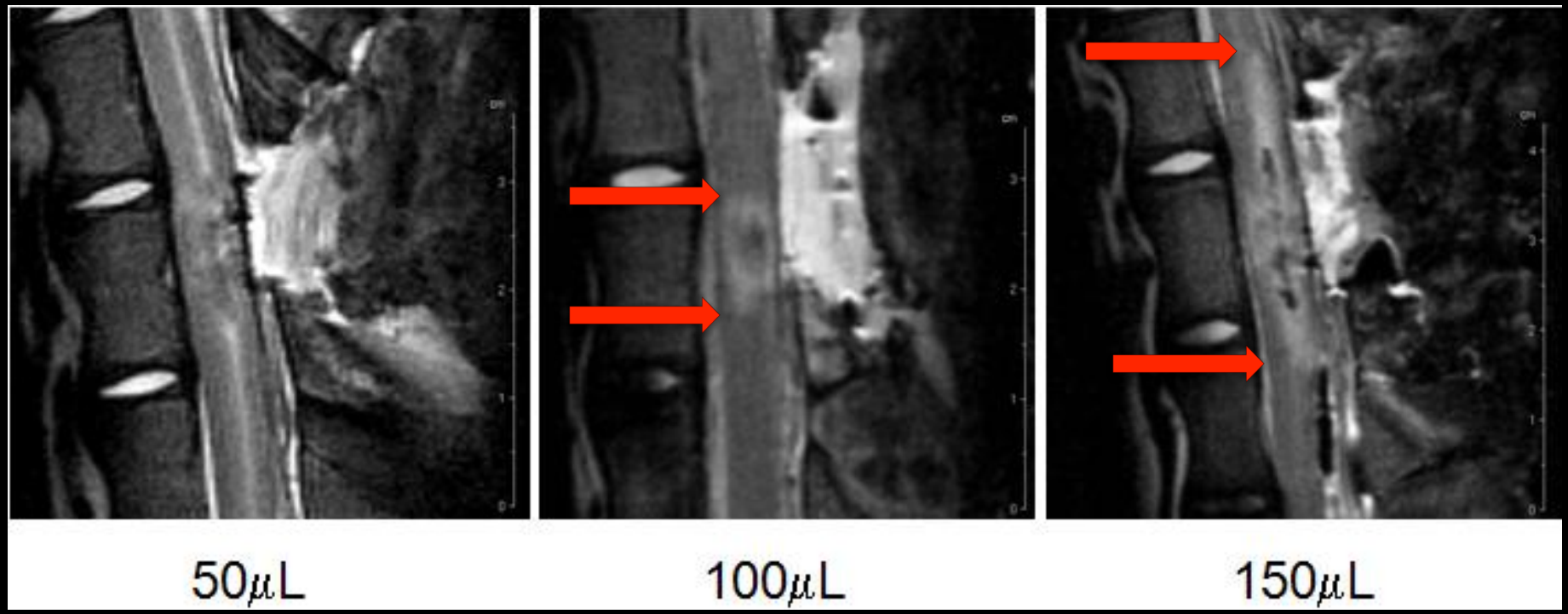




Day 0 Post contusion

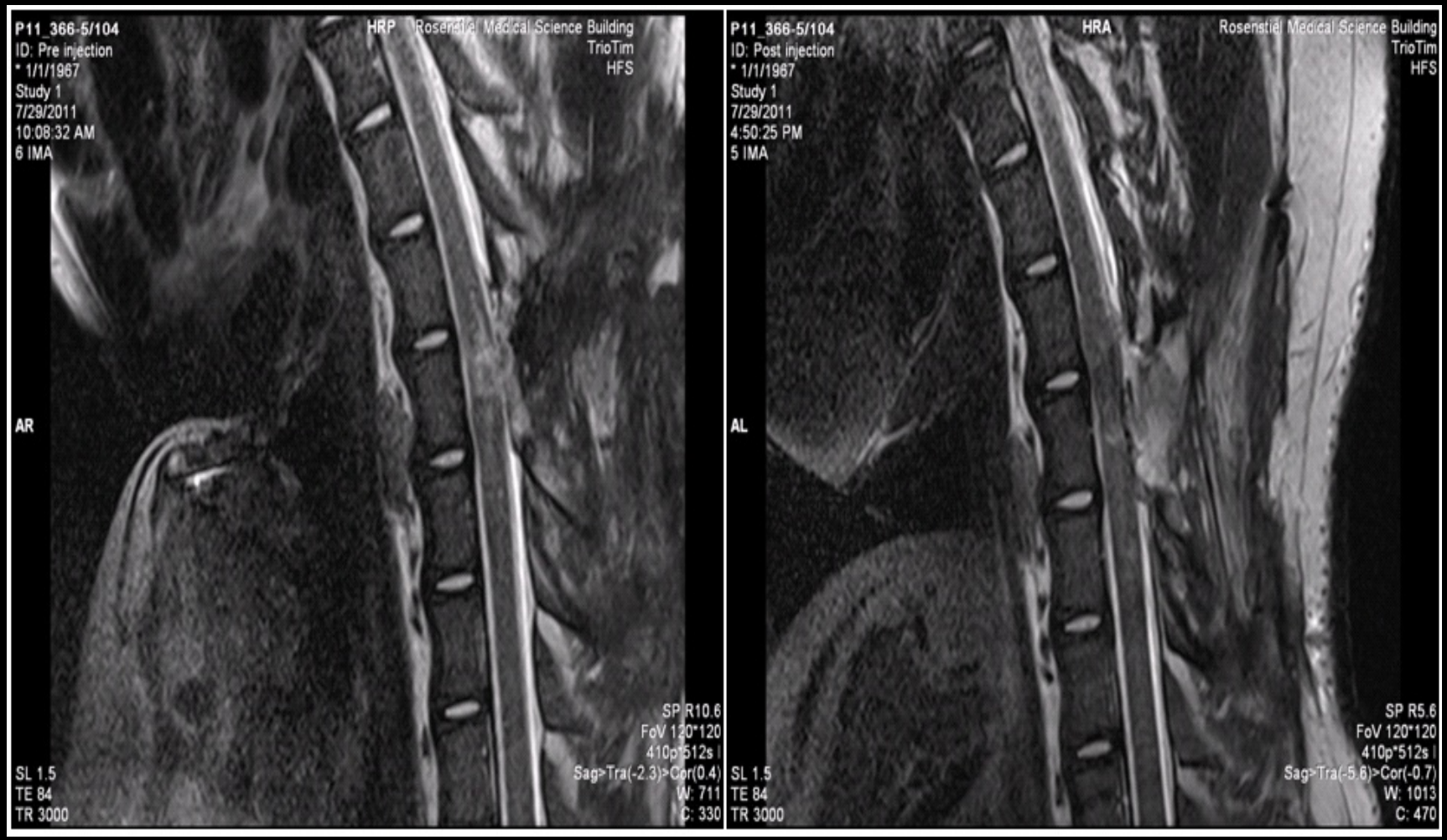
Post contusion +
100 ul SC injection 225,000 cells/ul day 24

Schwann Cell Dose/Volume Testing.

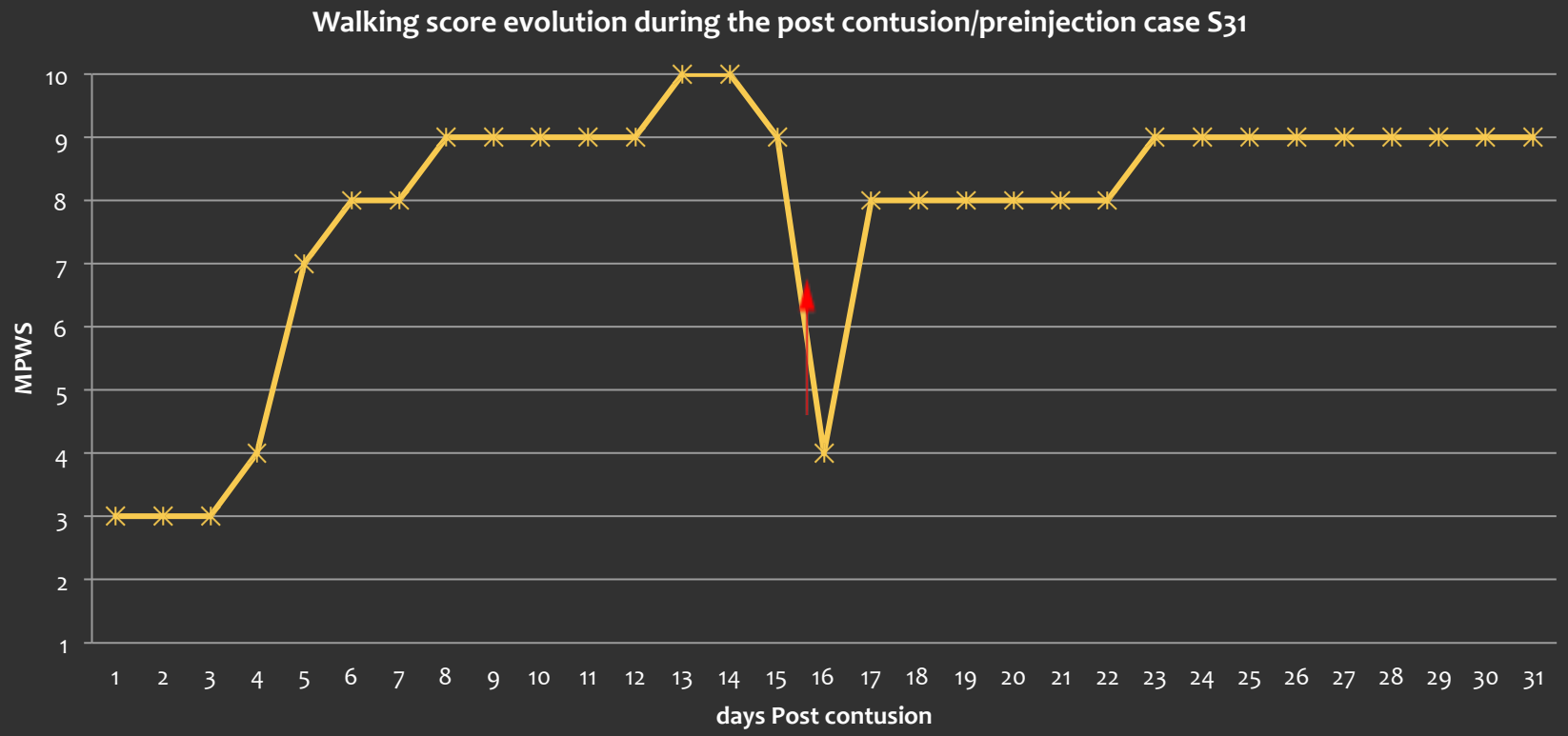


Increased injection volumes are associated with increased T2 signal and tissue dissection

No post-implant deficit and continued locomotor recovery

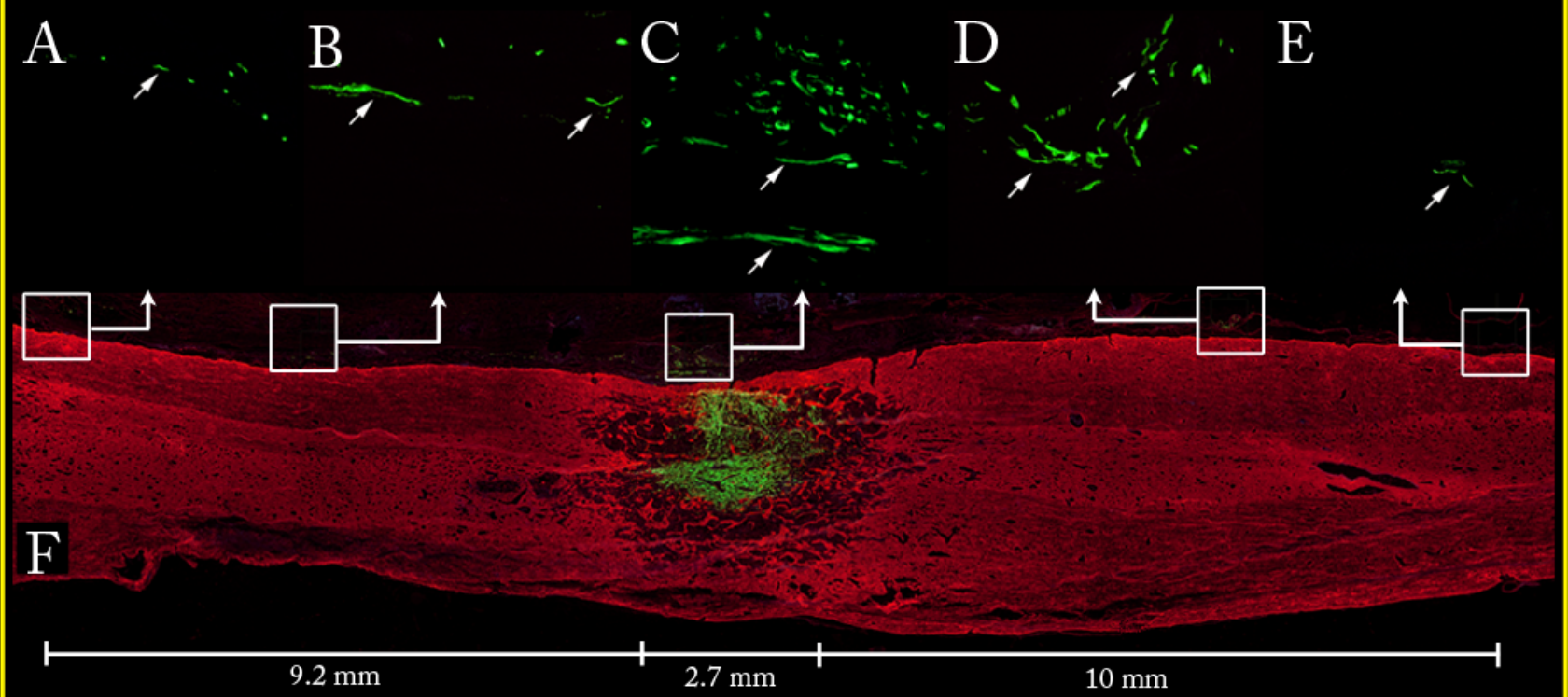


1	1	2	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5

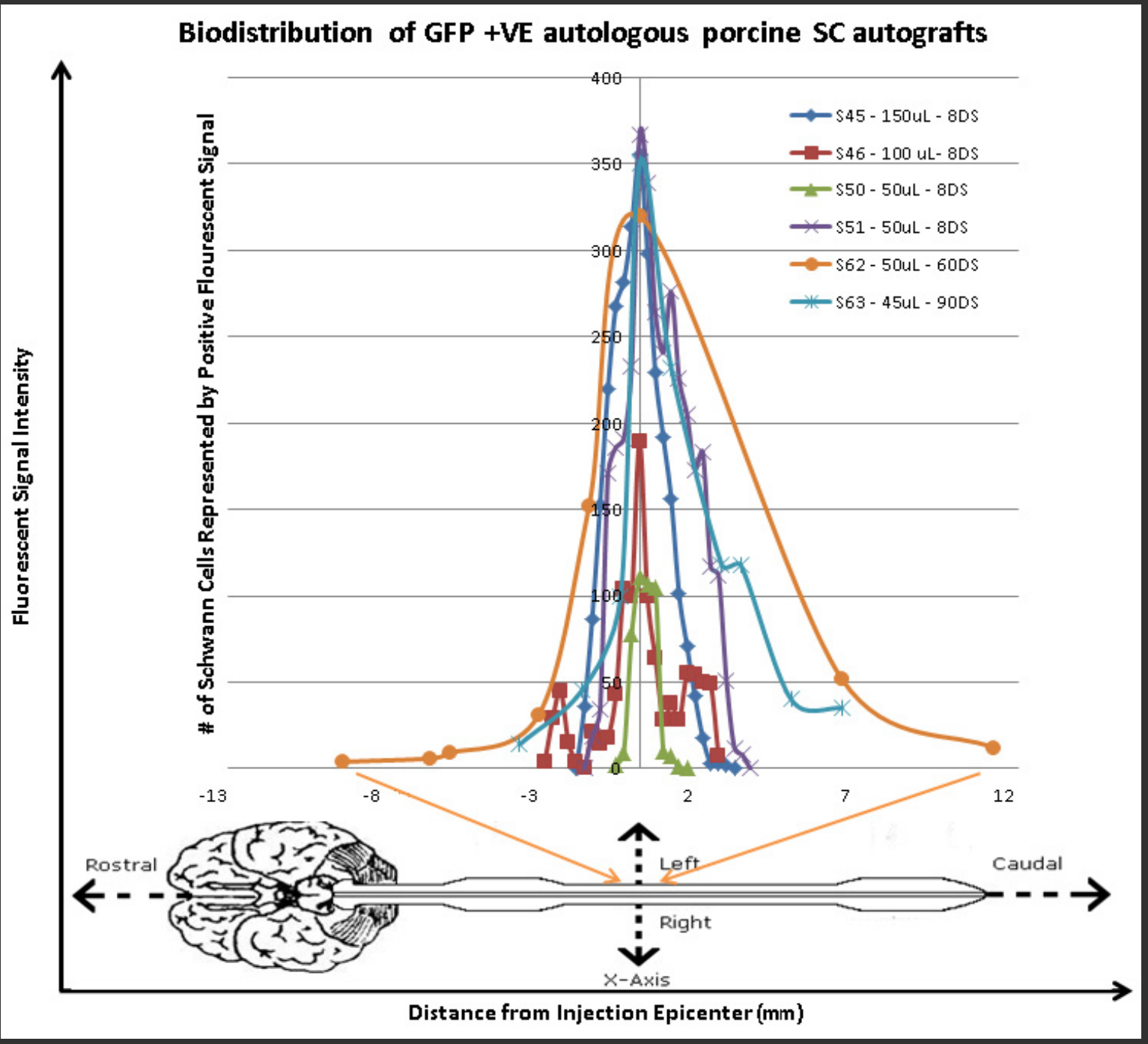

Walking score evolution during the post contusion/preinjection case S31
MPWS
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
days Post contusion

4. Graft Biodistribution

GFP. GFAP.Hoechst
27d post cont/8d post inj
50 µl GFP SC autograft

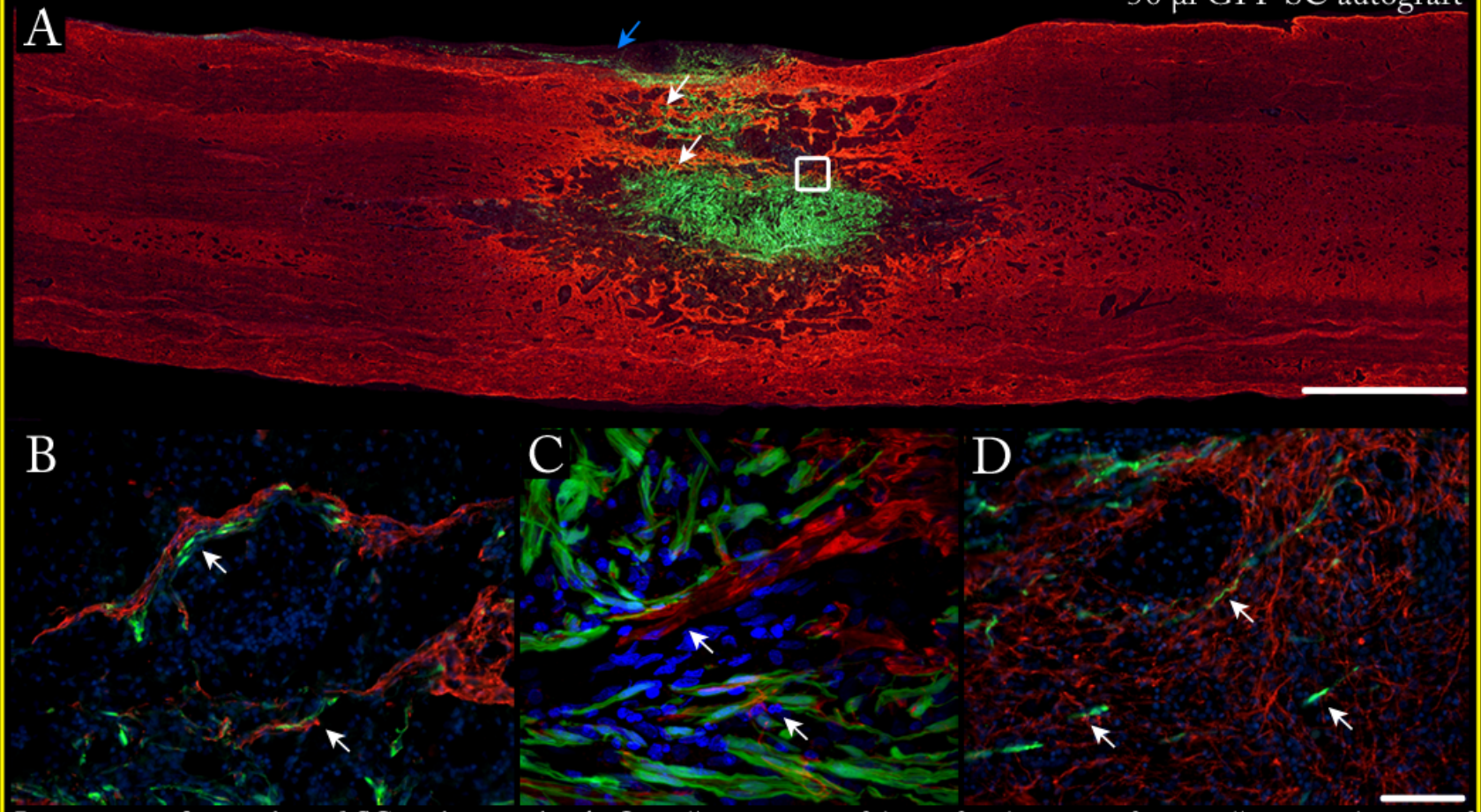


Biodistribution of GFP labeled SC. Section from a 30mm epicenter block in which transplanted cells where identified rostrally and caudally in the dorsal pia. **F**: GFAP immunolabelling allows identification of cavity margins, GFP immunolabelling shows filling of the injury cavity by grafted SC. In this minipig grafted SC were found at the greatest distance from the injection site. **A**, **B**, **C**, **D**, **E**. The 9.2mm distance at which GFP positive cells were observed in **A**, triggered further analysis of sections from the contiguous rostal block. Additional SCs were found at a maximum distance of 41.5mm from the graft margin. Although migration from a pocket of effluxed cells may account for this finding, the large distance is more likely attributable to movement within CSF.


Biodistribution of GFP +VE autologous porcine SC autografts
Fluorescent Signal Intensity
# of Schwann Cells Represented by Positive Flourescent Signal
400
350
300
250
200
150
100
50
0
S45 - 150uL - 8DS
S46 - 100 uL- 8DS
S50 - 50uL - 8DS
S51 - 50uL - 8DS
S62 - 50uL - 60DS
S63 - 45uL - 90DS
-13
-8
-3
2
7
12
Rostral
Caudal
Left
Right
X-Axis
Distance from Injection Epicenter (mm)

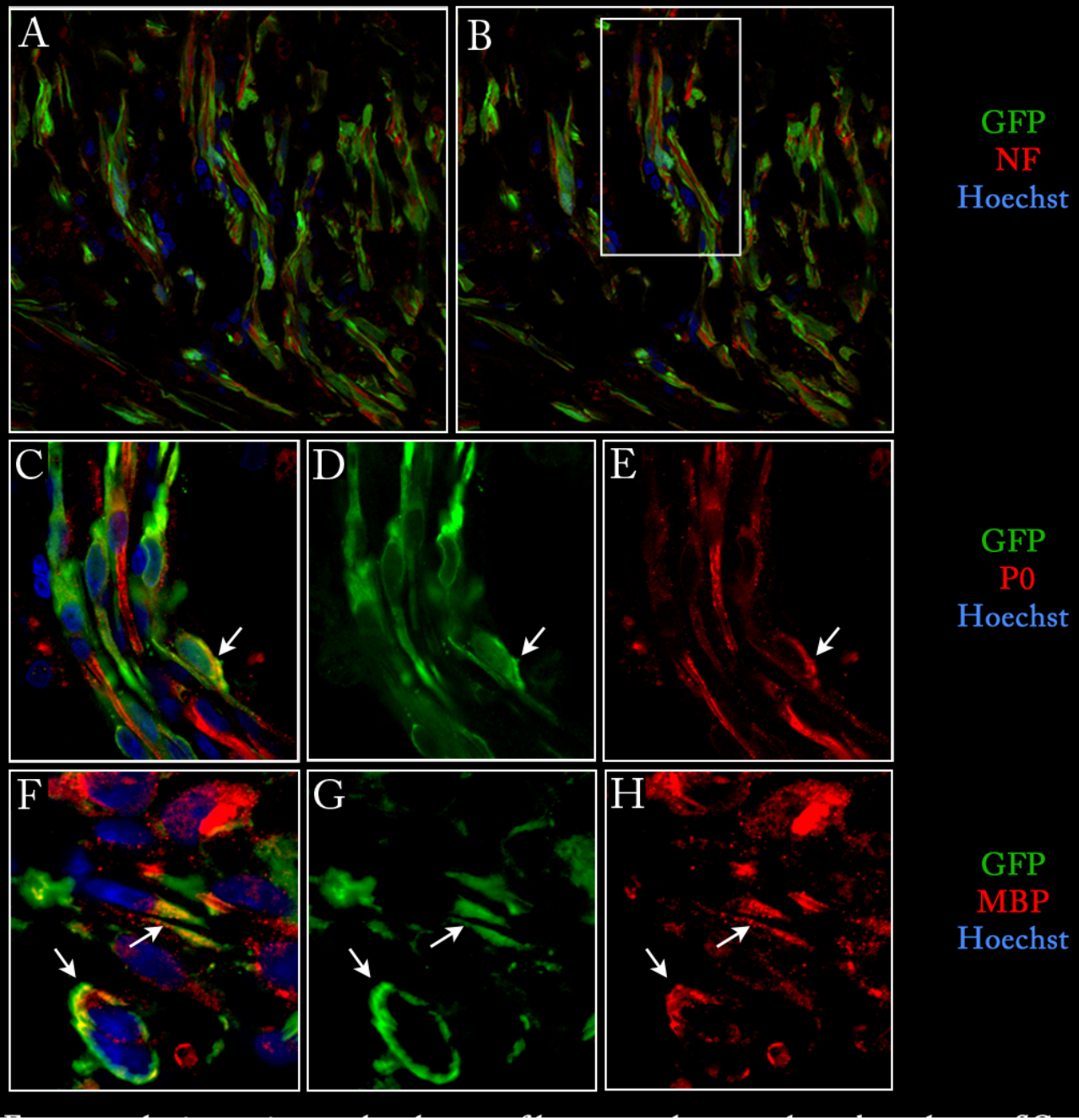
3. SC-Astroglial Integration

GFP. GFAP.Hoechst
27d post cont/8d post inj
50 µl GFP SC autograft



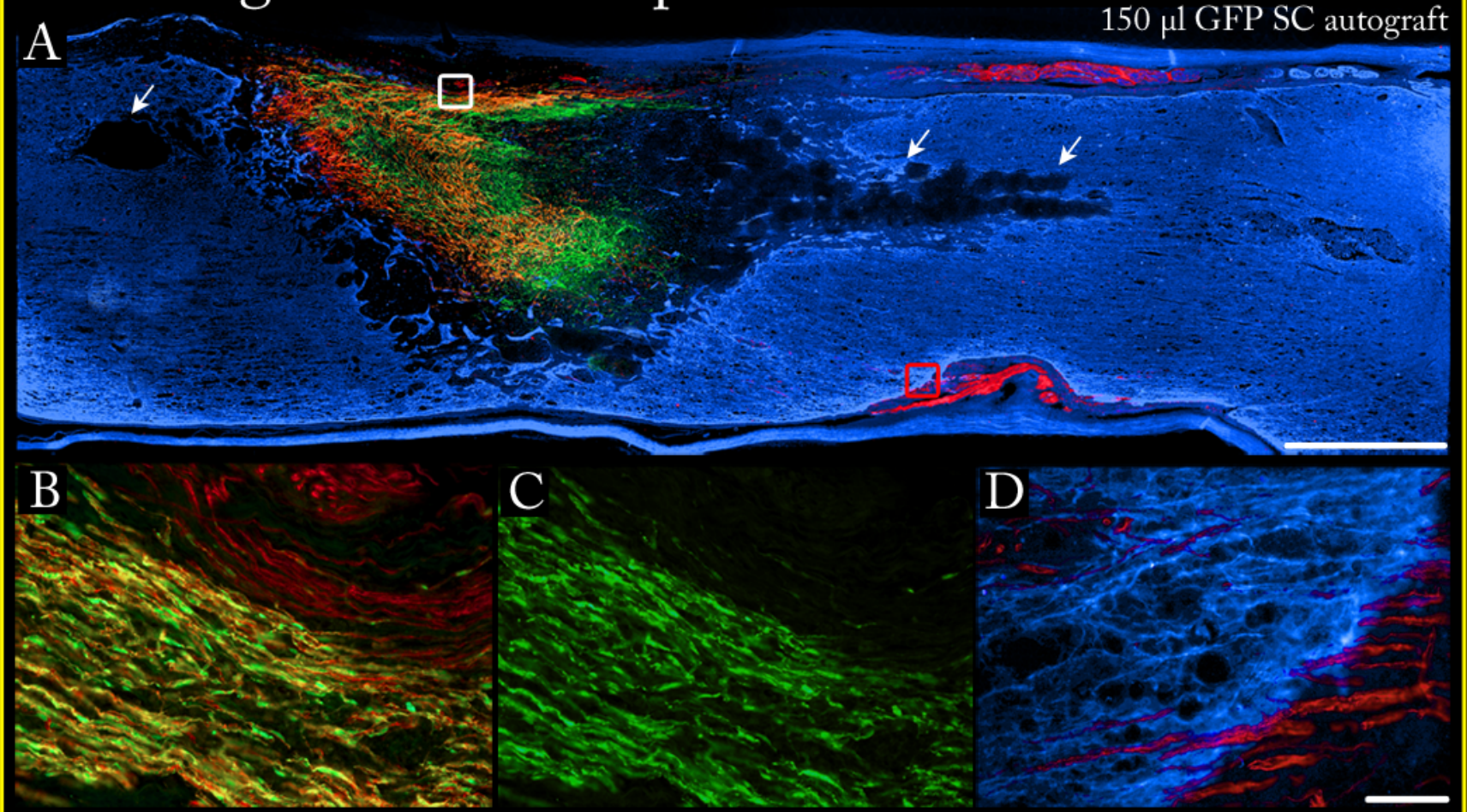
Integration of transplanted SC with astroglia, **A**: Overall appearance of the graft at low magnification allows visualization of the GFP+ cells within the injury cavity. Note that this 50 µl graft was not associated with extra-cavity extrusion as compared to panel 2. GFP + cells are also visible in the dorsal pia mater (blue arrow), white arrows point to sites of close interaction between GFAP positive processes and transplanted GFP+ SCs, see close alignment magnified in **B**. White square magnified in a confocal image at 60X (**C**) allows appreciation of this close interface where Hoechst nuclear dye signal reveals numerous other unlabeled cells, possibly inflammatory or fibroblastic. Similar integration of astrocytes and transplanted SCs was seen across all auto and allografts at early time points. **D**: A 150 µl injection animal showing GFP+ cells within GFAP positive parenchyma at 9d graft survival. Bar **A**: 2000 µm; **B**,**D**: 100 µm.

5. Ensheathment-Myelination

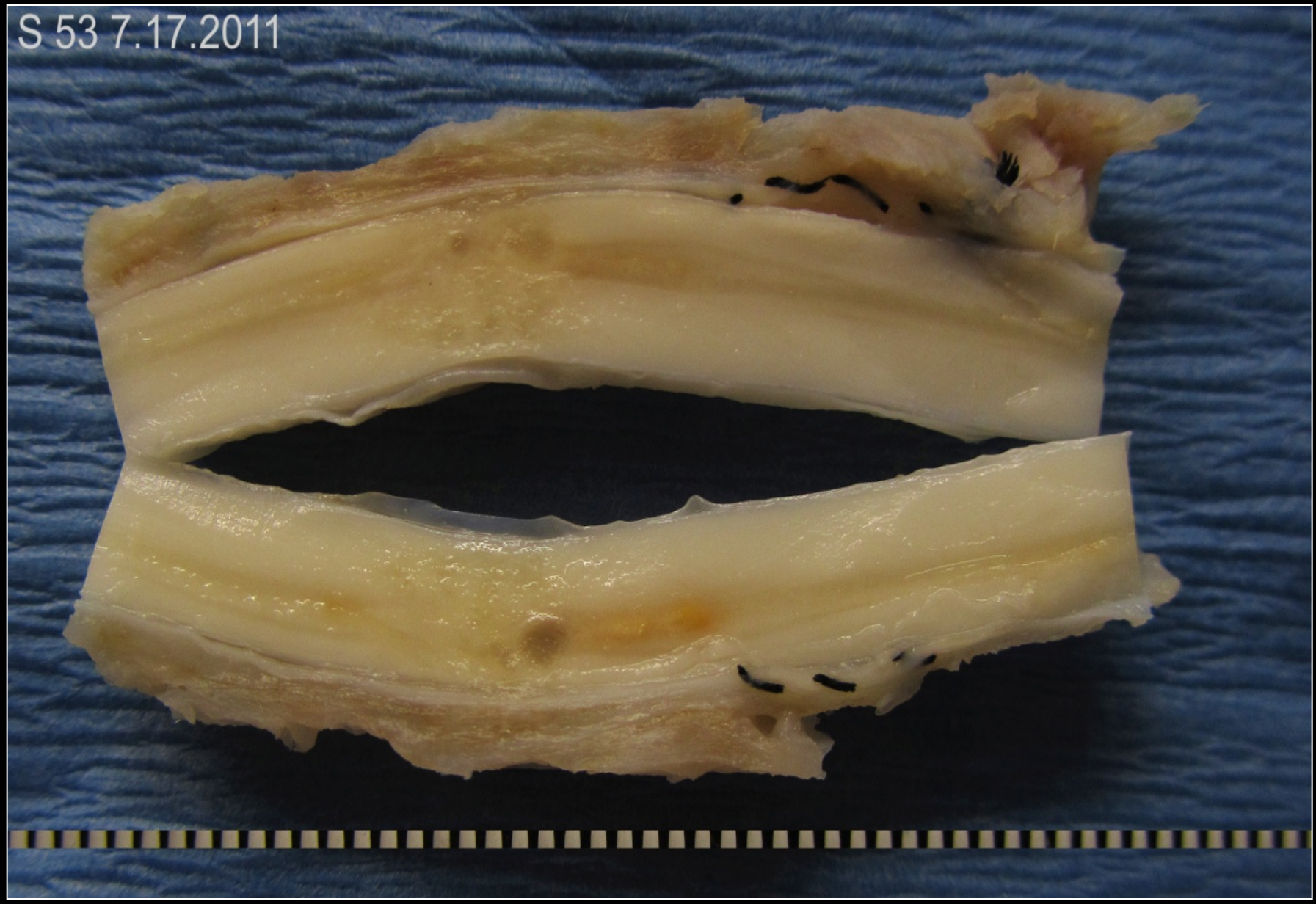


2. Endogenous SC Response

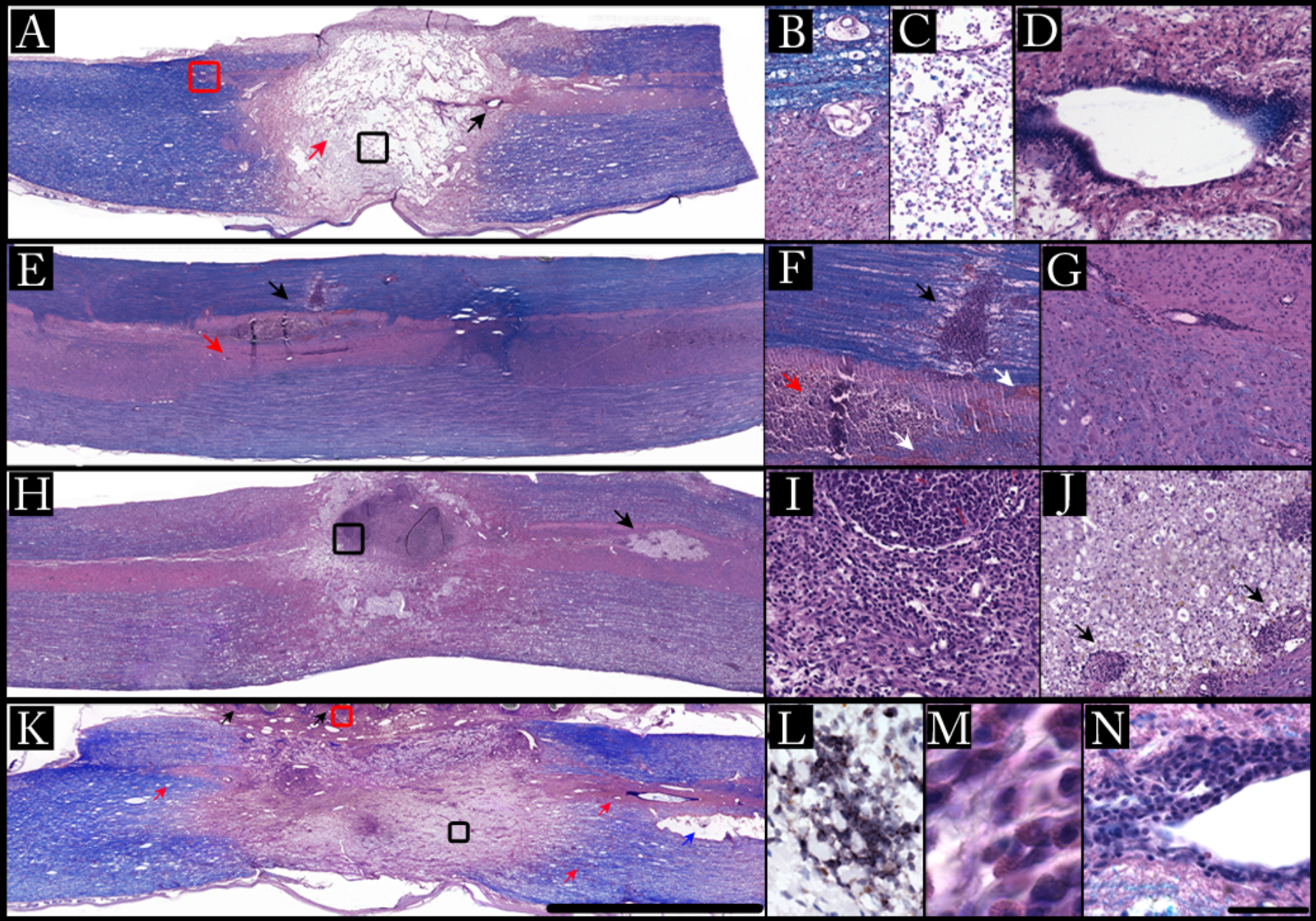
GFP. p75.GFAP
29d post cont/9d post inj
150 µl GFP SC autograft



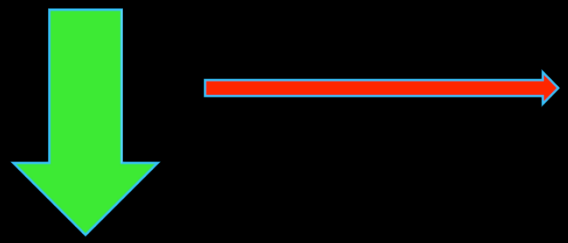
Endogenous and transplanted SC migration. **A:** p75+ SC from adjacent ventral and dorsal nerve roots migrated towards the injury cavity. Ventral root (red square) is magnified in **D** showing endogenous SC migrating through the GFAP+ve astrocytic parenchyma. Dorsal site of injury (white square) magnified in **B**, showing endogenous p75+ SC entering through the breached glia limitans, yellow signal is colocalization of GFP+ transplanted SC with p75, **C:** visualization of the GFP+ transplanted SC signal under single 488nm excitation. Note the overall distribution of the large graft in the cavity where pressure extrusion along grey matter is evident especially towards the caudal end (arrows). The cavity contains three SC phenotypes: GFP+ p75- (transplanted SC), GFP+ p75+ (transplanted SC) and GFP- p75+ (endogenous SC). Further studies will clarify the role of p75 expression in the SC ability to show extensive migration from nerve roots. Bar **A**: 2000 µm; **B,C,D**: 100 µm.


S 53 7.17.2011

6. Host Immune Responses

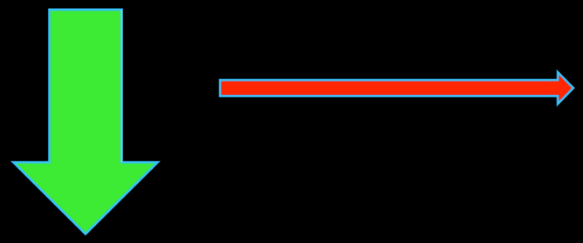


Primate model (chronic)



Pre-injury baseline behavioral training, MEP.

C0/C1 medullary pyramid radiofrequency lesion



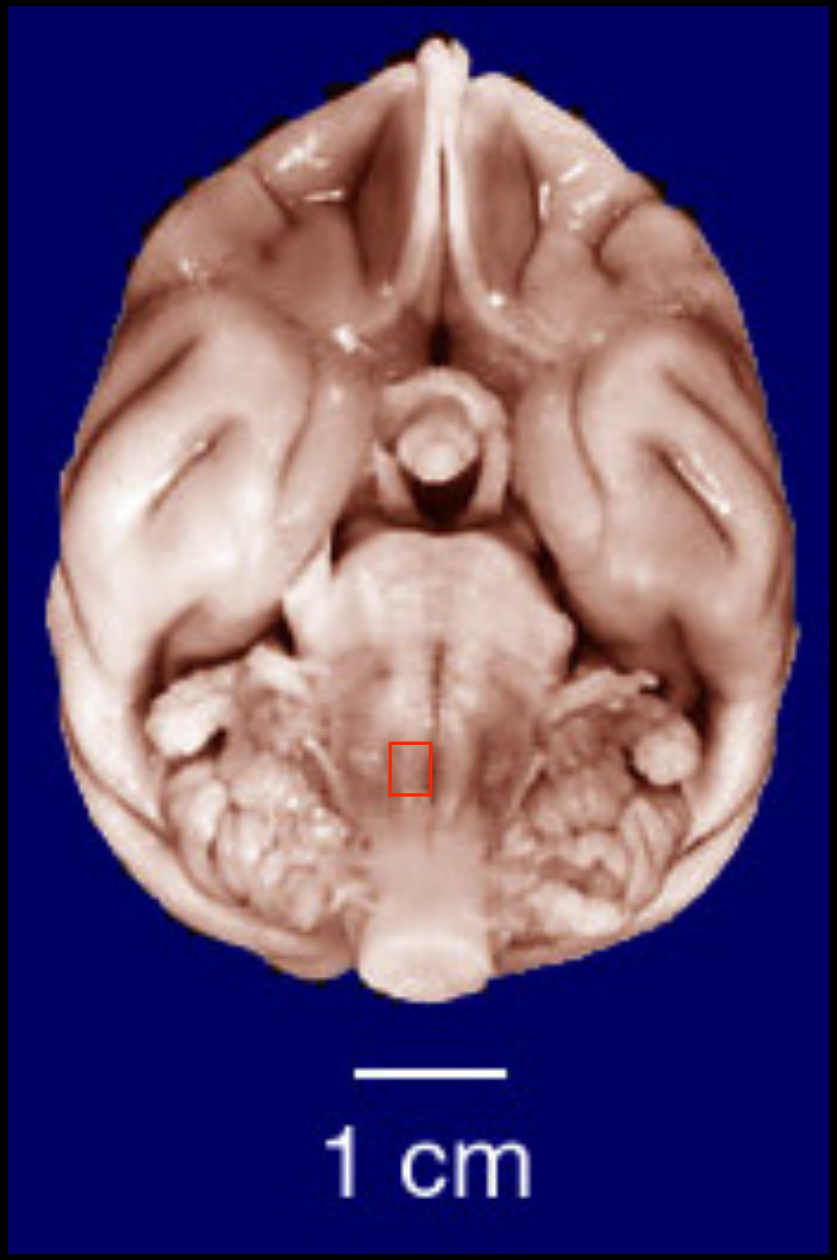
Peri-injury MEP, brainstem stimulation, post-lesion MRI, behavioral testing. Harvest of sural nerve- production of cells, labeling of cells.

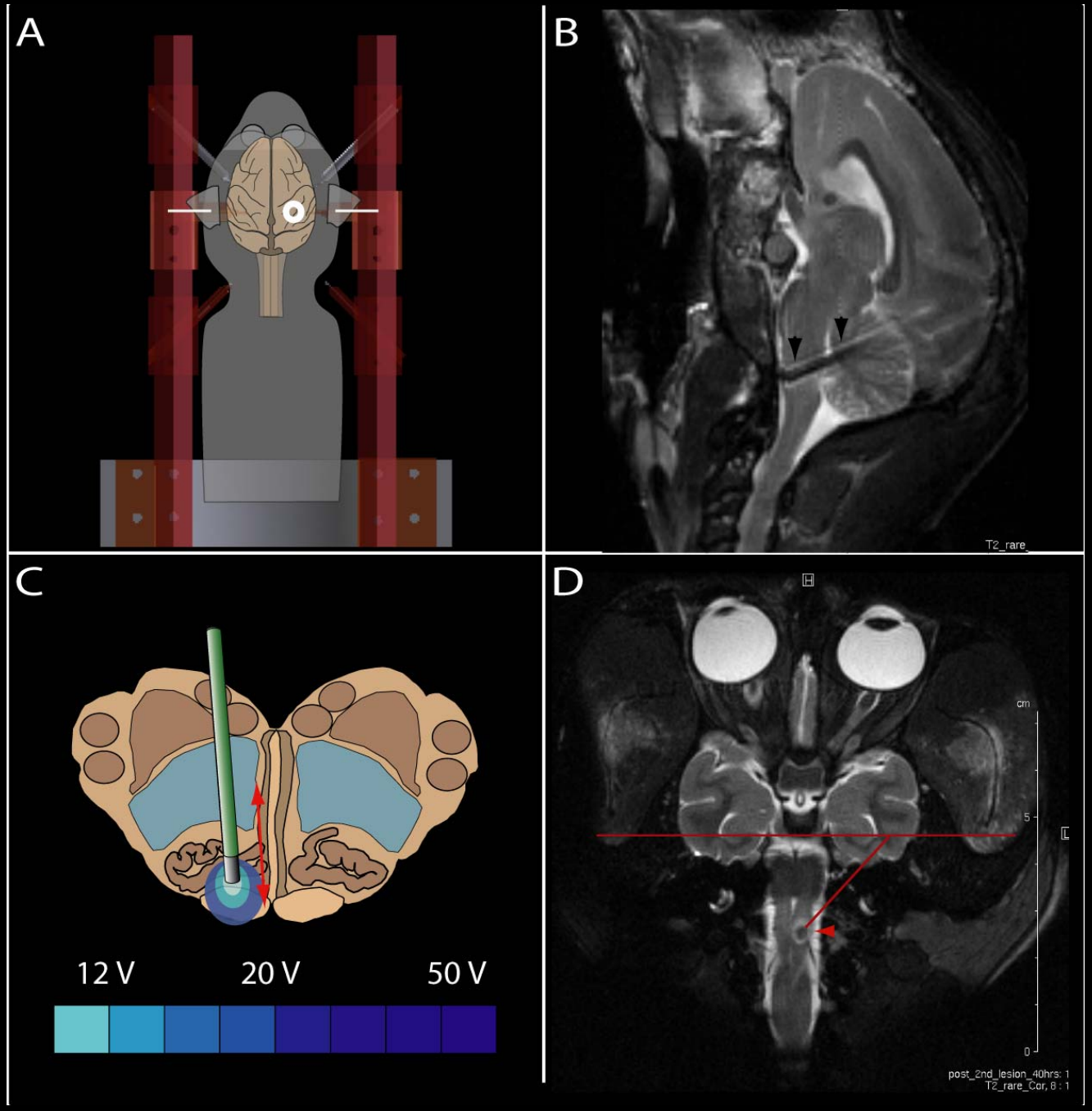
Cell injection procedure 1-2 years

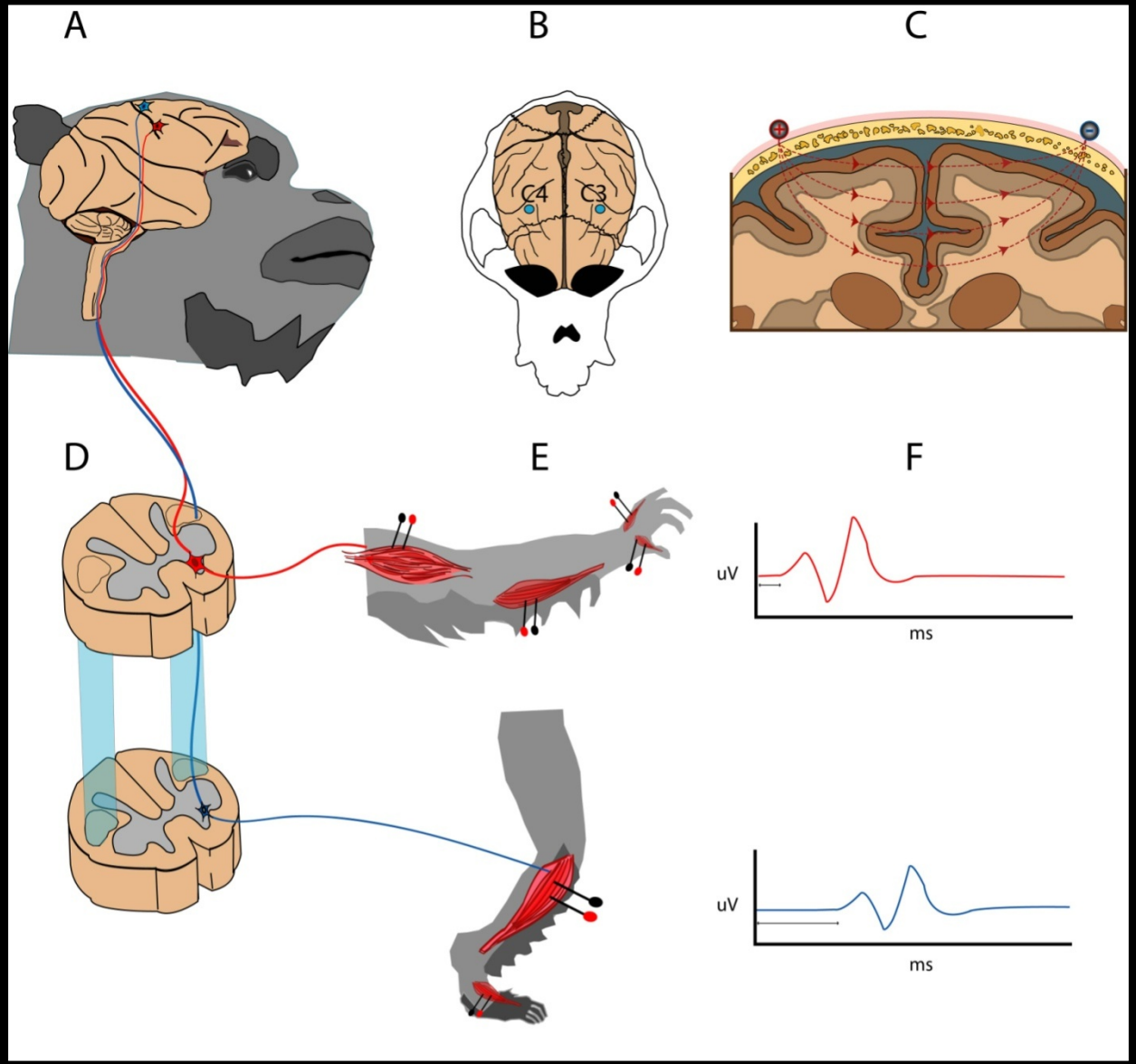
Pre- injection MRI, Peri- transplant MEP, cord stimulation, post-injection MRI, hand/arm and locomotor training and testing.

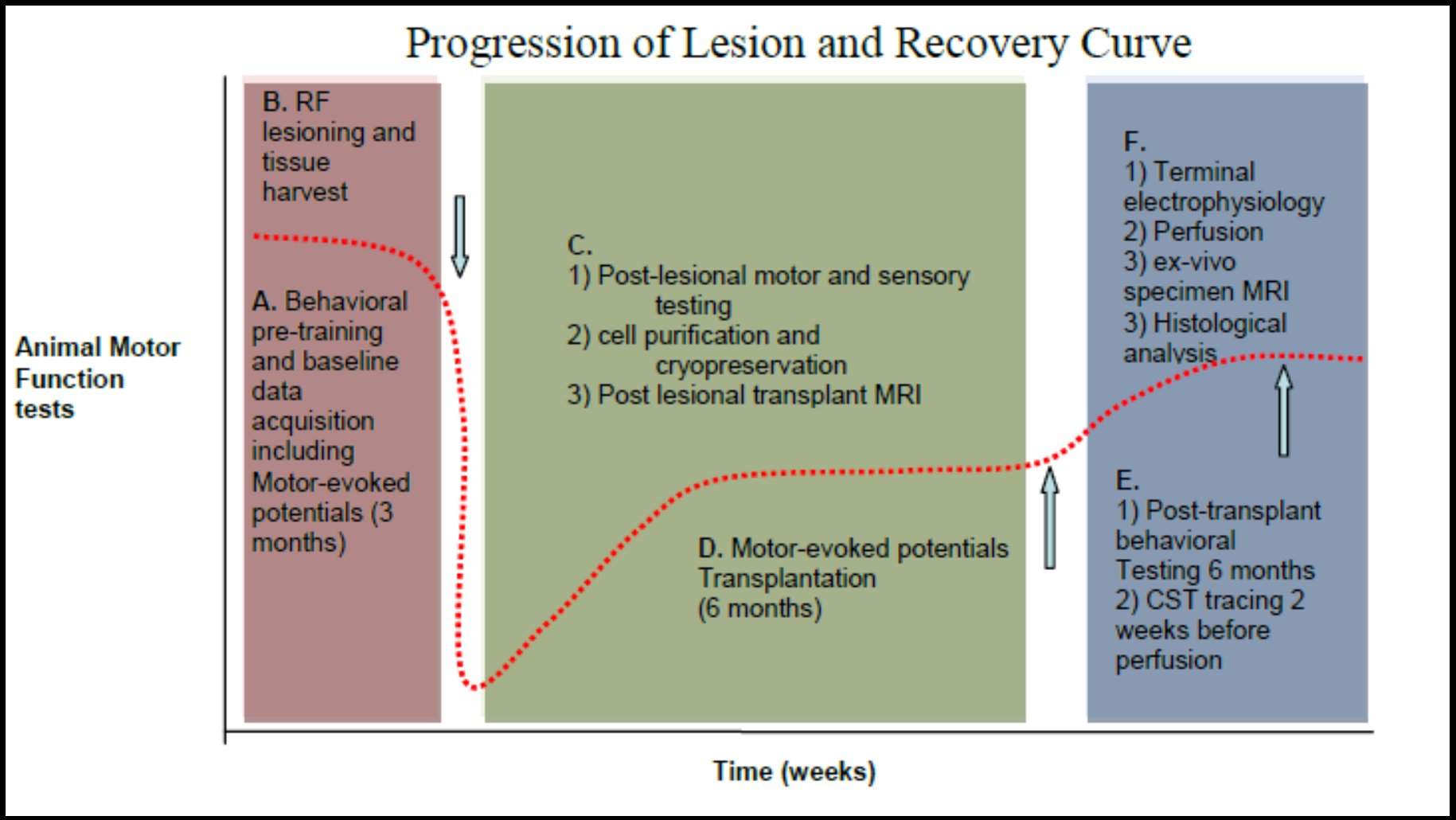
Perfusion

Histology

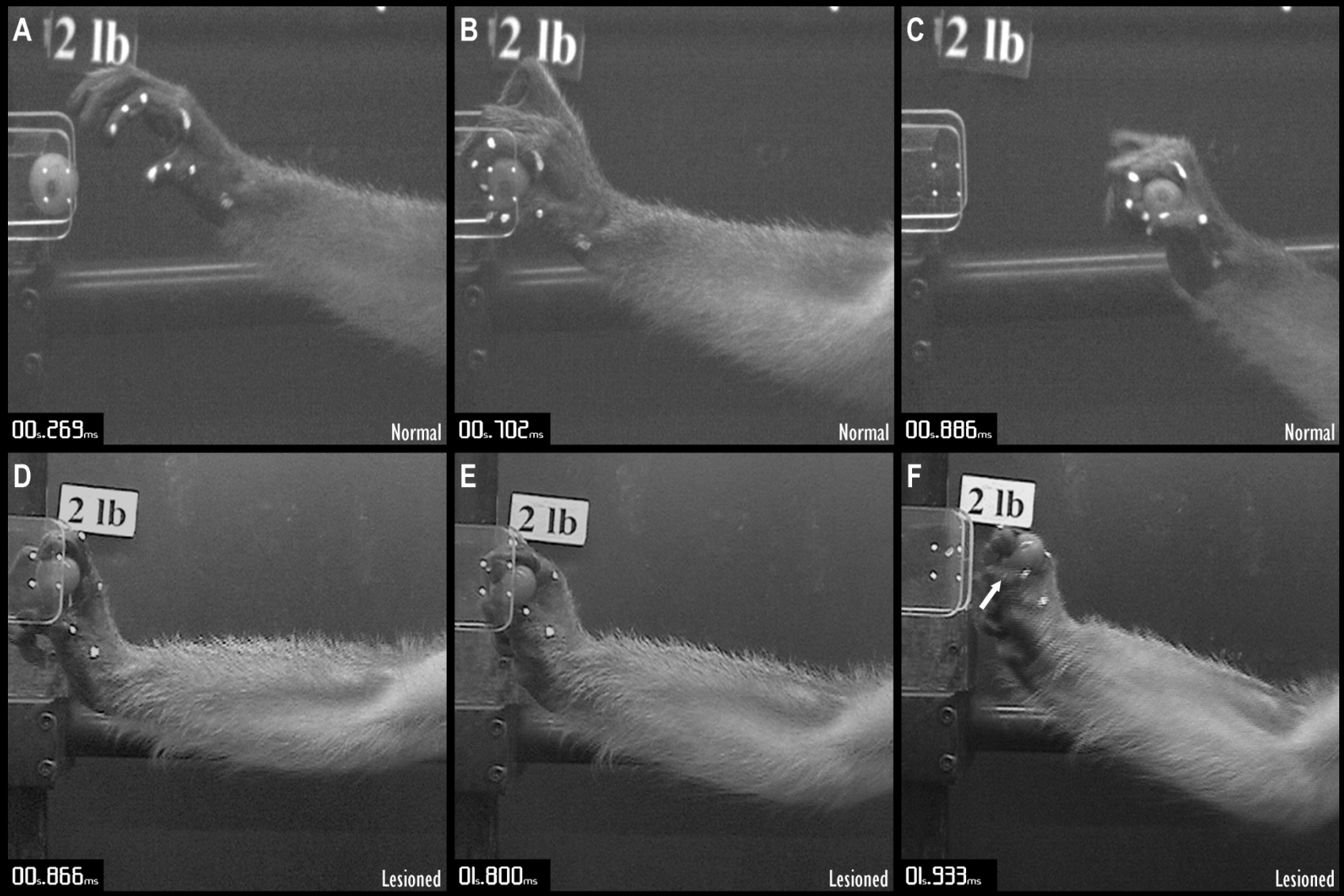

1 cm

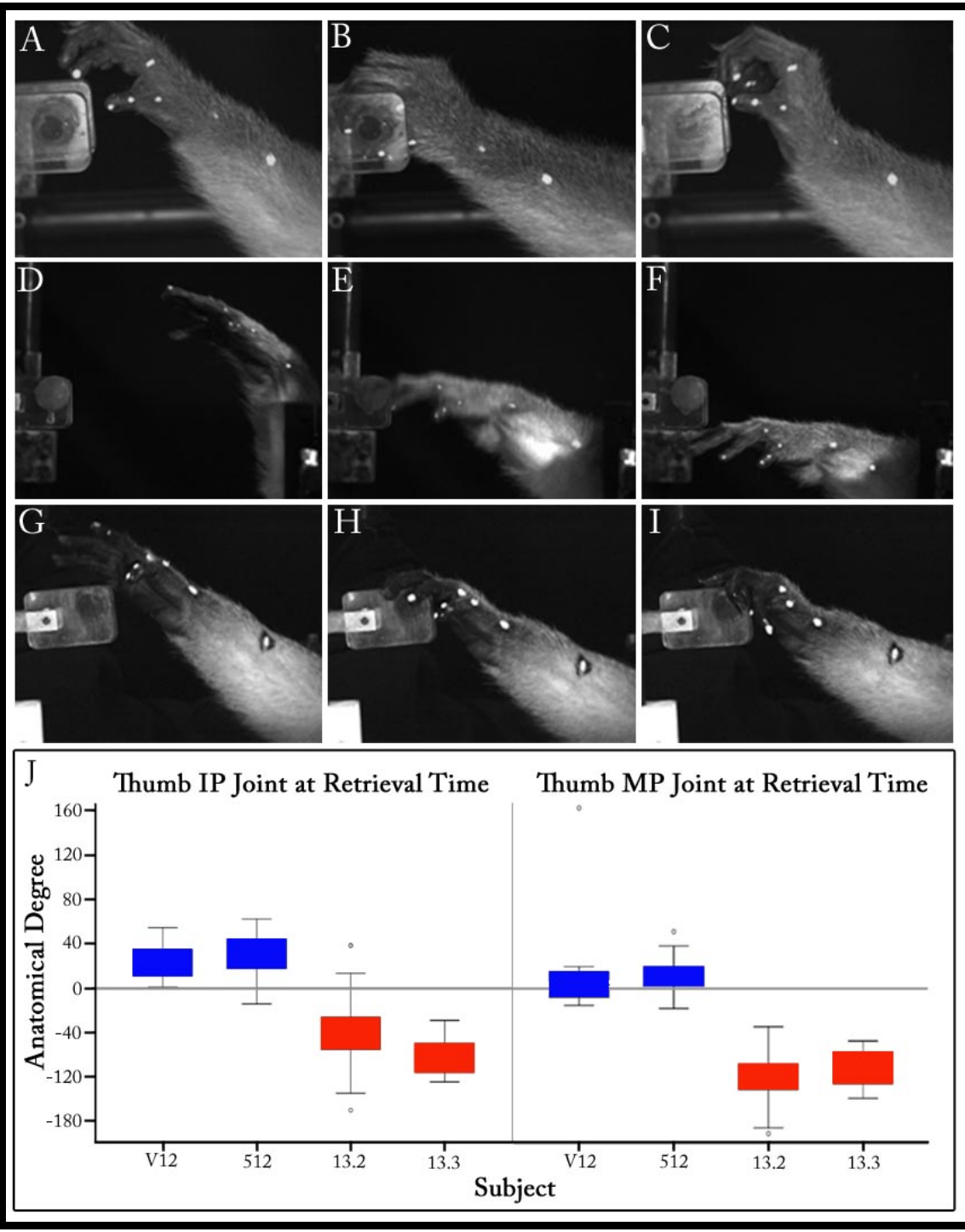

A
B
T2_rare_
C
12 V
20 V
50 V
D
cm
5
0
post_2nd_lesion_40hrs: 1
T2_rare_Cor, 8 : 1

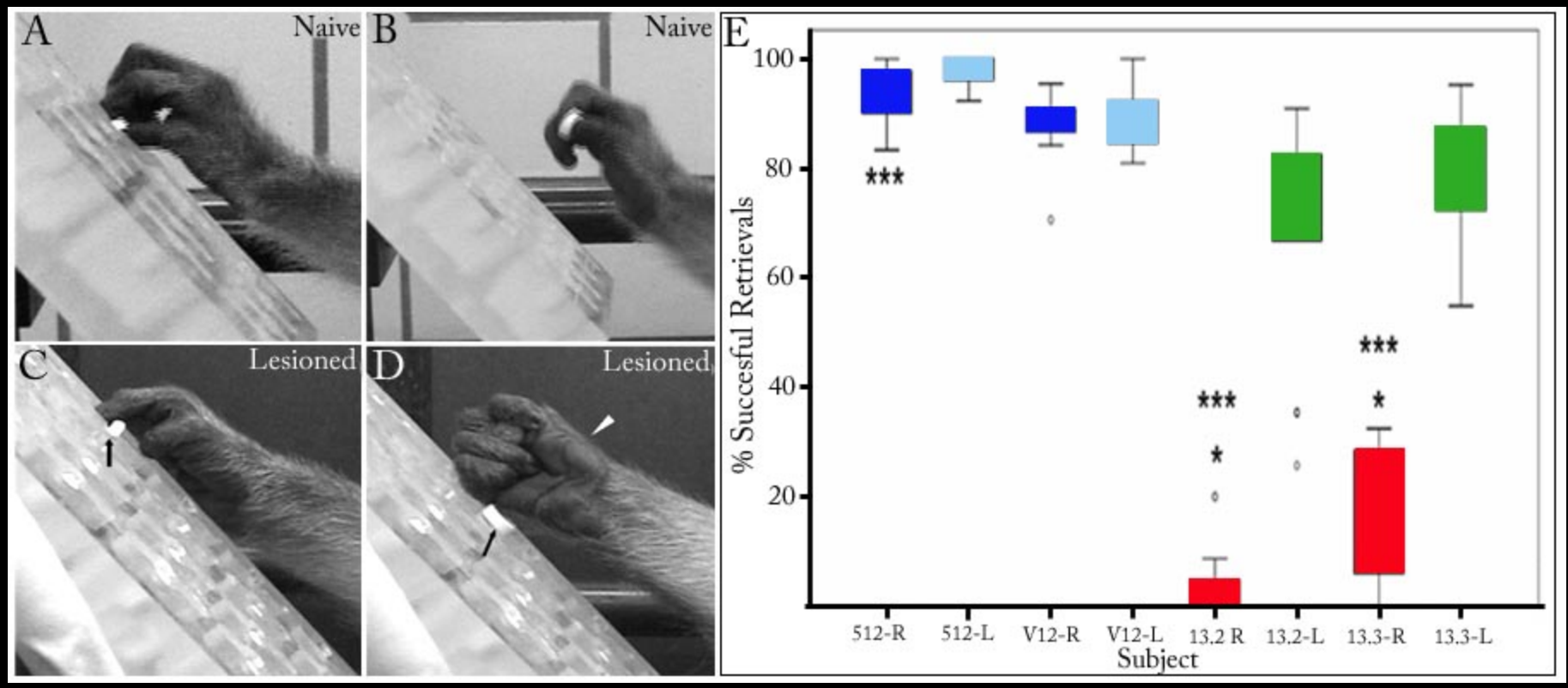

A
B
C
C4
C3
D
E
F
uV
ms
uV
ms


Progression of Lesion and Recovery Curve
Animal Motor Function tests
B. RF lesioning and tissue harvest
A. Behavioral pre-training and baseline data acquisition including Motor-evoked potentials (3 months)
C.
1) Post-lesional motor and sensory testing
2) cell purification and cryopreservation
3) Post lesional transplant MRI
D. Motor-evoked potentials Transplantation (6 months)
F.
1) Terminal electrophysiology
2) Perfusion
3) ex-vivo specimen MRI
3) Histological analysis
E.
1) Post-transplant behavioral Testing 6 months
2) CST tracing 2 weeks before perfusion
Time (weeks)

Primate hand function testing after C1 radiofrequency lesion of the corticospinal tract

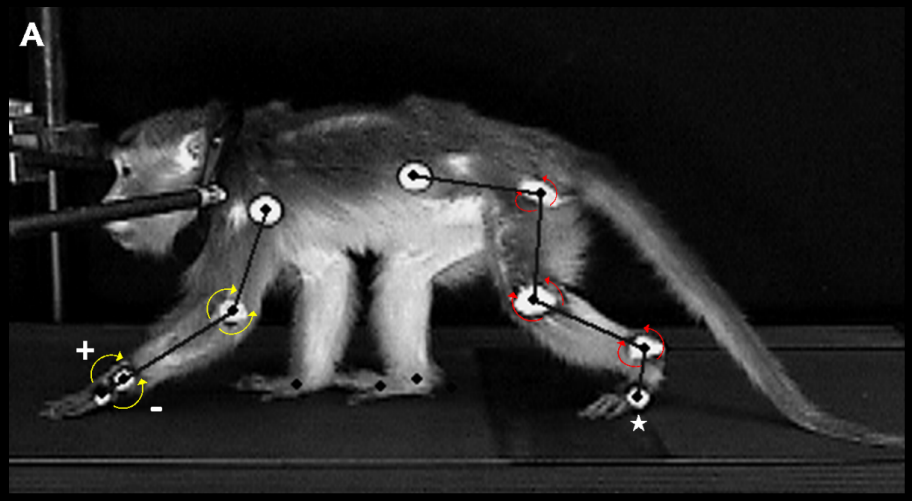


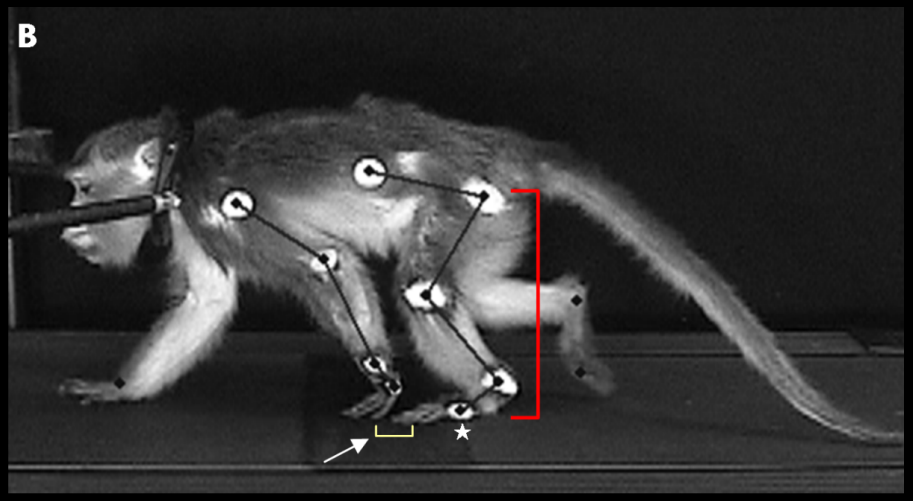

A
B
C
D
E
F
G
H
I
J
Thumb IP Joint at Retrieval Time
Thumb MP Joint at Retrieval Time
Anatomical Degree
160
120
80
40
0
-40
-120
-180
V12
512
13.2
13.3
V12
512
13.2
13.3
Subject


A
Naive
B
Naive
C
Lesioned
D
Lesioned
E
% Succesful Retrievals
100
80
60
40
20
***
***
*
***
*
512-R
512-L
V12-R
V12-L
13.2 R
13.2-L
13.3-R
13.3-L
Subject

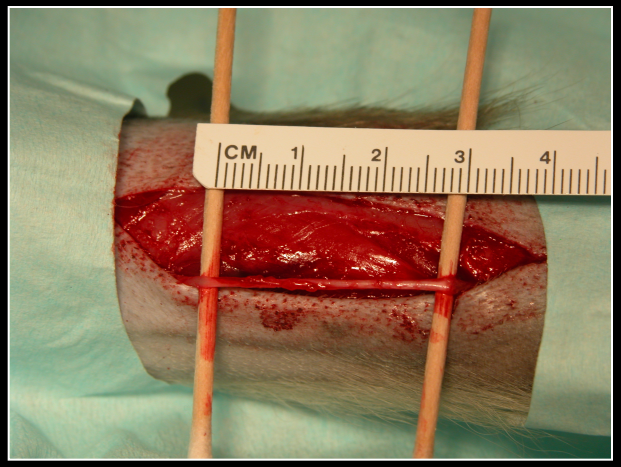
Autologous SC transplantation in chronic cervical injury in NHP

NHP 512 control



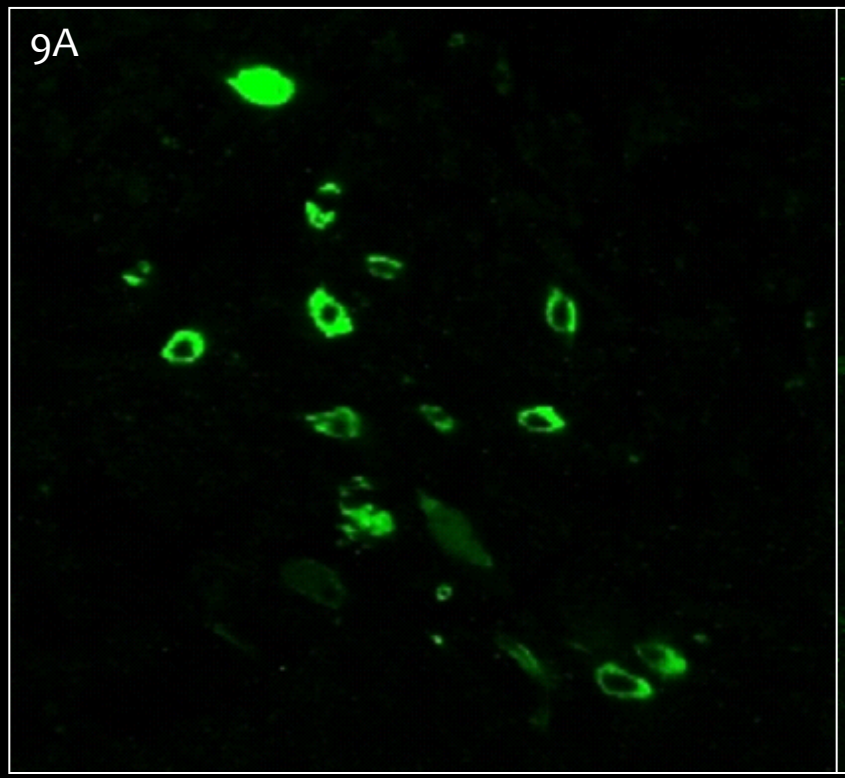


Primate 6. Sural nerve harvest

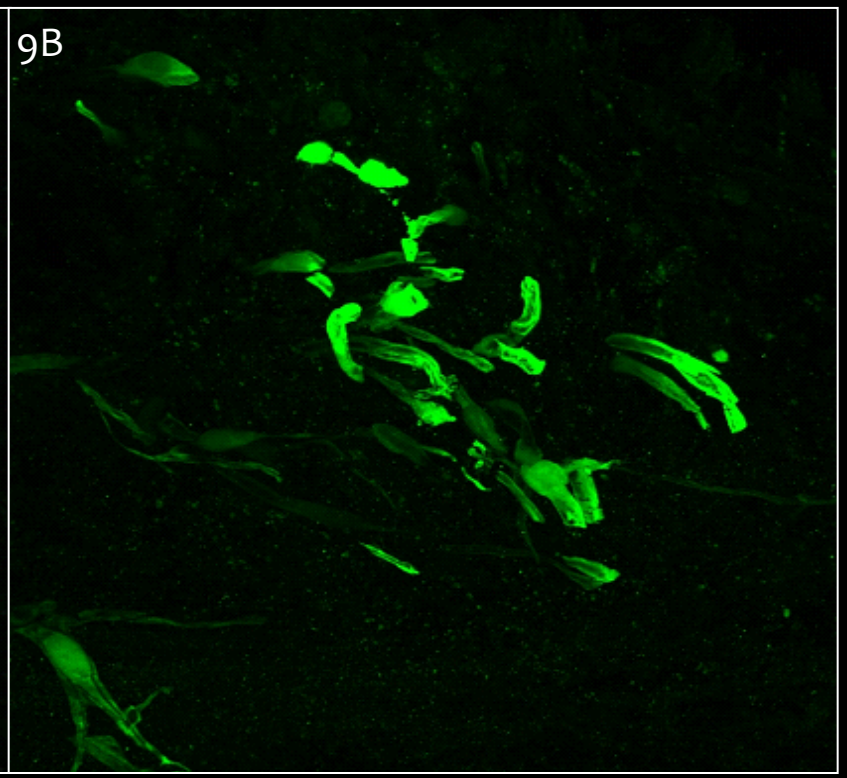


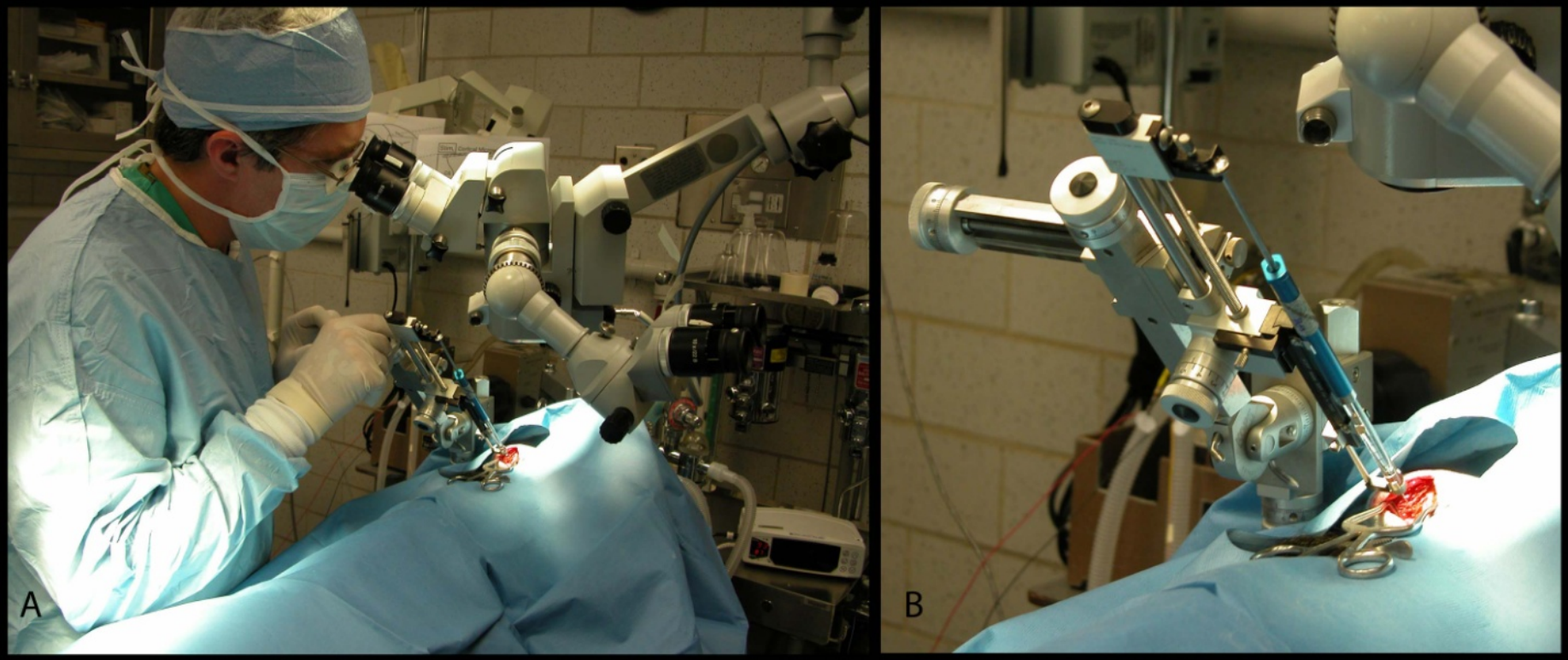
GFP +ve cells, 45d post-transplant.

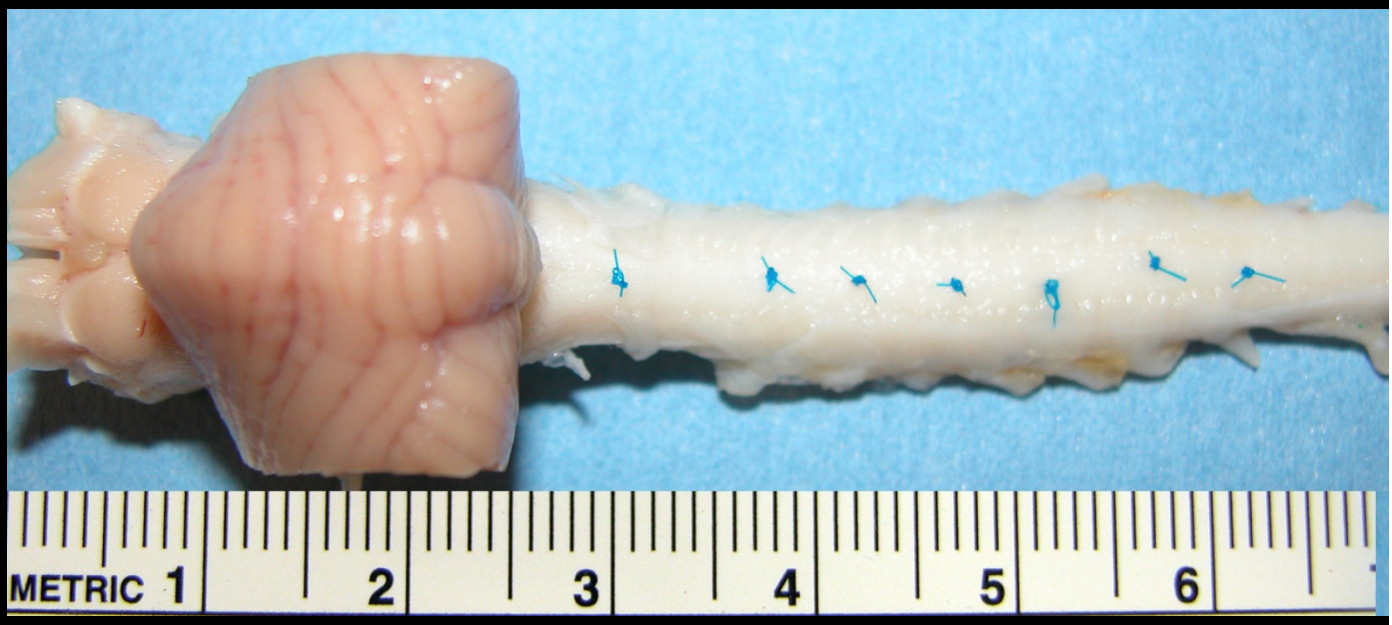
9A



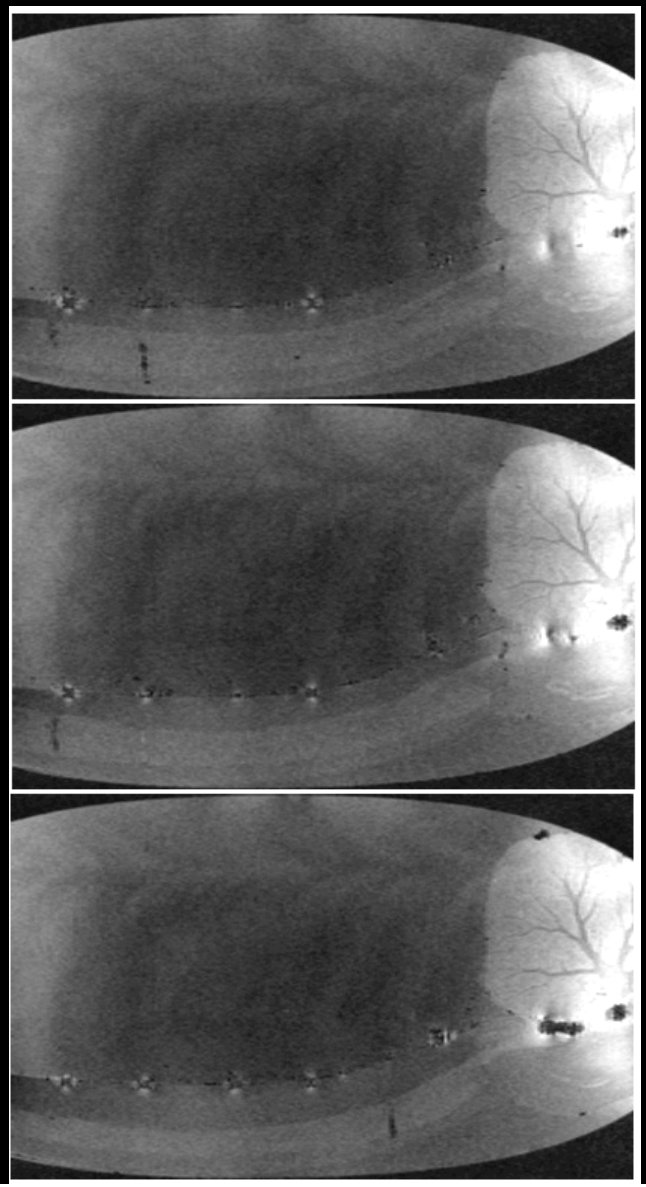
9B

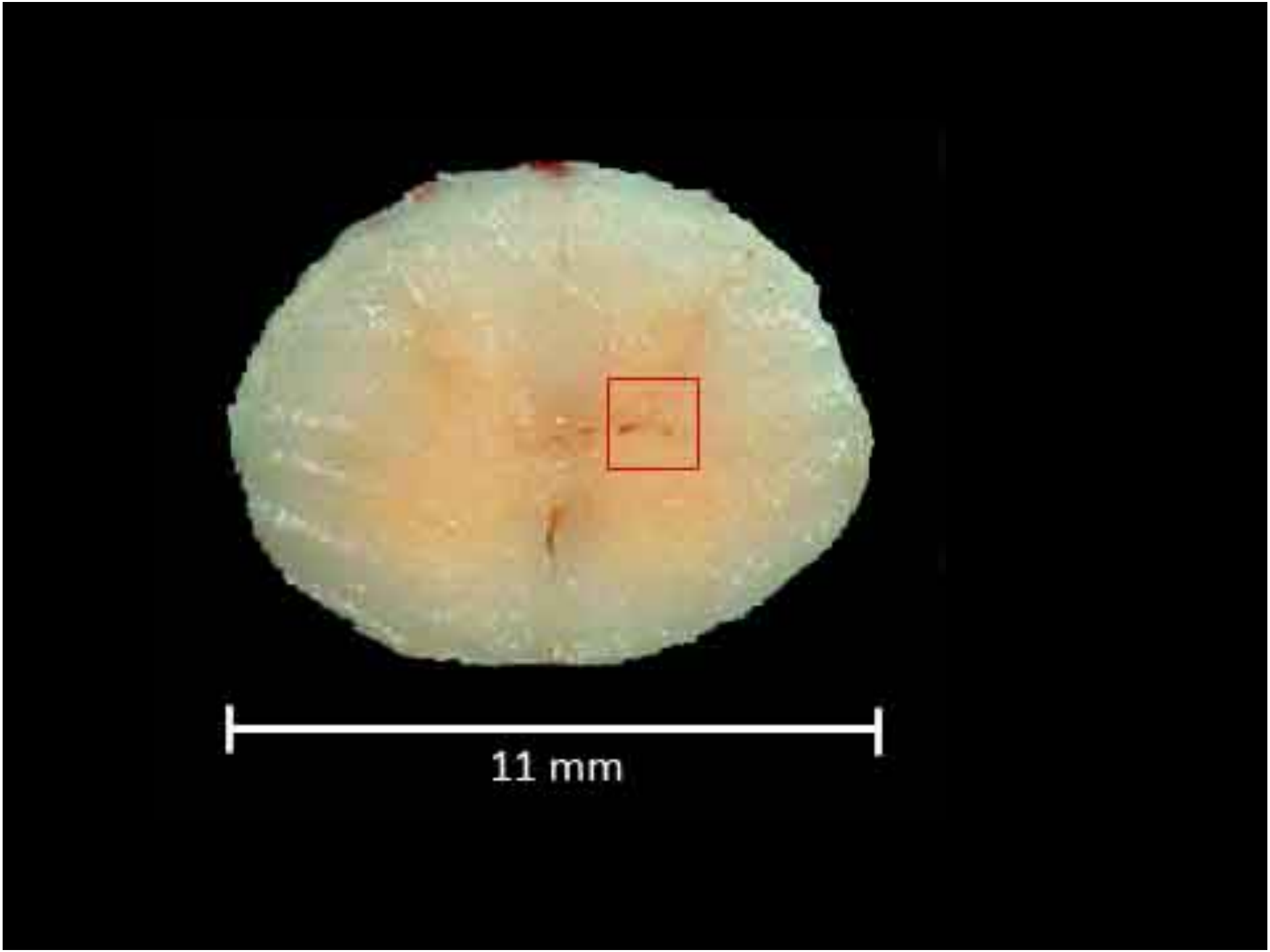


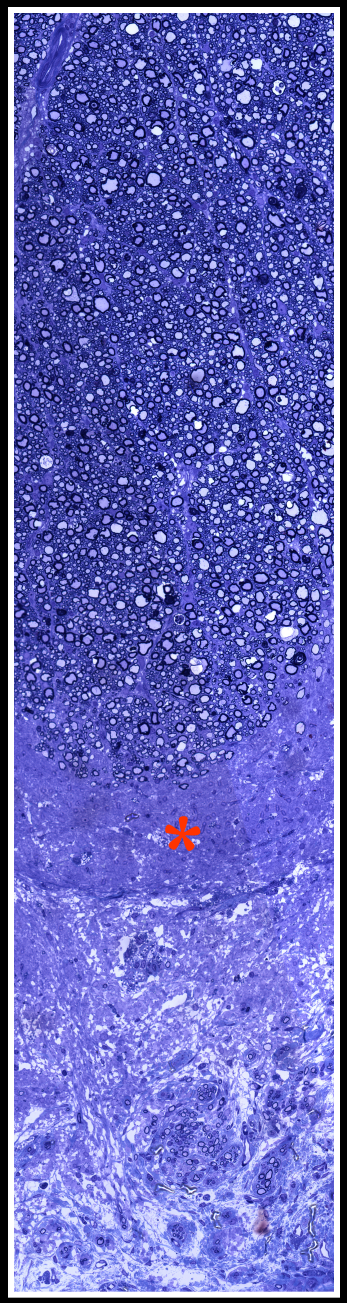

A
B

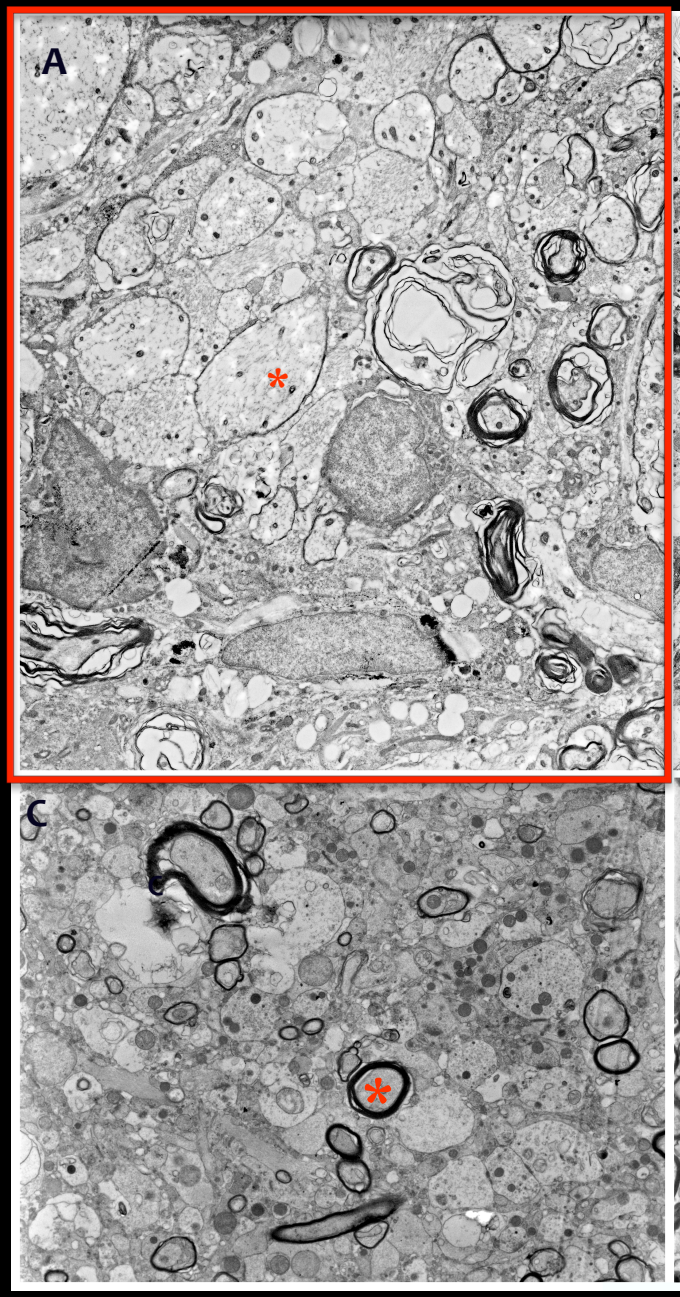

METRIC 1
2
3
4
5
6

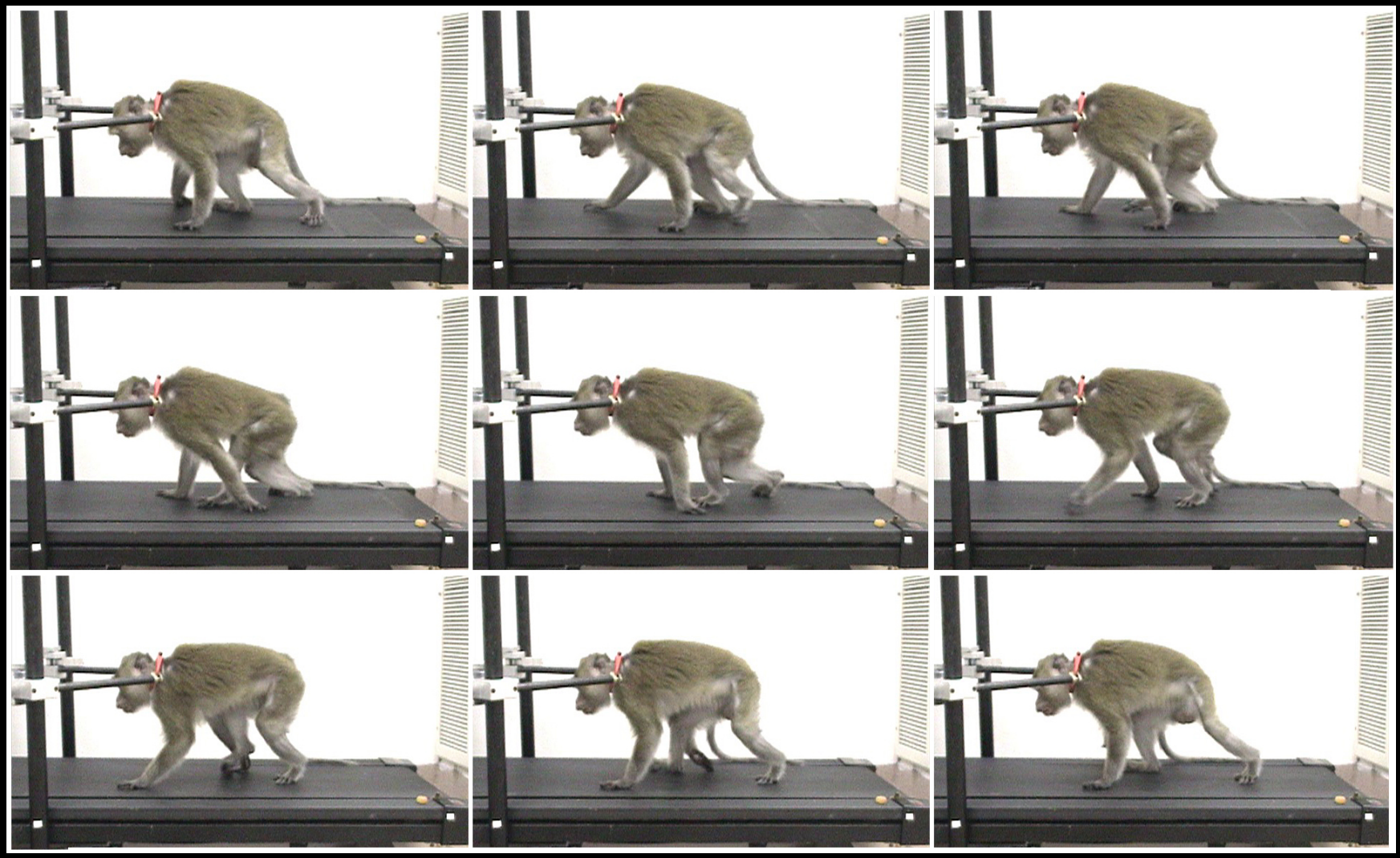
Ex vivo MRI of autologous Schwann cell trails 30D post-injection into the cervical spinal cord



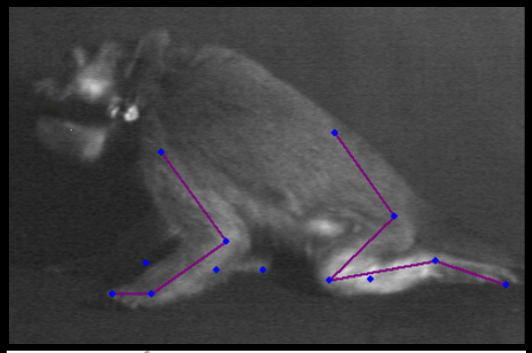

11 mm

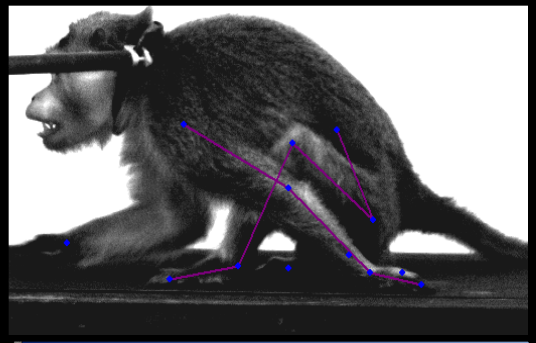


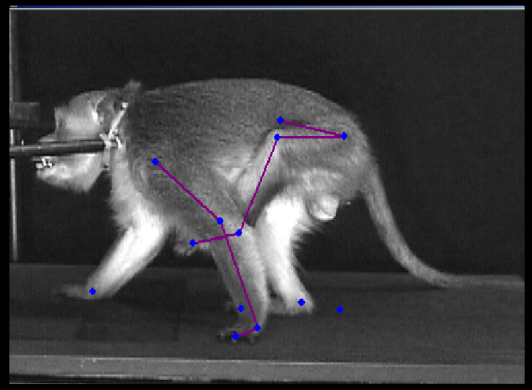

A
B
C
D

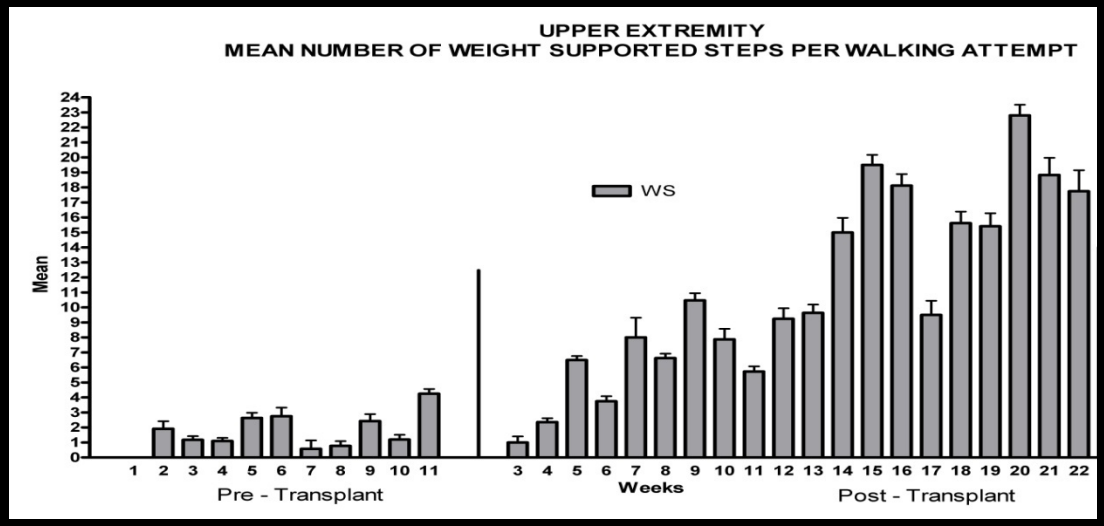


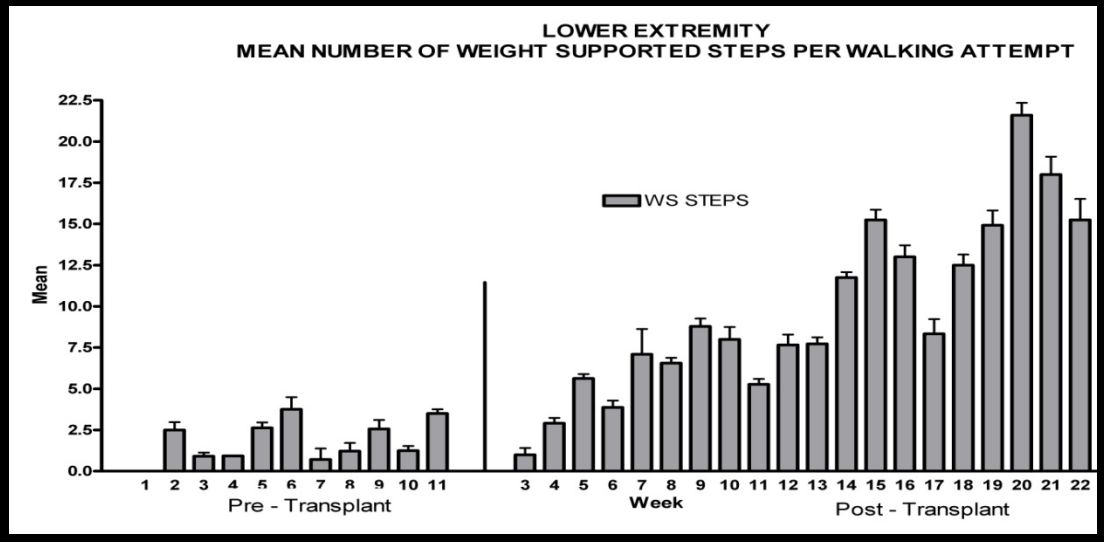
Gait assessment 2.3 years after RF lesion and 3 months after autologous SC transplantation



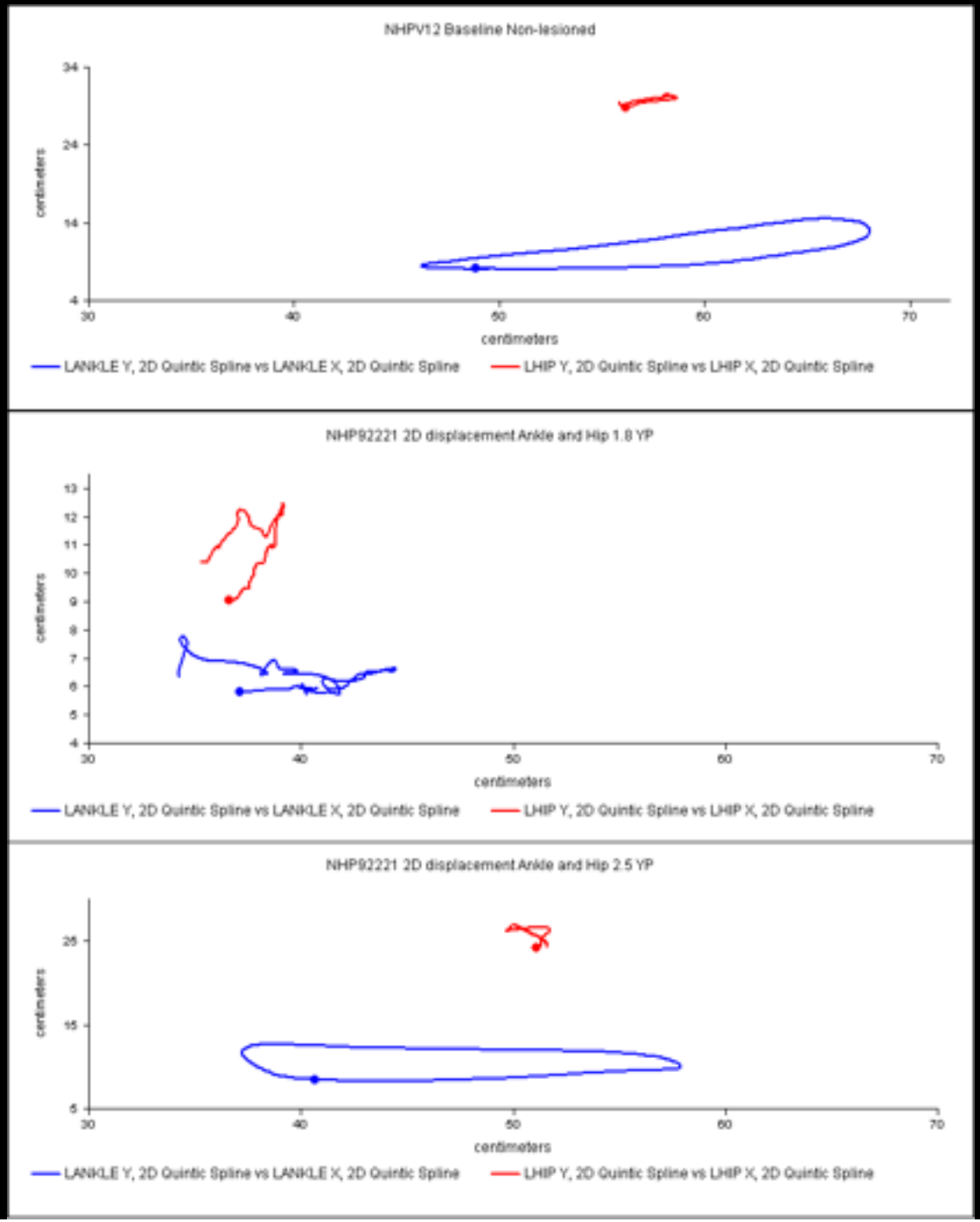




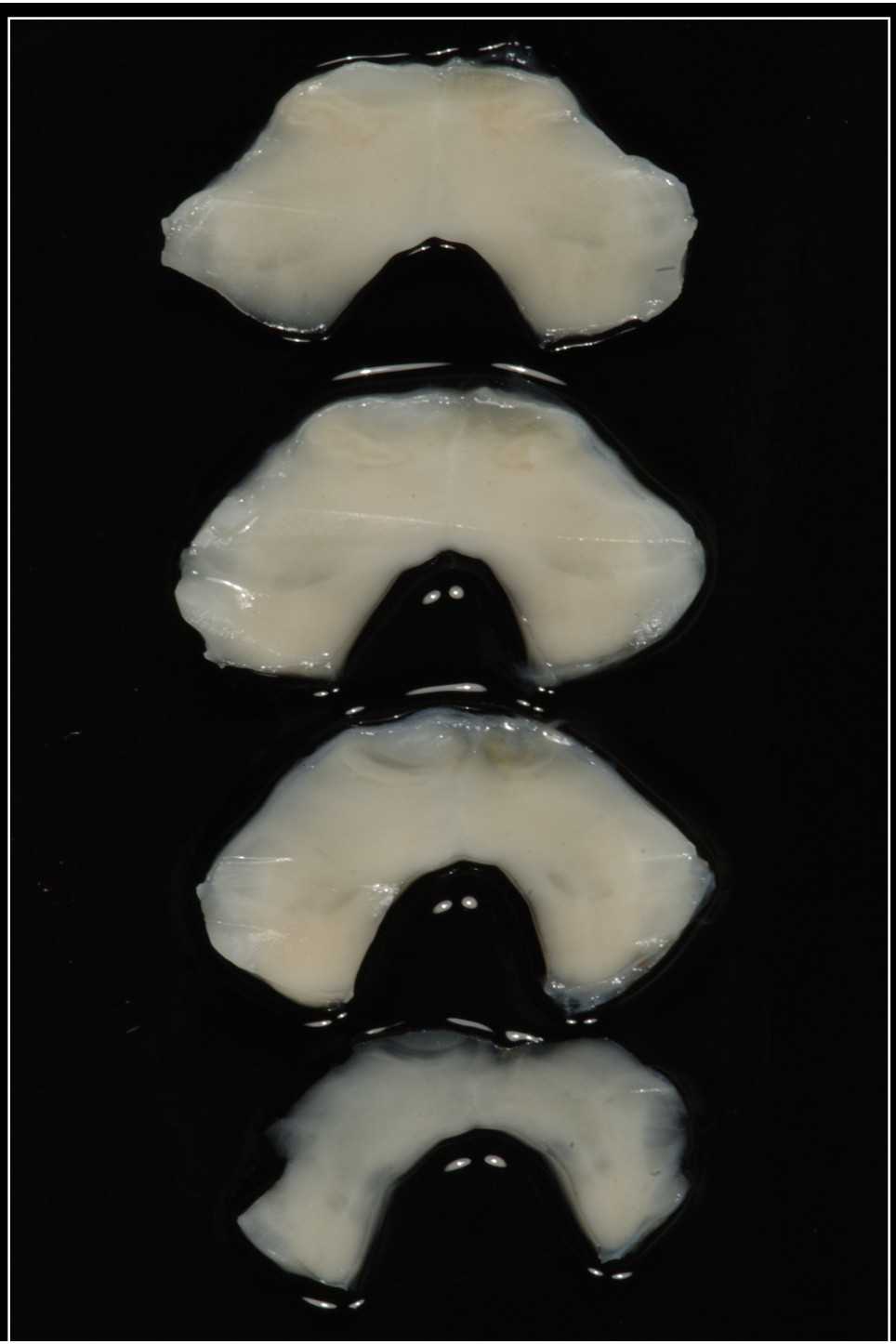


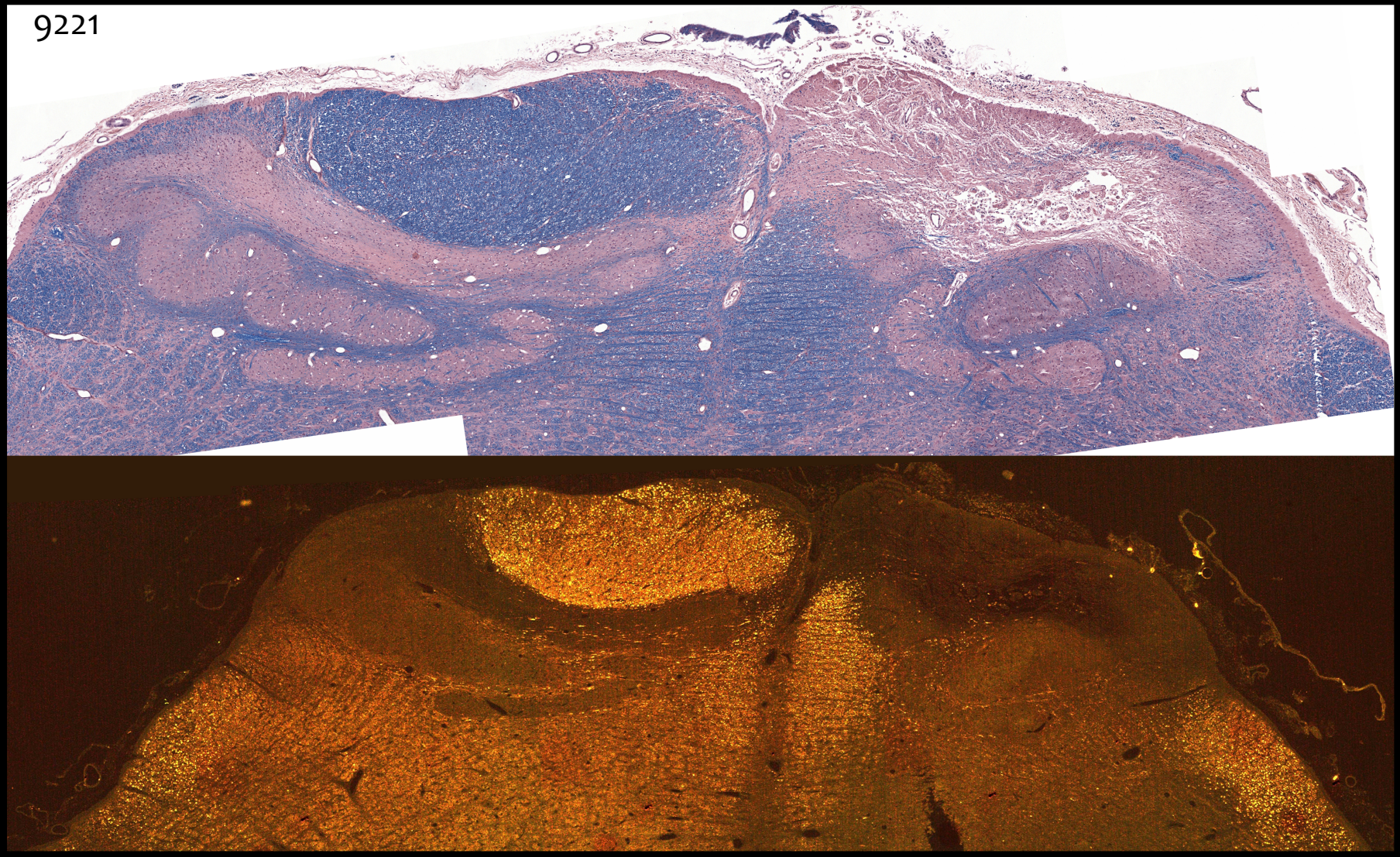


NHP 9221 transplanted 2.3 years post-injury

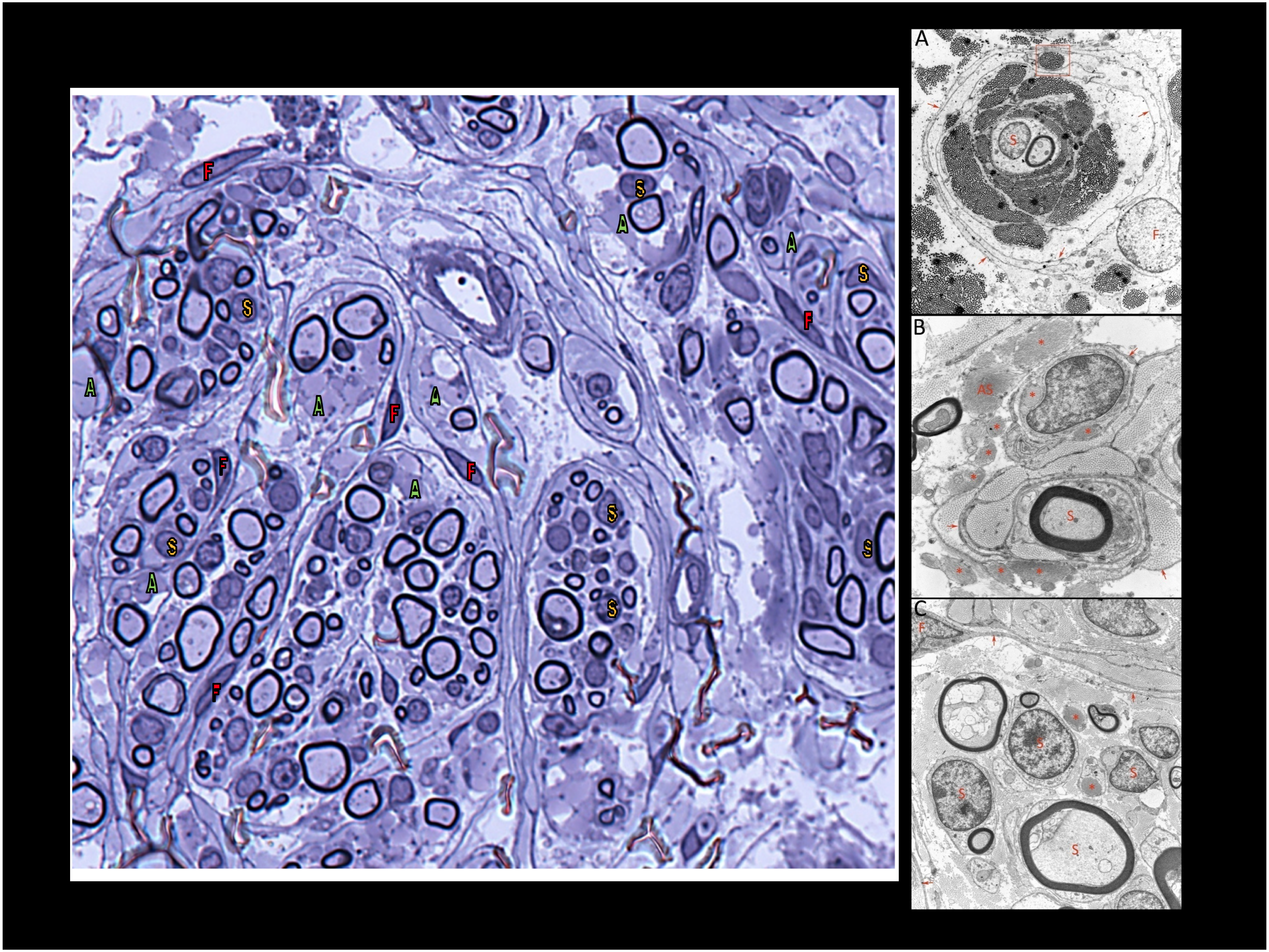

NHPV12 Baseline Non-lesioned
centimeters
34
24
14
4
30
40
50
60
70
centimeters
LANKLE Y, 2D Quintic Spline vs LANKLE X, 2D Quintic Spline
LHIP Y, 2D Quintic Spline vs LHIP X, 2D Quintic Spline
NHP92221 2D displacement Ankle and Hip 1.8 YP
centimeters
13
12
11
10
9
8
7
6
5
4
30
40
50
60
70
centimeters
LANKLE Y, 2D Quintic Spline vs LANKLE X, 2D Quintic Spline
LHIP Y, 2D Quintic Spline vs LHIP X, 2D Quintic Spline
NHP92221 2D displacement Ankle and Hip 2.5 YP
centimeters
25
15
5
30
40
50
60
70
centimeters
LANKLE Y, 2D Quintic Spline vs LANKLE X, 2D Quintic Spline
LHIP Y, 2D Quintic Spline vs LHIP X, 2D Quintic Spline

9221 post-perfusion

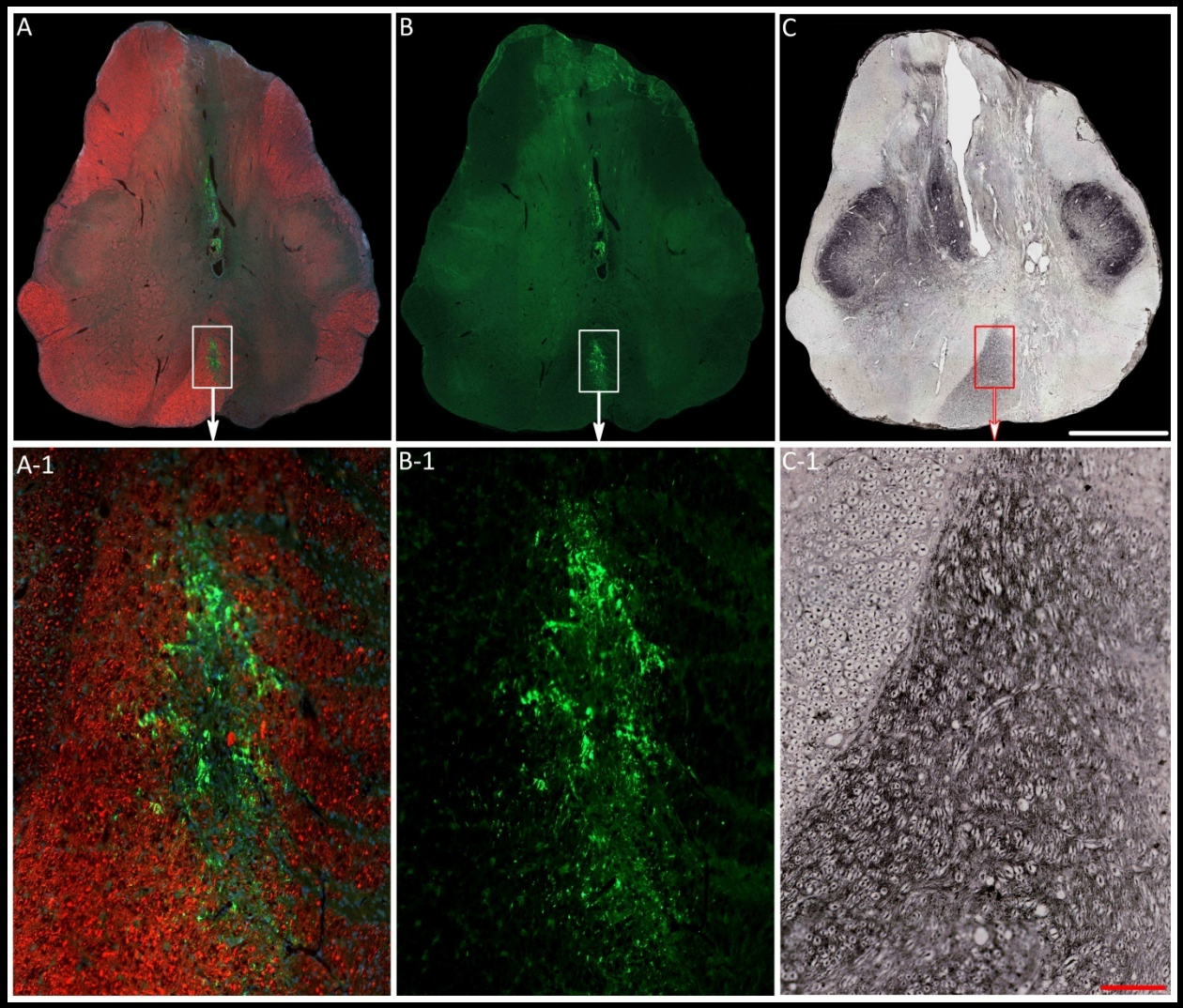




Anti-cam kinase II immunostaining



F
S
A
A
B
C
AS



Long term survival of GFP transplanted SC in the brainsteam of NHP 92221: A,B: anti Neurofilament red-594nm, anti GFP green-488nm, nuclei stained blue-Hoechst 33342; **C:** anti-CaM Kinase IIα nickel enhanced HRP precipitation. Once the animal reached a behavioral plateau, a transplant of aSCs was stereotaxically placed targeting the right pyramid after which primate presented improvement in its gait. Figures A, B show GFP positive signal at the pyramidal decussation 6 months after transplant (boxes). It is conceivable that improvement could be attributed to the activity of the SC including myelination. **C:** anti-CaM Kinase IIα specificity for the corticospinal tract stained on an adyacent section labeling the non lesioned left pyramid and its decussation confirm the SC localization. Bar C: 1500 µm, C-1:200 µm.

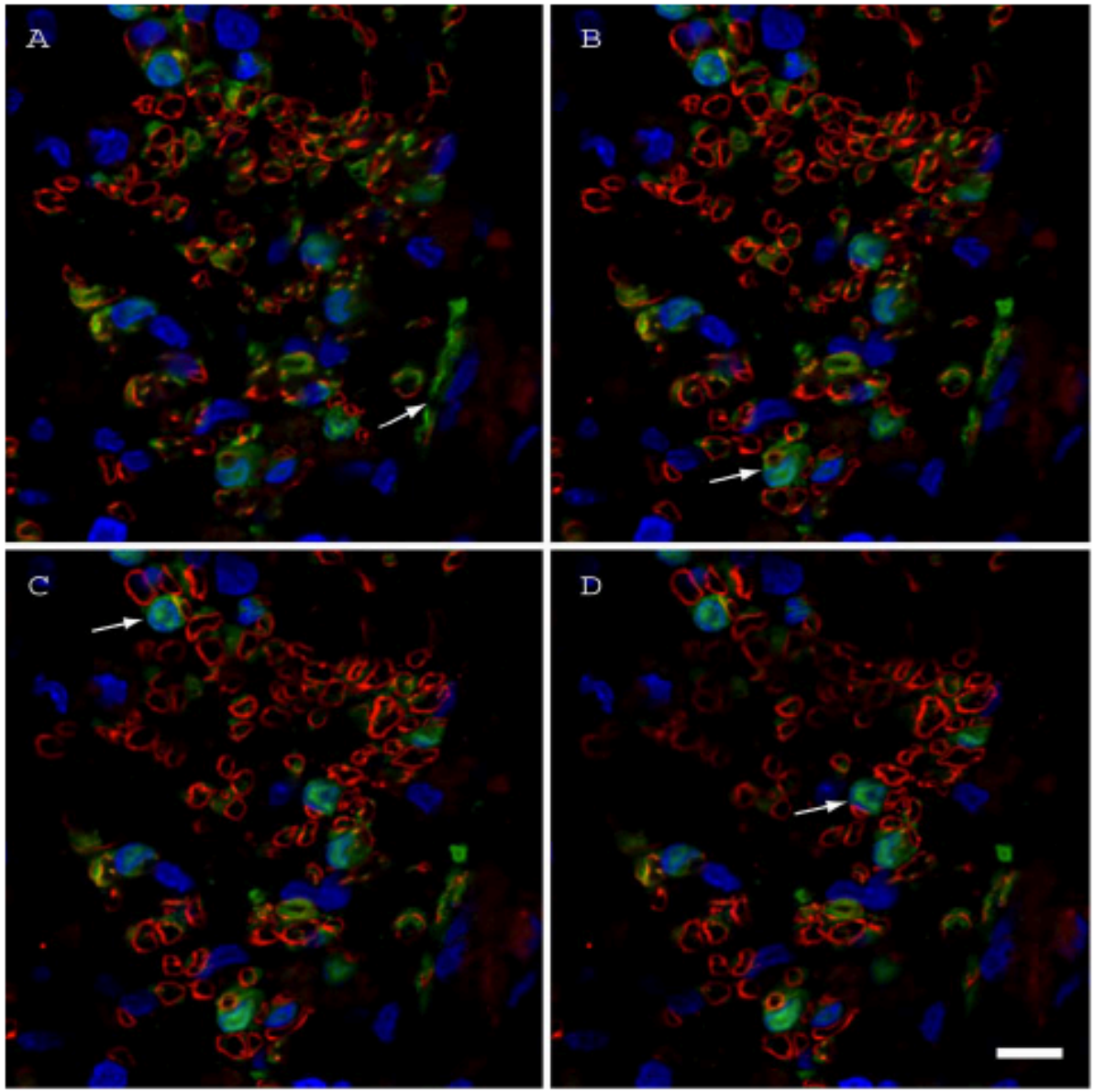


Figure 55. Myelination by transplanted SCs in NHP 92221. Confocal sequence. Micrographs, immunolabeled for green fluorescent protein, myelin protein zero (P0-red), and nuclei (Hoechst stain blue) . Several P0 positive rings are present that clearly colocalize with GFP+ve SC and their nuclei in a very characteristic signet ring appearance. The arrow in A pointing to an interrupted green longitudinal signal, aligned with P0 that resembles a node of Ranvier. Arrows in **B,C and D**, show several examples of signet ring colocalization classical for SC producing myelin. Scale bar 10um.

Human Schwann Cell Clinical Trial

Specific Aims

1. Conduct a Phase I clinical trial to determine whether or not there are any toxicities or other adverse effects produced by injecting the patient's own SCs into the spinal cord lesion.
2. Collect Safety and Efficacy data on a sample of 8 patients with complete (ASIA-A) SCI for submission in support of a Phase II randomized clinical trial.

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Design

* This Phase 1 clinical trial will employ an open label, unblinded, nonrandomized and non-placebo controlled dose-escalation design to evaluate the safety of transplantation of autologous human Schwann (ahSC) transplantation in subjects with subacute spinal cord injury (SCI).
* This will be a dose escalation study involving 3 dose cohorts.

Figure 1: Flow chart of trial design

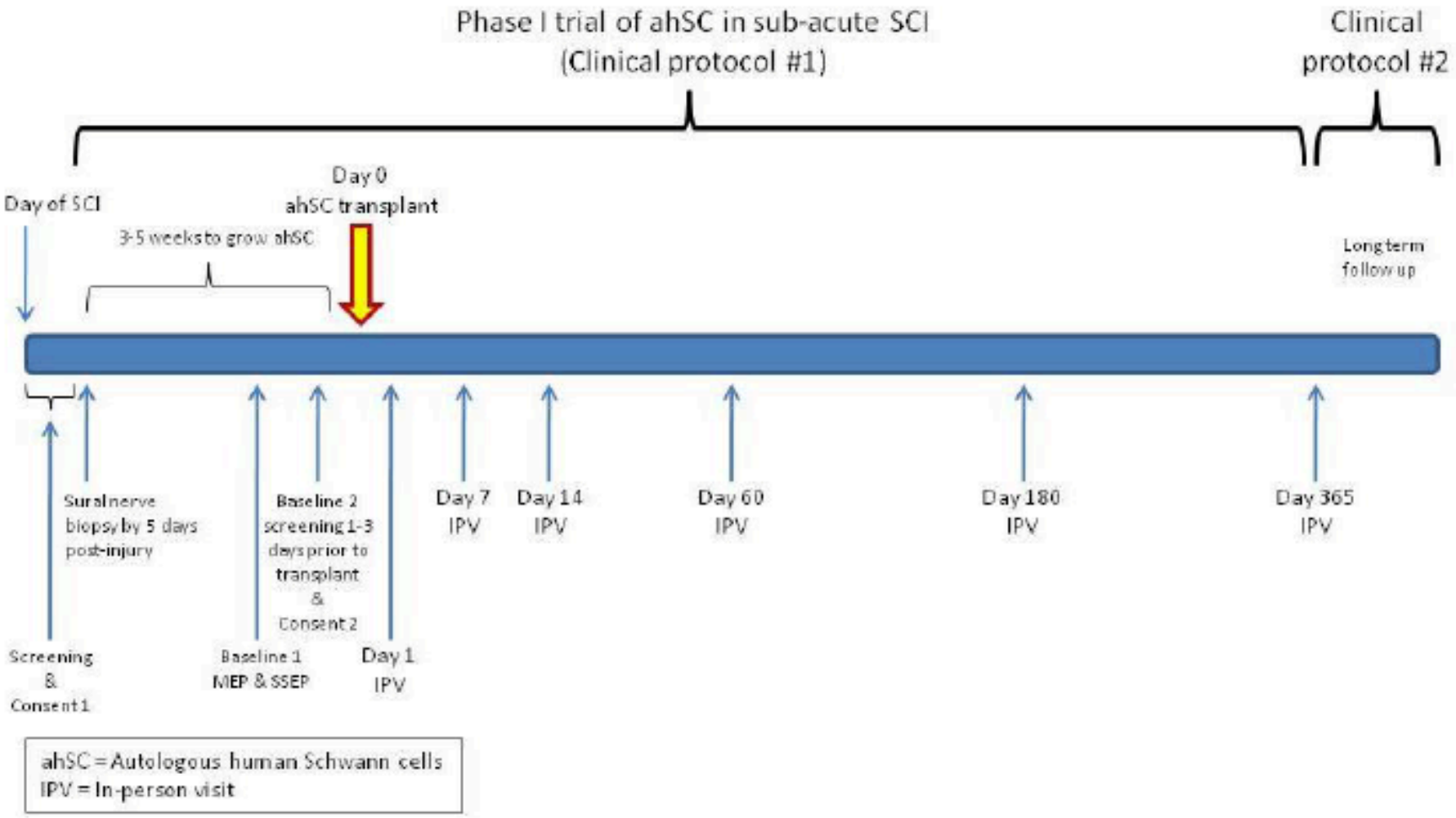
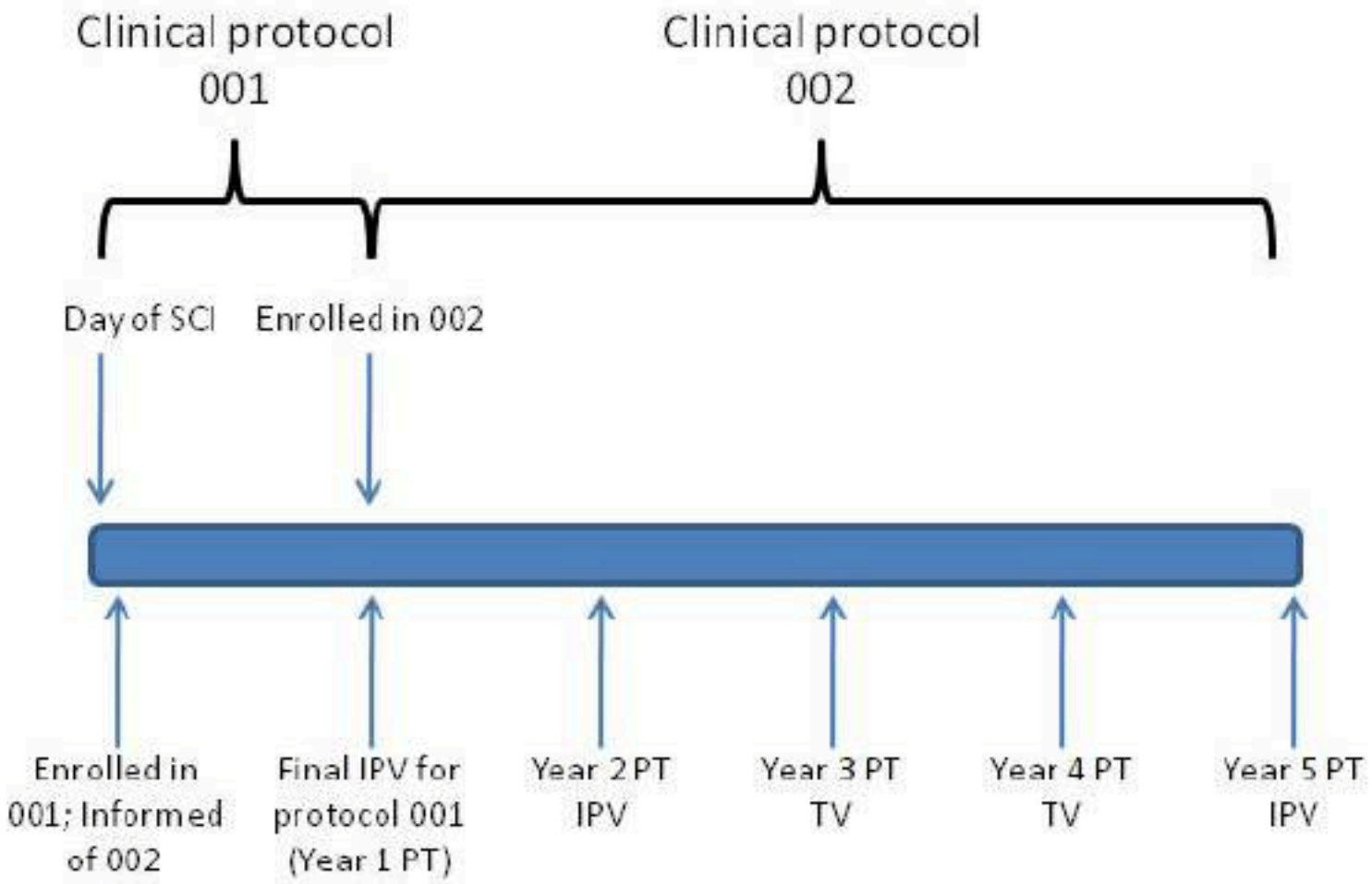


Figure 1: Flow chart of trial design



IPV = In-person visit
PT = Post-transplantation
TV = Telephone visit

12.2 *Dose to be studied:*

Cohort	Target dose (# cells)	# of injections	Total Volume	# subjects in cohort
1	5×10^6	1	50µl	2
2	10×10^6	1	100µl	3
3	15×10^6	1	150µl	3

Inclusion Criteria

1) The study will be open to patients who have either sustained a severe traumatic SCI. Subjects should meet the following criteria.

2) Between the ages of 18 and 50 at last birthday.

3) SCI at thoracic level between T3-T11, defined by the most caudal level of motor and sensory function on the ASIA scale.

4) Complete Grade A, as evaluated with (the Classification System and Prognosis factors for recovery of SCI) of the American Spinal Injury Association (ASIA).

5) Patient provides fully informed consent to obtain peripheral nerve biopsies, receive autotransplantation of SCs into a thoracic laminectomy performed under general anesthesia, and participate in a one-year follow-up to assess safety and efficacy outcomes and five year overall protocol.

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Main Outcome Measures

1. Serious adverse events
2. Successful autologous culture and transplantation
3. ASIA sensory and motor scores
4. Multiple secondary outcome measures include pain assessments.

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Key points

* Patient population (acute trauma in spinal cord (or TBI) versus subacute versus chronic disease); stage of disease for neurodegenerative disorders (e.g., early vs. advanced ALS, Parkinson's, Alzheimer's, Huntington's)
* Anatomic location
* Cell fate
* If it doesn't work, how do you figure out why?
* End points – e.g., is it walking or bladder/control of bodily functions for SCI
* Immune response and immunosuppression
* Dose & delivery
* Mechanism of action

Issues raised at our conference call

* In general the first-in-man clinical protocols are fairly conservative and tend to address a very severe end of the clinical spectrum of disease states
* Difficulty to show measurable change in severely injured subjects
* Can the efficiency of preclinical studies be improved
* How will data from clinical subjects influence preclinical requirements?

TRIAL OBJECTIVES AND PURPOSE

9.1 *Purpose/Objective:*

The purpose of this study is to assess the safety of ahSC transplantation in subjects with subacute SCI.

For humans with subacute SCI, we hypothesize that axons might show improved function if myelin repair is induced with the implantation of ahSC. In addition spinal cord cavitation may be reduced, and neural sprouting and plasticity may be enhanced.

9.2 *Core (null) hypothesis:*

We hypothesize that, in humans, implantation of autologous SC will not affect axonal function.

9.3 *Primary objectives:*

To evaluate the safety of ahSC administered at a single time-point to participants with SCI.

9.4 *Secondary objectives:*

To evaluate the potential trend towards efficacy of ahSC assessed by various neurological assessments and imaging methodology.

10.2 *Primary and secondary endpoints:*

10.2.1 Primary outcome measures for safety will include:

- Protocol compliance;
- Feasibility and;
- Adverse events.

10.2.2 Secondary outcome measures for safety will include:

- Emergence of clinically significant neuropathic pain no greater than expected for a historical cohort;
- Emergence of clinically significant muscle spasticity no greater than expected for a historical cohort;
- Absence of detectable mass lesion on MRI at 6 and 12 months follow-up.

10.2.3 Primary outcome measures for trend-toward efficacy will include:

- Assessment of motor and sensory function according to the ISNCSCI, including motor function assessment and light touch and pin prick sensation;
- Determination if there is a conversion of ISNCSCI from A to a higher grade.

10.2.4 Secondary outcome measures for trend-toward efficacy will include:

- Bowel and bladder function;
- Parameters of autonomic function;
- Quality of life improvement.

11.1 *Subject inclusion criteria:*

- 11.1.1 Persons with traumatic SCI that occurred within the previous 5 days;
- 11.1.2 Between the ages of 18 and 50 years at last birthday;
- 11.1.3 SCI at thoracic level between T3-T11 as defined by MRI and the most caudal level of intact motor and sensory function on the International Standards for Neurological Classifications of Spinal Cord Injury (ISNCSCI) scale;
- 11.1.4 Acute SCI with ISNCSCI grade A impairment at the time of enrollment.

11.2 *Subject exclusion criteria:*

- 11.2.1 Any failure to meet above criteria;
- 11.2.2 Persons with penetrating injury of the spinal cord or complete transection of the cord, including bone fragment lacerations, as identified by MRI;
- 11.2.3 Persons with a lesion in the conus medullaris, cauda equine, or lower extremity peripheral nerve;
- 11.2.4 Persons unable to safely undergo an MRI;
- 11.2.5 Persons in whom adequate MRI imaging cannot be obtained;
- 11.2.6 Persons who have developed a PE or a DVT;
- 11.2.7 Other traumatic injuries (e.g. CHI, another level of SCI) affecting the ability to provide informed consent and participate fully in rehabilitation;
- 11.2.8 Persons with self-reported persistent severe neuropathic pain, inadequately controlled by non-narcotic medication;

11.2.9 Persons with severe persistent mechanical or thermal hypersensitivity/allodynia at the neurological level or rostral to it as documented by clinical testing;

11.2.10 Pregnant women or a positive pregnancy test in those women with reproductive potential prior to enrollment;

11.2.11 Presence of systemic disease that might interfere with subject safety, compliance or evaluation of the condition under study;

11.2.12 Presence of any unstable medical or psychiatric condition that could reasonably be expected to subject the participant to unwarranted risk from participation in the study or result in a significant deterioration of his/her clinical course;

11.2.13 Body Mass Index (BMI)> 35;

11.2.14 History of active substance abuse;

11.2.15 Persons who have participated in other experimental treatments within the past 90 days deemed by the PI to represent a possible confound or enrolled in any other ongoing trial;

11.2.16 Persons with significant lower extremity injury, previous surgery, or amputation such that would preclude satisfactory sural nerve harvest;

11.2.17 Persons allergic to gentamicin;

11.2.18 Persons who test positive for HIV or Hepatitis B or C virus;

11.2.19 Baseline entry criteria for renal function, CBC, INR, and liver tests including serum albumin, total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphastase (ALP), and gamma glutamyl transpeptidase (GGT).

11.3 *Major exclusion criteria, transplant procedure:*

11.3.1 Persons with acute SCI whose ISNCSCI grade improves between initial admission and the scheduled implantation procedure, and are no longer classified as ISNCSCI A.

11.3.2 Presence of reproducible, valid MEP or SSEP signals below the level of injury within 2 to 5 days prior to transplantation;

11.3.3 Failure to obtain cultured SC that meet study criteria;

11.3.4 Active medical conditions precluding safe transplantation;

11.3.5 Unresolved post-surgical wound infection;

11.3.6 Ascending neurologic injury by three or more levels, persisting for one week, prior to transplant procedure;

11.3.7 Presence of sacral or ischial pressure sore, greater than stage II.

7.3.2 Donor Sural Nerve Collection

Participants enrolled in this phase I clinical trial will undergo a sural nerve harvest procedure at UM/JMH, pre-transplant. Upon enrollment, a minor surgery for collection of a portion of the peripheral (sural) nerve from the leg of the participant will be scheduled and initiated. The quantity of nerve collected, 10-20 cm, is a sufficient source of tissue for seeding the culture, while minimizing the disruption of standard medical care for participants. Each sural nerve harvest procedure will take <1 hour.

The harvested nerve will be transported in a sterile container with Belzer's transport media (on ice) to the Cell Processing Facility. Upon receipt, at the Cell Processing Facility, the nerve will be confirmed to meet Good Tissue Practices (21 CFR 1271) based screening criteria before processing.

Dose Enrollment Schedule

* First dose cohort 2 subjects receive a single 50µl injection of 5 million ahSC each.
* Waiting period of one month between each subject to observe for evidence of adverse effects.
* If no clinically significant transplant-related adverse events are observed for this cohort, then the second dose cohort will be initiated (n=3).
* First subject will receive a single 100µl injection of 10 million ahSC and be observed for one month.
* If no adverse events are observed, the second and third subjects in this cohort will receive the same dose, waiting one month between each subject.
* If none of these three subjects demonstrates clinically significant transplant-related adverse events, the third dose cohort will be initiated (n=3).
* The first subject in that cohort will receive a single 150µl injection of 15 million ahSC. The rate of enrollment for the remainder of the study will be one participant per month, provided that no transplant-related adverse events emerge.

Rehabilitation procedures:

* Subjects will undergo standardized assessment and conventional rehabilitation.

Follow-up assessments (post ahSC transplantation) include:

* - AE assessment, vital signs, transplantation and biopsy sites evaluation: at 1 day, 2-3 days, 1 week, 2 weeks, 2 months, 6months, 12 months
* - ISNCSCI exam: at 2-3 days, 1 week, 2 weeks, 2 months, 6 months, 12 months to monitor for any deterioration in neurological function;
* - Neurophysiologic assessments (MEP, SSEP): at 2 months, 6 months, and 12 months;
* - Pain assessments: at 1 week, 2 months, 6 months, 12 months will assess for development of, or changes in neuropathic pain;
* - MRI scans: at 1 day post-transplantation, 6 months and 12 months to assess for contusion size and location, signal changes associated with transplantation, tumorigenesis, and any neurological deterioration;
* - Functional assessments: at 2 weeks, 2 months, 6 months, 12 months;
* - QoL assessments: at 2 months, 6 months, 12 months;
* - Autonomic assessments: at 2 months, 6 months, 12 months
* - Serum chemistry panel: at 6 months, 12 months
* - Interview with clinical psychologist: at 6 months, 12 months.

Assessment of Safety

* Participants will be monitored throughout a one (1) year evaluation period (assessments will be performed at days 1-3 post-transplantation, weeks 1 and 2 post-transplantation, and months 2, 6, and 12 post-transplantation) for occurrence of AEs (acute, delayed, and/or cumulative), as well as for changes in clinical status, neurological status, and/or functional status. Safety will be determined using the following assessments: AEs, discontinuation due to AEs, physical examinations, vital signs, and laboratory determinations.

Major Inclusion Criteria:

* Persons with traumatic SCI that occurred within the previous 5 days.
* Between the ages of 18 and 50 at last birthday.
* SCI at a thoracic level between T3-T11 as defined by MRI and the most caudal level of intact motor and sensory function on the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI).
* Acute SCI with ISNCSCI grade A impairment at time of enrollment.

Major Exclusion Criteria, admission to study:

* Persons with penetrating injury of the spinal cord or complete transection of the cord, including bone fragment lacerations, as identified by magnetic resonance imaging (MRI).
* Persons with a lesion in the conus medullaris, cauda equina, or lower extremity peripheral nerve.
* Persons unable to safely undergo an MRI.
* Persons in whom adequate MRI imaging cannot be obtained.
* Persons who have developed a pulmonary embolism (PE) or deep vein thrombosis (DVT).
* Other traumatic injuries (e.g., CHI, another level of SCI) affecting the ability to provide informed consent and participate fully in rehabilitation.
* Persons with self-reported persistent severe neuropathic pain, inadequately controlled by non-narcotic medication.
* Persons with severe persistent mechanical or thermal hypersensitivity/allodynia at the neurological level or rostral to it as documented by clinical testing.
* Pregnant women or a positive pregnancy test in those women with reproductive potential prior to enrollment.
* Presence of systemic disease that might interfere with subject safety, compliance, or evaluation of the condition under study.
* Presence of any unstable medical or psychiatric condition that could reasonably be expected to subject the participant to unwarranted risk from participation in the study or result in a significant deterioration of his/her clinical course.
* Body Mass Index (BMI) > 35.
* History of active substance abuse.
* Persons who have participated in other experimental treatments within the past 90 days deemed by the PI to represent a possible confound or enrolled in any other ongoing trial.
* Persons with significant lower extremity injury, previous surgery, or amputation such that would preclude satisfactory sural nerve harvest.
* Persons allergic to gentamicin
* Persons who test positive for HIV or Hepatitis B or C virus.
* Baseline entry criteria for renal function, CBC, INR, and liver tests including serum albumin, total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphastase (ALP), and gamma glutamyl transpeptidase (GGT).

Major Exclusion Criteria, transplant procedure:

* Persons with acute SCI whose ISNCSCI grade improves between initial admission and the scheduled implantation procedure, and are no longer classified as ISNCSCI A.
* Presence of reproducible, valid MEP or SSEP signals below the level of injury within 2 to 5 days prior to transplantation.
* Failure to obtain cultured SC that meet study criteria.
* Active medical conditions precluding safe transplantation.
* Unresolved post-surgical wound infection.
* Ascending neurologic injury by three or more levels, persisting for one week, prior to transplant procedure.
* Presence of sacral or ischial pressure sore.

Dose to be Studied	
Cohort	
Dose	
(# cells)	
# of injections	
Total Volume	
# subjects in cohort	
1	
5 x 10^6	
1	
50 µL	
1	
2	
10 x 10^6	
1	
100 µL	
2	
3	
15 x 10^6	
1	
150 µL	
5	

Safety Evaluation

* Although preclinical data suggests ahSC transplantation is relatively safe, several adverse events are possible and will be noted. These events might include immune reactions, contamination by infectious or toxic agents, disruption of spinal cord by implantation of ahSCs, tumorigenesis, and aberrant neuroplasticity.
* Full ISNCSCI motor and sensory assessments done at admission, enrollment, prior to implantation, and at 1 and 2 weeks and 2, 6, & 12-months post-transplantation will monitor for any deterioration in neurological function during the study.
* Imaging studies (MRI) will be done at admission, within 1-2 days prior to implantation, the day following implantation, and 6 & 12-months after implantation to assess for tumorigenesis or unexpected changes in spinal cord structure. MRI will also be performed in the instance of clinically distinct neurological deterioration at the treating physician's discretion.
* Pain assessments will evaluate development of, or changes in, neuropathic pain: ISCI basic pain dataset, LANSS pain scale, and pain drawings.

Secondary outcomes

* Functional Independence Measure (FIM)
* Spinal Cord Independence Measure (SCIM III)
* Motor Evoked Potentials (MEP)
* Somatosensory Evoked Potentials (SSEP)
* Autonomic assessments: BP, HR, tilt table response, sympathetic skin responses, ISCI basic bowel dataset, ISCI basic lower urinary tract (LUT) dataset
* Quality of Life: SF-12, Guy Farrar Patient Global Impression of Change
* Modified Ashworth Scale