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New Jersey Commission on Spinal Cord Research Final Report

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Specific Aims

The overall goals of the proposed research are to standardize, characterize, and validate two models of graded spinal cord injury in mice, i.e. contusion by dropping a 10 gram rod 6.25, 12.5, or 25.0 mm onto T13 cord and ischemia by compressing the T13 spinal cord with a custom laser Doppler flow probe to stop blood flow for 10, 20, or 30 minutes.

Our specific aims are:

1. Develop standardized parameters of the two mouse SCI models. We will assess the feasibility of proposed animal and injury parameters, including anesthesia (isoflurane), age (10 weeks), body weight (18-25 g), strain (C57Bl/6), post-operative antibiotics (cephalosporin), antibiotics for recurrent bladder infections (Baytril), and treatments for autophagia (oral acetaminophen).
2. Characterize the outcomes of the two mouse SCI models. We will determine the time courses of acute tissue damage (using ionic lesion volumes), gene expression (NGEL mouse gene chip), histological changes (2-week histological lesion volume), neurophysiological (somatosensory evoked potentials), and locomotor recovery (Basso mouse locomotor score).
3. Validate the models. Determine the variability of the outcome measures and do power analysis to determine the number of mice required to detect expected changes of outcome measures, including ionic lesion volumes, gene expression, histological lesion volumes at 2 weeks, somatosensory evoked potentials and locomotor recovery.

Project successes

We have successfully established the mouse contusion model with a modified rat MASCIS impactor. We have also tested the feasibility of ischemia model with Laser Doppler probe compression. After establishing the mouse contusion model, we carried out a series of study on a transgenic knock-out mouse line: Osteopontin (OPN) deficient mice and published the result in the Journal of Neuroscience. This publication is the first demonstration of the importance of a standardized mouse contusion model and will open doors to other investigators to study spinal cord injury in different genetic background and compare data.

Surgery and impactor related instruments

First, we have made significant changes in mouse surgical procedures including instrument selection and anesthesia protocols. Second, we designed a mouse specific clamping system that is in high demand by spinal cord scientists (Figure 1). Third, MASCIS impactor devise is completely redesigned to accommodate mouse anatomic structure (Figure 2). Fourth, we have set up laser Doppler probe and detector for ischemia model with limited success (Figure 3).

Mouse contusion and ischemia models

First, we have validated that the modified mouse impactor can achieve the expected parameters from different heights including Impact Velocity, Compression Distance and Compression Time (Figure 4). Second, we have determined that 6.25 mm

drop will causes severe damage to the spinal cord and there is no difference among 6.25mm, 12.5 and 25 mm height drop from acute lesion volume (LV) study (Figure 5) and chronic locomotor scores (BMS). Third, the lesion volume data from ischemia model show that longer compression causes more tissue lose (Figure 6).

Project challenges

While we have quite success with the mouse contusion model, we had some difficulties with the ischemia model using a laser Doppler probe for compression. We have established the surgical, anesthesia, and instrumental procedures and carried out large number of study in three levels of injury. From the Doppler probe readout, we were able to establish consistent near-zero blood flow for 10, 20 and 30 minutes. But, when we evaluated the BMS score for these mice, the results were inconsistent. Some mice with 30 minutes compression showed significant higher BMS scores then that of 10-minute and 20-minute groups. We concluded that the Doppler probe compression could not generated reliable graded injury in mice.

Implications for future research and/or clinical treatment

The availability of many transgenic mouse strains is a powerful experimental tool for investigating to study gene function. By adapting from a well-standardized rat contusion model, we are able to establish a standard mouse contusion model. The availability of a well-standardized and reliable mouse SCI model will not only allow investigation of genetic mechanisms but also should help provide important information about potential transgenic gene therapy. In addition, many inbred strains are available, allowing cell transplantation studies without immunosuppression.

In fact, we have carried out the first study of spinal cord injury on an Osteopontin deficient line. Osteopontin (OPN) is expressed in many tissues during inflammatory responses. After spinal cord injury, microglia expresses OPN at the site of injury during the early to subacute stages. However, the function of OPN in spinal cord injury is not well understood. This study examines the responses of OPN knock-out (KO) and wild-type (WT) mice to spinal cord contusion injury. Lesion volume showed no significant differences between KO and WT mice at 24 h. RT-PCR indicated that KO mice had significantly less Bcl-2, tumor necrosis factor beta, interleukin-1-alpha, and interleukin 6 mRNA compared with WT controls. Western blot also showed that KO had significantly less Bcl-2 7 d after spinal cord injury. KO mice had significantly worse BMS locomotor scores than WT at 6 weeks. KO mice also had a significantly reduced area of spared white matter and fewer neuronal-specific nuclear protein-positive neurons in the spinal cord surrounding the impact site. This result supports a potential neuroprotective role for OPN in the inflammatory response to spinal cord injury (Publication attached)

Plans to continues research, including application submitted to other sources for ongoing support

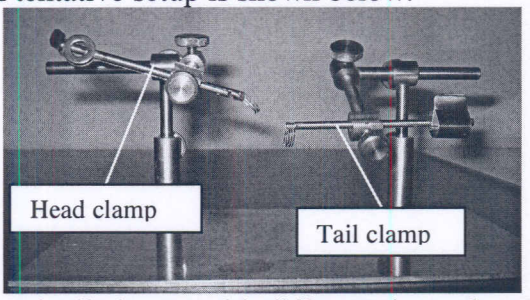
Many ongoing projects at the W. M. Keck Center for Collaborative Neuroscience will use the established mouse contusion model as a tool to study from single gene function to functional recovery of a therapy. We have applied a stem cell core program project to the Commission on Science & Technology of New Jersey, in which the mouse

contusion model will be used in several project. We are also testing several cellular therapies using mouse embryonic stem cells in the mouse contusion model.

List and include a copy of all publication emerging from this research

See attachment.

Figure 1. Clamping system: Due to the smaller size of mouse spinal column, the rat clamping system cannot be used. We have designed several clamps to accommodate the mouse spinal column. A tentative setup is shown below.



We have designed head and tail clamps with different tip angle. The head clamp's tip is designed in a way so it can reach the mouse thoracic spinal column without causing more damage. The tail clamp tip is more perpendicular to the holding rod to accommodate lumbar region. This setup securely holds the mouse vertebral column during the impact.

Figure 2. Mouse impactor. A mouse impactor tip was designed to have a smaller diameter (1.0 mm) than the one for rat. A shorter tip with a bend was put in for mouse impactor. We also replaced the spring for the positioning rod so that it will cause fewer traumas to mouse tissue. A handle was also put in place to reduce pressure on mouse spinal cord. A complete setup is shown below.

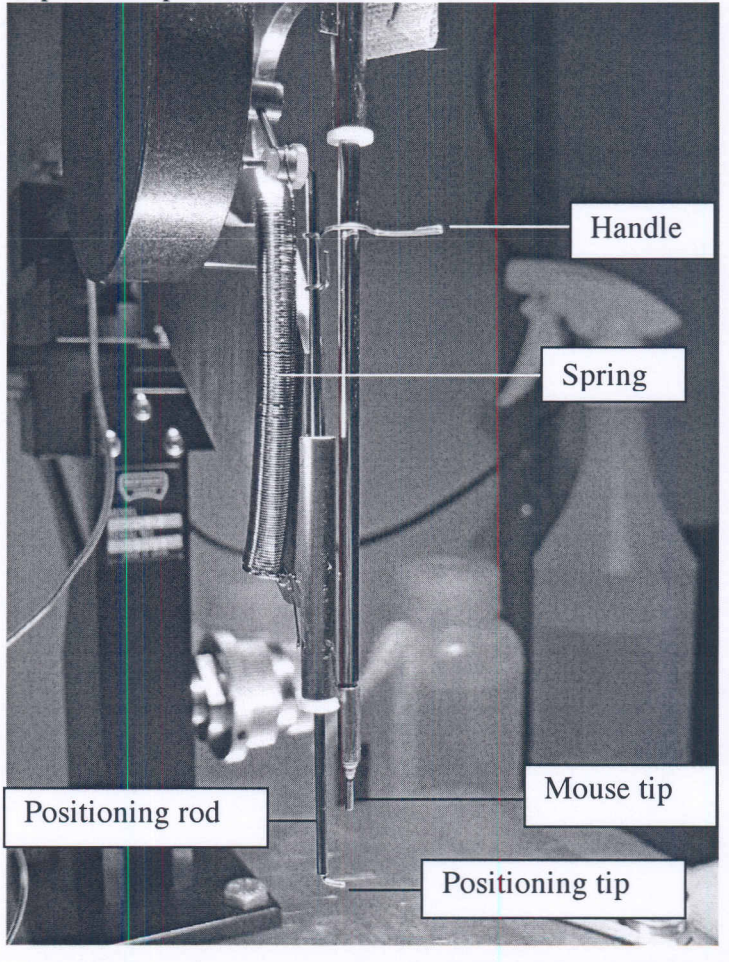
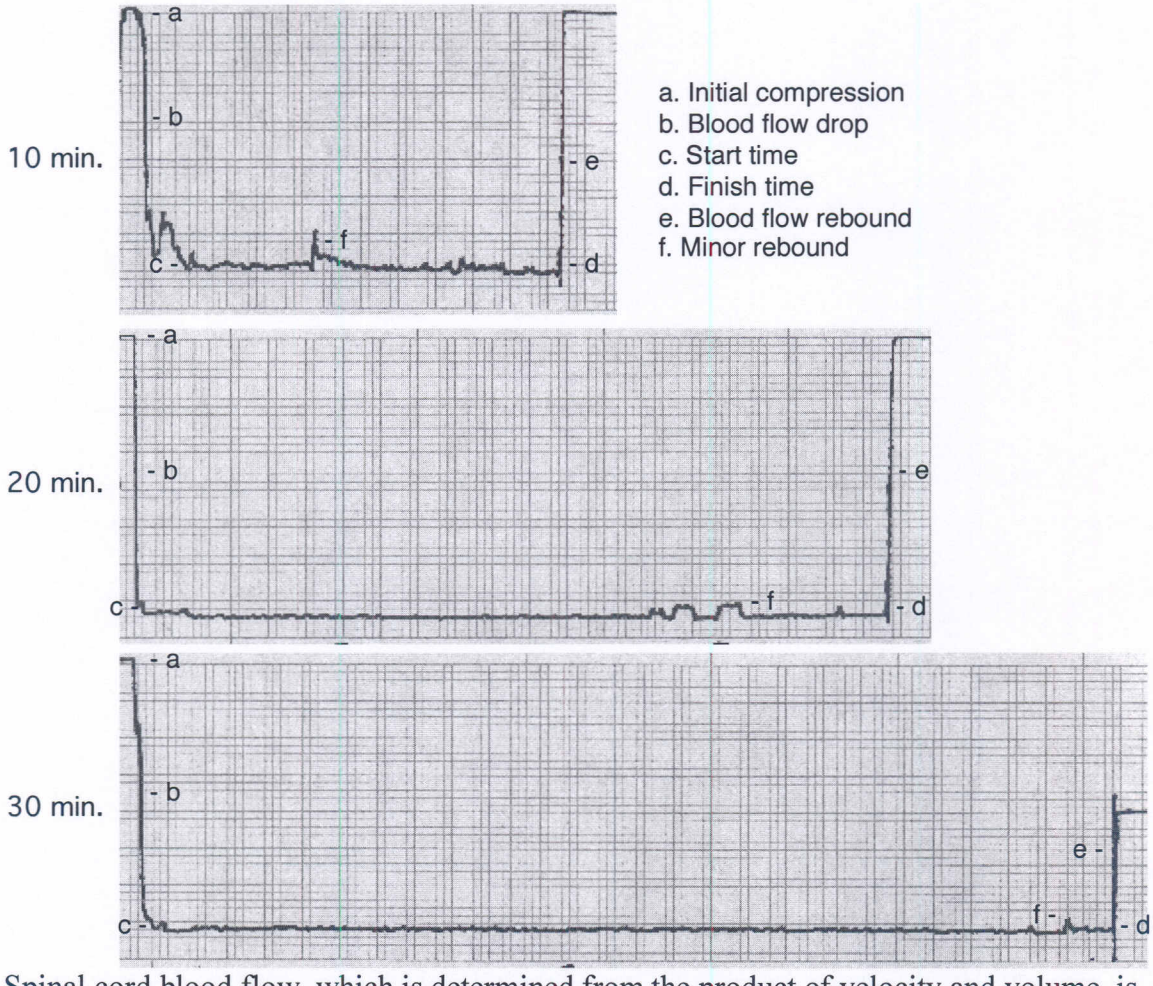


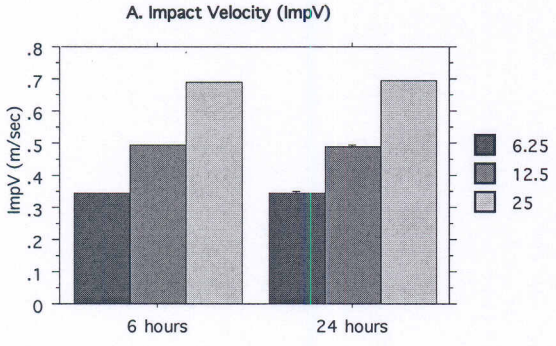
Figure 3. Three typical experiments were shown below with 10, 20 and 30 minutes compression.



Spinal cord blood flow, which is determined from the product of velocity and volume, is measured when the Doppler probe is in contact with tissue surface and throughout compression. Initial compression (a) on spinal cord produces a significant drop in flow measurement (b). Starting time (c) is recorded when there is no more drop in blood flow measurement (c) and finish time (d) is recorded at 10, 20 and 30 minutes respectively. After Doppler is released, there is significant blood flow rebound (e). There a few minor blood flow rebound during compression and many of them can be controlled by slowly advancing the probe.

Figure 4. Mouse contusion model

All the animals were injured with a 10 gram rod dropped from 6.25, 12.5 and 25.0 mm height onto the T13 spinal cord exposed with a T9-10 laminectomy. The Impactor device estimates the impact velocity (ImpV) by measuring the trajectory of the falling rod 2 msec before contact with the spinal cord and uses linear regression to estimate the velocity. Impact Parameters. (A) Mean impact velocity (ImpV) of contusion and (B) Cord compression rate (Cr) are segregated by sacrifice time (SacT) and drop height (DropHt). The error bars indicate standard errors of means. Cr=cord compression distance (Cd)/cord compression time (Ct). Our previous study has shown that Cr correlates with spinal cord lesion volumes. All drop height groups were statistically significant at $p<0.05$. The data indicate that all the animals received a graded contusion.

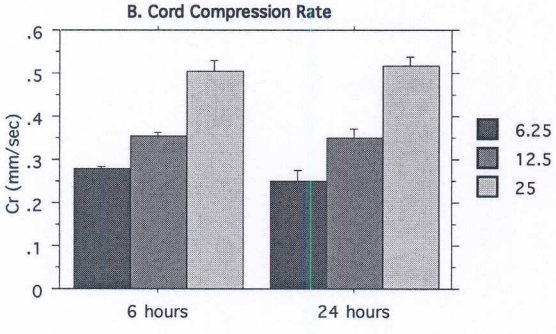


Means Table for ImpV

	Count	Mean	Std. Dev.	Std. Err.
6 hours, 6.25	5	3.438E-1	3.899E-3	1.744E-3
6 hours, 12.5	6	4.935E-1	2.258E-3	9.220E-4
6 hours, 25	4	6.900E-1	1.826E-3	9.129E-4
24 hours, 6.25	6	3.465E-1	4.593E-3	1.875E-3
24 hours, 12.5	5	4.894E-1	6.348E-3	2.839E-3
24 hours, 25	5	6.936E-1	2.966E-3	1.327E-3

ANOVA Table for ImpV

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sac T	1	4.090E-6	4.090E-6	2.568E-1	.6168	2.568E-1	7.664E-2
DropHt	2	5.892E-1	2.946E-1	1.850E4	<.0001	3.699E4	1.000
Sac T * DropHt	2	9.225E-5	4.612E-5	2.896	.0739	5.792	5.060E-1
Residual	25	3.982E-4	1.593E-5				



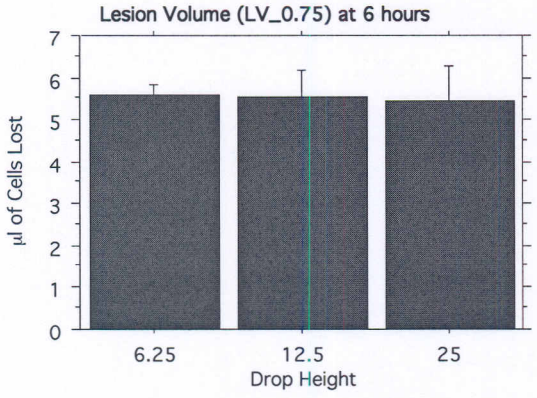
Means Table for Cr

	Count	Mean	Std. Dev.	Std. Err.
6 hours, 6.25	5	2.798E-1	1.230E-2	5.499E-3
6 hours, 12.5	6	3.547E-1	1.767E-2	7.213E-3
6 hours, 25	4	5.055E-1	4.871E-2	2.435E-2
24 hours, 6.25	6	2.495E-1	6.563E-2	2.679E-2
24 hours, 12.5	5	3.520E-1	4.449E-2	1.990E-2
24 hours, 25	5	5.185E-1	4.417E-2	1.975E-2

ANOVA Table for Cr

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sac T	1	3.380E-4	3.380E-4	1.815E-1	.6737	1.815E-1	6.891E-2
DropHt	2	3.031E-1	1.515E-1	8.140E1	<.0001	1.628E2	1.000
Sac T * DropHt	2	2.408E-3	1.204E-3	6.468E-1	.5322	1.294	1.425E-1
Residual	25	4.654E-2	1.862E-3				

Figure 5. Lesion Volume
Mice were euthanized 6 hours or 24 hours after injury and lesion volumes were calculated from potassium concentration of spinal cord samples. Mouse spinal cords were removed and 1 cm tissue centered on the impact site was preserved in Trizol solution. The figure below illustrates the mean lesion volumes, segregated by SacT and DropHt.
Lesion volume at 6 hours



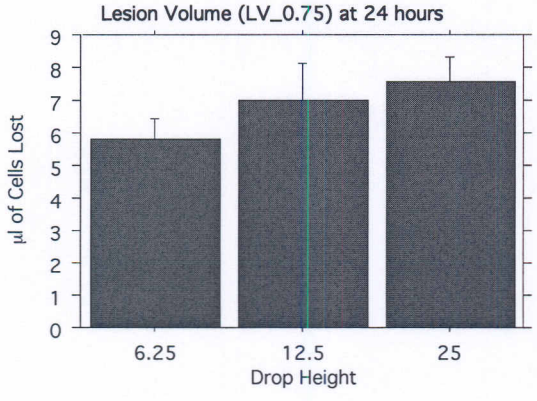
Means Table for Lesion Volume 6 hrs

	Count	Mean	Std. Dev.	Std. Err.
6.25	5	5.591	5.702E-1	2.550E-1
12.5	6	5.565	1.528	6.238E-1
25	4	5.453	1.602	8.009E-1

ANOVA Table for Lesion Volume at 6 hours

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
DropHt	2	1.381E-1	6.903E-2	4.217E-2	.9588	8.433E-2	5.508E-2
Residual	13	2.128E1	1.637				

Lesion volumes at 6 hours have shown no significant difference between drop height groups.
Lesion volume at 24 hours



Means Table for Lesion Volume 24 hrs

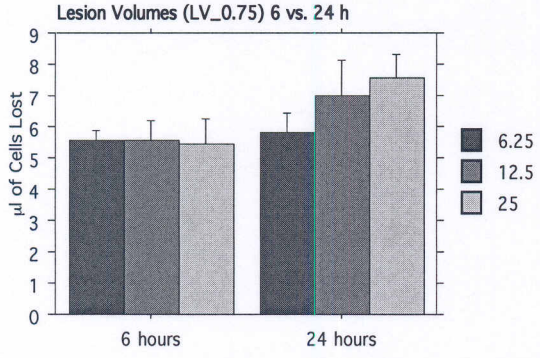
	Count	Mean	Std. Dev.	Std. Err.
6.25	6	5.839	1.522	6.215E-1
12.5	5	7.029	2.402	1.074
25	5	7.537	1.734	7.753E-1

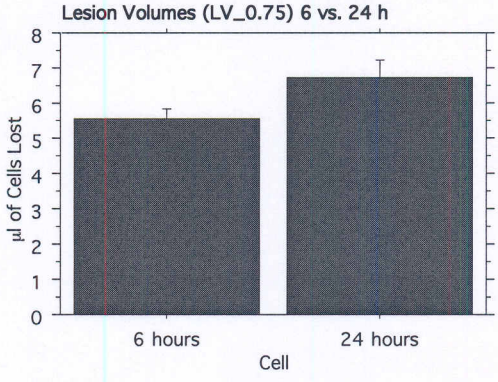
ANOVA Table for Lesion Volume at 24 hours

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
DropHt	2	8.468	4.234	1.179	.3385	2.358	2.080E-1
Residual	13	4.669E1	3.592				

Lesion volumes at 24 hour have shown no significant difference between drop height groups but a trend of more tissue damage with higher height group.

Combined lesion volume analysis





Means Table for Lesion Volumes 6 vs. 24 h
Effect: Sac T * DropHt

	Count	Mean	Std. Dev.	Std. Err.
6 hours, 6.25	5	5.591	5.702E-1	2.550E-1
6 hours, 12.5	6	5.565	1.528	6.238E-1
6 hours, 25	4	5.453	1.602	8.009E-1
24 hours, 6.25	6	5.839	1.522	6.215E-1
24 hours, 12.5	5	7.029	2.402	1.074
24 hours, 25	5	7.537	1.734	7.753E-1

Means Table for Lesion Volumes 6 vs. 24 h
Effect: Sac T

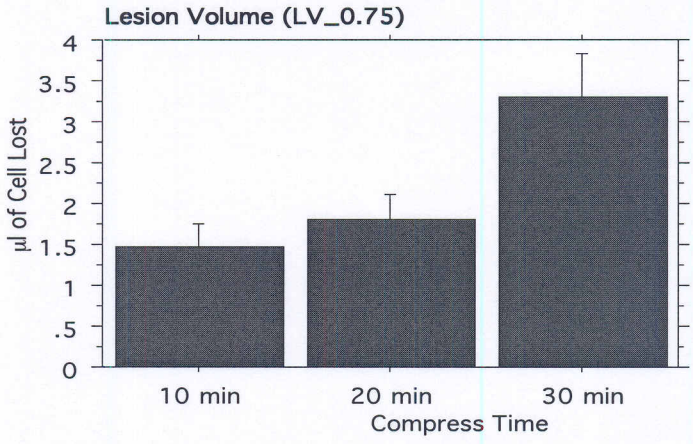
	Count	Mean	Std. Dev.	Std. Err.
6 hours	15	5.544	1.217	3.141E-1
24 hours	16	6.742	1.918	4.794E-1

ANOVA Table for Lesion Volumes 6 hours vs. 24 hours

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sac T	1	1.218E1	1.218E1	4.521	.0435	4.521	5.240E-1
DropHt	2	3.358	1.679	6.231E-1	.5444	1.246	1.389E-1
Sac T * DropHt	2	4.401	2.200	8.166E-1	.4534	1.633	1.687E-1
Residual	25	6.737E1	2.695				

ANOVA indicate a significant difference between 6 and 24 hour groups, with 24 hour group having more cell loss.
All lesion volume data indicated that there is no significant tissue loss differences among drop heights. These results raised several questions, which will be discussed below.

Figure 6 Ischemia model
A Doppler probe is compressed on mouse spinal cord for 10, 20 and 30 minutes. Spinal cord tissue is collected and lesion volume is calculated from potassium content and tissue wet weight.



Means Table for LV_0.75

	Count	Mean	Std. Dev.	Std. Err.
10 min	7	1.467	7.639E-1	2.887E-1
20 min	9	1.805	8.875E-1	2.958E-1
30 min	10	3.302	1.650	5.217E-1

Fisher's PLSD for LV_0.75

	Mean Diff.	Crit. Diff.	P-Value	
10 min, 20 min	-3.382E-1	1.273	.5880	
10 min, 30 min	-1.835	1.245	.0057	S
20 min, 30 min	-1.497	1.161	.0137	S

ANOVA Table for LV_0.75

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
CompT	2	1.710E1	8.550	5.734	.0095	1.147E1	8.244E-1
Residual	23	3.430E1	1.491				

Lesion volume data from ischemia model show that longer compression causes more tissue lose.