Research Article

A Novel Compression Rat Model for Developmental Spinal Stenosis†

Running title: *Developmental spinal stenosis rat model*

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Abstract

Developmental spinal stenosis (DSS) is characterized by pre-existing circumferential narrowing of the bony spinal canal which predisposes neural tissue to compression. This study aims to create a reproducible animal model mimicking DSS for investigation of its pathoanatomy. Developmental spinal canal constriction was simulated using circumferential compression. Eighteen female Sprague-Dawley rats (13.0-14.5 weeks-old) underwent circumferential compression at L4-L5 using silicone sheets; or dorsal compression using overlapping silicone sheets; or as controls. A series of outcome scores were used for locomotor function assessment, together with electrophysiological and histological assessment. Assessment time-points were at preoperative, postoperative 1-week, 2-weeks, 3-weeks, 1-month and pre-sacrifice. Statistical analyses were performed. At all postoperative time-points, circumferential group had the worst mean Basso, Beattie and Bresnahan locomotor scores with significant difference from Control group ($p<0.05$), as well as the lowest mean Louisville Swim Scale scores, as compared to the dorsal ($p<0.05$) and to control ($p<0.01$) group. Circumferential group had worse mean foot fault score for both hindlimbs ($p<0.01$ to $p<0.05$) and highest error rate in foot placement accuracy, especially higher than dorsal ($p<0.05$) and control ($p<0.05$) group at pre-sacrifice. Electrophysiological assessment revealed postoperative increase in P1 latency was higher in circumferential than dorsal compression. Highest postoperative mean P1 latency was observed for both paws at all postoperative time-points for circumferential group (except at postoperative 1-week). Circumferential group had lower myelin-to-axonal area ratio and higher g-ratio than both the dorsal and control group ($p<0.001$). For each study group, hindlimb P1 latency and P1-N1 amplitude were each correlated with g-ratio ($p<0.05$); and mean myelin-to-axonal area ratio correlated with P1 latency of both hindlimbs ($p<0.05$). Based on these more severe axonal demyelination and neurological deficits, a valid DSS rat model is created with somatosensory evoked potential neuro-monitoring technique.

Key Words: developmental spinal stenosis, compression, circumferential, rat model

Introduction

Developmental Spinal Stenosis (DSS) is defined as a pre-existing circumferential narrowing of spinal canal originating from the mal-development of dorsal spinal elements.[1] The anteroposterior diameter of the bony spinal canal is abnormally short and the resultant narrowed vertebral canal can predispose patients to nerve compression even with minor degrees of canal compromise such as posterior spur formation.[2] Hence, stenotic symptoms can be precipitated at an earlier onset than degenerative-type spinal stenosis.[1; 3; 4] DSS may involve multiple vertebral levels[3; 4] and has potential risk for symptom recurrence after decompression surgery for spinal stenosis.

One must appreciate the unique pathological characteristics of DSS that distinguish it from degenerative/acquired lumbar spinal stenosis. Previous studies have characterized DSS with both x-ray and magnetic resonance imaging (MRI) phenotyping,[5-7] having a paradoxical relationship with ligamentum flavum hypertrophy,[8] and possible genetic origins.[6; 8-10] Yet, further understanding of its pathophysiology is necessary to determine the clinical significance of DSS. Animal models are useful for testing patterns of nerve tissue response to duration of compression and timing of decompression. However, there is no existing circumferential compression model to study DSS. Existing models are limited as they simulate single directional compression with an anterior compression to mimic disc protrusions, posterolateral compressions by flavum hypertrophy or osteophytes, or single nerve root compressions seen

in foraminal stenosis.[11-14] Hence, this study aims to create and test a circumferential compression rat model for DSS. Validation of a method to perform intraoperative somatosensory evoked potential monitoring will also be established.

Methods

Model creation

A total of 18 female Sprague-Dawley (SD) rat of 13.0 - 14.5 week-old were utilized. All animal experiments involved in this study were approved by the Animal Ethics Committee. The rats were randomly allocated into 3 groups: circumferential compression (n=6), dorsal compression (n= 6), control (n=6). Circumferential compression was achieved using 0.51mm thick silicone sheet. The silicone sheet was guided circumferentially to the dural sac from the operator side, and the ends of the silicone sheet were approximated and secured by a non-absorbable suture over the dorsal aspect (**Figure 1(a)**). Dorsal compression was conducted by firstly removing the spinous process of L5, followed by insertion of silicone sheets dorsal to the dura, inducing compression at the region of L4-L5 region. Silicone sheets were doubled and overlapped to ensure occupation of space between spinous process and neural bundles. (**Figure1 (b)**) The sham control group underwent the same surgical exposure and manipulation without insertion of any compression medium.

Regarding the surgical procedure, the rats were weighed preoperatively, and were

anaesthetized with ketamine hydrochloride (100mg/ml) and xylazine (20mg/ml) at 60mg/kg via intra-peritoneal injection. All surgical procedures were performed by a single surgeon for consistency. All procedures were performed at the most commonly involved stenosis level, L4-L5.[15] Incision was introduced along the midline of the dorsum of the lumbar spine, and the paraspinal muscles were retracted. By using an operating microscope (Wild-Heerbrugg M691 stereo binocular microscope by Leica Microsystems, Bannockburn, IL, USA), a laminectomy was performed followed by insertion of a compression medium as described above.

After the compressive device was inserted and wound closed, ketoprofen (100mg/ml) was administered subcutaneously for postoperative pain control. All vital signs for breathing, pulse and body temperature were monitored throughout the entire surgery, and the animals were kept at recovery facility until anesthesia was completely worn off. At sacrifice, the rats were anesthetized using sodium pentobarbital (150-200mg/ml), then euthanized by transcardial perfusion of heparinized saline, followed by 10% buffered formalin for histology assessment.

Assessments

All rats were assessed by a research personnel who was blinded to the subject allocation. Assessments were performed at the following set time-points: Preoperative, Postoperative 1-week, Postoperative 2-weeks, Postoperative 3-weeks, Postoperative 1-month, and Pre-sacrifice (at 2.0 to 2.5 months postoperatively).

i) Behavioural Tests

Rats were assessed through a series of locomotor tests in order to reveal whether and how the spinal compression affected their behaviour and motor function over time. Both horizontal over-ground locomotion and locomotion during swimming were analysed. Rats were trained and tested on ladder walking, with an elevated horizontal ladder consisted of side rails and metal rungs on an approximately 40-centimetre platform at each side. To prevent the animals from anticipating the distances between metal rungs and familiarizing the pattern, the metal rungs were arranged randomly to achieve various spacing at each assessment time-point. The pattern also differed by having the rats walking from the left and then from the right. For the swimming test, there was prior training, and each animal was allowed to swim for 60 seconds at each test in a three-quarters full water tank filled with warm water.

For analysis, video recording of each swimming and locomotion test was performed. The outcome assessment used included: Basso, Beattie and Bresnahan (BBB) locomotor rating scale,[16] Foot fault scoring system,[17] Foot accuracy replacement analysis,[17] and Louisville Swim Scale (LSS) scoring.[18] The BBB locomotor rating scale is a 21-point scoring system, examining the joint movement, hindlimb movements, stepping, forelimb and hindlimb coordination, trunk position and stability, paw placement and tail position.[16] Lower BBB scores indicates worse locomotor function. A score of 14 to 21 represents forelimbs coordinating with hindlimbs, 8 to 13 represents the presence of uncoordinated stepping and 0 to 7 represents isolated joint

movements with little or no hindlimb movement. Foot fault scoring system is a qualitative evaluation of the accuracy of placement of foot or paw on the rungs according to their position and errors.[17] There are 7 categories, each with an assigned score: Total miss(0), Deep slip(1), Slight slip(2), Replacement(3), Correction(4), Partial placement(5), Correct placement(6). The foot placement accuracy is a quantitative assessment, represented by the foot slip frequency (any kind of foot slip, missteps, or total miss are considered as errors) over the total number of steps by each limb.[17] An average value from five trials was used for analysis and was expressed as error rate in percentage. LSS was used to evaluate performance based on three primary components of swimming: forelimb dependency, hindlimb activity and alternation, and body position.[18; 19] It has an 18-point scale divided into three ranges: 0-5 represents a poor swimmer with seldom/none hindlimb movement, seldom/none hindlimb alternation, frequent to consistent forelimb dependency, consistently moderate to severe trunk instability and moderate to severe body angle (the tail is at or just below the water surface), 6-11 indicates an intermediate swimmer, whereas 12-17 represents a good swimmer with frequent to consistent hindlimb movement, occasional to consistent hindlimb alternation, none/seldom forelimb dependency, none to moderate trunk instability and none/mild body angle.

ii) Electrophysiological Assessment

Electrophysiological tests provided objective assessment of any changes in the integrity of neural function after the surgical compression. Somatosensory-evoked potential (SSEP) was conducted through both front-paws and hind-paws with an established protocol by using evoked potential equipment (YRKJ-A2004; Zhuhai Yiruikeji Co, Ltd, Zhuhai, China).[20] SSEP signals were recorded at the skull via the sensori-motor cortex after a constant current stimulator with a 5.1 Hertz square wave at 0.2 millisecond (ms) duration. At each assessment time-point, the signals recorded allowed the assessment of P1 latency, N1 latency and P1-N1 amplitude. The percentage of increase in P1 latency was calculated by comparing the value at pre-sacrifice with baseline to validate the extent of the neural effects incurred by different types of compression. The change of P1 latency was calculated between each subsequent time-point. Front-paws were used as control.

iii) Histological Assessment

The section of lumbar spine was harvested and was directly immersed in 10% buffered formalin (containing 4% (w/v) formaldehyde and 0.075 M phosphate buffer), for 12 hours overnight at 4 °C. After adequate time of fixation, a section of dural sac at least 1cm cranial and caudal to the compression site was dissected from the vertebrae and washed with phosphate buffer solution. The specimens were fixed for approximately 120 minutes at room temperature

in 2% osmium tetroxide (OsO_4), and then dehydrated through an ethanol series starting with 30% to 100% ethanol, and ended with chloroform. Tissue samples were embedded in paraffin blocks.

The lumbar nerve bundles at the compression site were cut into transverse sections at a thickness of 5μm using a microtome (Leica RM2135, Nussloch, Germany). The sections were mounted onto glass slides, dried, and dewaxed. The slides were then stained by Masson's trichrome, which was used as a counterstain as it allowed connective structures in nerves to be detected effectively.[21] Images were viewed under light microscope (Nikon Eclipse 80i, Melville, New York, USA) and captured along with a scale for storage by computer imaging software (NIS-Elements F4.30.01 64 bit, Nikon, Japan) (**Figure 2**).

Histological analysis of the degree of axonal myelination of neural tissues was performed at the operated lumbar segment. For the nerve sample of each subject, at least three to five microscopic transverse sections, within which a total of 100 counts of axons were randomly selected for measurement. By using ImageJ 1.50i (RSB, NIMH, Maryland, USA), the outer edge and the inner boundary of the stained myelin sheath of each axon were outlined and traced manually. This enabled the calculation of areas of the myelin sheath and of the axon using the software. Demyelination was then evaluated by the ratio of myelin sheath area to axonal area derived from the histological quantitative measurement. A smaller ratio denoted higher degree of demyelination and vice versa. The myelin thickness and axonal diameter were

also derived. In addition, the g-ratio, the ratio of the inner axonal diameter to the total outer diameter, was calculated for each axon measured and an average value was obtained. Further examination of any association between electrophysiological parameters and histological quantification of myelination (ratio of areas of myelin sheath and axon, g-ratio) was performed.

Sample Size Calculation

As there is a lack of previous available data comparing rat models across three study groups (circumferential compression, dorsal compressions and control), we performed a pilot study of 10 subjects with 6 rats as DSS models, 2 rats for dorsal compression and 2 as controls, and they were assessed at each of the six time-points as set out. Based on the collected data from these first 10 rats from each of the behavioural tests at every time-point (as the number of data points analyzed in each behavioural test was the smallest), we found that a total of 18 subjects with an equal number of 6 rats per study group could achieve a power of >80% with a significance level of 0.05 to detect significant inter-group differences in each behavioural outcome measure as found in the pilot dataset. An attrition rate of up to 20% has been taken into consideration.

Statistical Analysis

Normality and linearity assessment of each parameter data was performed using Shapiro-Wilk test and scatterplots. Any significant differences of parameters between study groups were tested using one-way analysis of variance (ANOVA) with post-hoc Tukey's honest significant difference (HSD) for normally distributed data. For non-parametric data, Jonckheere-Terpstra test with post-hoc pairwise comparison was conducted, given the test of equal variance for each study group was satisfied. With a priori specific alternative hypothesis defined as locomotor dysfunction increases with larger extent of neural compression, the Jonckheere-Terpstra test was used not only for testing any ordered difference in medians of locomotor outcome scores, it also helped in determining the significance of a trend.[22] By assessing whether increasing extensiveness of neural compression (from none in control, to dorsal compression and to the most severe in circumferential compression) results in an increase or decrease in the outcome scores, this non-parametric test helped in understanding whether any score differences among study groups were based on the increasing extent of compression. When $p<0.05$, the above stated priori hypothesis of the trend would be accepted and considered significant. For non-parametric data which failed the equal variance test, Kruskal-Wallis test was used to test for any intergroup difference. In addition, Spearman's rank correlation test was used to investigate any association between electrophysiological parameters and histological quantification of myelination, and the strength of such association

was expressed as Spearman's rank correlation coefficient, rho, r_s. All mean values were calculated with standard deviation (SD). A p-value of <0.05 was considered statistically significant for all statistical tests. Data analyses were conducted using SPSS Windows 23.0 (IBM SPSS Inc., Chicago, IL, USA).

Results

Behavioural Tests

Among the study groups, circumferential compression had the lowest BBB scores (**Table 1**) with demonstrated progressive, gradual and continual deterioration of locomotor function (**Figure 3**). The trend of increasing extent of neural compression with increasing deterioration of BBB scores was significant at all postoperative time-points ($p<0.001$ to $p<0.05$). Dorsal compression and control group locomotor function deterioration (**Figure 4**) plateaued immediately at postoperative 1-week whereas circumferential group continued to deteriorate. For foot fault scoring, significant differences among study groups were evident with post-hoc tests indicating circumferential group having worse score than both dorsal compression ($p<0.05$) and control groups significantly at postoperative 1-month up until pre-sacrifice (right limb), and at postoperative 3-weeks (left limb)(**Table 2**). For foot placement accuracy analysis (**Table 3**), the significant trend of increasing neural compression group with increased error rate was demonstrated with circumferential group having the highest mean error rate of steps

from postoperative 2-weeks onwards, not only performing worse than control group, but higher mean error rate than both dorsal and control group at pre-sacrifice ($p<0.05$). For LSS (**Table 4**), the significant trend of increasing compromised scores was explained by increasing extent of neural compression. Circumferential group had the lowest mean scores at all postoperative time-points, with a significantly worse score than the dorsal as well as the control group ($p<0.01$ to $p<0.05$) throughout by a difference of mean score up to 8.8 ($p=0.008$). Circumferential group was the only group with continual deterioration of LSS scores since postoperative 1-week (**Figure 5**).

Electrophysiological Test

When comparing to baseline at pre-operation, percentages of increase in P1 latency at sacrifice were 24.7% (right hind-paws) and 20.5% (left hind-paws) for circumferential group, whereas dorsal group had 13.7% and 9.9% for right and left hind-paws respectively. Circumferential group was consistently having the largest mean P1 latency at all postoperative time-points except postoperative 1-week for right hind-paws (**Figure 6(a)**), and demonstrated a trend of increasing P1 latency for left hind-paws from post-operative 3-weeks as compared to the dorsal compression and control group. (**Figure 6(b)**). Pairwise significant difference was detected mainly between circumferential and control group (**Table 5**). When comparing change of P1 latency between time-points, only circumferential group had significant increase in P1

latency ($p<0.05$, one-way ANOVA) (mean change: 2.4ms) as compared to the control group (mean change: -1.6ms) between postoperative 3-weeks to 1-month ($p<0.05$) for left hind-paws, the dorsal group has a decrease in P1 latency also (mean change: -1.4ms). For P1-N1 amplitude (**Table 6**), circumferential group had higher P1-N1 amplitude than dorsal compression and control group (each at $p<0.05$) at pre-sacrifice for left hind-paws, and significant difference among 3 study groups for right hind-paws.

Histological analysis of demyelination

The mean ratio of areas of myelin sheath to axons were 0.98±0.58 (circumferential), 1.27±0.75 (dorsal compression) and 2.23±1.57 (control), with significant intergroup difference ($p<0.001$, Kruskal-Wallis one-way ANOVA). Post-hoc test found that the myelin-axonal area ratio of each group was significant different from each other at $p<0.001$. Circumferential group was much lower (by -0.29±0.05, $p<0.001$) than dorsal compression and control (by -1.34±0.12, $p<0.001$) group. Dorsal compression group was lower than control (by -1.04±0.12, $p<0.001$). For g-ratio, Kruskal-Wallis test revealed that each study group was significantly different to each other, with circumferential group having the highest value of 0.73±0.09, as compared to dorsal compression (0.68±0.09) and control (0.59±0.10) (all at $p<0.001$).

Electrophysiology and demyelination

For both hindlimbs at pre-sacrifice, P1 latency was found correlated with mean g-ratio for each group (r_s: 0.473, $p<0.05$), and also correlated with the mean myelin-axonal area ratio (r_s:-0.520, $p<0.05$). There was a significant correlation found for P1-N1 amplitude at pre-sacrifice with the mean value of g-ratio (r_s: 0.599, $p<0.05$) as well.

Discussion

This study has established and validated a novel rat model which simulates DSS. The use of circumferential compression makes a severe compression model, as evidenced by the more extensive deterioration in various functional and electrophysiological tests than dorsal compression, and not only when compared with control group. This is supported by the significant statistical trends found. The ability to display a range consisting of relative different levels of motor dysfunction among the 3 study groups, in particular the demonstration of circumferential compression having larger motor dysfunction than dorsal compression group in this same study, is crucial. That can be found in the locomotion and swimming test results, as well as electrophysiologically. The circumferential group suffers higher degree of motor dysfunction such as the inability to coordinate forelimbs and hindlimbs, dominant weight-supported plantar steps, higher rate of inaccurate paw placement, higher forelimb dependency for forward motion in water with minimal hindlimb movement, and truncal instability. The

chronic nature of spinal stenosis has been mimicked successfully, with circumferential compression causing motor function impairment for a sustained duration, with progressive, continual deterioration until sacrifice. Importantly, these behavioural outcomes and worst locomotion status at end-point correspond well with the worst histological change in the compressed neural tissues. As such, circumferential compression causes axonal demyelination to the largest degree as compared to dorsal compression and control subjects.

Our study is the first to utilize SSEP in assessing post-compression neural changes for a lumbar spinal stenosis model as to the existing use of SSEP in spinal cord trauma and injury protocols.[23] From our consistent findings, SSEP is feasible to detect changes with lumbar spinal canal compression. The P1 latency is a sensitive measure to depict the neurological effect of the circumferential compression. The circumferential group has the highest percentage of increase in P1 latency, which is well over the 10% threshold value for increase in latency indicative of possible tissue damage at a nerve injury event.[24] In addition, for the purpose of monitoring the effectiveness of the induced compression, it is crucial to investigate whether such electrophysiological assessment couples with histological changes. The significant correlations found between the P1 latency of both hind-paws and histological findings establish the relationship of conductive velocity and axonal demyelination. The role of SSEP in neuro-monitoring of this circumferential compression DSS model is therefore confirmed and validated, and indeed represent neurological changes occurring at tissue level with the

corresponding degree of demyelination (based on g-ratio, myelin-to-axonal area ratio). Interestingly, circumferential group exhibited the increasing trend in P1 latency still at postoperative 1-month until pre-sacrifice at the left hind-paws, as well as the significantly larger P1-N1 amplitude at both hind-paws at pre-sacrifice. The validation of neuro-monitoring in a DSS animal model is important for facilitating future testing, such as quantifying the magnitude of impact caused by circumferential compression and the effect of decompression intraoperatively.

The process of peripheral neural tissues to remyelinate after demyelination via Schwann cells has been taken into consideration,[25; 26] and histological findings have been interpreted at the most conservative level. Even some degree of remyelination did occur throughout the postoperative period prior to sacrifice, worst degree of demyelination caused by stenosis simulation can only be more severe than what was presented at the axons pre-sacrifice. Yet the largest degree of demyelination was significantly found with the circumferential group, with the mean g-ratio of control group (0.59±0.10) being comparable to the suggested value of 0.6 for peripheral nerve fibres.[27] For future investigation, it is necessary to examine the interplay of remyelination-demyelination for this DSS model. The axon sheath is likely restored through time, and the extent of such restoration requires detailed investigation through sacrifice at various time points.

Despite our findings, further testing of this model and comparing it to other compression models is necessary to better characterize the various mechanisms of nerve injury. Furthermore, this current model only serves as a mechanical compression model to mimic DSS. A true DSS animal model can only be created via genetic manipulation once better understanding of its genetic origins has been established. Nonetheless, this novel DSS rat model has been validated through a range of comprehensive behavioural tests, electrophysiological assessment and its relationship with histological analysis of axonal demyelination. Further pathological tests is feasible in its current form.

Conclusion

Our novel circumferential compression model for DSS has successfully reproduced the continual deterioration of hindlimbs locomotion, increasing trend of P1 latency, as well as the worst degree of demyelination of compressed neural tissues. The induced changes were progressive without recovery, simulating the chronic nature of DSS. These consistent findings suggest this circumferential compression model is a reliable and viable tool for mimicking DSS. We have also established and validated the use of neuro-monitoring via SSEP for lumbar stenosis. The development of this model provides a basic platform for future experimental studies including neural tissue changes with variable duration of circumferential compression and with the timing of decompression surgery.

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Figure Legends

Figure 1: (a) Schematic diagram of the circumferential compression model - silicone sheet was guided circumferentially (black arrow, left) to the dural sac and was secured by a non-absorbable suture (right) over the dorsal aspect. (b) Schematic diagram of the dorsal compression model – two silicone sheets were overlapped and inserted underneath the spinous process of L4 to achieve compression over the dorsal aspect of L4-L5.

Figure 2: Microscopic image (x40) of transversal section of neural tissue at circumferential compression – stained with Masson's trichrome, pre-treated with 2% osmium tetroxide (OsO_4).

Figure 3: Basso, Beattie and Bresnahan (BBB) locomotor rating scale scores for horizontal rung ladder over time. The lowest mean BBB scores were observed for the circumferential compression group at all time-points.

Figure 4: Change of Basso, Beattie and Bresnahan (BBB) locomotor rating scale scores (difference between each assessment time-point at x-axis with preoperative) for horizontal rung ladder over time. The mean change of BBB scores indicated locomotor function deterioration, which plateaued immediately for dorsal compression and control groups after postoperative 1-

week, whereas the circumferential group had a more gradual deterioration up to pre-sacrifice.

Figure 5: Louisville Swim Scale (LSS) scoring over time. Circumferential group was the only group with continual deterioration of mean LSS scores, with significantly lower LSS score than both dorsal compression and control group at all postoperative time-points.

Figure 6: (a) Mean latency (with standard deviation bars) of P1 for right hind-paws over time. Circumferential compression group had the highest mean P1 latency at all time-points except postoperative 1-week.

(b) Mean latency (with standard deviation bars) of P1 for left hind-paws over time. Circumferential compression group had the increasing trend of mean P1 latency at postoperative 3-weeks to pre-sacrifice. The dorsal and control groups, however, had reducing trend of P1 latency.

Table 1. Comparison of Basso, Beattie and Bresnahan (BBB) score for horizontal rung ladder

Groups / Time-points	BBB score (mean ± SD)			p-value^	Post-hoc pairwise comparison	
	Circumferential	Dorsal compression	Control			
Preoperative	18.5 ± 0.5	19.3 ± 0.8	18.5 ± 0.8	0.131		
Postoperative 1-week	11.3 ± 1.5	11.8 ± 1.9	14.8 ± 1.2	0.002*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.005 p=0.010 p>0.05
Postoperative 2-weeks	11.2 ± 2.1	12.5 ± 1.3	15.7 ± 1.6	0.002*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.009 p=0.026 p>0.05
Postoperative 3-weeks	10.0 ± 1.7	12.5 ± 1.7	15.5 ± 1.4	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.006 p=0.025 p>0.05
Postoperative 1-month	9.8 ± 1.6	12.3 ± 1.5	16.0 ± 2.5	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.006 p=0.046 p>0.05
Pre-sacrifice	9.8 ± 2.7	12.2 ± 1.3	15.5 ± 2.3	0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.023 p=0.031 p>0.05

^ Jonckheere-Terpstra test with post-hoc pairwise comparison, * statistical significance at p<0.05
Note: SD: standard deviation

Table 2. Comparison of foot fault scoring for flat rung ladder

Groups / Time-points	Foot fault score (mean ± SD)			p-value^	Post-hoc pairwise comparison	
	Circumferential	Dorsal compression	Control			
Right Limb						
Preoperative	3.0 ± 1.5	5.0 ± 0.0	4.8 ± 0.4	0.039*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p>0.05 p>0.05 p=0.029
Postoperative 1-week	0.8 ± 0.4	3.7 ± 1.4	3.2 ± 2.1	0.014*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p>0.05 p>0.05 p=0.004
Postoperative 2-weeks	0.7 ± 0.8	2.6 ± 1.7	3.5 ± 2.3	0.012*	Overall significant difference among 3 study groups but not pairwise	
Postoperative 3-weeks	1.0 ± 0.9	3.5 ± 1.9	4.2 ± 1.2	0.006*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.008 p>0.05 p>0.05
Postoperative 1-month	1.0 ± 1.3	4.8 ± 0.4	4.3 ± 1.2	0.007*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.010 p>0.05 p=0.007
Pre-sacrifice	0.8 ± 0.5	3.8 ± 1.3	4.3 ± 1.2	0.004*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.011 p>0.05 p=0.019
Left Limb						
Preoperative	2.8 ± 1.7	4.3 ± 1.2	3.5 ± 1.6	0.302		
Postoperative 1-week	1.0 ± 0.6	2.8 ± 1.5	2.7 ± 2.1	0.067		
Postoperative 2-weeks	0.7 ± 0.5	2.0 ± 2.0	4.7 ± 0.8	0.005*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.005 p>0.05 p>0.05
Postoperative 3-weeks	1.5 ± 0.8	4.5 ± 1.0	4.5 ± 0.8	0.003*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.004 p>0.05 p=0.011
Postoperative 1-month	1.2 ± 1.3	3.4 ± 2.1	4.0 ± 1.3	0.017*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.017 p>0.05 p>0.05
Pre-sacrifice	1.3 ± 1.0	2.8 ± 1.5	4.7 ± 0.5	0.001*	Between Circumferential and Control groups Between Dorsal and Control groups	p=0.012 p>0.05

					Between Circumferential and Dorsal groups	p>0.05

Note: SD: standard deviation; ^ Jonckheere-Terpstra test with post-hoc pairwise comparison, * statistical significance at p<0.05

Table 3. Comparison of foot placement accuracy analysis using error rate (percentage of steps)

Groups / Time-points	Error rate (mean ± SD)			p-value^	Post-hoc pairwise comparison	
	Circumferential	Dorsal Compression	Control			
Preoperative	4.3 ± 3.2	1.8 ± 2.2	1.4 ± 1.7	0.095		
Postoperative 1-week	15.4 ± 6.6	4.5 ± 4.3	7.9 ± 8.0	0.068		
Postoperative 2-weeks	13.1 ± 6.1	7.3 ± 8.3	1.5 ± 2.4	0.010*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.014 p>0.05 p>0.05
Postoperative 3-weeks	14.1 ± 6.7	3.3 ± 3.7	1.8 ± 2.1	0.003*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.006 p>0.05 p>0.05
Postoperative 1-month	11.0 ± 9.5	2.3 ± 2.7	3.0 ± 2.0	0.120		
Pre-sacrifice	18.0 ± 10.3	3.9 ± 2.8	2.1 ± 3.1	0.017*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.028 p>0.05 p=0.021

^ Jonckheere-Terpstra test with post-hoc pairwise comparison, * statistical significance at $p<0.05$

Note: error rate = ratio of number of errors per step in percentage, SD: standard deviation

Table 4. Comparison of Louisville Swim Scale (LSS) scoring

Groups / Time-points	LSS score (mean ± SD)			p-value^	Post-hoc pairwise comparison	
	Circumferential	Dorsal compression	Control			
Preoperative	16.5 ± 0.5	16.0 ± 1.7	16.4 ± 0.5	0.806		
Postoperative 1-week	4.8 ± 0.8	7.3 ± 1.6	10.8 ± 1.3	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.008 p=0.009 p=0.031
Postoperative 2-weeks	4.2 ± 1.5	7.0 ± 1.0	12.2 ± 3.3	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p=0.005 p=0.022 p=0.030
Postoperative 3-weeks	3.0 ± 1.3	6.8 ± 1.8	11.3 ± 3.7	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p= 0.005 p>0.05 p=0.014
Postoperative 1-month	2.2 ± 0.4	7.2 ± 2.6	10.2 ± 3.6	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p= 0.004 p>0.05 p=0.006
Pre-sacrifice	2.0 ± 0.7	7.2 ± 3.0	10.8 ± 3.3	<0.001*	Between Circumferential and Control groups Between Dorsal and Control groups Between Circumferential and Dorsal groups	p= 0.008 p>0.05 p=0.012

^ Jonckheere-Terpstra test with post-hoc pairwise comparison, * statistical significance at p< 0.05
Note: SD: standard deviation

Table 5. Comparison of P1 latency and N1 latency

Groups / Time-points		Circumferential	Dorsal	Control	p-value^	Post-hoc Tukey's HSD	Circumferential	Dorsal	Control	p-value^	Post-hoc Tukey's HSD
		Latency of P1 (ms, mean ± SD)									
		Right hind-paws					Left hind-paws				
Preoperative		18.0 ± 4.5	15.7 ± 1.0	16.0 ± 0.8	0.694		18.7 ± 3.4	15.3 ± 2.8	17.3 ± 0.3	0.430	
Post-operative	Immediate	21.1 ± 3.0	17.5 ± 2.3	16.4 ± 2.1	0.014*	Circumferential and Control, p=0.015	22.9 ± 6.3	18.2 ± 2.3	16.7 ± 2.4	0.052	
	1-week	18.2 ± 2.6	19.0 ± 3.1	15.5 ± 1.0	0.059		17.9 ± 2.8	20.6 ± 4.2	15.0 ± 1.0	0.019*	Dorsal and Control, p=0.015
	2-weeks	18.9 ± 2.3	16.8 ± 1.2	16.0 ± 1.4	0.036*	Circumferential and Control, p=0.033	18.4 ± 3.8	17.4 ± 1.7	16.6 ± 1.9	0.532	
	3-weeks	19.5 ± 4.8	17.8 ± 3.2	17.0 ± 1.9	0.468		18.2 ± 4.5	18.7 ± 3.1	17.0 ± 3.0	0.733	
	1-month	18.9 ± 3.2	16.8 ± 2.7	15.8 ± 1.2	0.122		20.6 ± 2.9	17.3 ± 3.1	15.4 ± 2.2	0.016*	Circumferential and Control, p=0.013
Pre-sacrifice		18.5 ± 3.3	17.6 ± 1.9	16.6 ± 1.3	0.397		20.0 ± 2.2	17.8 ± 2.2	16.8 ± 1.4	0.048*	Circumferential and Control, p=0.041
		Latency of N1 (ms, mean ± SD)									
		Right hind-paws					Left hind-paws				
Preoperative		23.6 ± 5.1	23.1 ± 5.2	24.0 ± 0.9	0.982		24.7 ± 5.7	21.2 ± 4.2	26.3 ± 3.2	0.609	
Post-operative	Immediate	27.4 ± 3.6	22.4 ± 3.2	21.3 ± 2.7	0.010*	Circumferential and Control, p=0.012 Circumferential and Dorsal, p=0.038	31.1 ± 7.2	26.7 ± 4.8	24.4 ± 4.5	0.146	
	1-week	24.3 ± 4.4	29.0 ± 6.4	22.5 ± 4.5	0.113		24.8 ± 5.6	28.3 ± 3.8	25.1 ± 1.3	0.272	
	2-weeks	24.5 ± 5.1	27.4 ± 7.5	26.6 ± 4.2	0.680		25.0 ± 5.0	27.4 ± 1.4	24.5 ± 3.6	0.425	
	3-weeks	25.6 ± 7.4	24.4 ± 4.5	23.6 ± 2.6	0.807		24.2 ± 6.3	25.0 ± 3.8	25.4 ± 4.2	0.915	
	1-month	25.4 ± 5.0	24.8 ± 6.4	22.9 ± 3.1	0.662		26.7 ± 4.6	25.1 ± 3.0	23.7 ± 4.9	0.505	
Pre-sacrifice		24.1 ± 5.5	24.8 ± 7.3	25.3 ± 3.3	0.939		22.4 ± 11.3	24.9 ± 5.4	23.7 ± 1.3	0.861	

^ one-way ANOVA with Post-hoc Tukey HSD (honest significant difference) test, * statistical significance at $p < 0.05$

Note: SD: standard deviation, ms: millisecond

Table 6. P1-N1 amplitude

Groups / Time-points	Circumferential	Dorsal	Control	p-value^	Post-hoc Tukey's HSD	Circumferential	Dorsal	Control	p-value^	Post-hoc Tukey's HSD
	P1-N1 amplitude (μV, mean ± SD)									
	Right hind-paw					Left hind-paws				
Preoperative	5.8 ± 2.9	5.3 ± 1.5	6.1 ± 0.3	0.952		6.2 ± 3.6	3.3 ± 0.4	5.7 ± 1.7	0.547	
Immediate postoperative	2.5 ± 1.8	6.1 ± 3.8	5.4 ± 3.7	0.149		2.9 ± 1.3	8.9 ± 8.1	4.3 ± 0.8	0.128	
Postoperative 1-week	3.8 ± 1.2	6.7 ± 4.3	4.0 ± 4.7	0.349		4.4 ± 2.4	7.9 ± 4.7	3.0 ± 1.4	0.058	
Postoperative 2-weeks	5.3 ± 3.0	3.2 ± 0.7	4.7 ± 3.8	0.472		5.3 ± 2.8	4.7 ± 4.9	3.2 ± 1.9	0.598	
Postoperative 3-weeks	4.9 ± 1.5	1.9 ± 1.1	4.3 ± 3.2	0.084		5.4 ± 1.1	5.5 ± 5.4	3.5 ± 0.7	0.538	
Postoperative 1-month	5.7 ± 1.2	3.7 ± 1.8	3.4 ± 2.3	0.087		5.4 ± 3.1	5.9 ± 4.6	3.4 ± 1.4	0.446	
Pre-sacrifice	5.0 ± 1.5	4.3 ± 4.4	3.3 ± 2.1	0.049*	Overall significant difference among 3 study groups, not pairwise	5.0 ± 1.3	2.6 ± 1.4	2.7 ± 1.0	0.015*	Circumferential and Control, p=0.030 Circumferential and Dorsal, p=0.025

^ one-way ANOVA with Post-hoc Tukey HSD (honest significant difference) test, * statistical significance at $p < 0.05$

Note: SD: standard deviation, μV: microvolt

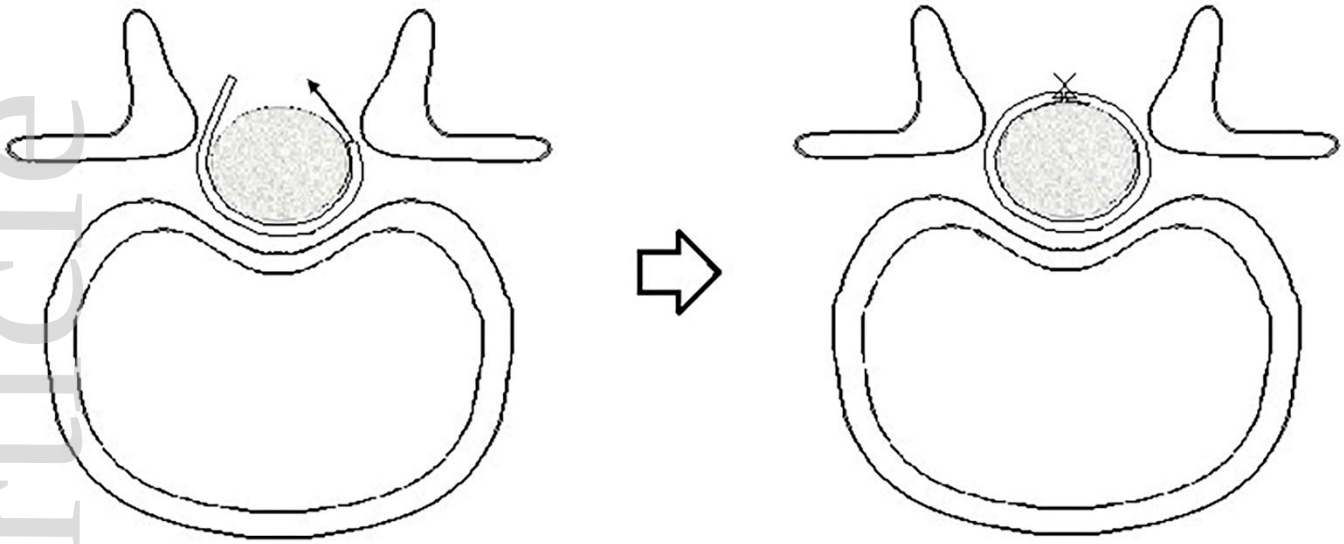

Figure 1(a)

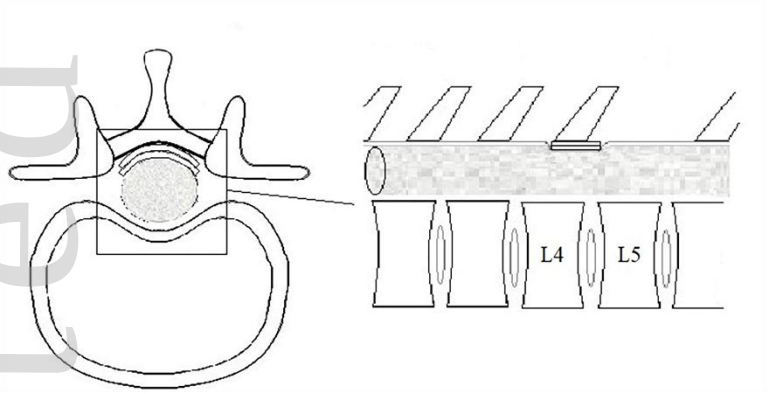

Figure 1(b)

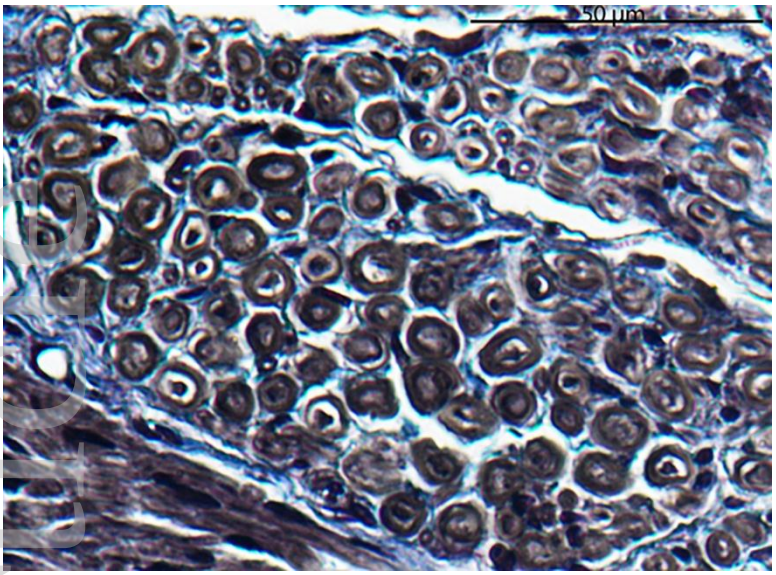

Figure 2

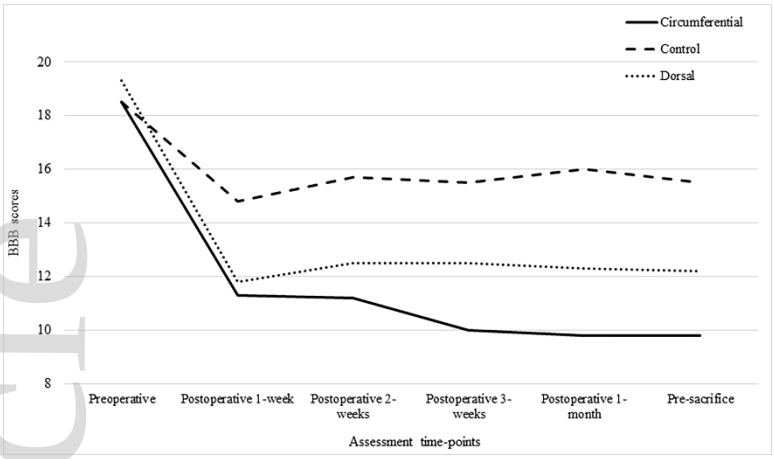

Figure 3

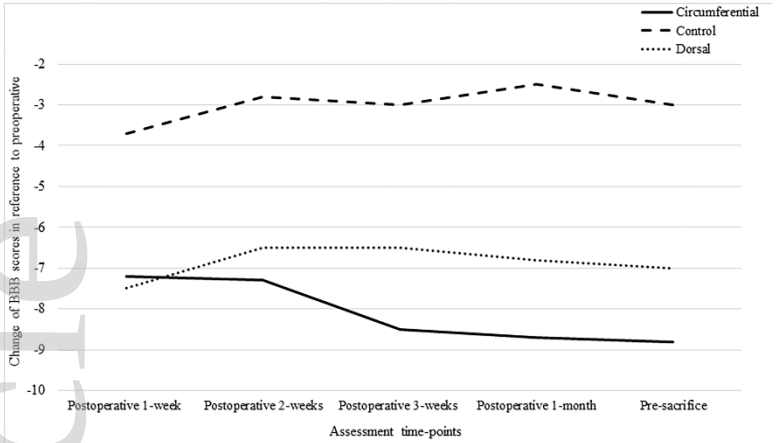

Figure 4

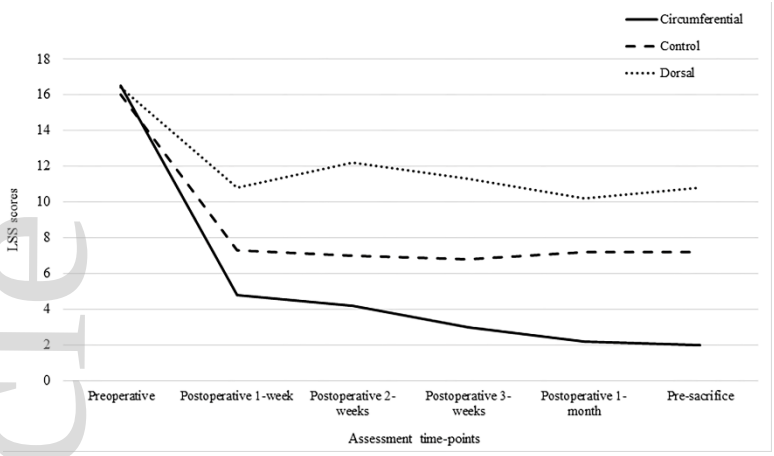

Figure 5

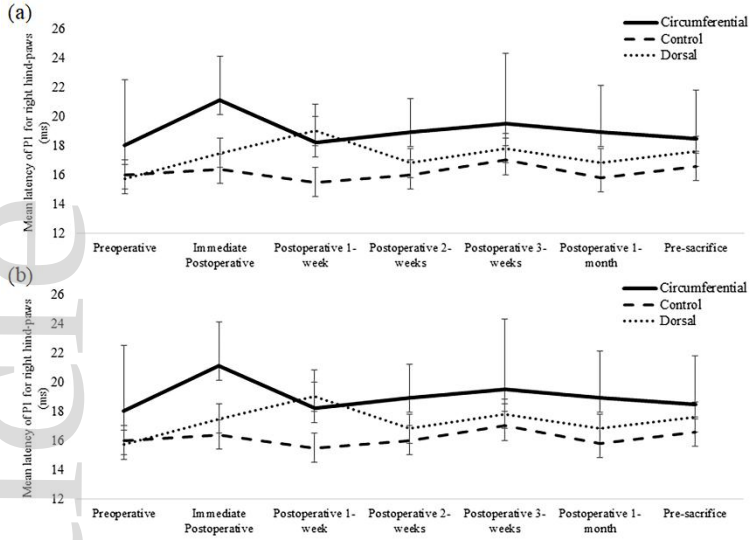

Figure 6