SPECIAL ISSUE

Large animal models of human cauda equina injury and repair: evaluation of a novel goat model

Wen-tao Chen[1], Pei-xun Zhang[1, *], Feng Xue[1, *], Xiao-feng Yin[1], Cao-yuan Qi[2], Jun Ma[2], Bo Chen[1], You-lai Yu[1], Jiu-xu Deng[1], Bao-guo Jiang[1, *]

1 Department of Trauma and Orthopedics, Peking University People's Hospital, Beijing, China
2 Department of Radiology, Beijing Tian Tan Hospital, Capital Medical University, Beijing, China

Correspondence to:
Pei-xun Zhang, M.D., Ph.D.,
zhangpeixun@126.com.
Feng Xue, M.D., Ph.D.,
drxuefeng@gmail.com.
Bao-guo Jiang, M.D., Ph.D.,
jiangbaoguo@vip.sina.com.

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Abstract
Previous animal studies of cauda equina injury have primarily used rat models, which display significant differences from humans. Furthermore, most studies have focused on electrophysiological examination. To better mimic the outcome after surgical repair of cauda equina injury, a novel animal model was established in the goat. Electrophysiological, histological and magnetic resonance imaging methods were used to evaluate the morphological and functional outcome after cauda equina injury and end-to-end suture. Our results demonstrate successful establishment of the goat experimental model of cauda equina injury. This novel model can provide detailed information on the nerve regenerative process following surgical repair of cauda equina injury.

Key Words: *nerve regeneration; spinal cord injury; goat; animal model; radiography; magnetic resonance imaging; diffusion tensor imaging; fiber bundle; diagnosis; injury; physiology; neuroimaging; NSFC grants; neural regeneration*

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Introduction
Attempts at repairing the injured cauda equina have had limited success (LeBlanc et al., 1969; Gertzbein et al., 1988). Nonetheless, there are a number of successful cases of cauda equina repair in both experimental studies and in the clinical setting (Cheng et al., 2004). Previous studies have shown that the transected nerve roots can be surgically repaired and axonal regeneration achieved (Conzen and Sollmann, 1985). However, it is difficult to repair the fragile nerve roots without attachment to an intact epineurium (LeBlanc et al., 1969; Konno et al., 1995). Little is known about cauda equina regeneration following transection. Most animal models of cauda equina injury use rats, and have a number of shortcomings (Blaskiewicz et al., 2009; Mackenzie et al., 2012). The cauda equina is much smaller in rats than in humans, making surgery and repair difficult. A novel experimental animal model mimicking cauda equina injury, allowing for surgical repair and monitoring of surgical outcome, was developed in our laboratory. There are three major aims of our study: (1) to establish a novel animal model that mimics intrathecal cauda equina injury and that allows for surgical repair of the spinal cord; (2) to identify the electrophysiological properties of muscles innervated by the cauda equina; and (3) to develop effective methods to evaluate cauda equina morphological and functional recovery using histology and MRI.

Materials and Methods
Animals
Three 6-month-old male goats, weighing 20, 24 and 27 kg (Lvyuanweiye Farm, Shunyi District, Beijing, China; license No. 2013-60), were used in this pilot study. The study protocol was carried out in strict accordance with the recommendations of the Institutional Animal Committee of Experimental Animals, Peking University People's Hospital (Beijing, China). Anesthesia was performed using intramuscular injection of Sumianxin II combined with ketamine (1:1, v/v), 0.2 mL/kg. Goats were raised as a closed herd and kept under a strict quarantine protocol.

Modeling procedure
The skin over the lumbosacral junction was shaved. The goats were fixed in the prone position for surgery. After routine disinfection and draping, a 5.0–6.0-cm longitudinal skin incision was made straight along the spinous processes originating from the posterior superior iliac spine. The subcu-

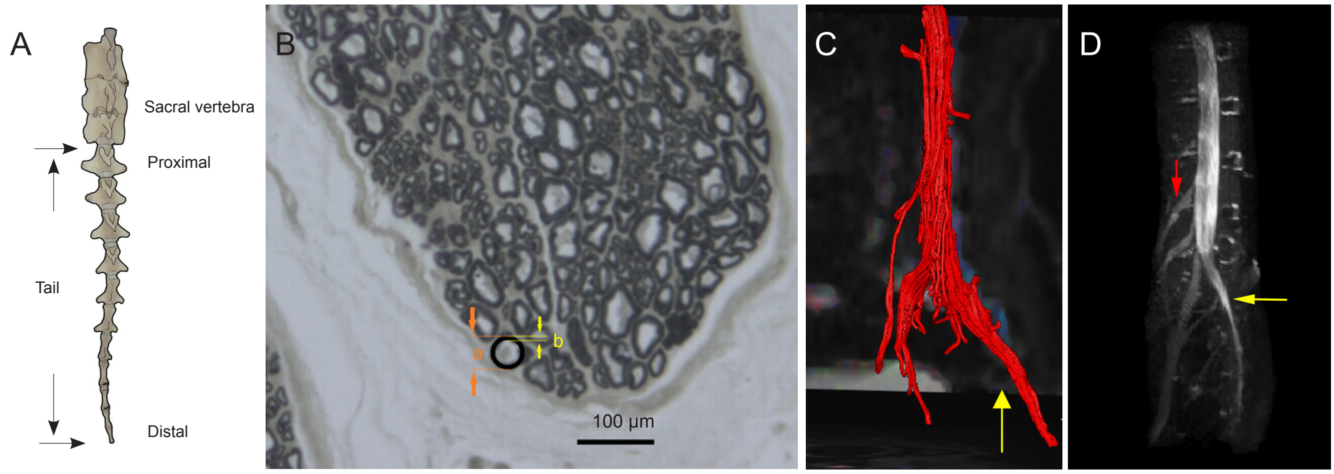

Figure 2 The gross skeletal anatomy of the goat tail, morphology of the goat cauda equina and magnetic resonance reconstruction images of the normal goat cauda equina.
(A) In the goat tail, the distal five vertebrae have no transverse process, and they diminish in size and form a dumbbell shape. (B) Intact myelinated axons were evident in the cauda equina, and it was feasible to count the number and diameter (light microscope, osmium tetroxide staining). The letter "a" (arrows) indicates the diameter of the nerve fiber, while the letter "b" (arrows) indicates the thickness of the myelin. (C) 3D diffusion tensor imaging reconstruction showing the continuous integrity of the cauda equina trunk (yellow arrows), which can be observed in any plane. (D) 3D SPACE-STIR image showing the distribution of the cauda equina (yellow arrow) as well as the nerve roots (red arrow).

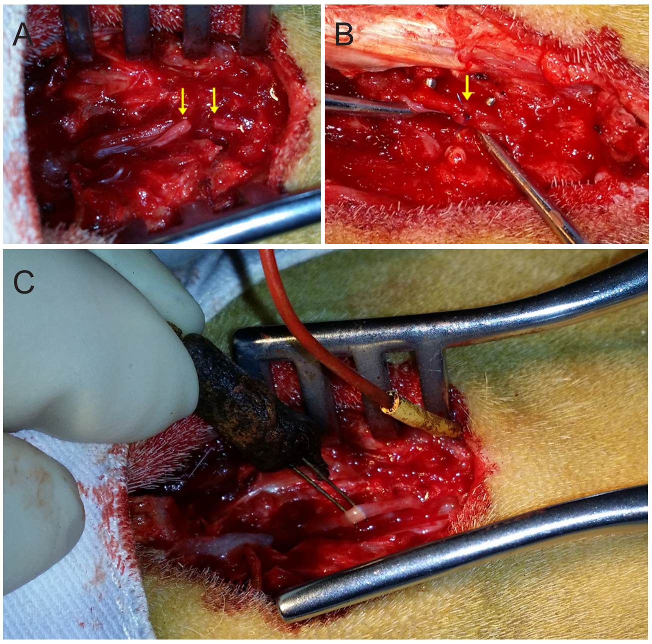

Figure 1 The surgical procedure for repairing cauda equina injury and placement of electrodes for electrophysiological examination of cauda equina nerves in goats.
The cauda equina was exposed and subjected to electrophysiological examination. The nerve was transected (A, yellow arrows indicate the end of the nerve) and then repaired with end-to-end suture *in situ* (B, yellow arrow). (C) The cauda equina was exposed, and a pair of wire hook electrodes was positioned on the nerve trunk, which was elevated slightly to avoid contact with cerebrospinal fluid. Single-pulse electrical stimulation was applied on the trunk, and electromyography activity in the tail muscles was recorded.

taneous tissue and fascia were incised to expose the lamina. The tissues were retracted laterally, and the posterior lamina was removed using a rongeur and micro-curette. Then, a window in the vertebral lamina was formed *via* the posterior approach under direct visualization of the spinal cord. The dura was then cut, and the cauda equina was exposed and isolated. After the electrophysiological examination was done, all cauda equina nerves were transected and repaired with end-to-end suture (**Figure 1A, B**). The dura was then closed with a 6-0 suture. The procedure was performed by the same senior doctor.

Electrophysiological examination
After exposure and isolation of the cauda equina, multichannel stimulus-evoked electromyography was used to monitor action potentials of tail muscles innervated by the cauda equina. A spaced (approximately 1-mm) pair of wire hooks were placed gently onto the cauda equina for bipolar stimulation. To avoid contact with cerebrospinal fluid, the hooks were elevated slightly (**Figure 1C**). Four pairs of parallel needle electrodes were inserted symmetrically along the tail and connected to preamplifiers for recording evoked electromyograhies. Every single cauda equina nerve was stimulated. Single-pulse electrical stimulation (0.09 mA in intensity, 0.1 ms in duration) (MedlecSynergy; Oxford Instrument Inc., Oxford, UK) was applied to the cauda equina. The initial intensity was 0.06 mA, and then adjusted to 0.09 mA to evoke the electromyography response from the tail muscles. When the test was completed, the nerve was transected. Stimulation was then applied to the proximal stump of the transected nerve, and the electromyography was recorded. Action potentials evoked by the electrical stimulation of the nerve were recorded. The amplitude and duration of action potentials preoperatively and postoperatively were compared.

Histological examination
Several tissue samples were obtained from the cauda equina and cut into 2-mm pieces. The samples were fixed in 4% formaldehyde in 0.1 mM phosphate buffer for 24 hours at 4°C

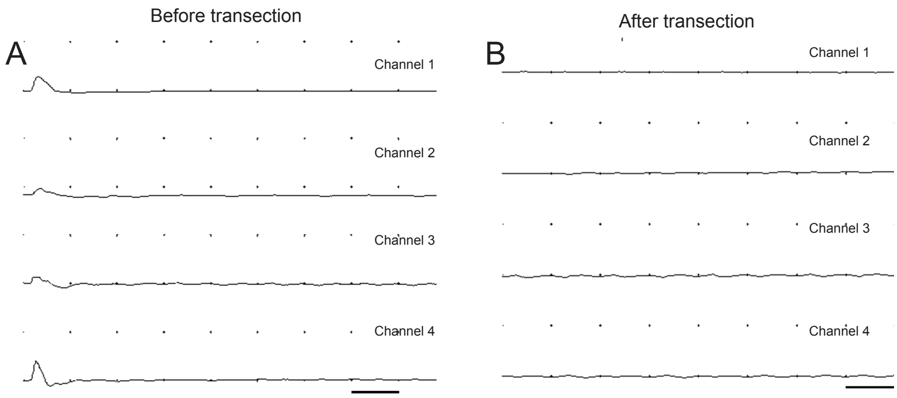

Figure 3 Electromyography recordings from the four recording electrodes (0.09 mA electrical stimulation) before and after transection of the cauda equina.

(A) Before transection, electrical stimulation on every single cauda equina site elicited an electromyography response in the four recording electrodes. (B) When all cauda equina nerves were transected, no evoked electromyography was recorded. Vertical calibration, 2.0 mV; horizontal calibration, 10.0 ms. The numbers 1–4 represent different recording electrodes.

Table 1 The fractional anisotropy values and apparent diffusion coefficients for different slices by magnetic resonance diffusion tensor imaging

Slice number	Fractional anisotropy	Apparent diffusion coefficient
1	0.41	2.09
2	0.44	1.65
3	0.55	1.88
4	0.44	2.60
5	0.56	2.17
6	0.33	2.43
7	0.44	3.21
8	0.33	3.55
9	0.24	3.25
10	0.34	2.95
11	0.41	2.27
12	0.38	1.78
13	0.56	1.33
14	0.52	1.09
15	0.36	1.66
16	0.41	1.93
17	0.43	2.10
18	0.42	2.04
19	0.39	2.37
20	0.39	2.99
21	0.53	1.64
22	0.30	3.27
23	0.33	3.12
24	0.31	3.56
25	0.24	3.83
26	0.40	1.26
27	0.34	1.18
28	0.34	1.45
29	0.37	1.09
30	0.37	1.30
31	0.35	1.53
32	0.37	1.25
33	0.32	1.03
34	0.46	0.94
35	0.46	0.84

The slices proceed rostrocaudally.

and then rinsed in water for 12 hours. After this step, the samples were stained in 1% osmium tetroxide for 12 hours, rinsed in water for 6 hours, immersed in neutral buffered formalin twice for 10 seconds, dehydrated through a graded ethanol series (50%, 70%, 90%, 95%, absolute), immersed in xylene twice for 40 seconds, and embedded in paraffin for sectioning. The blocks were cut into 2-μm-thick cross-sections.

The images were observed under a DFC 300FX color digital camera (Leica, Heidelberg, Germany). The total number and diameter of myelinated axons were measured (Wang et al., 2013). The thickness of the myelin was subsequently evaluated. The number and diameter of the proximal and distal ends of the transected cauda equina will be determined and compared at different postoperative time points in a future study.

MRI procedure

All images were acquired with a 3.0T MR scanner (Siemens Healthcare, Erlangen, Germany). The animals were placed on the surface coil in the supine position. The protocol consisted of spin-echo (repetition time = 4.0 seconds, echo time = 98 ms, field of view = 350 cm × 350 cm, slice thickness = 4.0 mm), half-Fourier acquisition single-shot turbo spin-echo, SPACE-STIR, MEDIC, and diffusion tensor imaging (DTI; repetition time = 4.0 seconds, echo time = 98 ms, field of view = 280 cm × 280 cm, slice thickness = 4.0 mm) sequences. The parameters for the DTI sequence were as follows: spatial resolution, 2.0 mm × 2.0 mm × 4.0 mm; coverage area, L2-coccygeal vertebra; *b*-value, 600; scanning time, 14 minutes 59 seconds. A volume coil and surface coil (Siemens Healthcare, Erlangen, Germany) were used in the scanning. The inside diameter of the volume coil was 49 cm, and was used for radiofrequency transmission. The diameter of the surface coil was 52 cm, and was used for receiving radiofrequency signals. Image reconstruction for tractography was performed using a workstation (Neuro 3D, Siemens, Germany) by selecting regions of interest. The region of interest was between the middle part of L_4 and the inferior part of S_1. Fractional anisotropy (0.2) and angle threshold (30 degrees) were used for tractography. The complete cauda equina could be visualized and compared in reconstructed tractography images.

Results

Gross anatomy of the goat cauda equina

Macroscopic anatomical visualization showed that the goat tail is composed of 11 vertebrae, of which the distal five have no transverse process that may serve to attach the tail muscles. The seventh coccygeal vertebra was relatively small and had a dumbbell shape that was quite different from the first to sixth vertebrae (**Figure 2A**). Six major erector spinae muscles covered the ventral side of the tail, and two the dorsal. There were intrinsic muscles and ligaments attaching the adjacent vertebra. Intrinsic tail muscles and musculus sacrococcygeus might control the movement of the tail. Gross anatomy indicated that the terminal part of the spinal cord extended into the cauda equina at the level of the posterior superior iliac spine. The region between the posterior superior iliac spine and the proximal end of the tail can be used to fully expose the cauda equina. In the goat, the cauda equina, with a comparatively large diameter, is more clearly visible than in the rat. The cauda equina was divided into several bundles (range 4 to 6) with an intact perineurium. Before the surgery, the goats often swung their tails in a circular arc, which did not appear to affect body stability or balance, which may be in contrast to the rat. All the goats tolerated the surgery and were returned to their herd for free movement. No obvious dysfunction of the hindlimb or bowel or bladder incontinence was found postoperatively.

Histology of the goat cauda equina

The cauda equina was stained with osmium tetroxide. The number and diameter of the nerves could be enumerated. Microscopic images revealed that the goat cauda equina had some similarities to the human cauda equina, in the structure of the myelin sheath and perineurium (**Figure 2B**).

MRI characteristics of the goat cauda equina

MR DTI reconstruction showed the continuous integrity of the cauda equina axons and myelin, and the distribution of the nerve roots (**Figure 2C, D**). The nerve bundles were colored red during image reconstruction, and were easy to distinguish. As shown in **Table 1**, the fractional anisotropy value and apparent diffusion coefficient of the proximal part seemed not very different from those for the distal cauda equina (**Table 1**). Changes in fractional anisotropy values and apparent diffusion coefficients can serve as adjunctive diagnostic and prognostic tools in the rehabilitation of cauda equina injury in the future.

Electrophysiological examination of the goat cauda equina before and after injury

Electrical stimulation was applied to different portions of the cauda equina, and the four recording electrodes were used to monitor action potentials. Electrical stimulation on every single cauda equina site produced an electromyography response in the four recording electrodes (**Figure 3**). When a portion of the cauda equina was cut, the recording electrode also monitored evoked Electromyograhies following electrical stimulation of the left cauda equina. When all cauda equina were transected, no evoked electromyography was recorded in the tail (**Figure 3**).

Discussion

Fracture of the spine can lead to spinal cord injury involving the cauda equina. The tone of the urinary and anal sphincter is controlled by the cauda equina in humans, and cauda equina injury can lead to severe back pain, saddle anesthesia, bladder and bowel dysfunction, weakness of the lower extremity muscles, and sexual dysfunction. Several attempts have been made to repair the damaged cauda equina. Some studies reported unsatisfactory outcomes (LeBlanc et al., 1969; Gertzbein et al., 1988). However, some studies have reported successful outcomes in experimental studies and in the clinical setting (Cheng et al., 1996; Cheng et al., 2004). It is necessary to evaluate outcomes following cauda equina injury and repair using animal experiments. Previous animal experimental studies focusing on the identification of extrathecal nerve roots in rats have shown promising results (Blaskiewicz et al., 2009; Mackenzie et al., 2012). However, the cauda equina in rats is difficult to observe without surgical microscopes. In contrast, the cauda equina in large animals is amenable to unassisted visual inspection, and the nerve diameters are similar to those in the human. Primates, such as the rhesus monkey, are ideal as models for cauda equina injury and repair. However, primates are expensive and their use is accompanied by major ethical considerations. In comparison, goats are widely available and inexpensive. However, little is known about the anatomy, histology or electrophysiology of the cauda equina in goats.

Previous studies have examined the electrophysiological characteristics of extrathecal nerve roots in the rat model (Blaskiewicz et al., 2009; Mackenzie et al., 2012). Stimulus-evoked electromyography is often used in spine surgery and peripheral nerve surgery (Bose et al., 2002; Holland, 2002; Luo et al., 2012). Furthermore, it is an effective technique for the diagnosis and prognosis of nerve injury. Accordingly, the recruitment patterns of the muscles controlling the goat tail were examined in this study using stimulus-evoked electromyography.

Electrophysiology showed that the muscles in the tail were not innervated exclusively by a single cauda equina nerve. Even with little cauda equina remaining, the function of the tail was partially preserved, and electromyography activity in the tail muscles could also be observed. Therefore, we postulate that the cauda equina nerves cross-innervate the tail muscles. Thus, if a part of the cauda equina is damaged, the function of the target muscles could still be restored. The cauda equina could serve as a nerve donor for nerve defect repair. Electrophysiology is useful for evaluating the recovery process following cauda equina repair after transection, and can be used to assess the functional recovery of tail muscles.

Using osmium tetroxide staining, the number and size of cauda equina nerves were clearly visible under the light microscope and calculated in the digital image. This staining technique can be used to assess morphological recovery. The nerve fibers in the proximal and distal parts of the nerve can be compared to evaluate nerve regeneration at different time points. This approach can help estimate recovery time after cauda equina injury.

MRI is an effective imaging technology in the diagnosis and management of spinal injury. MRI can help evaluate the arrangement and integrity of the spinal cord compared with CT angiography (Goldberg and Kershah, 2010). Hemorrhage and cord swelling detected by MRI can help predict the prognosis of spinal cord injury (Miyanji et al., 2007). Changes in water content due to altered axoplasmic flow secondary to neurotmesis produce signal changes on MRI sequences in peripheral nerve injury (Filler et al., 1996). MR DTI can provide additional insight into axon and myelin integrity (Kim et al., 2006). A number of studies have successfully used DTI in rats (Gullapalli et al., 2006; Ellingson et al., 2008; Kim et al., 2009; Zhang et al., 2009). Although less sensitive than electromyography, MRI may be a useful adjunctive diagnostic tool, with a sensitivity of 84% and specificity of 100% for detecting denervation (McDonald et al., 2000). MRI was also used in our pilot study to provide detailed information on the cauda equina in goats, which had not been reported before. Difference in fractional anisotropy and apparent diffusion coefficient values at different time points can be quantified for comparison, and can serve as adjunctive diagnostic and prognostic tools. Images constructed by MRI provide a clear visualization of the cauda equina in any plane, and can be helpful for diagnosing cauda equina injury and for evaluating postoperative repair outcome and the integrity of the axonal structure *in vivo* following surgical reconstruction. Our results demonstrate the feasibility of using MRI in the diagnosis and prognosis of cauda equina injury.

Our research shows that the target organ of the cauda equina in goats is the tail muscle, and that MRI-DTI is effective for the diagnosis and prognosis of cauda equina injury. The tail muscles were found to be cross-innervated by cauda equina nerves by electrophysiology. The functional recovery of tail muscles can be evaluated using electrophysiological testing by comparing the amplitude and interval of electromyography activity at different time points.

Although this pilot study is descriptive and qualitative, not quantitative, it provides fundamental histological, electrophysiological and MRI data on the cauda equina in goats. The degeneration and regeneration of the nerves and target tail muscles can be evaluated using the methods used in this study. Furthermore, our animal model is easy to establish and reproduce. Our findings demonstrate the feasibility of surgical repair of the cauda equina. It is ideally suited for morphological and functional analysis during the entire neurological recovery process.

Author contributions: *WTC, PXZ, FX and BGJ designed the study. WTC, PXZ, FX, CYQ, JM, BC, YLY and JXD performed experiments. WTC, PXZ, XFY and BGJ analyzed the data. WTC, PXZ, FX and BGJ wrote the paper. All authors approved the final version of the manuscript.*

Conflicts of interest: *None declared.*

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