Chapter 10

Animal Models in Traumatic Spinal Cord Injury

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1. Introduction

Traumatic spinal cord injury (SCI) causes high mortality, severe disability, expensive cure, extensive rehabilitation, and a high economic burden. There has been no definite treatment for SCI, but numerous studies including experimental modeling are being performed to assist resolving this fundamental problem.

The first reported SCI model was presented by Allen in 1911 where a mass was dropped from a prescribed height onto the dorsal surface of the canine dura. After that, the animal models of SCI from simple lamprey to non-human primates were used to develop pathophysiological knowledge on cell injury and repair process of spinal cord.

Currently, to choose an animal model, some factors are considered depending upon the proposed aim of the study. Transections and contusions of the spinal cord are the most commonly used methods for animal modeling of SCI. While transection models provide an idealized setting for studying spinal cord regeneration across a complete lesion, but transected spinal cords are rarely encountered in human SCI. In other words, most injured spinal cords maintain some tissue continuity across the area of injury. But contusion and compression models are more clinically relevant. These models can create graded injuries and characterized by hemorrhagic necrosis, ischemia, inflammation, and central cavitation. Besides, compression models contribute to simulate the persistent spinal canal occlusion that is common in human SCIs.

The ongoing development of SCI animal models reflects the need to review all types of them and gauge about their advantages or disadvantages. The purpose of this chapter is to review animal models in SCI from studies indexed in Medline.

2. Why animal models?

Animal model refers to the use of a living, non-human animal to simulate the human disease or injury, for better understanding the disease where it is practically or ethically difficult to use humans. It is used to learn more about a disease, its pathophysiologic changes, diagnosis and treatment. Animal models are often preferable for experimental disease or injury research because of their unlimited supply, ease of manipulation, the possibility to standardize the condition, the capability to use more invasive procedures to observe the effects of treatment, and no concern for the patients' safety [1, 2]. In fact, many potential therapies require testing for safety and efficacy in animals before it is possible to move to a clinical trial.

To serve as a useful model of a human condition, a modelled disease or injury not only must be similar in the etiology and function to the human equivalent but also has to offer advantages over direct clinical observation and experiment [2, 3].

On the other hand, spinal cord injury (SCI), as a fundamental problem in medicine, causes high mortality, severe disability, expensive cure, extensive rehabilitation, and a high economic burden. So far management of SCI is challenging and there has been no definite treatment for it. But numerous studies including experimental modeling are being performed to assist understanding the anatomical and biological consequences of injury and repair, and testing the efficacy and the risk-to-benefit ratio of a proposed therapy [3]. Animal models have been developed with the aim of recreating features of either complete or incomplete SCI to increase the knowledge about disease mechanisms and evolution of injury, and provides a clinically relevant platform for developing and evaluating therapies in SCI [4, 5]. Animal models have also some other benefits over their human equivalent; e.g. the specified tissue needed can be used and processed for histological purposes to investigate co-localization of proteins of interest, mRNA analysis (microarray) to give expression of proteins and protein analysis (western blotting) to give levels of protein [6].

3. History

Various methods for induction of experimental SCI have been used in the past. The first reported SCI model was presented by Allen in 1911 where a mass was dropped from a prescribed height onto the dorsal surface of the canine dura. He used a simple irrefutable logic that when a known weight dropped from a constant height shall produce same impact force on all occasions. Based on this concept, he prepared a metal tube with pores. A rod of 10 g was inserted into the tube and can be stopped at various heights using a pin inserted into the pores on the tube at regular intervals. By aiming the tube over a surgically exposed spinal cord and by withdrawing the pin holding the rod, a reproducible impact force would be created when the rod get dropped on the spinal cord. For unknown reasons, most data available concerning experimentally induced SCI are modifications of an injury model proposed by Allen [7].

In 1936, the load throw devices were used to make a spinal cord contusion [8]. In 1953, a model was created in which a dog had its spinal cord injured by an inflated balloon inside the spinal

canal [9]. In 1976, Eidelberg created an SCI model in rats caused by direct epidural compression [10]. New techniques were developed and improved, e.g. spinal cord stabilization and precise distribution of strengths involved on impact, the use of mechanisms able to measure the strength to which an animal's spinal cord is exposed, as well as the invention of pneumatic impact mechanisms [11].

Because the weight-drop techniques deliver a single, rapid blow to the spinal cord, neither model simulates ongoing cord compression secondary to residual spinal column displacement. Thus, in 1978, Rivlin and Tator introduced a clip compression model of SCI in rats, in which the spinal cord was compressed for variable durations between the arms of a modified aneurysm clip [12]. This model demonstrated the relation between the severity of neurologic injury and the length of compression.

Afterwards, more technical devices such as Ohio State University's electromagnetic spinal cord injury device (OSU impactor) and New York University (NYU) impactor came into use. In 1987, the researchers at Ohio State University applied a computer feedback-controlled electromagnetic force to create contusion and concussion in the spinal cord of rats [13]. In this model, after laminectomy, the OSU impactor probe is slowly screwed down to the dural surface, which it contacts and displaces 30 micrometers with a force of approximately 3000 dynes. This is meant to provide a consistent starting point from which to initiate the injury. The system then is triggered, and the device rapidly impacts the cord for a predetermined amount of displacement before releasing [14]. Because the OSU impactor is actively withdrawn, there is no bouncing of the impactor back onto the cord, which is a probable basis of variation in a weight-drop technique. NYU impactor was at first described by Gruner in 1992 and then refined by a consortium of eight spinal cord laboratories in the United States called MASCIS (Multicenter Animal Spinal Cord Injury Study). The NYU-MASCIS weight-drop model standardizes grades of contusive spinal cord injury by dropping a 10g rod from specific heights of 6.25 (mild), 12.5 (moderate), 25 (severe) or 50 mm (very severe) upon the exposed dorsal surface of the spinal cord [15]. Usage of the recent impactors requires intense training, extensive maintenance and sophisticated software which give more room to exclude the post-operative animals being used for the experiments.

In addition to traumatic SCI, spinal cord ischemia remains an underappreciated clinical dilemma which mostly occurs after aortic problems. Therefore, experimental models of spinal cord ischemia have been developed in different animals with variable reproducibility [16-19].

In the last decades, transection has been favored to study approaches of nerve fiber regeneration and cell transplantation that are likely to be most appropriate to the subacute stage.

4. Level of SCI

The majority of reported human injuries occurs at the cervical level, often secondary to vertebral fracture, producing compression or contusion of the spinal cord [20]. Functional deficits after cervical injury are a result of damage to both white and gray matter. At this level,

white matter disruption leads to spastic paralysis below the injury, sensory loss/chronic pain, cardiovascular, gastrointestinal, and sexual dysfunction. Motor neurons controlling the upper limb musculature reside there, and their loss induces flaccid paralysis [21]. But so far, thoracic SCI is the most commonly used location in animal models. Since gray matter loss at this spinal level causes less identifiable functional loss, thoracic SCI could contribute to isolate and study white matter deficits. In addition, high cervical levels can result in diaphragm dysfunction due to interruption of bulbospinal respiratory drive to phrenic motoneuron pools (C3–C5) [22, 23]. Thus thoracic SCI models are obviously reliable and easy to reproduce [24, 25].

However, due to differences in spinal cord diameter, the distance of injury from both the neuronal cell body and the original targets of innervations, the relative dedication of the cord to specific ascending and descending systems and their different termination sites, the degree of vascularization, the size of the sensory and motor neuron populations, the level of their importance in locomotion, and white/gray matter distribution, histological, behavioral, and therapeutic findings in the thoracic spinal cord, may not be so readily applicable to the cervical level [26].

On the other hand, rats do not use their hindlimbs as skilfully as their forelimbs. Also the hindlimb paw and digit use cannot be evaluated as carefully as the forelimb paws and digits. Thus, forelimb evaluation could superiorly assess the efficacy of potential therapies, especially in mild degrees of improvement. Therefore, some scientists tried to characterize cervical SCI in rats [26, 27]. In 2001, Soblosky et al. characterized a unilateral cervical contusion SCI model which allowed the contralateral side to serve as a within-subject control [24]. In this model, the injury did not cause overt bladder dysfunction, which significantly reduced the need for chronic intensive care after SCI. In 2005, this model has been further standardizes by Gensel et al. [21].

5. Injury paradigms

In general, experimental models can be naturally occurring (e.g. injured dogs in road traffic crashes), congenital disease (e.g. a spontaneous mutant), or induced (surgical, genetically engineered) that is similar to a human condition. SCI models are mostly created based on surgical methods which are determined by the experimental aims of a particular research. Every injury techniques concentrate on a special question, and hence each carries their own pros and cons:

- *Contusion:* If the pathophysiology of secondary injury is the main part of research interest, a contusion and/or compression model could be selected; because most human SCIs involve contusive or compressive injury [28]. Contusion is the oldest and most widely used for SCI models. The contusive models can create graded injuries and characterized by hemorrhagic necrosis, ischemia, inflammation, and central cavitation. It elicits both motor and sensory dysfunction, such as tactile allodynia, neuropathic pain, and thermal hyperalgesia.

Some devices exist to create contusion in a controlled way to limit the variation between animals and allow the comparison between results obtained in different laboratories. (Figure 1)

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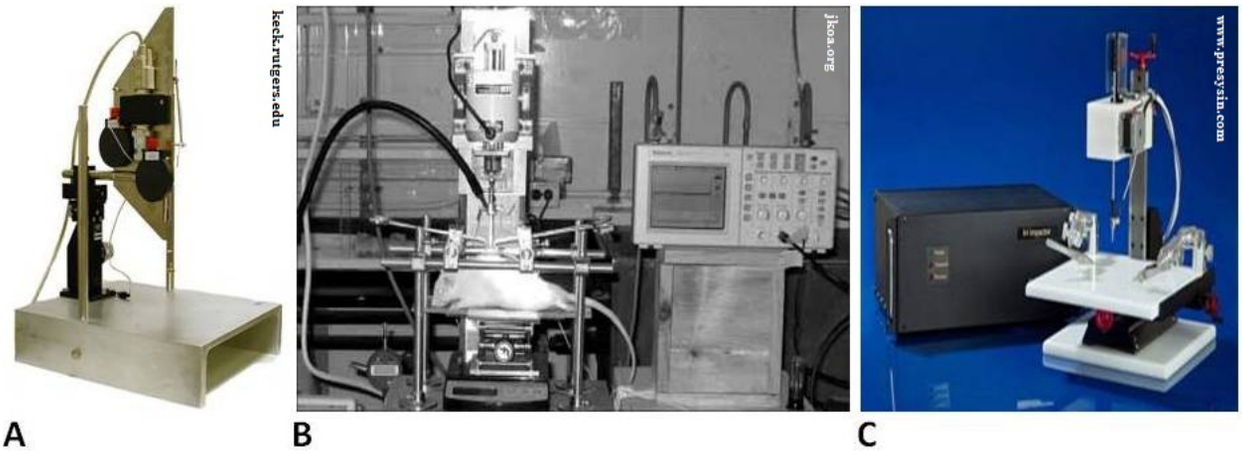

Figure 1. A: NYU Impactor. B: OSU Impactor. C: IH Impactor.

The most widely used device is the NYU impactor which concurrent recording of kinematic parameters of the impounded probe allows the validation of the injury process.

OSU impactor electromagnetically drives an impounder tip onto the cord until a desired displacement of the cord surface is reached. After a defined time, the tip is retracted and the pressure released [29]. This computer controlled contusion model consists of an animal trap that reproducibly delivers a defined weight to the exposed spinal cord, with a computer monitoring the dynamics of the impact [30].

In a similar way operates the only commercially available device, the Infinite Horizon (IH) impactor. A stepping motor applies a defined force to the cord. Once the force is reached, the impactor retracts [31].

The NYU impactor is rather easier to use, but the OSU impactor and IH impactor have more precision to produce lesions more reliably [32].

Hemicontusion: Hemicontusion or unilateral contusion is used in cervical spinal cord, because life-threatening adverse effects could occur in cervical contusion. Since motor dysfunction appears in the forelimbs, pain related behavior is difficult to estimate, and for this reason, cervical contusion is often utilized for motor functional analysis [21].

- *Compression:* Compression models contribute to simulate the persistent spinal canal occlusion that is common in human SCIs and investigate the effects of compression or the optimal timing of decompression. For this reason, a clip, balloon, spacer, or forceps compression model would be appropriate. (Figure 2)

Clip compression injury is similar to spinal contusion injury at the point of the injury caused by pressure to the spinal cord. Following laminectomy, a vascular clip is dorsoventrally closed over the entire cord. With this method, the spinal cord becomes ischemic and mimics common clinical injuries and outcomes. Compressive injury is induced with clips calibrated to exert a convinced force to induce mild, moderate or severe injury [33, 34].

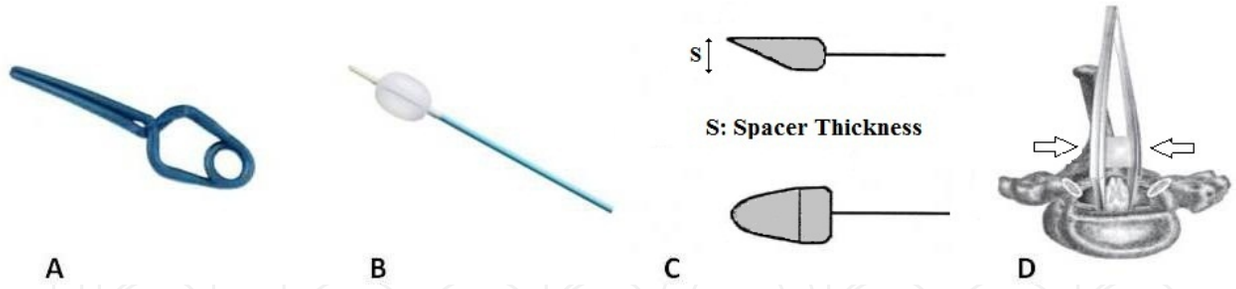

Figure 2. A: Aneurysm clips. B: Fogarty catheter. C: Spacer. D: Forceps [2].

The balloon-induced method has been used because it is a simple method that does not cause any damage to the surrounding structures. The volume of balloon inflation must be measured several times and used in combination with the size of the experimental animals when determining a sufficient amount of injury to inflict [35]. A Fogarty catheter is inserted into the dorsal epidural space through a small hole made in vertebral arch, advanced cranially to one or two higher spinal levels. Spinal cord damage is graded by increasing the volume of saline used to inflate the balloon.

To use a spacer, at first the average anteroposterior spinal canal diameter should be determined from the spines of animals of similar weight and age. This allowed for the determination of the spacer size needed to produce a precise degree of narrowing of the spinal canal diameter [36].

The calibrated forceps can produce a lateral compression injury by inserting on either side of the spinal cord and closing together to induce a central hemorrhagic necrosis and displacement of the centrally located, damaged tissue in cranial and caudal directions [2].

- *Transection:* The transected spinal cords are rarely encountered in human SCI, but transection models provide an idealized setting for studying hypotheses that concern regeneration, degeneration, tissue engineering strategies, or plasticity on an axonal level. These types of lesions are most usefully combined with neuroanatomical tract tracing and electrophysiological studies [32, 37]. Transection models are also increasingly used to model the effects of scaffolds, biomaterials, neurotrophic factors, and combinatorial therapies on axon regeneration after injury [6]. To allow for regeneration, sterile gel foams have been placed between the two ends of transected cords with variable degree of success [38]. Besides, if a device is to be implemented, a partial or complete transection model might be best suited for device placement. Many studies have reported bilateral muscle spasms, neuropathic pains, mechanical allodynia and thermal hyperalgesia at same, above, and/or below the level of the lesion following complete spinal transection model [38].

Spinal cord transection is performed after laminectomy with fine surgical scissors (iridectomy scissors) that allows the targeted interruption of a particular nerve fiber systems such as motor tracts (corticospinal tract, rubrospinal tract) or sensory tracts (dorsal columns), or even complete interruption of the spinal cord [32].

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For certain applications, partial transection can be a viable alternative to complete transection. In other words, because the lesion that results from a complete transection creates such a hostile tissue environment, injury paradigms have been developed that decrease the physical damage to the cord and the consequential cavitation and physical separation. Thus researchers can selectively interrupt certain pathways with partial transections to hold a tissue bridge between the proximal and distal ends of the cord, and maintain tissue continuity [39]. A dorsal hemisection for selective transection of the corticospinal tract can be performed with some feedback from the change in color and texture between the white and gray matter, giving a sign of the entirety of the hemisection [2, 37]. But dorsal hemisection cannot be used rigorously to assess true axon regeneration [39]. Dorsolateral quadrant lesions are used to interrupt the rubrospinal tract, and lateral hemisections disrupt all tracts on one side but spare some or all tracts on the opposite side.

- *Photochemical model:* This model was developed by Watson et al. in 1986 [40] and was proven to be one of the most reliable and reproducible graded ischemic experimental models of SCI [41]. With the exposed spinal column intact, irradiation of the translucent dorsal surface induces excitation of the systemically injected dye (e.g. rose Bengal) in the spinal cord microvasculature. The resultant photochemical reaction leads to vascular stasis, hemorrhagic necrosis of the central grey matter, edematous pale-staining white matter tracts and vascular congestion. The main benefit of this technique is that the resulting injury does not induce mechanical trauma to the cord, because there is no need for laminectomy. On the other hand, an intravascular photochemical reaction occurs through the use of a dye that is activated by an argon ion laser to produce single oxygen molecules at the endothelial surface of spinal cord vessels. This leads to a severe platelet reaction, subsequent vessel occlusion, and parenchymal tissue infarction. Also, the degree of injury is hard to control [38].

- *Ischemic model:* Initial studies used the methods described by Lang-Lazdunski et al. [16]. This method uses an anterior sternotomy with temporary aortic occlusion created by aneurysm clips sited at the aortic arch plus left subclavian artery [42].

- *Excitotoxic model:* Following intraspinal or intrathecal injection of some excitotoxins (e.g. A-metabotropic receptor agonist quisqualic acid or other excitatory amino acids such as glutamate, N-methylasparate, and kainic acid), the cascade of events described following ischemic and traumatic SCI, including prominent inflammation, neuronal loss, astrocytic scaring, cavity formation, syringomyelia, long-lasting spontaneous pain, and mechanical allodynia occur. This model can correlate specific areas of tissue damage with behavioral changes. But almost all animals develop varying degrees of hypersensitivity to mechanical and thermal stimuli [38, 43].

- *Combination:* For some particular goals, a combination of models might be designed. For example, the early stages of an experimental study that explores axon regeneration may use transection paradigms to definitely reveal regenerated axons and recognize the most promising therapies, which can then be examined in contusion models [37].

6. Species of animals used

Rodents are the most common type of mammal employed in SCI experimental studies, and widespread research have been conducted using rats, mice, gerbils, guinea pigs, and hamsters [1]. Other animal experiments include cats, non-human primates, goats, pigs, and dogs [1, 4, 35, 44-49]. Of course, larger mammals such as cats, dogs, or pigs are also used but very rarely and are less experienced models based in SCI research, requiring expensive after care and housing as well as stringent ethical considerations [6, 37]. Other models include invertebrates, such as eels [50], whose unique regenerative capacities have been studied in efforts to apply novel strategies to human SCI.

- *Rat:* Rat models are most widely used to study SCI. They are inexpensive, friendly, easy to care for, and can be studied in large numbers. They have a well understood anatomy and few surgical infections. There are also well-established functional analysis techniques in rats. Early mortality of them is not costly [37, 38]. In addition, rats develop large fluid-filled cystic cavities at the injury site, similar to the human pathology. Therefore those are preferable for studies where mimicking the human pathology is important, including preclinical studies that focus on the efficacy of novel cellular and/or pharmacological therapies [51]. Rats can be used when the size is of less importance [52]. The corticospinal tract of rat is mostly dorsal. As two disadvantages of the rat models, the corticospinal tract lesions would not significantly create disability, and rats are quadrupeds not bipeds.

- *Mouse:* In SCI research, mouse models have also been implemented increasingly, but the small working size prohibits many surgical maneuvers and device implantations [37, 38]. The injury site in mice is densely packed with cells and actually decreases in size over time (that do not have a cyst). Thus to gain mechanistic insights into the basic cellular and molecular biology of SCI, mouse models may have more to offer [51, 53].

Among rodents, the majority of genetic studies, especially those involving disease, have employed mice, not only because their genomes are so similar to that of humans, but also because of their availability, ease of handling, high reproductive rates, and relatively low cost of use [30, 54, 55]. Using mice with a knockout of a target molecule has become the gold-standard for functional testing, and Cre-Lox technology along with increasing numbers of transgenic mice have provided greater temporo-spatial control of the knockout strategy that has proven invaluable for providing mechanistic insights into the cellular and molecular processes of axon regeneration [51].

- *Cat:* Use of cats can clarify the histopathologic features of acute and chronic stages of SCI. Their larger size allows implementation of more intensive therapeutic regimens, such as implantation of electrical stimulators, than is possible when smaller animal models are studied [52]. Cats have been a popular model for spinal cord electrophysiologists [56].

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- *Pig:* Because of large size and greater likeness to human physiology, pig models are becoming more important as a preclinical model that is intermediate in size between rodents and humans [51].

- *Dog:* Dogs can be surveyed after naturally occurring SCIs e.g. following road traffic accidents or disc degenerations. The mechanisms of injury in clinical SCI in dogs are similar to those in human patients: vertebral fracture–luxation and disc extrusions – both of which produce the mixed contusion-compression lesion to the ventral aspect of the cord that is problematical to model in the laboratory [45]. To date, dogs have been used to study spinal cord injuries because neurological examinations could be carried out easily, and more detailed pathophysiological studies could be conducted [35, 46]. Compared to analysis of trials in human patients, dogs have the advantage that there is less of an ethical problem.

- *Non-human primate:* Non-human primate models are limited by extremely high costs related to the intensive animal care and ethically challenging, but may be imperative to prove safety and efficacy on a small scale prior to human experimentation, particularly for strategies involving device implantation [25]. Because of similar anatomy and pathology to human, a primate model could provide greater positive predictive value to human therapies, and lead to basic discoveries that might not be identified in rodent models [4].

7. Outcome assessments

- **Behavior**

Behavioral outcome in experimental SCI models is the most important factor for evaluating the extent of injury and treatment efficacy. It is directly related to the extent of neuronal damage in the gray matter at the injury site, the loss of ascending and descending axons in the white matter, and the reorganization of the remaining nervous system [57, 58]. Sedy et al. categorized the behavioral tests as: locomotor tests (testing the locomotor apparatus of the animal), motor tests (analyzing the strength, coordination and other abilities of the skeletal muscles), sensory tests (evaluating proprioception, touch, pain or temperature sensing), sensory–motor tests (testing the proper connection between the sensory and motor systems), autonomic tests (evaluating the function of the sympathetic and parasympathetic systems), and reflex-response based tests [58]. (Table 1)

Rahimi-Movaghar et al. showed usefulness of the tail-flick reflex in the prognosis of functional recovery in paraplegic rats [59]. Although there has been an abundant interest in locomotion in animal studies, the connection between locomotion and spinal cord integrity at the site of injury in the animal is not at all easy. In particular, behavioral measurements in the context of lateral or dorsal hemisection are even more difficult [2]. Table 2 shows recommended testing methods for SCI models.

Behavioral tests	Tests	Reflects	Lesion severity			Pros	Cons
			Mild	Moderate	Severe		
Locomotor tests	Primary open-field	Locomotion		■		Simple, cheap	Low sensitivity
	BBB	Locomotion	■	■		Simple, cheap	Subjective
	Open-field activity	Locomotion	■	■		Unique data	Depends on motivation
	Automated walkway	Locomotion	■	■		Precise	Equipment
	Footprint analysis	Motor coordination	■	■		Precise	Environment-dependent
	Kinematic analysis	Locomotion	■	■		Detailed	Equipment
	Thoracolumbar height	Weight support		■		Examines only one characteristic	Equipment
	Swim	Swimming ability	■	■		Spontaneous locomotion	Subjective
	Eshkol–Wachmann notation	Locomotion	■	■		Detailed	Requires training of scientist
Motor tests	Inclined plane	Muscle strength	■	■	■	Simple, cheap	Not standard among laboratories
	Limb hanging	Grasping	■	■		Unique data	Not for severe injuries
	Limb grip strength	Muscle strength	■	■		Precise	Equipment
	Forelimb asymmetry	Paw preference	■	■		Sensitive to chronic deficits	Not for severe injuries
	Rearing	Paw preference	■	■		Sensitive to selective limb use	Not for severe injuries
	Food pellet reaching	Motor coordination	■			Fine motor function test	Food deprivation
Sensory tests	Hot plate-based	Temperature	■	■	■	Simple	Risk of injury

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Behavioral tests	Tests	Reflects	Lesion severity			Pros	Cons
			Mild	Moderate	Severe		
	Cold sensitivity-based	Temperature				Simple	False positivity
	Von Frey filaments	Mechanical allodynia				Simple	Low sensitivity
	Paw compression	Pain				Simple, cheap	High chance of mistakes
	Withdrawal reflexes	Reflex				Simple	Low sensitivity
Sensory–motor tests	Rope walk testing	Balance				Simple, cheap	Low sensitivity
	Narrow beam	Balance				Uncovers discrete changes	Requires training
	Grooming	Sensory–motor connection				Simple, cheap	Subjectivity
	Foot slip	Sensory–motor coordination				Uncovers discrete changes	Requires training
	Grid walking	Sensory–motor coordination				Uncovers discrete changes	False-positives or negatives
Reflex response-based tests	Toe spread reflex	Reflex				Simple, cheap	Low sensitivity
	Contact placing response	Reflex				Simple, cheap	False positivity
	Righting reflex	Reflex				Simple, cheap	Low sensitivity
Autonomic tests	Ex copula erection	Erection				Unique data	Subjectivity
	Non-contact erection	Erection				Unique data	Low sensitivity
	Mating	Erection				Unique data	Subjectivity
	Telemetric monitoring	Micturition erection				Precise	Equipment
	Autonomic dysreflexia	Autonomic dysreflexia				Unique data	Equipment

* Modified by: Mahdi Sharif-Alhoseini

Table 1. Main behavioral methods for testing SCI models* [58]

Level of injury		First choice	Second choice	Third choice
Cervical		Forelimb asymmetry	Footprint analysis	BBB
Thoracic	Compression	BBB	Hot plate	Inclined plane
	Contusion	BBB	Electrophysiology	Von Frey, Hot plate
	Transection	BBB	Electrophysiology	Kinematic analysis
	Hemisection	BBB	Electrophysiology	Hot plate, Von Frey
	Excitotoxic	Hot plate	Cold testing	Von Frey
	Ischemic	BBB	Electrophysiology	Inclined plane, Hot plate
Other		BBB	Electrophysiology	Hot plate, Grid walk

Table 2. Recommended testing methods for SCI models [58].

- **Electrophysiology**

Electrophysiological assessments via the evoked potentials are useful to survey the neural substrates underlying deficits and functional recovery. They are also used to examine neural pathway integrity [58, 60].

Somatosensory evoked potentials (SSEP) are valuable for the assessment of sensory spinal axon conduction. They involve electrical stimulation of the paws with electrodes temporarily inserted into them, and the recording of evoked potentials from electrodes previously implanted in the cranium over the somatosensory cortex [61].

Magnetically evoked inter-enlargement responses (MIER) are helpful for the evaluation of propriospinal conduction. The MIER procedure involves noninvasive magnetic stimulation at the animal's hip or knee and the recording of evoked potentials with EMG electrodes temporarily inserted into forelimb and masseter muscles [62].

Motor evoked potentials (MEP) assess supraspinal axon conduction with EMG electrodes temporarily inserted into hindlimb muscles [63]. The MEP offers a precious insight into the physiological status of motor tracts within the spinal cord and is appropriate to animal studies. It is seen as complementary to SSEP monitoring rather than an alternative for it [64].

All evoked potential methods take a few minutes and cause only slight pain and distress and so could be done without anesthesia. But there are the restricted information content, and the need for rigorous electrophysiological interpretation of the resulting signals [64].

Electromyography (EMG): EMG can be elicited both by intramedullary manipulation and rapidly applied transaxial spinal cord compression. Presumably, rapid deformation of spinal motor tracts generates descending volleys which can bring to firing threshold lumbar motor neurons [65]. It can also be used to survey autonomic dysreflexia [66].

- **Neuroimaging**

Functional magnetic resonance imaging(fMRI): fMRI is an accurate but challenging technique which could measure the anatomic functional/metabolic correlates of sensory-motor activities

[67]. It should be done under anesthesia and mechanically ventilation [58]. After the stimulation of the limb electrodes, a signal in the somatosensory cortex and/or subcortical sensory areas can be recorded. This method makes it possible to distinguish between the recovery of sensory and motor function [68].

Magnetic Resonance Imaging (MRI): MRI findings of parenchymal hemorrhage/contusion, edema, and spinal cord disruption in acute and subacute SCI may contribute to the understanding of severity of injury and prognosis for neurological improvement [67].

MRI-Diffusion Weighted Imaging (MRI-DWI): It is an MRI-based imaging modality that determines the free diffusion of water molecules, enabling the recognition of imaging information beyond the resolution of conventional MRI methods [69]. MRI-DWI can be utilized to measure response to various cellular therapy interventions after experimental SCI [67].

Computerized Tomography (CT): The assessment of the bone loss following SCI in an animal model could be done by high-resolution CT images [70].

- **Neuroanatomical tracing**

Recently, several studies used neuroanatomical tracing procedures to study axonal remodeling after cell transplantation in experimental SCI models [71-76].

- **Histology**

Histological outcome measures, including sparing at the lesion epicenter, sparing throughout the extent of the lesion, quantification of myelin loss rostral and caudal to the lesion, and motor neuron counts, are demonstrated via staining sections of the spinal cord [21].

Hematoxylin and Eosin (H&E) is useful as a general structural stain in most tissues. But the high lipid content of nervous tissue makes it less suited to H&E than most others.

Cresyl violet stains both neurons and glia. It bonds well with acidic parts of cells such as ribosomes, nuclei and nucleoli and demonstrates the nissl substance. It stains cell bodies a blue/violet.

Luxol Fast Blue gives particularly good delineation of nerve tracts in the CNS. It is probably one of the most popular stains for the demonstration of normal myelin.

Osmium tetroxide is both a stain and a fixative. While it's primarily used these days as a fixative in electron microscopy, since it binds to lipids strongly, it's particularly well suited to reveal the details of myelin in nerves.

Eriochrome cyanine (EC) staining protocol for differentiation of white matter and cell bodies is used to calculate the amount of spared tissue in sections of injured cords.

8. Considerations

To choose an animal model, the proposed aim of the study must precisely be noted. The researchers involved in scientific work with animals should know the ethical standards in

animal experiments and investigate what animals are appropriate for each area of study in their models. Reproducible experimental SCI requires suitable training, animal care, experience with animal spine surgery, and proper surgical equipment. A standard housing environment with ad libitum access to food and water is a necessity for animal experiments. Pretraining and habituation of animals are important. When a behavioral testing is planned, animals must be trained inadequate sessions pre-operatively. Anesthetizing, surgery, and/or sacrificing have to been performed based on confirmed methods, attentively. All animals should be inspected regularly for wound healing, weight loss, dehydration, infection, autophagia and any discomfort [77]. Animal models, particularly complete SCI ones, need to serious care including preparation of supportive fluids, analgesia, and antibiotics, and also continuous bladder and bowel care. Appropriate veterinary care was provided as needed. All behavioral, histological, etc. analysis should be precisely selected before beginning a study and conducted by personnel blind to groups of study.

9. Conclusion and future perspectives

Animal models of SCI have confirmed to be helpful for the development of experimental therapies, and certainly will continue to play an essential role in the studies related to SCI. They give researchers an opportunity to discover the characteristic pattern of cell death and sparing, and measurement of any neuroprotection, regeneration, collateral sprouting, demyelination, and recovery of locomotor or other deficits. All injury paradigms are useful, but differ in the information that can be gained. The contusion models better simulate the biomechanics and neuropathology of human injury. The transection models, either completely or partially, are valuable for investigating the anatomic regeneration. The conclusions of rodent studies should examine in other animal models to survey their biological responses. In parallel, controlling and monitoring the injury mechanism within the surgical field, and evaluation of behavioral and histological outcomes have to be enhanced by applying technological improvements. Finally, more experimental studies should be designed to quantify neuronal damage after ischemic SCI.

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