

REVIEW

Autonomic assessment of animals with spinal cord injury: tools, techniques and translation

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Study design: Literature review.

Objectives: To present a comprehensive overview of autonomic assessment in experimental spinal cord injury (SCI).

Methods: A systematic literature review was conducted using PubMed to extract studies that incorporated functional motor, sensory or autonomic assessment after experimental SCI.

Results: While the total number of studies assessing functional outcomes of experimental SCI increased dramatically over the past 27 years, studies with motor outcomes dramatically outnumber those with autonomic outcomes. Within the areas of autonomic dysfunction (cardiovascular, respiratory, gastrointestinal, lower urinary tract, sexual function and thermoregulation), not all aspects have been characterized to the same extent. Studies focusing on bladder and cardiovascular function greatly outnumber those on sexual function, gastrointestinal function and thermoregulation. This review addresses the disparity between well-established motor-sensory testing presently used in experimental animals and the lack of standardized autonomic testing following experimental SCI. Throughout the review, we provide information on the correlation between existing experimental and clinically used autonomic tests. Finally, the review contains a comprehensive set of tables and illustrations to guide the reader through the complexity of autonomic assessment and dysfunctions observed following SCI.

Conclusions: A wide variety of techniques exist to evaluate autonomic function in experimental animals with SCI. The incorporation of autonomic assessment as outcome measures in experiments testing treatments or interventions for SCI should be considered a high, clinically relevant priority.

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Keywords: spinal cord injury; cardiovascular; respiratory; bladder; bowel; sexual

Introduction

Although it is generally appreciated that spinal cord injury (SCI) disrupts all types of communication between the brain and periphery below the lesion, the outcome of SCI is still commonly described in terms of motor function. The pervasive mental association between SCI and paralysis is reflected in recent headlines announcing a study on experimental SCI published in *Nature Medicine*.¹ The headlines read ‘Scientists move toward helping paralysis patients’² and ‘paralysis cure’.³

In keeping with this perspective, an overwhelming number of clinical studies have focused on the effects of SCI on voluntary movement and the role of the somatic

nervous system. Despite the widespread effects of SCI on autonomic control, it is only recently that autonomic function following SCI has received significant attention in clinical research.^{4–6} The delay in addressing the autonomic effects of SCI has not only limited their appreciation among basic scientists and clinicians, but also efforts to develop new treatments or rehabilitation strategies targeting autonomic function following SCI. These shortcomings are not insignificant, as autonomic dysfunctions represent the primary causes of morbidity and mortality following SCI.^{7,8} In addition, individuals with SCI have identified recovery of autonomic functions as a high priority for improving their quality of life.⁹ Recent data demonstrate that autonomic function is not reliably predicted by the degree of residual motor or sensory function.^{10,11} Together these results suggest that there is an imbalance between clinical priorities and the general focus of SCI research.

A similar imbalance exists in animal research. This is highlighted by a systematic review of the animal SCI

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literature (Figure 1; see 'Methods'). We reviewed published studies of animals with experimental SCI, and identified studies with functional outcomes designed to evaluate motor, sensory or autonomic function. While the total number of studies increased dramatically over the past 27 years, published studies with motor outcomes dramatically outnumber those with sensory or autonomic outcomes. The disproportionate number of studies focusing on motor recovery after experimental SCI represents a significant mismatch between the clinical priorities of improved autonomic function and the direction of SCI research in animals.

When the functional autonomic outcomes are separated according to organ system dysfunction, it is immediately apparent that not all aspects of SCI-induced autonomic dysfunction have been examined to the same extent. The available data characterize mainly bladder and cardiovascular functions in SCI animals, whereas sexual function, gastrointestinal (GI) function and thermoregulation remain essentially uninvestigated. These findings are particularly alarming, as recovery of sexual function in particular has been identified by both paraplegics and quadriplegics as an urgent priority.⁹

There are several likely reasons for the paucity of research addressing autonomic dysfunctions following SCI. The complex organization of the autonomic nervous system, and its involvement in the control of almost every system in the body, makes it difficult to select appropriate functional tests. There is also some confusion surrounding the opera-

tional definitions of autonomic dysfunctions that are present in animals following SCI. Finally, there may be a lack of agreement on (and awareness of) well-designed, clinically relevant tests that have been validated to evaluate autonomic functions in animals with SCI.

The main goal of this review is to provide the scientific community with an overview of the current methods used to assess the autonomic function of animals with SCI. For six aspects of autonomic function—cardiovascular function, respiratory function, GI function, lower urinary tract (LUT) function, sexual function and thermoregulation—we review the innervation of the system in humans and rats, the clinical implications of dysfunction following SCI and the tests or techniques that are currently available to evaluate function after experimental SCI. We also include functional tests that have been developed in other animal models, but that appear to be applicable to animals with SCI. Each test is reviewed in terms of its methodology, the type of information that it provides and the available data in animals with SCI. We hope to improve the understanding of functional tests used to evaluate autonomic function and to increase their incorporation in SCI experiments, particularly those studies testing potential therapeutic agents (see Table 9). We also hope that this review will be useful to veterinarians in clinical practice, to add to their arsenal of assessments of animals with naturally occurring SCI. Throughout the review we also highlight clinical assessments that are similar to the experimental methods we describe. The use of similar tests in the clinic and the laboratory is valuable because it facilitates

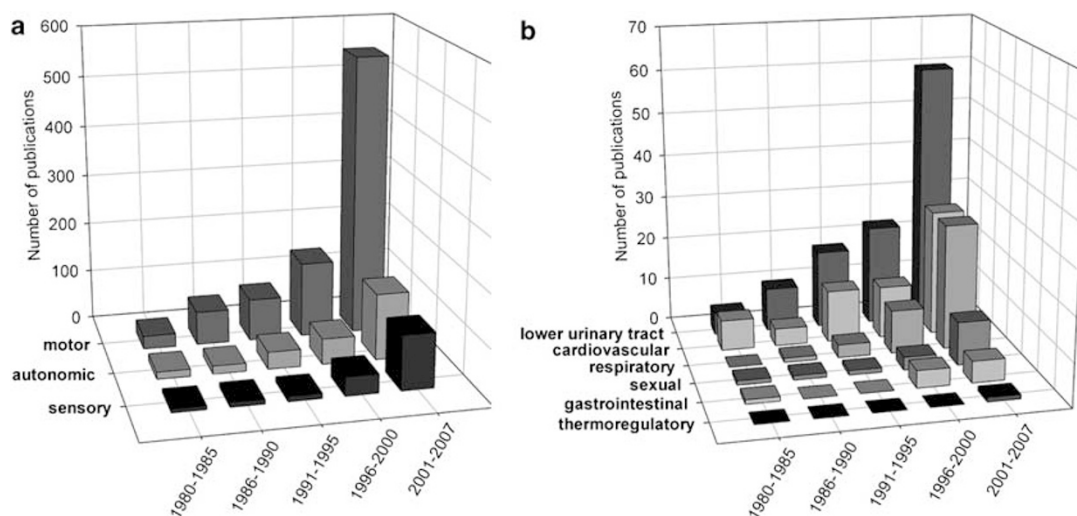


Figure 1 Autonomic dysfunction remains underrepresented in experimental spinal cord injury (SCI). (a) The total number of publications reporting the functional outcome in animal models of SCI has increased steadily since 1980. However, the rate of increase is dramatically different between studies characterizing function/dysfunction of different divisions of the nervous system. At every time period examined, published studies with motor outcomes far outnumber published studies investigating autonomic or sensory function. The disparity is particularly pronounced in the past 7 years, when studies incorporating a motor outcome outnumber publications with any autonomic outcome by more than four times. (b) When published data on autonomic function are categorized according to organ system dysfunction after SCI, it is clear that not every area is equally represented. Lower urinary tract (LUT) function/dysfunction is the best-characterized component of experimental SCI, whereas experiments studying sexual, gastrointestinal and thermoregulatory function remain comparatively scant. However, when we compare the number of published studies characterizing motor outcome in the past 7 years (554) with the number of published studies characterizing LUT function in the same period (60), it is obvious that every aspect of SCI-related autonomic dysfunction should be considered a priority in animal research.

Table 1 Search terms used to identify studies of motor, sensory and autonomic outcome of experimental SCI

Motor	Sensory	Autonomic					
		Autonomic; sympathetic; parasympathetic					
		Lower urinary tract	Cardiovascular	Respiratory	Sexual	Gastrointestinal	Thermoregulation
Motor	Sensory	Urinary	Cardiovascular	Respiratory	Sexual	Gastrointestinal	Thermoregulation
Movement	Sensation	Bladder	Blood pressure	Breathing	Uterus	Bowel	Sweating
Walking	Touch	Micturition	Hypertension	Lung	Vagina	Colon	Piloerection
Stepping	Proprioception	Renal	Hypotension		Menstruation		
Locomotor	Pain		Dysreflexia		Menstrual		
Locomotion	Allodynia		Heart		Penis		
Postural	Hyperalgesia		Arrhythmia		Erection		
Spasticity	Dysaesthesia				Ejaculation		
	Paresthesia						

Each search term was used in combination with 'spinal cord injury'. Searches were limited to animal studies published in English within specified date ranges (Figure 1). Abstracts and manuscripts were reviewed to obtain the final publication count in each area: see Methods for details and Table 2 for inclusion/exclusion criteria.

the translation of our knowledge about autonomic function and dysfunction following SCI between the bench and the bedside.

Methods: literature review

A systematic literature review was conducted using PubMed to identify studies that incorporated functional assessment after experimental SCI. Each search was limited to animal studies published in English, and to specified publication date ranges. Search terms were always used in combination with 'spinal cord injury'. A separate search was performed using each of the terms listed in Table 1. The abstracts returned by each search were reviewed to identify studies with relevant functional outcomes; inclusion and exclusion criteria are listed in Table 2. If the outcome measure(s) used in the study were not evident from reading the abstract, the 'Methods' section of the paper was reviewed.

Publications were not counted twice in the same section (that is, no paper was counted as both locomotor and movement), but some were considered as spanning two sections (that is, some papers contained both motor and sensory functional assessments). All studies incorporating the functional outcome of interest were included, regardless of the objective of the study. For example, studies aimed at characterizing the effects of a treatment on pain after SCI that also included motor testing were counted as both motor and sensory. Finally, publications were categorized according to the intent of the outcome measure. For example, if authors used a ladder-walking test to assess locomotor function, the study was counted as motor (even though there are presumably proprioceptive components to the performance).

Cardiovascular function

Autonomic innervation of the cardiovascular system

In humans and animals with an intact neuraxis, both tonic neurogenic and reflex autonomic control of the cardiovascular system ensure adequate regional blood supply under a wide range of physiological conditions. The autonomic

Table 2 Criteria used to identify studies with relevant functional assessment after experimental SCI

Included	Excluded
All experimental models of SCI (for example, traumatic, ischemic) in all species	<ul style="list-style-type: none"> ● Clinical (veterinary) SCI ● <i>In vitro</i> and <i>ex vivo</i> preparations ● Review articles
Studies incorporating functional assessments of SCI, including those that: <ul style="list-style-type: none"> ● Characterize an outcome measure ● Characterize function/dysfunction ● Use functional outcome to assess manipulation or treatment 	<ul style="list-style-type: none"> ● Studies detected by the search criteria that contained no functional assessments (For example, motor neuron atrophy after SCI) ● Studies that only monitored vital signs during surgery or treatment ● Electrophysiological studies of individual neuron properties (versus neurological function) ● Studies of spinal cord blood flow/lesion perfusion or microperfusion (versus systemic cardiovascular function) ● Studies testing the effects of vasopressor therapy as a treatment for SCI

Abstracts returned by literature searches were reviewed; if necessary, manuscripts were also reviewed to determine what outcome measures were included in the study. Studies were included/excluded according to the listed criteria.

innervation of the cardiovascular system has important ramifications for the pattern of cardiovascular dysfunction that emerges after SCI. Here we review the most relevant features of cardiovascular innervation (Figure 2), the details of which have been extensively reviewed elsewhere.^{12,13}

Clinical implications of cardiovascular dysfunction following SCI

Unlike dysfunction of the LUT, which can be described in general terms for suprasacral SCI, cardiovascular dysfunction varies dramatically with level of injury,^{14,15} its severity determined by the relative loss of supraspinal control over spinal sympathetic outflow.¹⁶ Cervical SCI disrupts supraspinal connections to preganglionic sympathetic innervation of the heart and blood vessels, eliminating tonic excitatory input to these organs. If the injury occurs at T5 or above,

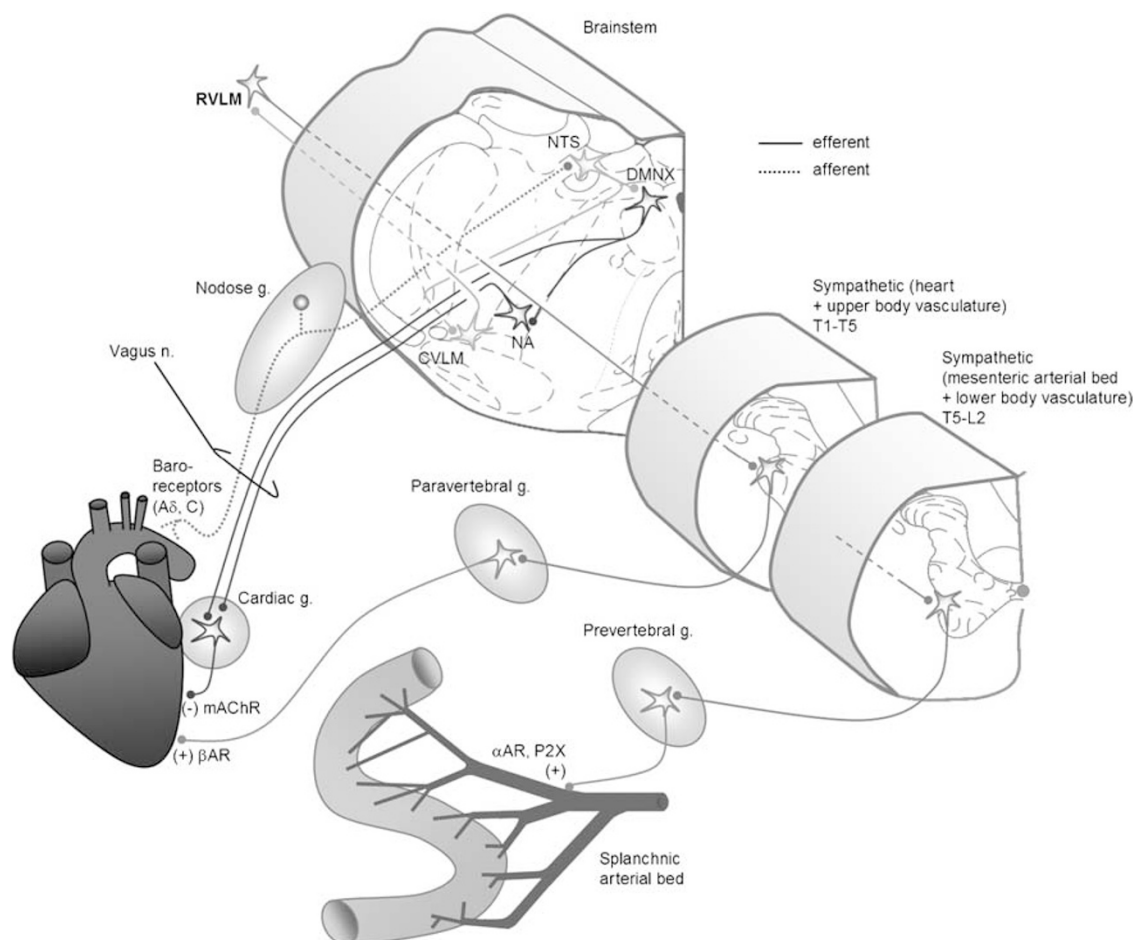


Figure 2 Autonomic innervation of the cardiovascular system. The major organs of the cardiovascular system are the heart and the blood vessels. The heart receives both parasympathetic and sympathetic innervation. Parasympathetic efferents travel to the heart in the vagus nerve, which exits the central nervous system (CNS) at the level of the medulla. The vagus nerve innervates the atria, nodes and Purkinje fibers via local cardiac ganglia, and vagal activity decreases heart rate, contractility and conduction velocity. Sympathetic activity has an opposite, stimulatory effect on the heart. All tissues of the heart receive sympathetic input from the upper thoracic (T1–T5) cord. Blood vessels are under sympathetic control, and vessels supplying the splanchnic region—the liver, spleen and intestines—are most important in cardiovascular control. The splanchnic bed is densely innervated, highly compliant and contains approximately one-fourth of the total blood volume in humans at rest.⁴⁰³ As such, it is the primary capacitance bed in the body. Sympathetic outflow to the splanchnic bed exits the thoracolumbar cord (T5–L2) and provides tonic vasoconstriction. The relative amount of sympathetic and parasympathetic activity governing cardiovascular control is determined (in part) by information from two types of afferents: baroreceptors and chemoreceptors. Baroreceptors in the aortic arch, carotid sinus and coronary arteries detect changes in arterial pressure, and chemoreceptors in the carotid bodies respond to changes in partial pressures of oxygen and carbon dioxide in the blood. Baroreceptor activity is the primary drive for rapid blood pressure adjustment. Baroreceptor afferents travel primarily in the vagus nerve and the glossopharyngeal nerve to reach the medulla. *Abbreviations:* AR, adrenergic receptors; CVLM, caudal ventrolateral medulla; DMNX, dorsal vagal motor nerve; g, ganglion; mAChR, muscarinic cholinergic receptors; NA, nucleus ambiguus; n, nerve; NTS, nucleus of the solitary tract; P2X, purinergic receptors; RVLM, rostral ventrolateral medulla; (+) denotes excitatory synapses; (–) denotes inhibitory synapses.

supraspinal control over sympathetic innervation of the splanchnic vascular bed is lost, jeopardizing blood pressure control. For this reason, injuries at and above T5–T6 are most likely to precipitate severe cardiovascular dysfunction.^{14,17}

The acute phase of clinical SCI is marked by neurogenic shock, a period of profoundly altered cardiovascular control.¹⁸ This phenomenon is particularly pronounced following cervical SCI, which frequently induces severe hypotension and bradycardia.^{14,17} After neurogenic shock resolves, basal cardiovascular parameters remain altered in people with cervical and high-thoracic SCI.¹⁹ People with mid-thoracic SCI typically have elevated heart rates,²⁰ whereas people with higher injuries often present with low

heart rate and resting blood pressure.²¹ Cervical SCI also abolishes circadian blood pressure rhythms^{22–24} and blunts the cardiovascular responses to exercise.²⁵ In addition to low resting blood pressure, many people with high SCI experience orthostatic hypotension (OH), a decrease in blood pressure that occurs with assumption of a sitting position.²⁶ These individuals are also prone to autonomic dysreflexia (AD), episodes of paroxysmal hypertension, often accompanied by baroreflex-mediated bradycardia, induced by sensory stimulation below the level of the injury.¹⁷ As a group, cardiovascular disorders are the most common underlying or contributing causes of death in people with SCI.^{8,27}

Assessment of cardiovascular function following experimental SCI
SCI alters the connectivity of autonomic circuits between the brainstem and spinal cord that governs cardiovascular control and modifies sympathetic intraspinal and neurovascular transmission resulting in altered vascular smooth muscle responses.^{28–34} These phenomena (as well as others) are likely to contribute to SCI-induced cardiovascular dysfunction. Here we focus on the systemic cardiovascular outcome of experimental SCI. Several aspects of SCI-induced cardiovascular dysfunction have been successfully modeled in experimental animals. Cardiovascular parameters of animals with SCI are monitored from direct arterial blood pressure measurements: indirect measurements of blood pressure (collected using a tail cuff, for example) have never been used in animals with SCI. Arterial blood pressure is taken either directly from a fluid-filled cannula or via radiotelemetric monitoring of animals with chronically implanted cannulae.

Short-term cardiovascular assessment using fluid-filled cannulae

The bulk of available cardiovascular data from animals with SCI has been collected using arterial cannulae implanted acutely prior to data collection. Typically, the cannula is placed in one carotid or femoral artery hours or days before data collection, although it is possible to maintain cannulae for at least 1 week with daily flushing. Cannulae are filled with heparin solution to preserve patency and tunneled subcutaneously for externalization. After arterial cannulae are implanted, animals are housed singly to protect cannulae from cage-mate chewing. Cardiovascular parameters can be monitored directly in conscious animals in their home cage by connecting the cannulae to a pressure transducer: this method has been used frequently to study many types of cardiovascular dysfunction in animals with SCI.

The aspect of cardiovascular dysfunction that has been best characterized in rodent models of SCI is AD, episodic hypertension that has been described in both rats and mice, and is induced by visceral and somatic stimuli (see Table 3 for detailed references). The nature of rodent AD and its inciting stimuli is very similar to that described in humans. Rats, like humans, typically experience AD when SCI occurs at or above T5–T6. In one study, bladder filling induced AD in rats with severe contusion SCI at T4, but not in rats with severe T10 contusion.⁶⁸ The most common experimental stimulus for AD is colorectal distension (CRD), in which a balloon catheter is inserted into the colon through the anus in the conscious animal.²⁸ Air is infused into the balloon to mimic the pressure of several fecal boli in the colon. Distension is typically maintained for 1 min, and arterial pressure is monitored before, during and after CRD.

Formerly considered to be associated with chronic SCI, AD may also emerge during the acute stages in both animals and humans.⁷⁴ Rats with complete transection of the spinal cord at T5 develop AD in response to CRD as early as 24 h after injury.²⁸ The severity of AD subsequently decreases during the first post-injury week, and AD is relatively mild at 7 days following T5 SCI.^{28,38} Thereafter, the severity of AD increases. Although the actual rise in blood pressure varies

with the level and severity of injury, as well as the experimental conditions, the majority of experiments are conducted at least 2 weeks after SCI when AD is pronounced. This well-characterized model has been used extensively to identify mechanisms contributing to the development of AD; still, only a few studies have examined the severity of AD as an outcome measure when testing therapies for SCI (Table 9).

Compared to its hypertensive counterpart, OH has received little attention in experimental SCI. Some clues to mechanisms of OH are provided by animal models of microgravity-induced cardiovascular deconditioning, in which microgravity is simulated by hindlimb unloading (HU).^{75–77} Animals exposed to HU experience a pronounced drop in blood pressure when subjected to head-up tilt.⁷⁸ Considering this, we are currently developing an animal model of OH after SCI. We have recently observed OH in rats with complete transection injury at T3–T4 (JAI, LMR and AVK, unpublished observations). When these unanesthetized rats are subjected to passive head-up tilt 1 month after injury, they experience a pronounced drop in blood pressure (Figure 3); uninjured rats subjected to the same maneuver exhibit either a rise in blood pressure or very little change.

Blood pressure data collected directly from arterial cannulae have also been used to monitor animals in the early phases of recovery from SCI. Neurogenic shock is generally considered to be less pronounced in experimental animals than in human SCI. This notion might stem from the fact that the overwhelming majority of experiments are performed in animals with thoracic SCI. Animals with cervical SCI may experience neurogenic shock that is more comparable to the clinical situation. Rats with complete transection between C7 and T1 developed both hypotension and bradycardia.⁷⁹ Mean arterial pressure fell precipitously (by approximately 30 mm Hg) in the first 24 h after injury, and slowly recovered to preinjury levels (around 100 mm Hg) by 9 days after injury. Heart rate also decreased dramatically (from about 360 b.p.m. to about 300 b.p.m.) and remained significantly lower than preinjury heart rate for 9 days (the duration of the study). Owing to the challenges involved in animal care, there are very few data on animals with high, severe SCI. These animal models are currently being developed (for example Pearse *et al.*⁸⁰) and should facilitate our understanding of cardiovascular control following cervical SCI.

Cardiovascular data collected from animals with acutely implanted arterial cannulae have also been subjected to spectral analysis. This type of analysis exploits the relationship between frequency of spontaneous fluctuations in cardiovascular parameters and the activity of systems (local, sympathetic and parasympathetic) governing cardiovascular control. Spectral analysis has been validated in humans,^{81,82} dogs^{82–84} and rats,⁸⁵ and provides indices of autonomic nervous system activity from relatively short segments of continuous recordings of blood pressure (and in humans, electrocardiogram signals). Spectral analysis has been applied to examine blood pressure variation in rats with acute SCI.⁷⁰ At 1 and 6 days after complete transection at T4–T5, rats exhibited reduced power in the low-frequency range,

Table 3 Overview of studies characterizing or targeting cardiovascular dysfunction after experimental SCI

Technique used	Species	Injury model	Time range post-injury	References
Acute arterial cannulation				
Basal cardiovascular parameters	Rat	T1–T4 clip compression	<4 h	35–37
			1.5 months	38
		C6/7 Tx; T2 Tx; T9 Tx	4 h	38
			≤1 week	39
		T4–T7 Tx	0 h–6 weeks	40
			≤1 week	41
		T5–T8 contusion	<1 h	42–44
		T9 clip compression	1, 2 h	45
		C7 Tx (ligature crush)	<1 h	46
		Cat		
Autonomic dysreflexia	Rat, cat Cat Dog Monkey	T6 contusion	≤24 h	47
		C4 Tx	<1 h	48
		T5 balloon compression		49
Colorectal distension	Mouse Rat	T2 clip compression	2 weeks	50
		T2 Tx	2 weeks	50–52
		C7–T1; C8 Tx	3 days	53,54
			2–3 months	55
		T4–T5 clip compression	≤1 week	38,56
			2–4 weeks	38,57–59
			4–6 weeks	38,59
		T3–T5 Tx	≤24 h	40,60,61
			≤1 week	28,40,60
			2–4 weeks	28,34,40,60–65
Bladder distension	Rat	C7–T1 Tx	2–3 months	55
			24 h	66
			≤1 week	67
		T4 contusion	4 weeks	68
		T5 Tx	≤1 week	28
		T2 clip compression	2 weeks	50
		T2 Tx		50–52
		T4 clip compression	2–6 weeks	59
		T7 Tx	1–3 weeks	69
		T1–T5 Tx	≤1 week	70,71
Somatic stim.	Mouse Rat Rat Rat	T2 clip compression	2 weeks	50
		T2 Tx		50–52
		T4 clip compression	2–6 weeks	59
		T7 Tx	1–3 weeks	69
		T1–T5 Tx	≤1 week	70,71
Vag.-cerv. stim.	Rat	T1–T5 Tx	≤1 week	70,71
Spectral analysis	Rat	T1–T5 Tx	≤1 week	70,71
Telemetric monitoring	Rat	C7–T1 Tx	2–3 months	55
		T4–5 Tx	≤24 h, ≤1 week	40
			4–6 weeks	40,72
			2–3 months	73
		T4 clip compression	1.5 months	38

Abbreviations: stim., stimulation; Tx, transection; vag.-cerv., vaginocervical.

Studies have been classified by the technique used to assess cardiovascular function. Studies employing a combination of techniques are referenced under both headings. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

indicative of reduced sympathetic tone (or increased parasympathetic tone⁸⁵) that affects blood pressure control. There are currently no data applying spectral analysis to more chronic experimental SCI. However, as this analysis is related to clinical measures of autonomic control following human SCI,⁸⁶ it represents a readily translatable tool for autonomic assessment in experimental animals and humans with SCI.

Advantages/disadvantages. Blood pressure measurements collected directly from fluid-filled cannulae provide high-resolution cardiovascular data with a relatively modest investment in equipment. However, preserving catheter patency for repeated measurements in the same animals can present a challenge. For this reason, this method typically only provides a snapshot of information about cardiovascular function in each animal (that is, taken at a single time point). The presence of investigators at the time

of data collection, as well as possible effects of recent surgery for cannula implantation, may contribute to stress in the animals during data collection.

Telemetric monitoring of cardiovascular parameters

Telemetric monitoring provides continuous physiological information about the cardiovascular function of conscious, freely moving animals over a long time course. This technique was first applied to animals with SCI in our laboratory (Figure 4),³⁸ and allowed us to characterize changes in resting blood pressure and heart rate in rats during recovery from high-thoracic SCI. Rats were instrumented with a telemetric transducer (catheter implanted into the descending aorta) 1 month prior to SCI, and arterial pressure data were transmitted as a radiofrequency signal to a receiver under the cage. The recovery time after transducer implantation was critical, as the effects of simultaneous

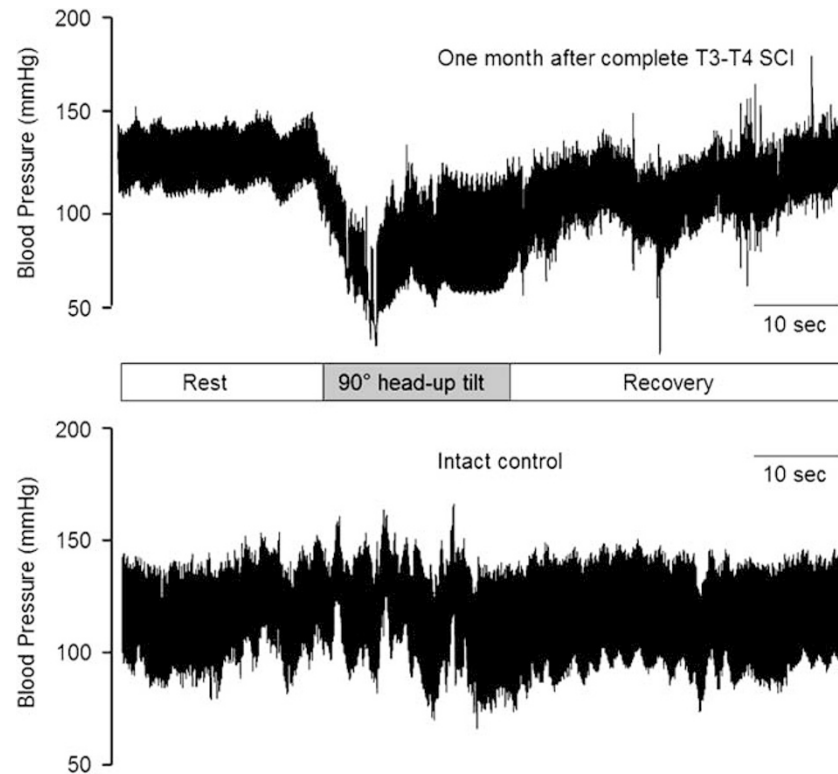


Figure 3 Representative tracings using fluid-filled cannulae to assess blood pressure during head-up tilt. One month after complete T3–T4 transection injury, conscious rats with a left carotid artery cannulation subjected to a passive 90° head-up tilt experienced a pronounced drop in blood pressure. Uninjured rats subjected to the same maneuver exhibited either a rise in blood pressure or very little change (JAI, LMR and AVK, unpublished observations).

transducer implantation and SCI can impede blood supply to the hindlimbs.³⁸

Telemetric monitoring of animals with SCI suggests that SCI-induced alterations in basal cardiovascular control are similar between rats and humans. Rats with T5 clip compression SCI experience transient hypotension and persistent tachycardia following injury.^{38,87} Telemetric monitoring revealed that blood pressure recovered to preinjury levels by 48 h after SCI, and remained equivalent to preinjury levels for at least 6 weeks following injury. Heart rate was increased relative to preinjury levels by 3 days after SCI, and remained elevated for at least 6 weeks. Diurnal fluctuations in blood pressure and heart rate returned approximately 5 days after injury.³⁸ Interestingly, recent telemetric data demonstrate that rats with more rostral injuries (complete T4 transection) experience persistent hypotension and tachycardia, with altered basal blood pressure and heart rate for at least 6 weeks following SCI.⁴⁰ Thus, it seems that in rats, as in humans, severity of cardiovascular dysfunction varies with both level and severity of SCI. Radiotelemetry has also been used to investigate AD induced by CRD, which is similar in progression between rats with T5 clip compression and rats with T4 complete transection (Figure 4).^{38,40}

Advantages/disadvantages. As data collection is continuous, telemetric monitoring is extremely informative. Since it avoids the potential stressors of intra-arterial catheterization,

catheter maintenance, or restraint for catheter connection, telemetry can be considered the gold standard for cardiovascular assessment in conscious experimental animals. It does not eliminate stress due to handling when animals must be loosely restrained for cardiovascular measurements (such as during CRD to induce AD), so this remains a consideration in telemetric studies. Most significantly, adopting telemetric monitoring entails considerable investment in equipment. Perhaps for this reason, data in animals with SCI remain scant (Table 3).

Respiratory function

Innervation of the respiratory system

Coordinated activity of somatic (diaphragm and accessory respiratory skeletal muscles) and autonomic (smooth muscles of the bronchial tree) nervous systems is crucial for normal respiration. Here we review efferent and afferent innervation as it pertains to respiratory dysfunction that emerges following SCI (Figure 5). For simplicity, we omit many important aspects, and the interested reader is directed to comprehensive reviews on the subject (for example, see Brading¹² and Canning and Fischer⁸⁸).

Clinical implications of respiratory dysfunction following SCI

Respiratory dysfunction after SCI is determined by relative loss of descending autonomic innervation to the respiratory

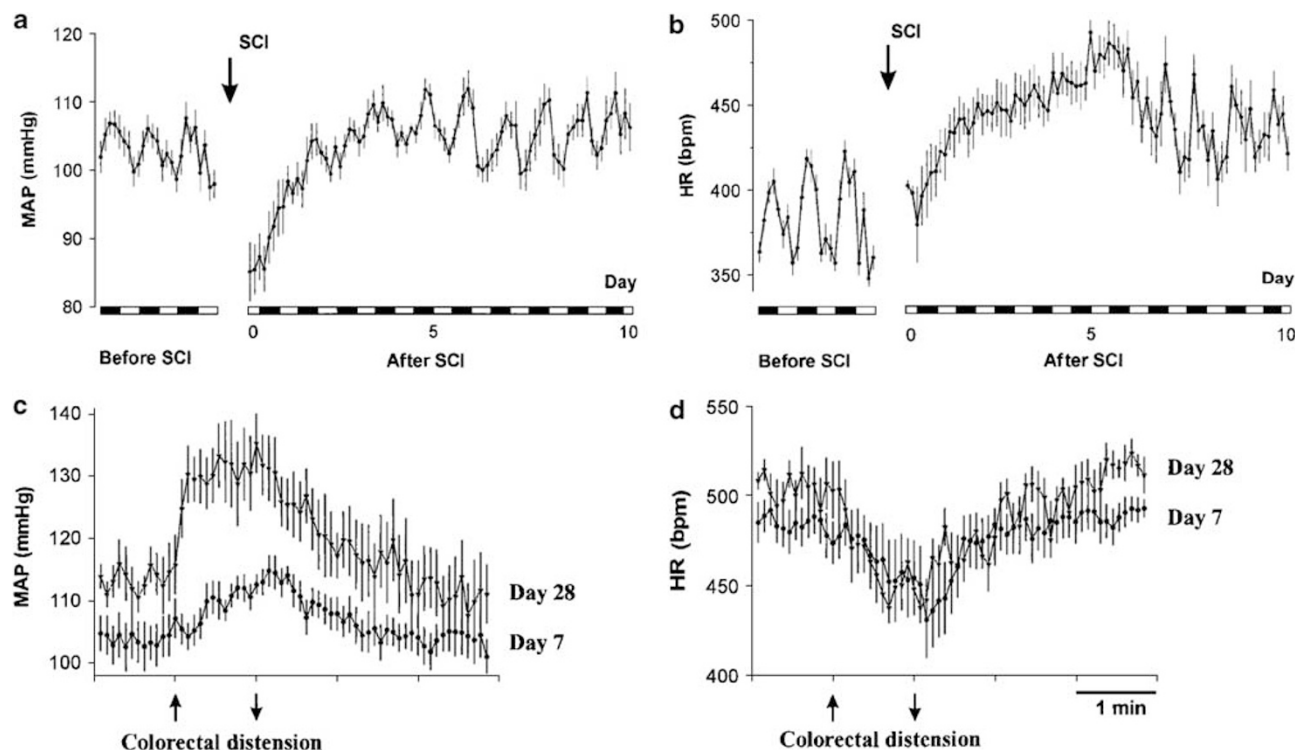


Figure 4 Use of telemetric monitoring to assess basal cardiovascular parameters (a, b) and autonomic dysreflexia (c, d) after experimental spinal cord injury (SCI). Rats were instrumented with radiotelemetric pressure transducers and cannulae were implanted into the descending aorta 1 month prior to T5 clip compression SCI. (a, b) Telemetric monitoring permitted data collection in freely moving rats for 3 days prior to SCI (to establish reliable uninjured baseline responses) and for the first 10 days following SCI. Mean values (\pm s.e.m.) of 3 h intervals for five rats are shown, and light and dark periods are indicated on the x axes. (c, d) Autonomic dysreflexia (AD) was examined at 7 and 28 days following SCI. Average responses (\pm s.e.m.) to colorectal distension in five rats are shown. This figure is reprinted from Mayorov *et al.*³⁸

muscles. Injuries above C3 paralyze the diaphragm, and ventilatory support is typically required to sustain life. After C3–C5 SCI, the diaphragm is partially denervated, and inspiration is compromised; still, most individuals with C4–C5 SCI do not require artificial ventilation (or can be weaned during recovery⁸⁹). In lower cervical SCI, innervation of the primary (and some accessory) inspiratory muscles is preserved, but ventilation is still impaired, because denervated intercostal muscles do not coordinate chest expansion with diaphragm descent.⁹⁰ Finally, any SCI above L1–L2 denervates abdominal muscles, reducing the effectiveness of coughing.

The clinical ramifications of respiratory dysfunctions following SCI are severe, and encompass pneumonia, atelectasis, bronchitis, reduced lung volumes and compliance, sleep apnea and respiratory insufficiency or dyspnea, particularly during exercise.^{91–95} The incidence and severity of respiratory dysfunction increases with the level of SCI. Respiratory complications are the leading cause of death in acute SCI.⁹⁶ In chronic SCI, respiratory dysfunction contributes significantly to mortality,⁸ and is associated with reduced quality of life.⁹⁷

Assessment of respiratory function following experimental SCI

The most common experimental model used to study SCI-induced respiratory dysfunction is rat cervical hemisection.

This injury reliably disrupts innervation to one-half of the diaphragm. Recently, cervical hemicontusion models have been developed that also induce respiratory deficits due to hemidiaphragm paralysis.^{98,99} Data from both injury models have increased our understanding of endogenous plastic processes that might improve respiratory function following SCI (Table 4; reviewed in Zimmer *et al.*¹³⁴). A few studies have also examined the potential for regenerative therapies to restore diaphragm function (Table 9). The obvious limitation of these models is that they are incomplete injuries, and thus do not model all aspects of clinical SCI. As more models of severe cervical SCI are developed, our understanding of respiratory dysfunction after SCI is likely to improve. Fortunately, outcome measures used to assess respiratory function are similar between experimental and clinical SCI, facilitating translation of these data.

Phrenic nerve conduction

Phrenic nerve conduction testing is used clinically for planning phrenic nerve or diaphragmatic pacing for assisted activation of the diaphragm.^{135–137} In animals, phrenic nerve responses during spontaneous breathing or spinal cord stimulation are examined in anesthetized, paralyzed (mechanically ventilated) animals.^{101–103,121,122} These data have been used extensively to characterize respiratory

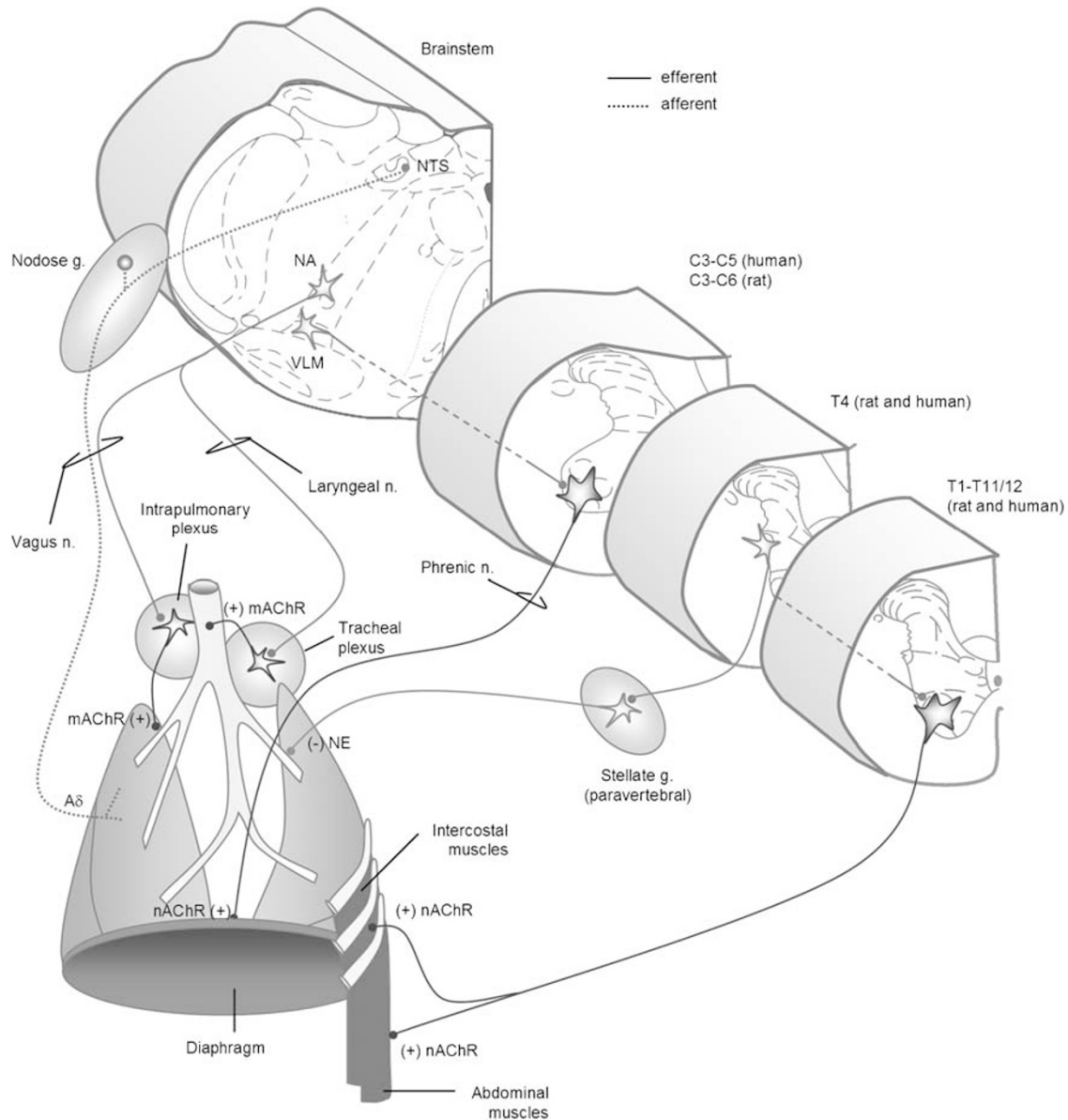


Figure 5 Innervation of the respiratory system. The main respiratory muscles are the diaphragm, intercostals and abdominals. The diaphragm is the major inspiratory muscle and is innervated by phrenic motor neurons that lie in the cervical spinal cord (C3–C5 in humans; C3–C6 in rats⁴⁰⁴). Innervation of respiratory intercostal and abdominal muscles exits the thoracolumbar spinal cord, from T1–T11 and T7–L2, respectively. Activity of these muscles (as well as that of accessory muscles) is modulated by autonomic premotor neurons in the VLM, which project to motor neurons in the spinal cord. The airways receive both parasympathetic and sympathetic inputs. The parasympathetic nervous system provides the most important innervation to the smooth muscle of the airways, and is thus most important in controlling its diameter. Preganglionic parasympathetic neurons originate in the NA, and innervate the trachea and the bronchi via the laryngeal and vagus nerves (respectively). Parasympathetic innervation is predominately cholinergic and its action is excitatory, reducing airway diameter (via mAChR). Sympathetic innervation of smooth muscle is comparatively scant. Preganglionic sympathetic axons exit at T4 and travel to paravertebral ganglia, and post-ganglionic adrenergic fibers elicit bronchodilation, acting through β -AR. The airways also have extensive afferent innervation. The most important afferents regulating respiration are vagal A δ , with cell bodies in the nodose ganglia and central axons projecting to the NTS. *Abbreviations:* A δ , mechanoreceptors; β -AR, β -adrenergic receptors; C, cervical spinal cord; g, ganglion; L, lumbar spinal cord; mAChR, muscarinic cholinergic receptors; NA, nucleus ambiguus; nAChR, nicotinic cholinergic receptors; NE, norepinephrine; n, nerve; NTS, nucleus of the solitary tract; T, thoracic spinal cord; VLM, ventrolateral medulla; (+) denotes excitatory synapses; (–) denotes inhibitory synapses.

plasticity after SCI, particularly during the crossed phrenic phenomenon (CPP).^{134,138}

The CPP relies on a functionally latent bulbospinal pathway that innervates the diaphragm bilaterally. When the hemidiaphragm is paralyzed by C2 hemisection, hypoxia induced by contralateral phrenic nerve section activates the

latent pathway to restore diaphragmatic function. This pathway also becomes spontaneously active over time following SCI, but this spontaneous return of activity may not be sufficient to restore diaphragmatic function.¹⁰⁴ A series of phrenic nerve function studies has revealed that systemic delivery of xanthines (adenosine receptor

Table 4 Overview of studies characterizing or targeting respiratory dysfunction after experimental SCI

Technique used	Species	Injury model	Time range post-injury	References
Phrenic nerve conduction	Rat	C2 hemicontusion	1 week	99
			4–6 weeks	99,100
		C2 hemisection	≤24 h	101–118
			≤1 week	104,116,117,119,120
			2–4 weeks	103,119,121–124
			4–8 weeks	121,125
Pneumotachometry	Rat Dog Turtle	C2 hemisection	2–4 months	116,117,126
			2–11 weeks	100
		T2, T4, T8 (seg. epid.)	1–2 months	127,128
			Immediate	129
		D8–D10 Tx	4, 8 weeks	130
Diaphragm EMG	Rat Dog Mouse	C2 hemisection	≤24 h	105,117,131
			≤1 week	119
			2–4 weeks	119
			90 days	117
		C2 hemicontusion	7 days, 1 month	99
			Immediate	129
Plethysmography	Rat	C2 hemisection	1–48 h	132
		C4/5 hemicontusion	2, 3, 5 weeks	122
			24 h, 1, 2, 4, 6 weeks	98
		T8 contusion	24 h, 7 days	133

Abbreviations: EMG, electromyography; seg. epi., segmental epidural; Tx, transection.

Studies have been classified by the technique used to assess respiratory function. Studies employing a combination of techniques are referenced under both headings. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

antagonists) can activate this pathway without phrenic nerve section, to restore respiratory drive to phrenic motor neurons following SCI.^{105–107,123,126} These data, and preliminary clinical experience, suggest that xanthine treatment may represent a viable therapeutic option in weaning individuals with SCI from ventilatory support.¹³⁹

Phrenic nerve recording has also been used to test the efficacy of olfactory ensheathing cell (OEC) transplantation in improving the respiratory outcome of experimental SCI^{140,141} (Table 9). In these experiments, rats with high cervical hemisection (that abolished ipsilateral phrenic nerve activity) received OEC transplantation at the time and site of injury. In one study, rats that received OECs recovered spontaneous respiratory rhythm in the ipsilateral phrenic nerve by 2 months after SCI.¹⁴⁰ In another set of experiments, ipsilateral phrenic nerve activity recovered to approximately 80% of contralateral nerve activity by 3–6 months post-SCI.¹⁴¹ In the latter set of experiments, the authors transected the contralateral spinal cord to demonstrate that a significant proportion of this recovery was due to ipsilateral projections. However, the underlying mechanism of recovery is not known.

Pneumotachometry

Pneumotachometry is the evaluation of the respiratory volumes and rate that can be readily applied in conjunction with phrenic nerve recording. This type of assessment was important in identifying altered breathing patterns in rats with unilateral cervical SCI.¹²⁷ Rats with C2 hemisection exhibit a reduced expiratory volume and an increased respiratory rate to preserve total (minute) ventilation.¹²⁷

Pneumotachography was also applied to verify the functional significance of crossed phrenic pathways.¹²⁸

Diaphragmatic electromyography

Similar to clinical practice, electromyography (EMG) of the diaphragm can be performed in conjunction with phrenic nerve conduction studies in animals with SCI. For example, rats that received OECs at the time of C2 hemisection recovered both ipsilateral phrenic nerve activity and ipsilateral diaphragm activity 3–6 months after treatment and injury.¹⁴¹ A recent study used diaphragmatic EMG to test the effect of administering a *N*-methyl-D-aspartic acid (NMDA) receptor antagonist (MK-801) in acute cervical SCI.¹³¹ In these experiments, i.p. MK-801 administration after C2 hemisection was associated with both recovery of ipsilateral diaphragm function and upregulation of NMDA receptor subunit NR2A.

Advantages/disadvantages (phrenic nerve conduction, pneumotachometry and diaphragmatic EMG). Although diaphragmatic EMG, phrenic nerve recordings and pneumotachometric evaluations have clinical correlates, they are much more invasive procedures in the experimental laboratory than in the clinical setting. These experiments are technically demanding and terminal preparations. Although they provide a quantitative and informative index of diaphragmatic function, other outcome measures may be more suitable when respiratory function is not the sole or primary focus of an experiment.

Plethysmography

An alternative outcome measure for assessing respiratory function in experimental SCI is whole-body plethysmography (WBP), in which respiratory function is determined in conscious animals, without invasive instrumentation. Prior to experimentation, rats are typically trained to become acclimated to the plethysmography chamber to prevent confounds of stress. In a recent study, WBP was used to characterize respiratory function in rats with a C5 hemi-contusion SCI.⁹⁸ These rats exhibited respiratory deficits that are reminiscent of clinical SCI:¹⁴² specifically, their ability to augment tidal volume in response to hypercapnic challenge was impaired 4 weeks after severe SCI. This technique has also been used to verify the altered breathing pattern induced by C2 hemisection previously reported in anesthetized rats.¹²² Although spirometry is more commonly used in the clinic, WBP has recently been validated for respiratory evaluation of people with SCI.^{143,144}

Advantages/disadvantages. The most obvious advantage of WBP is that it is not a terminal experiment. Rather, respiratory data can be collected in the same animals at different time points following SCI. It does not provide a direct index of diaphragm function, but does provide clinically relevant indices of respiratory function in experimental animals. As it also requires less technical expertise than other methods, WBP may represent an attractive method for many laboratories investigating respiratory dysfunction after experimental SCI.

Gastrointestinal function

Innervation of the gastrointestinal system

The GI system control involves a complex interaction between the somatic nervous system (anal sphincters), both divisions of the autonomic nervous system, and the unique intrinsic enteric nervous system (ENS). The ENS controls the secretion, motility, blood flow, storage and evacuation of the GI tract, and its basic organization and function is similar across species. The autonomic nervous system modulates the intrinsic activity of the ENS, and is especially important at the proximal and distal ends of the GI tract (Figure 6). More detailed information about the interactions between the autonomic nervous system and ENS can be found in recent reviews.^{145–147}

Clinical impact of gastrointestinal dysfunctions following SCI

Although the ENS does have some intrinsic functional capacity, this is significantly impaired when it loses central coordination following SCI. The disruption of autonomic innervation to the GI tract is primarily revealed by abnormal motor function as opposed to changes in secretory or absorptive function.¹⁴⁸ GI problems are prevalent in both the acute and chronic periods following SCI, and are a significant cause of rehospitalization and morbidity.^{149–153} Although there is some controversy over the effect of SCI on the upper GI tract, there is evidence of gastric dilation, delayed gastric emptying (GE), gastric ulceration and

prolonged orocecal transit time (OCTT) following SCI.^{154–157} The most common complications are lower GI tract dysfunctions and most research has focused on their identification and resolution. These typically present as constipation, compaction and fecal incontinence, and can trigger both physical and psychological problems that restrict lifestyle choices and disrupt rehabilitation and overall quality of life of individuals with SCI.^{158,159}

The term ‘neurogenic bowel’ describes the loss of neuronal control to the colon, and resulting dysfunction.¹⁶⁰ The neurogenic bowel can be divided into two main types, each with characteristic colonic dysfunctions; supraconal lesions result in damage to descending supraspinal pathways (upper motor neuron bowel syndrome) and infraconal (cauda equina or pelvic nerve lesions) damage to motor and parasympathetic innervation to the colon (lower motor neuron bowel syndrome).¹⁶⁰ The upper motor neuron bowel, or hyperreflexic bowel, is associated with spastic activity in the colon and external anal sphincter (EAS), which interferes with the voluntary ability to defecate but leaves the bowel reflex intact—the basis for bowel emptying using chemical or mechanical stimuli. The lower motor neuron, or areflexic, bowel is associated with a relaxed colon, perturbed peristalsis, slow stool propulsion and constipation.¹⁶⁰

Assessment of gastrointestinal function following experimental SCI

Given that SCI primarily affects GI motor control, the majority of GI assessments following SCI consist of tests of functional motility or contractile activity. This section focuses on functional GI assessments used in experimental animal models (Table 5), some of which have been used as outcome measures for assessing autonomic function of the GI tract in humans following SCI and for testing clinically relevant therapies for restoring GI function.

Gastrointestinal transit with oral markers

GI transit can be assessed in experimental animals by segmental dye recovery along the GI tract following oral marker delivery.^{170,171} In the experimental animal, a non-toxic, nonabsorbable dye is administered by gavage feeding to the stomach. After the desired time interval, animals are euthanized, clamps are secured between each GI segment and marker recovery is detected by spectrophotometry to quantify the amount of dye in each segment, an indicator of movement through the GI tract.¹⁷⁰

Dye recovery has been used as an outcome measure in rats with SCI at various levels.^{161,172} Rats with cervical or thoracic spinal cord transection showed increased dye recovery in the stomach and decreased recovery in the small intestine throughout the first week after injury, indicating decreased GE and overall GI transit.¹⁶¹ However, after 10 days post-SCI, there were no differences in dye recovery between the stomach and intestine, indicating that there is a recovery of GE and overall GI transit.¹⁶⁴ Unlike humans, these rats also showed concurrent spontaneous recovery of bowel function.¹⁷² A follow-up study revealed that large bowel empty-

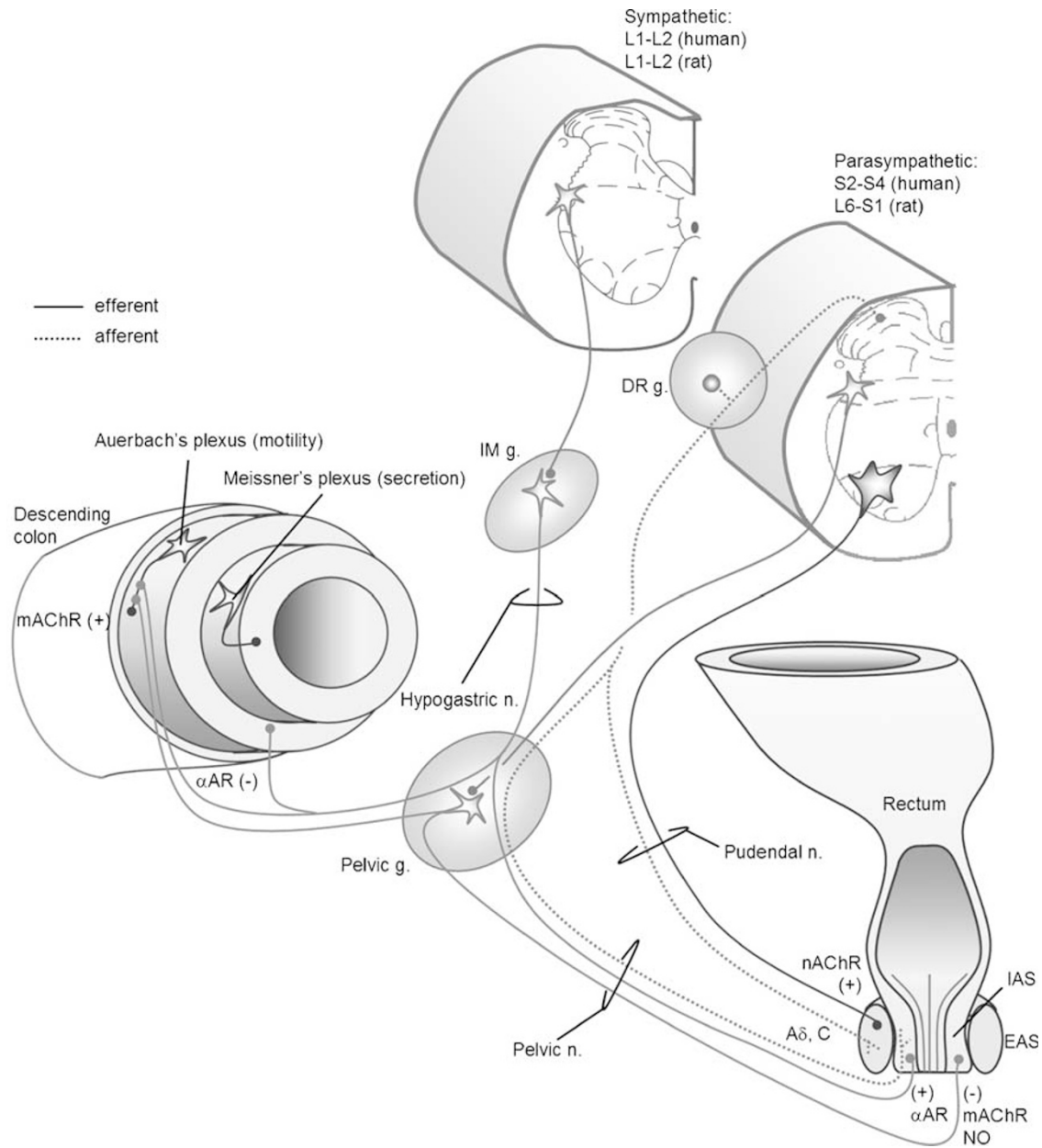


Figure 6 Innervation of the distal gastrointestinal (GI) tract. The ENS is composed of two main ganglionated plexuses that lie between the longitudinal and circular muscle layers (myenteric or Auerbach's plexus) and within the submucosal layer of the GI tract (submucosal or Meissner's plexus). The myenteric plexus is continuous throughout the entire length of the GI tract, and is primarily involved in the coordination of smooth muscle activity, whereas the submucosal plexus is present primarily in the small and large intestines, where it controls secretion and absorption.¹⁴⁵ Autonomic innervation of the GI tract is required to modulate the intrinsic activity of the enteric nervous system (ENS). This modulation is especially important in the distal GI tract (illustrated here), where the ANS coordinates storage and evacuation by regulating colon motility and resting anal sphincter tone.^{405,406} Parasympathetic innervation of the distal colon and rectum originates in the sacral cord (S2–S4), whereas the upper GI tract (to the level of the splenic flexure) is innervated by the vagus nerve (not illustrated here). Preganglionic parasympathetic neurons synapse directly on Auerbach's plexus that enhances smooth muscle activity (via mAChR). Sympathetic innervation is mainly postganglionic, and arises from paravertebral and prevertebral ganglia of the abdominal and pelvic cavities—celiac, superior and inferior mesenteric and pelvic ganglia. Sympathetic neurons in prevertebral ganglia inhibit muscle and secretory activity indirectly, by noradrenergic modulation of activity in both the Meissner and Auerbach's plexuses. The IAS receives both sympathetic and parasympathetic innervation, whereas the EAS is innervated by somatic fibers traveling in the pudendal nerve (S2–S4 in humans, L6–S1 in rats). Afferent information from this area travels in both the pelvic and pudendal nerves. *Abbreviations:* Aδ, C, mechanosensitive primary afferents; αAR, α-adrenergic receptors; DR g, dorsal root ganglion; EAS, external anal sphincter; g, ganglion; IAS, internal anal sphincter; IM g, inferior mesenteric ganglion; mAChR, muscarinic cholinergic receptors; n, nerve; nAChR, nicotinic cholinergic receptors; NO, nitric oxide; (+) denotes excitatory synapses; (–) denotes inhibitory synapses.

Table 5 Overview of studies characterizing or targeting gastrointestinal dysfunction after experimental SCI

Technique used	Species	Injury model	Time range post-injury	References
GI transit with oral markers	Rat	T4–T5 Tx	30 min–7 days	161
			1 day	162,163
			1–30 days	164
	Cat	C7–T1 Tx	30 min; 6 h; 1, 3, 7 days	161
		T4 hemostat clamp	1–2 weeks	165
Manometry	Rat	T4 Tx	1 day–2 weeks	166
	Cat	T4 hemostat clamp	1–2 weeks	165
	Dog	T10 Tx	2–6 weeks	167
EMG	Rat	T9–T10 Tx	≤ 24 h	168
		T9–T10 contusion	2 days–6 weeks	168,169
			2 days–6 weeks	169

Abbreviations: EMG, electromyography; GI, gastrointestinal; Tx, transection.

Studies have been classified by the technique used to assess gastrointestinal function. Studies employing a combination of techniques are referenced under both headings. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

ing prevented the development of delayed GE and GI transit following SCI.¹⁶² Although the effects of SCI on the upper GI tract are clinically still somewhat controversial, with some groups reporting GE delays and others demonstrating normal GE,^{155–157,173} these results suggest that delayed GE is in fact secondary to lower GI delayed motility.¹⁶² This corroborates previous research showing that the GE reflex is mediated by vagovagal reflexes and is not disrupted by sympathetic denervation of the upper GI tract.^{174,175}

Visualization techniques, similar to orally administered radionucleotides used to assess GI motility clinically,^{156,176,177} can also be applied in the experimental setting. Video fluoroscopy has been recently used to assess the functional benefits of colonic electrical stimulation as a treatment to improve colonic transit in rats^{178,179} and SCI cats.¹⁶⁵ These results suggest that colonic transit times are improved with the use of colonic electrical stimulation.¹⁸⁰ Magnetic resonance imaging (MRI) has also been used to visualize GI motility by detection of solid food labeled with trace amounts of nontoxic iron oxide particles in rats.¹⁸¹

Advantages/disadvantages. Oral marker delivery for evaluation of GI transit can require training and animal habituation, particularly if gavage feeding is required. The detection of the markers can be performed as a terminal preparation, or by indirect visualization. The latter type of detection techniques (MRI or X ray) are attractive as they could be easily combined with sensory or motor outcomes; however, these techniques are more expensive and technically demanding.

Electromyography

EMG recording of EAS activity has been used as a tool to investigate the physiology and pathophysiology of the EAS in rats with SCI.¹⁶⁸ This preparation allows for the measurement of both baseline EMG activity in the EAS and the contractions stimulated by EAS distension.¹⁶⁸ Animals are restrained in a supine position using a loose-fitting cylinder, or masking tape, to secure their torso, hind limbs and

tail.^{168,169} Temporary bipolar EMG electrodes are implanted in the EAS, with the external wire attached to the tail and connected to a preamplifier. After recording baseline EAS EMG activity, EAS distension is initiated with a plastic probe (used to mimic fecal bolus), and the resultant EAS contractions are recorded.¹⁶⁸ Anesthetics are not normally used in this assessment, as they significantly attenuate EAS hyperreflexia.

This technique has also been used to assess functional recovery of autonomic reflexes after the period of spinal shock, and the development of hyperreactive autonomic reflexes.¹⁶⁹ EAS hyperreflexia, reflected in prolonged burst duration of EAS activity, developed 2 days post-contusion at T9–T10 and resolved to preoperative levels, not significantly different from controls, by 6 weeks post-injury. In contrast, spinally transected animals developed EAS hyperreflexia 7 days post-injury and did not demonstrate any EAS reflex recovery.¹⁶⁹ This research demonstrates the sensitivity of EAS EMG recording and its potential as an objective assessment of pelvic autonomic reflexes.

EMG recording from the jejunum has been recently adapted for use with telemetry, allowing for recording in awake and mobile rats.¹⁸² In this technique, an EMG transmitter is implanted dorsally between the shoulder blades and connected to the electrodes in the jejunum.¹⁸²

Advantages/disadvantages. EMG is technically demanding, usually terminal, and requires both training and specialized equipment. However, it represents the most sensitive and direct evaluation of the activity in the GI tract.

Manometry

Manometry, the measurement of pressure changes within different parts of the GI tract, has also been used to assess colonic motility in rats following SCI.¹⁶⁶ Similar to the clinical technique^{183–185} a fluid-filled catheter is inserted into the colon and is attached to recording probes at different regions along the colon; however, in the rat these probes are secured with ligatures to allow for chronic

recording.¹⁶⁶ To quantify motor activity, a motility index can be calculated that incorporates the amplitude, duration, frequency and number of contractions. Using this technique, spinally transected rats showed a reduction of distal colonic motility acutely after injury, which returned to preoperative values after 7 days.¹⁸⁶ Unfortunately, the combined motility index does not provide an indication of the functional motility in the colon—an important consideration given that one of the main problems following SCI is the lack of coordinated peristalsis rather than lack of overall contractile activity.¹⁵⁵ However, when used in combination with oral marker delivery, the effects of reduced motility can be more clearly assessed.^{165,179,187}

Strain gauge transducers

Strain gauge transducers have also been used to continuously record gastric, small intestinal and colonic motility in awake rats.¹⁸⁸ In contrast to manometry, this technique measures the pressure changes on the extraluminal surface of the GI tract.^{189–191} Although each transducer has only uniaxial sensitivity, by placing transducers at right angles to each other in the same segment, both longitudinal and circular contractile muscle activities can be recorded—a good indicator of coordinated motility.¹⁸⁹ This technique has been used to model postoperative GI tract paresis,¹⁸⁸ assess neurochemical effects on GI motility¹⁹² and examine the functional effects of altered pacemaker activity in the gut.¹⁹³ Although this technique has not yet been used in experimental SCI, its relative noninvasiveness and capacity for chronic use makes it a promising way to measure functional GI motility.

Advantages/disadvantages (manometry and strain gauge transducers). Both manometry and strain gauge techniques require a fairly significant investment in training and equipment. Manometry is ideal for acute measurements, but its use is restricted to the colon. The use of strain gauges is surgically demanding, but does not interfere with normal GI motility, and is therefore preferable for chronic recording.¹⁹⁴

Hydrogen breath test

Hydrogen breath tests are commonly used to assess the pathophysiology of clinical GI disorders. As there is no bodily source of hydrogen other than that produced by bacterial metabolism in the cecum, an increase in hydrogen expiration following carbohydrate administration indicates the arrival of the nutrient bolus in the cecum, and can be used as a measure of OCTT.^{195–197} Using this test, individuals with SCI show significantly delayed OCTT compared to controls—an effect that is more pronounced in quadriplegic than paraplegic patients.¹⁹⁸ Although this technique has also been validated in feline, canine and rodent models,^{195,199,200} it has not yet been used in experimental SCI. However, it may prove to be a useful outcome measure to assess GI function following SCI—at both the bench and the bedside.

Advantages/disadvantages. The hydrogen breath test is minimally invasive and could easily be used in conjunction with other assessments in SCI animals. It can be used at repeated intervals in the same animals. However, specialized equipment is necessary for detection of hydrogen in the breath.

Urinary bladder function

Innervation of the lower urinary tract

Although interspecies differences exist, the basic organization and innervation of the LUT is similar among common experimental species and humans (Figure 7). In general terms, storage of urine is sympathetically mediated, whereas micturition is elicited by parasympathetic activation: however, normal LUT function requires the coordinated activity of the sympathetic, parasympathetic and somatic nervous systems. Here we provide only the basic scheme of LUT innervation, as neural control of the LUT and associated reflexes are comprehensively reviewed elsewhere.^{201–207}

Clinical implications of lower urinary tract dysfunction following SCI

The manifestation of LUT dysfunction after SCI is similar in experimental animals,^{67,208} (Table 6) and humans,^{205,206,268–270} and is broadly termed neurogenic bladder. The initial period following SCI is marked by bladder areflexia and urinary retention. When SCI occurs at or below the sacral level (that is, infraconal; a lower motoneuron injury) bladder areflexia persists. If the lesion extends rostrally to the thoracolumbar region to involve sympathetic preganglionic neurons, the bladder neck may also become hypoactive. Suprasacral (supraconal) SCI (an upper motor neuron lesion) typically produces hyperreflexia of the smooth (detrusor) muscle of the bladder and tonic activation of the striated urethral sphincter. Therefore, sphincter contractions are dyssynergic with detrusor contractions. Detrusor hyperreflexia and detrusor–sphincter dyssynergia result from both a loss of tonic supraspinal inhibition and the emergence of aberrant spinal reflexes. The clinical profile of LUT dysfunction is highly variable between individuals with SCI, but can be generally described in terms of impaired continence (most common in supraconal SCI), impaired emptying (most severe in infraconal SCI) and impaired sensation of bladder filling (a component of dysfunction for most people with SCI). Clinically, complications of LUT dysfunction remain the leading cause of rehospitalization among people with SCI.²⁷¹

Assessment of lower urinary tract function following experimental SCI

SCI induces profound changes in bladder innervation, particularly afferent, circuitry,^{206,217,272,273} morphology^{209,213,274} and structure,^{275–278} all of which likely contribute to neurogenic bladder. We limit this discussion to functional assessments only, with an emphasis on tests used in clinical SCI. Although an animal model of cauda equina/conus medullaris injury (lumbosacral ventral root avulsion in rat) has been recently developed,^{279,280} available experimental data describe

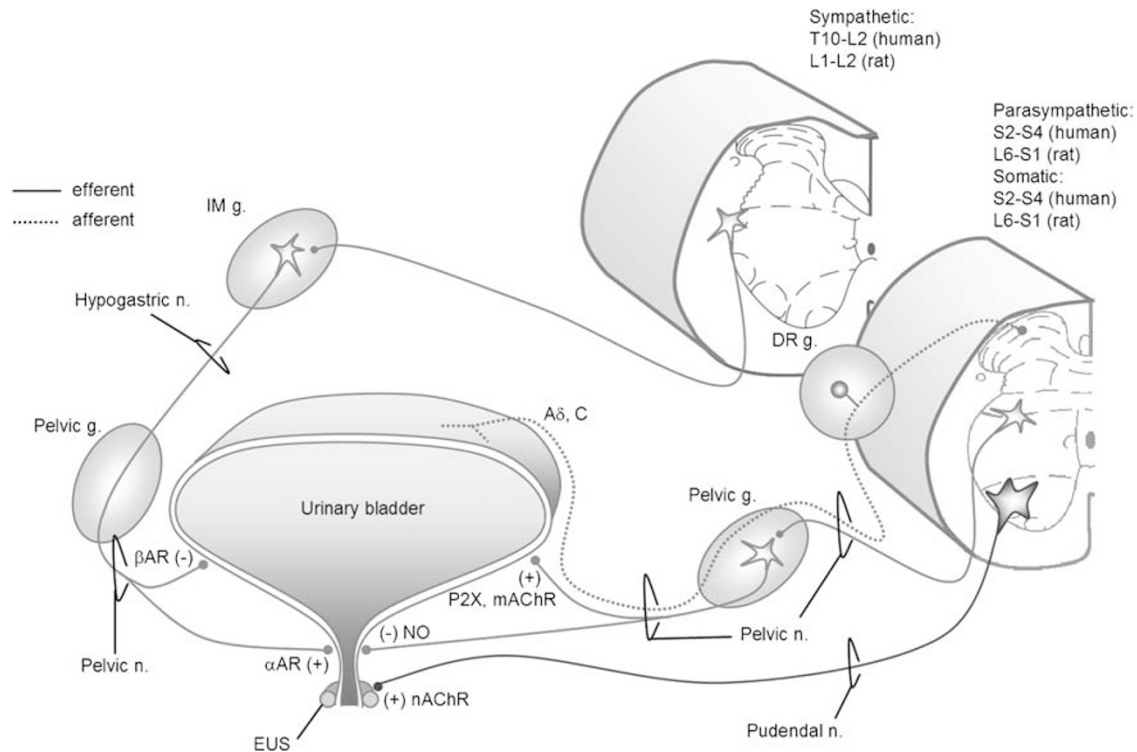


Figure 7 Innervation of the lower urinary tract (LUT). The LUT is comprised of the bladder, urethral sphincter and urethra. The LUT receives the bulk of its innervation from three nerves. The hypogastric nerve carries sympathetic innervation to the LUT; contributing spinal nerves exit the spinal cord (SC) between L1 and L2. Muscle activity for storage is mediated by α -AR expressed in the trigone, bladder neck and urethra (excitatory), and by β -AR expressed in the bladder dome (inhibitory). The pelvic nerve contains parasympathetic input originating in the sacral cord (L6–S1 in rat⁴⁰⁷) and controls micturition via cholinergic muscarinic receptors (mAChR) expressed throughout the LUT. The human pudendal nerve exits the sacral SC, and provides somatic innervation to the striated muscles of the external urethral sphincter; in rats, the pudendal nerve originates in the L6–S1 cord. In addition to their efferent function, each of these nerves carries afferent input from the LUT. Information about bladder distension is carried by mechanosensitive afferents (A δ , C) found primarily in the pelvic nerve. These afferents signal the coordinated switch between storage and micturition. The pudendal and hypogastric nerves mostly contain nociceptive afferents, which are not depicted here. *Abbreviations:* AR, adrenergic receptors; DR g, dorsal root ganglion; EUS, external urinary sphincter; g, ganglion; IM g, inferior mesenteric ganglion; L, lumbar spinal cord; mAChR, muscarinic cholinergic receptors; n, nerve; nAChR, nicotinic cholinergic receptors; NO, nitric oxide; P2X, purinergic receptor; S, sacral spinal cord; (+) denotes excitatory synapses; (–) denotes inhibitory synapses.

bladder dysfunction following supraconal (not infraconal) SCI. Although SCI-induced LUT dysfunction has been well-characterized in experimental animals, few studies have included LUT assessment as an outcome measure in testing therapies for SCI (Table 9).

Cystometric urodynamic analysis

The most common method for assessing LUT function in experimental animals is cystometric urodynamic analysis. Cystometry can be performed using a urethral or transvesical catheter implanted into the bladder dome: although the latter method is more invasive, it does not partially obstruct the urethra and permits EMG of the external urethral sphincter (see 'External urinary sphincter electromyography') to be performed concurrently. Cystometry is routinely conducted in both conscious and anesthetized animals, with the caveat that anesthesia does affect micturition reflexes,²⁸¹ particularly in animals with chronic SCI.^{235,236,257} This undesirable effect may be partially addressed by reducing the dose of anesthetic.²³⁶ Whether transvesical or transure-

thral, conscious or unconscious, cystometry in experimental animals is essentially similar to clinical cystometry: the bladder is filled with saline while recording intravesical pressure to examine the relationship between bladder volume and pressure during filling and micturition.

Detrusor hyperreflexia and detrusor–sphincter dyssynergia create a similar urodynamic pattern in rats^{209,237,218} and humans^{206,269,282,283} with suprasacral SCI. Post-injury cystometrograms are characterized by increases in volume threshold for inducing micturition, pressure during micturition, volume expelled during micturition and residual volume after micturition. In addition, spinal-cord-injured rats and humans often exhibit nonvoiding detrusor contractions during bladder filling, a hallmark of detrusor hyperreflexia. Rats assessed via conscious transvesical cystometry 2–3 weeks after complete thoracic (T8–T10) spinal transection had larger volume thresholds for micturition (1.43 versus 0.34 ml), larger micturition pressures (48 versus 26 mm Hg), increased voided volumes (0.72 versus 0.31 ml) and increased residual volumes (0.71 versus 0.03 ml) compared to uninjured controls.²¹⁸

Table 6 Overview of studies characterizing or targeting lower urinary tract dysfunction after experimental SCI

Technique used	Species	Injury model	Time range post-injury	References		
Cystometry	Rat	T8–T10 contusion	≤1 week	209–211		
			2–4 weeks	209,211,212		
		T8–T11 Tx	8 weeks	210,213		
			Immediate	214		
			24 h	215,216		
			≤1 week	217		
			2–4 weeks	209,214,216–234		
			4–8 weeks	225,231,235–245		
		Rabbit Cat	T12 heat injury	8–12 weeks	232,246,247	
				1 month	248	
			L3/4; L6/S1 Tx	2–5 weeks	214	
			T10 Tx	Immediate; 2–5 weeks	249	
			C6–T1 Tx	1, 2, 7–21 days	250	
			T1 clip compression	10 weeks	251	
	T8 Tx; T8 contusion		2–3, 5–7 weeks	252		
	T10 d. fun. Tx		3 weeks	253		
	T10–T12 Tx	Immediate	254			
	Dog	T8–T11 Tx	≤24 h	255,256		
			2–4 weeks	255–261		
		T8–T11 Tx	4–8 weeks	257,260		
6–12 months			262			
1–8 weeks			263,264			
1–8 months			263,264			
External urinary sphincter EMG	Rat	T8 contusion	≤1 week	209,213		
			2 weeks	209		
		T8–T9 Tx	6–8 weeks	210,213		
			Immediate	214		
			2–4 weeks	209,214		
			4–6 weeks	214,241,243		
		T8–T11 Tx	2–4 weeks	219		
			4–8 weeks	236,237,239,240		
			8–12 weeks	246		
			Rabbit Cat	L3/4; L6/S1 Tx	Immediate; 2–5 weeks	214
					2–5 weeks	249
				T10 Tx	1, 2, 7–21 days	250
		C6–T1 Tx		10 weeks	251	
		T1 clip compression		2–3, 5–7 weeks	256	
	T9–T12 Tx	2–4 weeks		253,258,261		
	Dog	T8–T10 Tx		6–8 weeks	251	
				1–8 weeks	264	
		T8–T10 Tx	1–8 months	264		
			Bladder volume	Rat	T8; T9/10 contusion	≤1 week
		2–4 weeks				265–267
4–6 weeks		265,266				
Cat	T10 Tx T8 Tx; T8 contusion	0–20 days		217		
		2 weeks		252		
		Videofluoroscopy		Cat	C6–T1 Tx	10 weeks
T1 clip compression	2–7 weeks		251			
Dog	T8–T9 Tx		1–8 weeks	262		
Pressure recording (c. spongiosus)	Rat	T9/10 contusion	≤1 week; 2–4 weeks	266		

Abbreviations: compr., compression; c. spongiosus, corpus spongiosus; d. fun., dorsolateral funiculus; EMG, electromyography; Tx, transection. Studies have been classified by the technique used to assess lower urinary tract (LUT) function. Studies employing a combination of techniques are referenced under both headings. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

Preclinical urodynamic analysis has been used extensively to characterize mechanisms of and identify therapeutic candidates for bladder dysfunction following SCI (Table 6). Such experiments continue to be informative even after treatments enter clinical practice: for example, rats were recently treated with botulinum-A to examine the effects of acute versus delayed therapy following SCI.²³⁸ To date, only

a few experimental studies testing regenerative, plasticity-promoting or protective treatments for SCI have incorporated urodynamic analysis as an outcome measure (Table 9). In one such study, rats that received transplants of immortalized neural stem cells at the site of thoracic SCI (T8 contusion) exhibited reduced micturition pressure and reduced residual urine compared to untreated controls.²⁸⁴

Two subsequent studies in the same injury model demonstrated that transplantation of neural precursor cells²⁸⁵ or genetically modified fibroblasts²⁸⁶ accelerated recovery from bladder areflexia and reduced micturition pressure and the number of nonvoiding detrusor contractions.

External urinary sphincter electromyography

In both clinical and experimental assessment of SCI, external urethral sphincter EMG (EUS EMG) can be performed in conjunction with cystometry to provide a direct measurement of detrusor–sphincter dyssynergia. Wire electrodes are inserted into the muscle of the EUS, and EUS activity during bladder filling and micturition is recorded. The data are typically expressed as mean EMG activity, mean EMG power frequency, mean EMG-spiking activity (ESA) and durations of activity or contractions.^{213,236} Also, the relationship between EUS EMG amplitude and detrusor contractions is examined to determine the extent of coordination between detrusor and sphincter activities. Changes in EUS EMG throughout the micturition cycle can be examined by power spectrum analysis using the fast Fourier transform algorithm.²¹³

In rats^{209,213,237} and humans^{206,269,287,288} with suprasacral SCI, EUS EMG reveals sphincter activity that is not associated with detrusor contraction or micturition. In rats, the micturition-induced increase in external sphincter activity (dESA) is lost following thoracic SCI: as dESA is negatively correlated with SCI severity, it can be used as an index of detrusor–sphincter dyssynergia.²¹³ Other intriguing indices of bladder–sphincter synergy have been identified by analyzing fractal dimensions and power spectra of EUS EMG and cystometrograms.^{239,240} Akin to urodynamic analysis, EUS EMG has been used as an outcome measure in mechanistic studies of LUT dysfunction in experimental SCI (Table 6). However, this technique is rarely included in preclinical studies that assess the functional benefits of therapies for SCI (Table 9).

Advantages/disadvantages (Cystometric urodynamic analysis and External urinary sphincter electromyography). Cystometry and EUS EMG applied in combination definitely represent the most informative and clinically relevant assessment of LUT function following SCI. However, these are invasive, technically demanding procedures that can only be performed at a single time point (that is, at the end of the experiment). Laboratories that lack expertise in these techniques may wish to adopt another method of monitoring bladder function after SCI.

Functional bladder volume

In rats, increases in bladder volume are proportional to severity of SCI.^{213,274} Bladder volume is estimated by measuring the volume of urine expressed at micturition, either by using a metabolic cage^{267,284–286} or by measuring the volume of urine that can be manually expressed.²⁸⁹ Bladder volume has been used as an outcome measure in experiments testing chondroitinase (a bacterial enzyme) as a regenerative therapy for SCI.²⁸⁹ In these experiments,

intrathecal chondroitinase treatment reduced the volume of urine that could be manually expressed in rats with moderate thoracic SCI.

An attractive alternative to estimating bladder function by expressed urine is transabdominal ultrasound, which has been recently used in rat SCI.²⁶⁵ In this study, a handheld digital ultrasound imaging system was used to measure bladder volume following severe thoracic (T10) contusion. Bladder volume was 3.51 ± 0.47 ml (compared to 0.089 ± 0.04 ml in uninjured rats) on day 4 post-injury and decreased to 1.83 ± 0.50 ml by day 8; it did not change significantly for the duration of the experiment (46 days). The authors found that ultrasonic measurements of bladder volume were comparable to estimates based on manual expression of urine.

Advantages/disadvantages. Measurements of volume of urine per micturition represent a cost-saving alternative for monitoring bladder function, but are tedious and lack precision. Transabdominal ultrasound is noninvasive, less stressful than other methods, does not disrupt concomitant motor and sensory testing and permits assessment in the same animals throughout the recovery period. As this is a common clinically used test, it could be recommended for more frequent use in animal experiments. This method will require some investment in technology and training.

Videofluoroscopy

In videofluoroscopy, the bladder is filled with radio-opaque medium and imaged by X-ray video recording. This approach is commonly used to evaluate a variety of organ functions in the clinical setting. Fluoroscopy has been used in preclinical investigations of electrical stimulation to improve bladder function following SCI (Table 6). These studies characterize the effects of sacral spinal stimulation,²⁶² direct bladder stimulation,^{251,290} and more recently, neuroprosthetic microstimulators targeting the bladder wall and plexus^{291,292} in dogs and cats with SCI.

Advantages/disadvantages. Videofluoroscopy is a clinically relevant technique and provides useful information for specific applications (those mentioned above) in larger species; however, it has never been used in rodent SCI. It also requires specialized equipment and expertise that is not likely to be available to most investigators.

Corpus spongiosum pressure recording

Another method of examining LUT function that has been recently developed involves telemetric monitoring of pressure within the corpus spongiosum of the penis (CSP).^{266,293} Traditionally used to study sexual function in experimental animals^{294–296} (see ‘Sexual function’), CSP pressure has been recently validated for assessing LUT function in spinal-cord-injured rats.²⁹³ In this study, the authors found that volume of urine expelled per micturition was highly correlated with micturition duration recorded by CSP pressure: thus, telemetric CSP pressure monitoring can provide information about voiding volume and frequency of micturition in freely

moving, conscious animals throughout their recovery from SCI. At 21 days after thoracic (T10) contusion, rats with telemetric pressure monitoring had micturition CSP pressure waveforms that were increased in duration, mean pressure and peak frequency compared to those observed before SCI.²⁶⁶

Advantages/disadvantages. Pressure recording from erectile tissue is technically challenging, but has the benefits of being able to be used chronically and in unanesthetized, freely moving animals. It is of particular interest for outcome assessment post-SCI because it allows for simultaneous recording of micturition and erection events—both of which are perturbed following SCI.

Sexual function

Innervation of the pelvic organs and genitalia

The autonomic innervation of the pelvic organs is essentially similar across mammalian species, and generally similar between males and females.²⁹⁷ Here we review only the most pertinent features of tissue innervation in the pelvic organs and genitalia (Figure 8). More detailed reviews can be found elsewhere.^{298–300}

The role of the autonomic nervous system in sexual function

Normal sexual function requires a combination of local spinal reflexes and descending cortical control.^{300,301} Genital arousal is a combined neurovascular and neuromuscular response that can be initiated reflexively or psychogenically. In both sexes, reflex sexual arousal results from increased parasympathetic activity, which causes nitric-oxide-mediated vasodilation,^{302–304} accompanied by inhibition of sympathetic adrenergic activity. Together these two changes lead to an increase in genital blood flow, engorgement of erectile tissues and intracavernous pressure increase and, in women, lubrication of the vaginal surface. Unlike reflex arousal, psychogenic arousal appears to be facilitated by the sympathetic nervous system.^{305–308} In the male rat, and probably also in humans, the contraction of the somatic striated perineal muscles (bulbospongiosus and ischio-cavernosus) also contributes to penile rigidity during erection.^{309–312}

Sexual climax appears to be mediated by a spinal reflex:³¹³ in response to genital stimulation, anesthetized, acutely spinalized rats show a urethro-genital response similar to that seen in human sexual climax.^{307,314–316} This reflex includes clonic contractions of the perineal muscles, rhythmic firing of the cavernous nerve, penile erections and ejaculation in the male, and rhythmic vaginal and uterine contractions in the female.³¹⁶ This response is mediated by efferent output from hypogastric and pelvic nerves, suggesting that both parasympathetic and sympathetic nervous systems are involved.³¹⁷ With regards to the autonomic nervous system, ejaculation itself is considered to be a sympathetically mediated event;³¹⁸ however, normal antegrade ejaculation

requires the coordination of both the somatic and sympathetic nervous systems.

The autonomic nervous system also has a role in the maintenance of reproductive capacity, although there is comparatively little research in this area.³¹⁹ The activity of the epididymis, whose main functions include sperm transport^{320,321} and fluid resorption,³¹⁹ is primarily regulated by the sympathetic nervous system, with particularly important innervation arising from the inferior mesenteric ganglion.^{320,321}

Clinical impact of sexual dysfunctions following SCI

Low sexual satisfaction and sexual dysfunction are both well documented after SCI, and the resolution of these problems has been identified as a high priority.^{9,322} The disruption to autonomic circuits following SCI can result in a number of different sexual changes and the sexual function subgroup of the ASIA/ISCOS working group is currently developing international autonomic standards for documenting these changes.³²³ Here we focus primarily on changes in sexual function rather than reproductive function. The effect of SCI on sexual function is highly dependent on injury level and the most commonly affected sexual responses are arousal and orgasm.

Individuals with upper motor neuron lesions, and preserved S2–S5 roots, generally have preserved reflex genital arousal, as the reflexes mediating erection are located in the spinal cord. However, these individuals generally are unable to initiate genital arousal psychogenically. On the other hand, lower level SCI (intraconal or cauda equina) tends to disrupt reflex vasocongestion, but can leave sympathetically mediated psychogenic arousal intact.^{306,307} These psychogenic erections are comparable in duration, rigidity and tumescence to the preserved reflex erections in men with higher lesions.³⁰⁶

Many women with SCI maintain the ability to reach orgasm. However, men often have difficulty maintaining erection, ejaculating and sensing orgasm. As a result, male reproductive function is significantly affected by SCI. Penile vibratory stimulation and electroejaculation have been used to successfully manage infertility in many cases.^{324–326} Most women maintain reproductive capacity, and are able to become pregnant and undergo normal pregnancy after a short period of amenorrhea acutely after SCI.^{327,328}

AD must also be included in the discussion of sexual function, as it can be triggered by sexual activity and sperm retrieval in those with injuries above T5.^{329–334} During periods of AD, blood pressure can reach levels that are potentially life threatening. However, despite these extreme blood pressure levels, the symptoms of AD are not always subjectively detected by the individual (termed silent AD).³³⁵ Therefore, it is imperative that blood pressure be assessed in both private sexual activity and during sperm retrieval procedures to assure that patients are not at risk.³³⁵ Interestingly, although AD interferes with sexual activity for some individuals, the symptoms of AD during sexual activity can also be perceived as pleasurable or arousing.^{333,336}

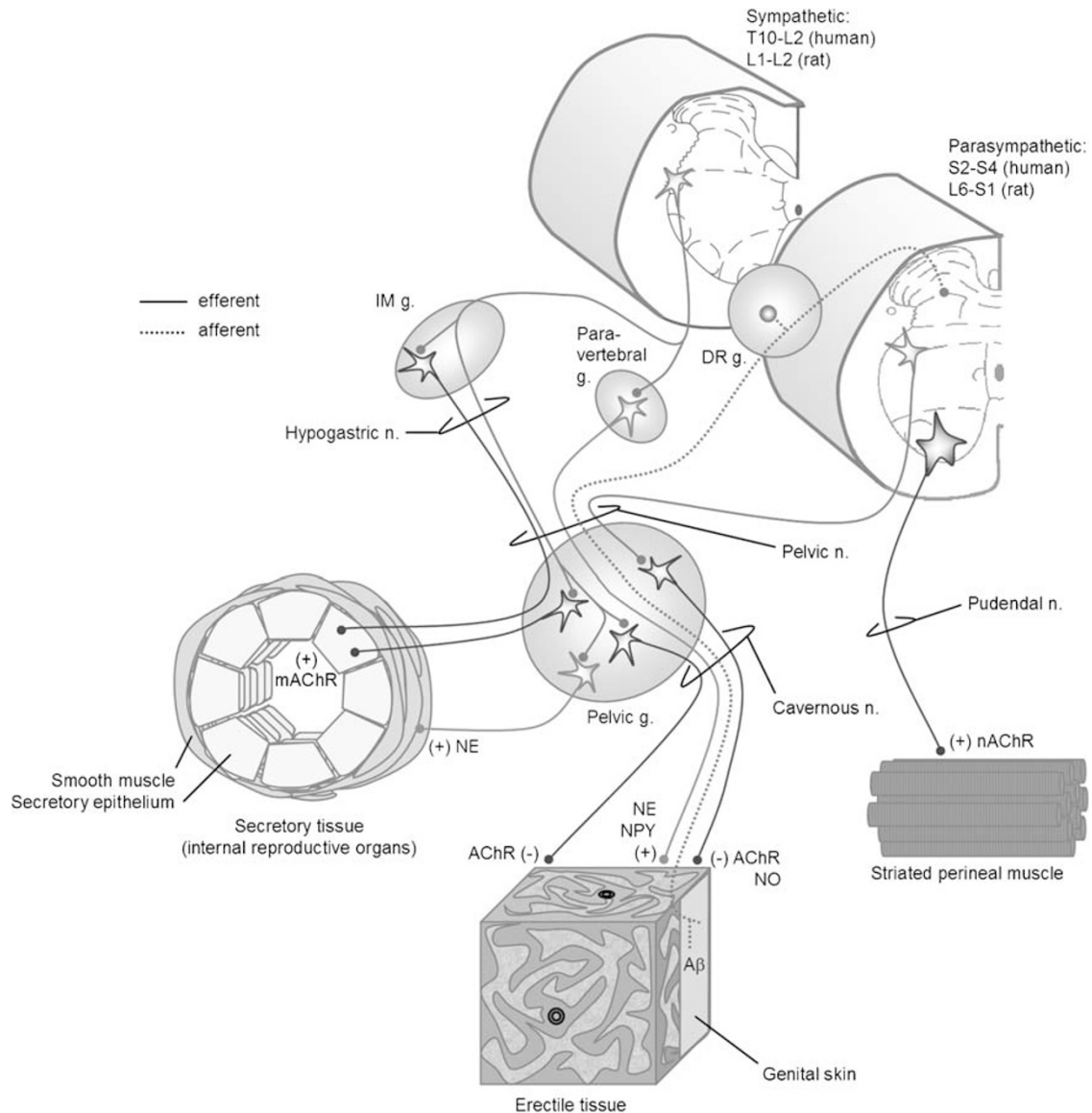


Figure 8 Innervation of the pelvic organs and genitalia. In the pelvic organs and genitalia there are three main tissue types: secretory, erectile and striated muscle. The majority of the autonomic innervation to these tissues comes from the bilateral pelvic ganglia (PG), which contains both sympathetic and parasympathetic neurons. Parasympathetic preganglionic neurons originate in the sacral cord (S2–S4 in humans; L6–S1 in rodents) and travel in the pelvic nerve to the PG. Sympathetic innervation originates in the lumbar cord (L1–L2) and travels via the hypogastric nerves to innervate the PG—in rodents, sympathetic nerves also travel to the PG via the pelvic nerve, which is mixed sympathetic and parasympathetic. Unlike human PG, which form a diffuse plexus on either side of the prostate (men) or cervix (women), rat PG are more condensed, and form two true ganglia. In both sexes, the largest nerve exiting from the PG is the cavernous nerve (also called penile nerve in males). In humans, somatic innervation of the striated perineal muscles, which include the ischiocavernosus, bulbocavernosus and levator ani, originates in the sacral cord (S2–4), whereas in the rats, this is shifted rostrally (L6–S1). Afferent information from the pelvic organs is relayed to the spinal cord via the ‘genitospinal’ nerves (pelvic, hypogastric and pudendal; for simplicity, only the pelvic nerve is illustrated here) and sensory pathways ascend bilaterally in the dorsal quadrant of the spinal cord.⁴⁰⁸ *Abbreviations:* AChR, cholinergic receptors; DR g, dorsal root ganglion; g, ganglion; IM g, inferior mesenteric ganglion; n, nerve; nAChR, nicotinic cholinergic receptors; NE, norepinephrine; NO, nitric oxide; NPY, neuropeptide Y; (+) denotes excitatory synapses; (–) denotes inhibitory synapses.

Assessment of sexual response and reproductive function following experimental SCI

Although the subjective responses from human studies are indispensable to fully elucidate sexual functioning following SCI,³³⁷ physiological data can be obtained from experimental animal models regarding normal sexual function³³⁸ and

recovery of autonomic circuits after injury.²⁶⁶ Spinal transection models have been used to study the neurophysiology of spinal sex reflexes in the absence of supraspinal control,³¹⁶ and to study infertility post-SCI.^{339–341} However, sexual function has been rarely used as an outcome measure in testing therapies for SCI (Table 7). Like human research,

Table 7 Overview of studies characterizing or targeting sexual dysfunction after experimental SCI

Technique used	Species	Sex	Injury model	Time range post-injury	References
Behavioral scoring	Rat	M	Mid-T Tx	28–52 days	342
			T8–T9 Tx	1–7 days	343
			T6 Tx	3, 4 weeks	344
Pressure recording					
c. cavernosus		M	T8–T9 Tx	2 weeks	345
c. spongiosus		M	T10 contusion	≤1 week, 2–4 weeks	266
Doppler flowmetry		F	T10 Tx	6–8 weeks	346
Epididymal sperm transport		M	T9 contusion	10 days	347

Abbreviations: c. cavernosus, corpus cavernosus; c. spongiosus, corpus spongiosus; Tx, transection.

Studies have been classified by the technique used to assess sexual function. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

experimental animal research has been dominated by the study of male sexual function, and there are comparably few validated experimental animal models of female sexual function.³⁴⁸ This section focuses on functional assessments of autonomically mediated components of sexual and reproductive function, with a focus on those that have been used in experimental SCI.

Ex copula visual scoring

Visual scoring has long been used to assess sexual function in experimental animals. This semi-quantitative technique includes observation, grading and quantification of sexual arousal and copulatory events (mounts, intromissions and ejaculations in the male; lordosis in the female animals).³⁴⁹ *Ex copula* tests have been primarily used in experimental SCI models, as functional copulatory behavior is unrealistic given the associated motor deficits. Animals are generally tested in a supine position with their legs and torso restrained by straps.³⁵⁰

To test reflex sexual function, penile reflexes are elicited by the retraction of the penile sheath and light pressure on the base of the penis,³⁵¹ mechanical stimulation of the urethra^{316,352} or by electrical stimulation of the dorsal penile nerve.³⁴³ The stereotyped responses occur in clusters and include erections (reddening of the glans), cups (flaring of the glans into a trumpet shape) and flips (anteroflexions of the glans of varying lengths).^{351,353,344} After SCI, these reflex erections occur more quickly and more frequently than in uninjured animals.³⁴²

Visual scoring can also be used to investigate psychogenic erectile function. Penile reflexes triggered by central stimulation of the medial preoptic area (a brain region with a well-established role in the facilitation of sexual behavior) revealed that the capacity for centrally mediated erections is preserved in a rat model of cauda equina injury.³⁵⁰ As erectile function has been traditionally associated with parasympathetic activation, this research revealed that the presence of thoracolumbar sympathetic activity is sufficient to mediate erection, and likely forms a component of normal erectile function.³⁵⁰ Similar results have also been found clinically.³⁵⁴

Like many of the other assessment methods described herein, visual scoring is most valuable when used in

conjunction with other physiological measures, such as EMG recording. Together, these two techniques have been recently used as outcome measures for assessing the effects of pharmacological manipulation to facilitate penile reflexes and ejaculation after experimental SCI.³⁴⁴ Similarly, the combination of visual scoring and blood pressure recording following penile reflex stimulation in a rodent model of SCI could be used to address the important clinical issue of vibrostimulation-triggered AD in men undergoing fertility treatment (JAI, LMR and AVK, unpublished observations).

Corpus spongiosum and cavernosus pressure recording

During erection, dilation of arteries and erectile tissue relaxation increase blood flow and result in increased intrapenile pressure. Pressure recording of the corpus cavernosum (CC) or corpus spongiosum (CSP) has been used to study sexual function and erectile function in intact rats,^{294,355,356} and is the most common assessment of erectile function used in preclinical trials.³⁵⁶ In this technique, the CC or CSP is catheterized using a polyethylene tubing, or a hypodermic needle attached to tubing, connected to a pressure transducer.³⁴⁵ The parameters that are commonly measured include the baseline, peak and plateau pressures, total area under the curve, as well as erection latency and total number of erections.³⁵⁶ These outcome measures have been used to characterize the sexual response changes that occur after SCI and the effectiveness of drugs in restoring normal responses.

Recent validation in rats with SCI showed that CSP pressure recording is a reliable method to evaluate erectile function over extended periods of time in conscious and freely moving animals as well as in restrained animals.^{266,293} Akin to previous reports using reflex erection tests on SCI rats,^{311,351} SCI rats exhibited shorter time to first observed erectile event compared to baseline values.²⁶⁶ CSP pressure recording was sensitive to early changes in SCI rats: the number of CSP pressure peaks increased in SCI rats as early as 1 day post-injury, even though at this time the total number of erectile events was not different from baseline values.²⁶⁶ Furthermore, SCI rats had many more CSP pressure peaks per erectile event, revealing that although erections may be qualitatively similar after SCI, their physiology may be significantly altered.²⁶⁶ Pressure recording has been recently

used as an outcome measure for evaluating the effectiveness of pharmacological manipulation to restore erectile capacity after SCI.³⁴⁵

Advantages/disadvantages. Described above; see Urinary bladder function ('Assessment of lower urinary tract function following experimental SCI' and 'Corpus spongiosum pressure recording').

Perineal muscle electromyography

Physiological responses and the role of perineal muscles can be measured during sexual behavior using EMG recording. This technique was used in the discovery of the urethro-genital reflex, a model used to study human sexual climax.³¹⁶ In the men, wire electrodes are placed in the bulbospongiosus muscle, which is attached to the CSP; in women, this recording is usually taken from the smooth muscle of the vagina.³¹⁶ In both sexes, mechanical stimulation of the urethra elicits clonic contractions of the perineal muscles and rhythmic cavernous muscle activity, with expulsion of the urethral contents in the men.³¹⁶ This technique has been validated in uninjured rats as well as spinalized, anesthetized animals.^{316,357}

Advantages/disadvantages. Like EMG in other systems, perineal EMG is generally a terminal preparation and is conducted on anesthetized animals. However, it offers a readily quantifiable measure of the sexual response.

Flowmetry

As signs of female genital arousal are quite subtle, experimental research in this area relies heavily on physiological recording. Vaginal photoplethysmography offers a reliable clinical measurement of vaginal blood volume and pulse amplitude.^{307,358–360} Experimentally, vaginal blood flow recording by laser Doppler flowmetry has been recently established as a method to investigate sexual arousal in rats.^{348,361,362} In this technique, capillary blood flow is measured by a surface probe, placed on the ventral surface of the vagina or clitoral glans, which is connected to a flowmeter.^{346,348,361} Using a bipolar hook electrode, electrostimulation is applied to the clitoral nerve or pelvic plexus to elicit a vascular response.³⁶¹

After suprasacral SCI, unlike male rats, female rats exhibited decreased response to electrostimulation; only 50% of animals showed increased clitoral blood flow, and even then, the increase was weak and nonsustained.³⁴⁶ In contrast, uninjured female rats showed immediate and sustained increases in both clitoral and vaginal capillary blood flow.³⁶¹ Recently, this technique has been used as a functional outcome to evaluate the effectiveness of pharmacological agents to restore sexual arousal following experimental SCI.³⁴⁶

Advantages/disadvantages. This technique offers a new way to investigate the underrepresented issue of female sexual dysfunction following SCI. Flowmetry itself is noninvasive, but it is often used in combination with nerve stimulation,

in invasive and terminal preparations. It is conducted in anesthetized animals, as the recording is very sensitive to movement.

Epididymal sperm transport assay

Unlike the techniques described above, which assess sexual function, this assay is utilized to assess reproductive function following SCI. Epididymal sperm transport is assessed by labeling of spermatozoa followed by quantification of labeled sperm throughout the length of the epididymis.³⁴⁷ This technique offers an indirect way to assess the preservation of sympathetic innervation to the epididymis, as sperm transport through the epididymis is sympathetically mediated.^{320,321} Following SCI, rats show decreased epididymal sperm transport.³⁴⁷

Advantages/disadvantages. This assay is a terminal preparation that demands some level of technical skill—for both the injection of the label and the epididymal dissection.

Thermoregulatory function

Autonomic control of thermoregulatory effectors

The thermoregulatory role of the autonomic nervous system is primarily mediated by the sympathetic nervous system and its vasomotor, sudomotor and pilomotor effectors. Although each of these effectors can also be activated by emotional stimuli, for the purposes of this review, we focus only on their thermoregulatory function. The preganglionic neurons of these effectors are cholinergic, and lie in the thoracolumbar cord (T1–L2); the ganglionic neurons are also cholinergic, and lie in the paravertebral ganglia.

Vasomotor efferents that innervate cutaneous vascular beds regulate the amount of heat that is dissipated to the surrounding environment. Unlike the visceral vasoconstrictors, cutaneous vasoconstrictors are not under strong baroreflex control, but are strongly affected by core temperature changes. Some areas of the skin are more specialized to perform thermoregulatory roles; areas with arteriovenous anastomoses allow for quick transfer of blood from the arteries to veins. Although the location of anastomoses varies from species to species, their function is similar.

In contrast to most sympathetic efferents, sudomotor efferents are cholinergic. When activated, these neurons act via muscarinic receptors to increase sweat gland secretion. In humans, there are two types of sudomotor neurons, apocrine or eccrine. Eccrine glands (present over the entire skin surface, with especially high levels on the palms, soles, face and axillae) are primarily used for diffuse thermoregulatory sweating; apocrine glands are associated with hair follicles, and are present in the genital, axillary and mammary regions. Unlike humans, rats only have apocrine glands, present on the plantar and palmar paw surfaces. These glands are activated by psychogenic rather than thermal stimuli, and do not have a role in thermoregulation.³⁶³

Pilomotor neurons are noradrenergic, and innervate piloerector muscles, which are found throughout the hairy skin of experimental animals and skin of humans. When

these muscles contract, they raise their associated hairs and ultimately augment insulation. The rostrocaudal innervation pattern of piloerector muscles is similar to that of the sensory dermatomes.

Clinical implications of thermoregulatory dysfunction after SCI

SCI can disrupt the descending sympathetic input, leaving large areas of skin and associated blood vessels, sweat glands and hair follicles disconnected from supraspinal sympathetic control.³⁶⁴ As a result, sympathetic mechanisms do not respond adequately to adjust core temperature and maintain equilibrium. The resulting thermoregulatory instability in both the acute and chronic periods can be severely debilitating.

Clinically, there are three main phenomena associated with temperature dysregulation reported following SCI: poikilothermia, acute hyperthermia and exercise-induced hyperthermia.⁵ Poikilothermia is also called 'environmental fever', but can refer to both the hypo- and hyperthermia experienced by individuals with SCI as a result of the ambient environment that they are exposed to. 'Quad fever' is used to refer to hyperthermia present in the first few weeks or months after SCI that is unrelated to infection or other identifiable causes.

In general, individuals with high injuries are predisposed to more severe temperature dysregulation than those with lower injuries.^{365,366} However, even individuals with low-level SCI can exhibit up to a 50% reduction in whole-body sweating capacity compared to able-bodied controls—a reduction that correlates with their reduced skin surface area for sweating.^{367,368} Although there is some evidence of increased productivity of sweat glands above the injury as a mechanism of compensation for decreased sweating below the injury,³⁶⁹ individuals with SCI remain at a significant risk for heat illness due to increased heat storage.³⁷⁰ Heat storage is particularly problematic in wheelchair athletes,³⁷¹ but recent evidence suggests that precooling or cooling during exercise can reduce the development of a high core temperature.^{372,373}

As well as being at increased risk for thermoregulatory dysfunction, individuals with high-thoracic and cervical injuries are also at risk for AD. During periods of AD, when reflex sympathetic adrenergic vasoconstriction below the level of injury elevates systemic blood pressure, reflex sympathetic cholinergic activity can also cause excessive sweating. In combination with thermoregulatory dysfunction,

profuse sweating can contribute to a lowering of body temperature, which can result in hypothermia.³⁷⁴

Assessment of thermoregulatory function following experimental SCI

In this section we focus primarily on functional tests of thermoregulatory control that are currently employed in experimental SCI research. These studies most commonly aim to characterize the thermoregulatory changes that occur after SCI, and a small number of them test approaches to mitigate these dysfunctions (Table 8). Molecular approaches to thermoregulation are beyond the scope of this review and have been recently reviewed elsewhere.^{377,378}

Core temperature recording

Core temperature recording provides a rough indication of the thermoregulatory capacity. Clinically, this type of information is easy to obtain, as it is part of routine care of individuals following SCI. In animals, core temperature is also quite easily obtained using a rectal thermometer. Acutely after cervical and high-thoracic SCI, rat core temperatures decline significantly and are vulnerable to changes in ambient environmental temperatures.^{40,379} Although the use of heating mats and raised environmental temperatures can be used to maintain body temperature in the acute setting,³⁷⁹ at 6 weeks post-injury core temperatures remained significantly lower in SCI rats compared to preoperative levels.⁴⁰

Advantages/disadvantages. Core temperature recording is minimally stressful for SCI animals with limited colorectal sensation. The tools are easily available and the technique itself requires minimal training. However, investigators must be aware that rectal probe insertion could induce AD, which can alter core temperature.^{40,374}

Cutaneous temperature recording

Cutaneous temperature recording provides a simple indirect way to investigate cutaneous vasoconstrictor activity. Despite the fact that skin blood flow is important in heat loss during exercise in individuals with SCI,³⁸⁰ there has been little research investigating cutaneous blood flow after experimental animal SCI.⁴⁰ The skin of the tail is the most important thermoregulatory organ in the rodent, and has been targeted in many investigations of thermoregulatory function.^{381,382} Infrared thermography can be used to record the surface temperature of the rat tail and hindlimb.^{40,383,384}

Table 8 Overview of studies characterizing or targeting thermoregulatory dysfunction after experimental SCI

Technique used	Species	Injury model	Time range post-injury	References
Core temperature recording	Rats	C6/7 Tx	6 h	375
Infrared thermography		T4 Tx	1 day–6 weeks	40
Flowmetry		T2; T9 Tx	1 h–7 days	39
Microneurography		T12–T13 Tx	0–72 h	376

Abbreviation: Tx, transection.

Studies have been classified by the technique used to assess thermoregulatory function. Studies employing a combination of techniques are referenced under both headings. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

Following SCI, the skin temperature of the mid-tail and the hindpaw increased. These changes correlated with the decrease in core temperature, suggesting that the two are potentially related.⁴⁰

Advantages/disadvantages. Cutaneous temperature recording is noninvasive, causes no stress to the animals and can be used chronically. However, specialized equipment is required for both the data acquisition and analysis.

Flowmetry

Blood flow recording provides a noninvasive way to assess sympathetic vasomotor pathways following SCI. Clinically, this method has been used to show that individuals with high SCI have reduced skin vasoconstriction in response to cutaneous cold compared to controls, and reduced vasodilation in response to local heating below the injury.^{385,386} This method is also used in experimental animal research, where ultrasound flow probes are surgically implanted around the artery, tissue or organ of interest, and flow is recorded using a flowmeter.^{39,382} Hong and colleagues have recently looked at organ system microcirculation, including the skin in the forepaw and hindpaw, after acute SCI at high and low thoracic levels.³⁹ In the acute period, there was a significant decrease in forepaw blood flow, which may be related to changes in regional peripheral vascular resistance.³⁹

Advantages/disadvantages. Flowmetry can be minimally invasive (when recording from the skin surface), but requires a significant investment in equipment. Although the equipment is quite sensitive to movements, caused by breathing for example, there are ways to reduce these artifacts.³⁹

Microneurography

In vivo microneurography allows for the direct intraneural recording of sympathetic neuronal activity.³⁸⁷ Changes in sudomotor and vasoconstrictor activity can be recorded in response to thermoregulatory stimuli, or nerve activity can be evoked to investigate the function of the peripheral effectors. Microneurography has been recently used to assess whether therapeutic electrical stimulation can facilitate skin sympathetic nerve activity following SCI in rats.³⁷⁶

Advantages/disadvantages. Microneurography is both invasive and technically challenging. However, it provides a sensitive and direct measurement of sympathetic nerve activity.

Sympathetic skin response

Clinical sudomotor tests have proven successful in the identification and localization of autonomic nervous system lesions.³⁸⁸ The sympathetic skin response (SSR) is a widely used clinical technique that assesses the integrity of sympathetic cholinergic pathways. In humans, it is typically recorded from the palmar and plantar surfaces, areas rich in eccrine glands, and measures changes in skin conductance in response to electrical nerve stimulation.³⁸⁹ SSR has been suggested as one way to assess autonomic completeness of

SCI clinically, as it requires supraspinal input.³⁹⁰ However, no animal studies of SCI have included assessment of sudomotor function as an outcome measure of autonomic nervous system function.

Advantages/disadvantages. SSR is a noninvasive technique, but it has not yet been developed for use in animals.

Visualization of sudomotor function

The visualization of sudomotor function is used in both clinical and in experimental research. Thermoregulatory sweat testing with alizarin red has been used to define the extent of peripheral neuropathies.^{391,392} Preliminary results show that this technique can also be used as a tool to map the preserved function of cholinergic sympathetic pathways in individuals with SCI.³⁹³ The starch iodine technique is an analogous assessment that is used in rats to visualize sweat gland function.^{394,395}

Advantages/disadvantages. This technique is minimally invasive and the results could be easily translated between the clinic and the experimental laboratory. However, in rodents, it can only inform about the presence or absence of sympathetic innervation of the palmar and plantar surfaces. Furthermore, it does not reveal any information about the thermoregulatory response.

Conclusion

Historically, SCI was synonymous with paralysis, and the ultimate goal of therapy was recovery of movement. Today, SCI is more correctly recognized as a potential disruption of all nervous system functions, including motor, sensory and autonomic. Although motor impairments are the most obvious manifestation of disability, they may not be the most catastrophic: injury to the spinal autonomic pathways results in functional deficits of the major organ systems, manifesting as bladder, gastrointestinal and sexual dysfunctions, and disordered cardiovascular function, thermoregulation and respiration. These aspects of autonomic dysfunction are fast emerging as priorities in clinical management of SCI.^{5,323}

To address these priorities, discovery scientists must incorporate autonomic assessments as outcome measures in their experimental SCI research. Here we have reviewed a wide variety of techniques that are available to evaluate autonomic function in experimental animals, including some that have not yet been used in experimental SCI, but appear to be feasible. For some laboratories, incorporating autonomic assessment may involve adding new techniques to their existing battery of outcome measures. For others, collaboration may be required to attain the necessary expertise. In some cases, it might be necessary to add additional groups of animals to maintain scientific rigor.

Whatever the practical considerations of incorporating autonomic assessment, the gain—an improved understanding of autonomic dysfunction after SCI—is indisputably worth the effort. There is an urgent need to include

Table 9 Overview of studies testing protective or regeneration/plasticity promoting therapy for SCI

	Technique used	Species	Injury model	Time range post-injury	Treatment tested	References
Bladder	Cystometry	Rat	T8/9 compression	2 weeks; 1 month	Neural stem cell transpl. (9 days after SCI)	284
			T8–T11 contusion	8 weeks	Neural precursor transpl. (9 days after SCI)	285
					Modified fibroblast transpl. (9 days after SCI)	286
				2 months	Schwann cell transpl. (1 h after SCI)	396
	Bladder volume		T8/9 compression	2 weeks; 1 month	Neural stem cell transpl. (9 days after SCI)	284
			T8–T12 contusion	0–24 h; 1–8 weeks	Neural precursor transpl. (9 d after SCI)	285
				8 weeks	Modified fibroblast transpl. (9 d after SCI)	286
				0–100 days	Intrathecal chondroitinase ABC (for 2 weeks from the time of SCI)	289
Cardiovascular	Autonomic dysreflexia (colorectal distension)	Rat	T4 clip compression	2, 6 weeks	Anti-CD11d antibody and methylprednisolone (singly and in combination; i.v.; 2, 24 and 48 h post-injury)	397
				2, 6 weeks	Anti-CD11d antibody (i.v.; 2, 24 and 48 h post-injury)	398
				5 weeks	Anti-CD11d antibody (i.v.; 6 h post-injury)	399
				6 weeks	Polyethylene glycol and magnesium sulfate (i.v.; 15 min and 6 h post-injury)	400
Respiratory	Phrenic nerve conduction	Rat	C2 hemisection	3–6 months	OEC transpl. (at injury site)	141
			High C hemisection	2 months		140
	Diaphragm EMG		C2 hemisection	3–6 months		141
	Plethysmography		T8 contusion	24 h, 7, 28, 35 days	FGF infusion (at injury site)	401

Abbreviations: EMG, electromyography; FGF, fibroblast growth factor; i.v., intravascular; OEC, olfactory ensheathing cells; transpl., transplantation; Tx, transection.

Studies have been classified by the autonomic system that they assess, as well as by the technique used to assess that system. Studies using similar experimental animals, injury models and time range of study post-injury are grouped together.

autonomic evaluations in experiments testing therapeutic (that is, regenerative or protective) agents after SCI, as there are very few experiments currently doing so (see Table 9).

At present, treatments are moving to clinical trial without any animal data that might predict their effects on autonomic function.⁴⁰² If we continue to neglect autonomic function after SCI in the laboratory, the consequences for people with SCI could be disastrous.

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