A Systematic Review of Exercise Training To Promote Locomotor Recovery in Animal Models of Spinal Cord Injury

Camila R. Battistuzzo,¹ Robert J. Callister,² Robin Callister,² and Mary P. Galea¹

Abstract

In the early 1980s experiments on spinalized cats showed that exercise training on the treadmill could enhance locomotor recovery after spinal cord injury (SCI). In this review, we summarize the evidence for the effectiveness of exercise training aimed at promoting locomotor recovery in animal models of SCI. We performed a systematic search of the literature using Medline, Web of Science, and Embase. Of the 362 studies screened, 41 were included. The adult female rat was the most widely used animal model. The majority of studies (73%) reported that exercise training had a positive effect on some aspect of locomotor recovery. Studies employing a complete SCI were less likely to have positive outcomes. For incomplete SCI models, contusion was the most frequently employed method of lesion induction, and the degree of recovery depended on injury severity. Positive outcomes were associated with training regimens that involved partial weight-bearing activity, commenced within a critical period of 1–2 weeks after SCI, and maintained training for at least 8 weeks. Considerable heterogeneity in training paradigms and methods used to assess or quantify recovery was observed. A 13-item checklist was developed and employed to assess the quality of reporting and study design; only 15% of the studies had high methodological quality. We recommend that future studies include control groups, randomize animals to groups, conduct blinded assessments, report the extent of the SCI lesion, and report sample size calculations. A small battery of objective assessment methods including assessment of over-ground stepping should also be developed and routinely employed. This would allow future meta-analyses of the effectiveness of exercise interventions on locomotor recovery.

Key words: exercise; locomotion; recovery; spinal cord

Introduction

SPINAL CORD INJURY (SCI) is a devastating and costly human condition with a worldwide incidence of 10–40 cases per million people (Norton, 2010). In the past four decades, significant progress in acute care has led to an increased life expectancy in spinal cord-injured patients (Strauss et al., 2006); however, recovery of function remains a major challenge for the clinical and research communities.

Following SCI some spontaneous recovery of motor function may occur in humans (Kirshblum et al., 2004; Waters et al., 1993), and in animal models (Forssberg et al., 1980). In animals, spontaneous recovery of motor function may be sufficient to enable the animal to step. In humans, the extent of spontaneous motor recovery varies, but is usually extremely modest and humans may remain substantially disabled, even

when there is evidence of some continuity across the lesion site (Bunge et al., 1993; Kakulas, 1999). A breakthrough set of experiments in spinalized cats some 30 years ago showed that exercise, in the form of treadmill training, resulted in marked restoration of stepping in the paralyzed hindlimbs compared to the disorganized movement present prior to training (Forssberg et al., 1980). This work provided the first behavioral evidence for a beneficial role of exercise in promoting motor recovery after SCI. Subsequently, exercise training strategies aimed at improving locomotor function have been investigated extensively in humans and in various animal models of SCI.

In this review we summarize the evidence of the effectiveness of exercise training in promoting locomotion recovery in animal models of complete and incomplete spinal cord injury.

¹Department of Physiotherapy, Melbourne School of Health Sciences, The University of Melbourne, Melbourne, Victoria, Australia.

²School of Biomedical Sciences and Pharmacy, Faculty of Health and Hunter Medical Research Institute, The University of Newcastle, Callaghan, N.S.W., Australia.

Methods

Literature search

A systematic literature search was conducted using three bibliographic databases (Medline, Embase, and Web of Science). The terms "locomotion," "exercise," and "spinal cord" were used as key terms or Medical Subject Headings where appropriate. Searches were limited to animal studies. Reference lists of included articles were also searched to identify additional studies.

Selection criteria

Primary research articles that compared animals given any type of exercise training intervention (to improve locomotion) to an untrained group of animals with thoracic spinal cord injury and with a locomotor outcome measure were included in this systematic review. Studies or study arms using genetically modified animals, *in vitro* spinal cord preparations, or electrical stimulation in addition to exercise training were excluded. All searches were limited to English-language journal articles published before March 2011.

Identification of articles

Two investigators (C.R.B. and M.P.G.) independently evaluated the titles and abstracts of the identified studies according to the selection criteria. Thereafter, the full text of all eligible studies was retrieved and assessed for inclusion or exclusion by the two investigators. In case of disagreement about a study's eligibility, decisions were made by consensus among investigators.

Data extraction

The following data were extracted from the included studies: name of first author, year of publication, animal strain, animal age (as reported in the study), gender, number of animals in each group, method used to induce spinal cord injury, level of spinal cord injury, type of exercise intervention, exercise duration, when exercise training was initiated, number of training sessions per day, and how motor recovery was measured. In studies with multiple intervention arms, only data from the untrained (control) and exercise-trained groups were considered in this analysis. The outcome of the treatment (positive or negative) was based on the study's conclusion.

Methodological quality assessment of studies

To our knowledge, there is no established valid and reliable tool to assess the methodological quality or bias of animal studies. Therefore we used the literature (Vesterinen et al., 2011) to develop a 13-item checklist to assess the quality of reporting and study design. For the purpose of this review, we deemed studies to be of higher quality if they reported at least randomization, blinded outcome assessments, and histological analysis of the extent of the SCI lesion.

Data analysis and synthesis

In order to facilitate data synthesis, studies were grouped according to type of spinal cord injury (complete or incomplete). Agreement between investigators on inclusion of

studies was assessed using the kappa statistic and percentage agreement (SPSS software version 17.0; SPSS Inc., Chicago, IL). Findings were summarized using descriptive statistics.

Results

Selection of studies

The process followed for article selection is shown in Figure 1. A total of 614 publications were identified by our search strategy. After full-text screening of 64 publications, 41 met the inclusion criteria. Of the 23 publications excluded at full-text level, 22 were excluded because they lacked an untrained comparison group. There was almost perfect agreement (Sim and Wright, 2005) between the two investigators who undertook article screening (% agreement = 0.98, kappa score = 0.92).

Characteristics of included studies

The characteristics of the studies assessing the effect of exercise training in animals with complete and incomplete spinal cord injury are shown in Tables 1 and 2, respectively.

Complete spinal cord injury

Seventeen studies were performed in completely transected spinal cord-injured animals. In all these studies a surgical incision was used to sever the spinal cord. The majority of these studies used rats (65%), and the remainder used cats (23%) or mice (12%). In 13 studies (76%) the animals were reported as adults, and in four studies (24%) neonates or young animals were used. Female animals were used in 13 studies (76%), whereas males were only used in one study (6%; Ung et al., 2010). In three studies (18%) the gender of the animals was not reported (Lovely et al., 1986; Smith et al., 1982; Timoszyk et al., 2005). The level of the spinal cord injury in completely transected animals ranged from T6–T13, with 47% between T9 and T11.

Exercise training was mostly delivered with some type of assistance, using either body weight-supported treadmill training (41%), or robotic devices to assist stepping (35%). There was a wide range in the time between surgery and initiation of training (1–64 days). In 53% of the studies exercise training commenced within 1–7 days, and all but one of the remaining studies commenced training 16–30 days after injury. Training regimens most often consisted of one training session per day (82%); only three studies (18%) used two or three sessions per day (Foret et al., 2010; Moshonkina et al., 2002; Nothias et al., 2005). The duration of each session was 5–15 min (53%), or 20–30 min (47%). Training was provided 5 days per week in 15 studies; two of the remaining studies did not report this information (Fong et al., 2005; Foret et al., 2010). Training duration varied widely, from 1–36 weeks (12% < 4 weeks, 53% 4–9 weeks, and 35% > 10 weeks).

Incomplete spinal cord injury

A total of 24 studies used an incomplete spinal cord injury model to assess recovery of locomotion. The majority (83%) were carried out in rats, with mice used in three studies (Engesser-Cesar et al., 2005, 2007; Goldshmit et al., 2008), and cats in one (Barriere et al., 2008). For the rat studies, 65% of the

FIG. 1. Flow chart of the procedure used for study selection.

animals were described as adults, but in three the age of the animals was not reported. The animals used in the incomplete SCI studies were predominantly female (67%). Males were used in five studies (21%; Carvalho et al., 2008; Goldshmit et al., 2008; Oh et al., 2009; Park et al., 2010; Siegenthaler et al., 2008), and three studies (12%) used both female and male animals in their experiments (Barriere et al., 2008; Fouad et al., 2000; Singh et al., 2011).

The method applied to induce an incomplete spinal cord lesion varied widely. Injuries were generated by either crushing to induce a contusion, compressing the cord, or by a defined surgical incision (e.g., hemisection in the dorso-ventral plane). Crushing involved delivering a defined force onto the exposed spinal cord; the force delivered determined the severity of the spinal contusion (Rosenzweig and McDonald, 2004). Of the reviewed articles, 42% of the studies described the SCI as a

TABLE 1. CHARACTERISTICS OF STUDIES ON COMPLETELY TRANSECTED SPINAL CORD-INJURED ANIMALS

Study	Animal (Strain)	Number of animals (t, u)	Age at SCI	Training characteristics			Method of locomotor analysis	R	B	Result
				Type of exercise	Time to training after surgery (days)	Duration of training				
De Leon et al., 1998	Cat	(6,6)	Adult	BWST	7	12 weeks	Stepping ability, EMG, kinematics	N	N	Positive
Smith et al., 1982	Cat	(7,5/5,4)	2 or 12 weeks	BWST	21–27	16 weeks	EMG, kinematics, 9 point scale	Y	N	Positive
Lovely et al., 1986	Cat	(8,13)	Adult	BWST	30	16–36 weeks	Treadmill velocity, number of full-weight-bearing steps	Y	N	Positive
Boyce et al., 2007	Cat	(4,3)	Adult	Treadmill	7–14	12 weeks	Kinematic	N	N	Positive
Moshonkina et al., 2002	Rat (SD)	(5,5)	Adult	Treadmill	1	9 weeks	5 point scale	N	N	Positive
Moshonkina et al., 2004	Rat (SD)	(7,5)	Adult	Treadmill	1	9 weeks	BBB	N	N	Positive
Lee et al., 2010	Rat (SD)	(10,10)	Adult	Robotic assisted treadmill	21	24 days	Kinematic, EMG	Y	N	Negative
De Leon et al., 2006	Rat (SD)	(7,7)	Adult	Robotic assisted treadmill	21	16 weeks	Kinematic	N	N	Negative
Nothias et al., 2005	Rat (SD)	(10,6)	Adult	Cycling	5	1 week	BBB	Y	N	Negative
Kubasak et al., 2008	Rat (W)	(9,9)	Adult	BWST	30	20 weeks	Kinematic	N	Y	Negative
Foret et al., 2010	Rat (W)	(7,9)	Adult	BWST	1	4 weeks	BBB	N	Y	Positive
Zhang et al., 2007	Rat (W)	(10,10)	Adult	BWST	5	40 days	BBB, ACOS	N	Y	Positive
Petruska et al., 2007	Rat (SD)	Not reported	Neonate	Robotic assisted treadmill	16	6 weeks	Kinematics	N	N	Positive
Tillakaratne et al., 2010	Rat (SD)	(14,14)	Neonate	Robotic assisted treadmill	26	8 weeks	Maximum number of consecutive steps, kinematics	N	Y	Positive
Timoszyk et al., 2005	Rat (SD)	(9,6)	Neonate	Robotic assisted treadmill	64	40 days	Stepping ability, kinematics	Y	Y	Negative
Fong et al., 2005	Mouse (SW)	(6,6)	5 weeks	Robotic assisted treadmill	5	4 weeks	Kinematic	Y	N	Positive
Ung et al., 2010	Mouse (CD-1)	(10,12)	Not reported	BWST	3	5 weeks	AOB, ACOS	Y	N	Negative

R, reporting of randomization; B, reporting of blinding; SD, Sprague-Dawley; SW, Swiss-Webster; W, Wistar; t, u, number of animals in trained and untrained groups; BWST, body weight-supported treadmill; EMG, electromyography; BBB, Basso-Beattie-Bresnahan scale; ACOS, average combine score; AOB, Antri-Orsal-Barthe motor scale; SCI, spinal cord injury.

TABLE 2. CHARACTERISTICS OF STUDIES ON INCOMPLETE SPINAL CORD-INJURED ANIMALS

Study	Animal (strain)	Number of animals (t,u)	Age at SCI	Type of injury	Training characteristics			Method of locomotor analysis	R	B	Result
					Type of exercise	Time to training after surgery (days)	Duration of training				
Barreire et al., 2008	Cat	(3,2)	Adult	Lateral hemisection, transection	Treadmill	Not reported	3–4 weeks	Kinematics, EMG	N	N	Positive
Fouad et al., 2000	Rat (L)	(7,7)	Adult	Dorsal hemisection	Treadmill	3	5 weeks	BBB, grid walk test, narrow beam test, footprint, kinematics, exploratory activity	N	Y	Negative
Multon et al., 2003	Rat (SD)	(7,7)	Adult	Incomplete compression	BWST	2-4	12 weeks	BBB	N	Y	Positive
Robert et al., 2010	Rat (SD)	(8,8,8)	Adult	Incomplete compression	Treadmill, swimming	14	2 weeks	BBB, Tarlov scale	N	N	Positive
Maier et al., 2009	Rat (SD)	Not reported	Adult	T-shape transection	BWST	7	8 weeks	Kinematics, inclined climbing	Y	Y	Positive
Smith et al., 2009	Rat (SD)	(6,6)	Adult	Contusion	Swimming	3	6 weeks	BBB, kinematics	N	N	Negative
Stegenthaler et al., 2008	Rat (SD)	(12,6)	Young, Aged	Contusion	Voluntary wheel-running	14	40 weeks	BBB	N	N	Positive
Park et al., 2010	Rat (SD)	(6,9)	Adult	Contusion	Treadmill	3	4 weeks	BBB	Y	Y	Positive
Oh et al., 2009	Rat (SD)	(49,22)	Not reported	Contusion	Treadmill	7	1–4 weeks	BBB, MFS, grid walk test	N	Y	Positive
Van Meeteren et al., 2003	Rat (W)	(9,8,10)	11 weeks	Contusion	EE, Voluntary wheel running	Not reported	8 weeks	Grid walk test, catwalk	Y	N	Positive
Lankhorst et al., 2001	Rat (W)	(15,15)	Adult	Moderate contusion	EE	1	8 weeks	BBB, BBB subscale, grid walk test, TLH, catwalk	Y	Y	Positive
Kuerzi et al., 2010	Rat (SD)	(6,4)	Adult	Moderate contusion	Shallow water	10	3–9 weeks	BBB, kinematics	Y	Y	Positive
Fischer and Peduzzi, 2007	Rat (SD)	(16,16)	Adult	Moderate contusion	EE	90	4 weeks	BBB, CBS	Y	N	Positive
Liu et al., 2008	Rat (SD)	Not reported	Not reported	Moderate contusion	Treadmill, cycle	8	12 weeks	BBB	Y	N	Positive
Smith et al., 2006	Rat (SD)	(7,8,7)	Adult	Moderate contusion	Swimming, with cutaneous feedback	14	4–9 weeks	BBB, LSS	Y	Y	Positive (cutaneous feedback only)
Singh et al., 2009	Rat (SD)	(11,11)	Not reported	Moderate contusion	Staircase climbing	7	8 weeks	BBB, catwalk, grid walk test	Y	Y	Positive

(continued)

TABLE 2. (CONTINUED)

Study	Animal (strain)	Number of animals (t,u)	Age at SCI	Type of injury	Training characteristics			Method of locomotor analysis	R	B	Result
					Type of exercise	Time to training after surgery (days)	Duration of training				
Stevens et al., 2006 Erschbamer et al., 2006	Rat (SD) Rat (SD)	(8,8) (12,8,12)	Adult Neonate	Moderate contusion Moderate contusion	Treadmill EE, voluntary wheel running	7 7	5 days 12 weeks	BBB BBB, grid walk test, exploratory activity	N Y	N Y	Positive Negative
Ichiyama et al., 2009 Heng and de Leon, 2009	Rats (SD) Rat (SD)	(14,14) (13,13)	Adult 8 weeks	Severe contusion Severe contusion	BWST BWST	30 42	8 weeks 8 weeks	Kinematic Stepping ability	Y N	Y N	Negative Positive
	Rat (SD)	(9,9)	Adult	Severe contusion	Swimming	Not reported	6 weeks	BBB	Y	Y	Negative
Carvalho et al., 2008 Engesser-Cesar et al., 2005	Mouse (C10)	(6,7,9)	Not reported	Moderate contusion	Voluntary wheel-running, flat surface	7	8-18 weeks	BBB, BMS	Y	N	Positive (flat surface only)
Engesser-Cesar et al., 2007	Mouse (C10)	(10,10)	Not reported	Moderate contusion	Flat-surface wheel-running	30	15 weeks	BBB, BMS, kinematics of treadmill walking	Y	Y	Positive
Goldshmit et al., 2008	Mouse (C6)	(11,12)	Adult	Lateral hemisection	Treadmill	7	5 weeks	BBB, horizontal grid test, kinematics, angled grid climbing, footprint	Y	N	Positive

R, reporting of randomization; B, reporting of blinding; L, Lewis; SD, Sprague-Dawley; W, Wistar; C10, C57BL/10; C6, C57BL/6; t, u, number of animals in trained and untrained groups; BWST, body weight-supported treadmill; EE, environmental enrichment; EMG, electromyography; BBB, Basso-Beattie-Bresnahan scale; MFS, motor function score; TLH, thoracolumbar height test; CBS, combined behavior score; LSS, Louisville swim scale; BMS, Basso mouse scale.

TABLE 3. METHODS USED FOR LOCOMOTOR ANALYSIS IN THE INCLUDED STUDIES

<i>Method for locomotor analysis</i>	<i>Description</i>
Stepping ability	Maximum speed at which full weight-bearing steps are achieved on the plantar surface of one hindlimb, and the number of full weight-bearing plantar surface steps performed by two hindlimbs (De Leon et al., 1998)
Basso-Beattie-Bresnahan scale (BBB)	Observational open field test based on grading hindlimb locomotion of rats from 0 (no spontaneous activity) to 21 (normal movement) (Basso et al., 1995)
Basso Mouse Scale (BMS)	Observational 9-point open field test adapted from the BBB to assess recovery of hindlimb locomotion in mice (Sedy et al., 2008)
Antri-Orsal-Barthe motor scale (AOB)	Observational 22-point scale designed to assess hindlimb movements in mice (Sedy et al., 2008)
Average Combined Score (ACOS)	Semi-quantitative method to assess hindlimb movement in mice; combines non-locomotor movement, locomotor-like movement, and movement amplitude, to quantify and score hindlimb movement (Sedy et al., 2008)
Motor Function Score (MFS)	Observational 10-point scale to assess hindlimb movement in mice (Oh et al., 2009)
Tarlov scale	Observational 4-point scale designed to assess hindlimb movement and weight support (Sedy et al., 2008)
Exploratory activity	Open field test using 9 fields, in which the number of fields crossed in 5 min is counted and compared to baseline data (Erschbamer et al., 2006)
Combined Behavior Score (CBS)	Scoring method consisting of 8 tests that measure hindlimb deficits; the score ranges from 0 (normal) to 100 (complete paralysis) (Fischer and Peduzzi, 2007)
Louisville Swim Scale (LSS)	Eighteen-point scale that assesses swimming performance (Smith et al., 2006)
Footprint analysis	Method used to assess hindlimb rotation and/or coordination; the animal's hind paws are inked and footprints are recorded on runway paper (Goldshmit et al., 2008)
Thoracolumbar height test (TLH)	Test that differentiates between full and partial weight bearing; uses thoracolumbar kyphosis height to define the rat's ability to support weight (Lankhorst et al., 2001)
Grid walk test	Test to evaluate limb placement and precise motor control; animals are allowed to walk in a long grid and the number of limb displacements is counted (Lankhorst et al., 2001)
Angled grid test	Test with the same purpose as the grid walk test, but uses an angled grid to provide a greater challenge to the animal (Sedy et al., 2008)
Narrow beam test	Method used to assess the ability of rats to balance on 1-m-long elevated beams with different cross-sectional profiles (Sedy et al., 2008)
Electromyography (EMG)	Method to evaluate and record electrical activity in muscles; can be used to compare changes in activation within selected muscles (De Leon et al., 1998)
Kinematic analysis	Quantitative description of limb movement without considering the forces involved; provides spatiotemporal characteristics of gait and measurements of joint angles (Field-Fote, 2003)
Catwalk	Gait analysis system for assessing subtle differences in gait; animals are filmed from below while crossing a glass walkway, and paw print locations are recorded and subsequently analyzed with the Catwalk software (Sedy et al., 2008)

moderate contusion, 12% as a severe contusion, and 21% did not describe contusion severity. A compression injury induced by inflation of a subdural balloon was used in 8% of the studies, and a surgical incision such as a hemisection was used in 17% of the studies. The segmental-level of injury varied between T7 and T13 in these studies. In 35% and 25% of the rat studies, the level of injury was T8 or T9, respectively. The two studies (67%) performed in mice had SCI at the level of T9 or T12.

A wide range of exercise training techniques was used in the incomplete SCI studies. The most frequent types of intervention applied were treadmill training (33%), followed by

body weight-supported treadmill training (BWSTT; 17%), voluntary wheel running (17%), and swimming (17%). Four studies (17%) employed environmental enrichment (EE) to enhance locomotion; at the very least a running wheel was provided in addition to tunnels and climbing frames. In the rat studies, exercise generally (63%) commenced within 14 days after injury. In 42% of the studies training commenced within a week, whereas in three studies (13%) they waited 30 days or more before commencing training.

Exercise training regimens in the animals with incomplete injuries consisted mostly of two or more sessions per day

FIG. 2. Methodological quality of the included publications.

(38%; Fouad et al., 2000; Goldshmit et al., 2008; Kuerzi et al., 2010; Liu et al., 2008; Maier et al., 2009; Multon et al., 2003; Park et al., 2010; Smith et al., 2006,2009), and seven studies (29%) reported one session per day (Carvalho et al., 2009; Heng and de Leon, 2009; Ichiyama et al., 2009; Oh et al., 2009; Robert et al., 2010; Singh et al., 2011; Stevens et al., 2006). In 30% of the studies, the number of sessions per day did not apply due to the type of intervention used (i.e., environmental enrichment or voluntary wheel running). Studies providing more than one training session per day had shorter sessions (3–10 min) than those with a single session per day (30–60 min). Like the complete SCI experiments, many (46%) of the studies employing incomplete SCI had exercise training 5 days per week, but three studies (Carvalho et al., 2008; Park et al., 2010; Smith et al., 2009) provided training on 6 days per week. The duration of the training program varied widely (1–40 weeks), although in most (80%) of the rat studies the animals were trained for 4–12 weeks.

Assessment of locomotor recovery

Overall, 20 methods of locomotor assessment were identified among the 41 included studies. A brief description of some of these methods is shown in Table 3. Twenty-four studies (59%) used more than one method to assess locomotor recovery. The most commonly used methods were: the BBB scale (56%), kinematic analysis (44%), and the grid walk test (17%). Sixteen studies (39%) only used functional scales (Carvalho et al., 2008; Engesser-Cesar et al., 2005; Fischer and Peduzzi, 2007; Foret et al., 2010; Liu et al., 2008; Moshonkina et al., 2002,2004; Multon et al., 2003; Nothias et al., 2005; Park et al., 2010; Robert et al., 2010; Siegenthaler et al., 2008; Smith et al., 2006; Stevens et al., 2006; Ung et al., 2010; Zhang et al., 2007); importantly, these scales assess a specific aspect of locomotion or hindlimb movement, and depend on observation and interpretation. In the complete SCI studies, kinematic analysis was used in 55% of rat, 75% of cat, and 50% of mouse

studies; the BBB scale was used in 36% of the rat studies. In the incomplete rat studies, the BBB was used in 80% of the studies, with 30% using the BBB as the only measure; 25% used a kinematic analysis, and 30% used a grid walk test.

Twenty-one studies (51%) used objective methods to assess recovery of locomotion. Most of these studies (86%) evaluated the animals' performance in the trained task (e.g., treadmill stepping or weight-supported stepping). Only three studies (14%) evaluated whether the effect of training can induce improvement in over-ground stepping using the Catwalk system (Lankhorst et al., 2001; Singh et al., 2011; Van Meeteren et al., 2003).

Assessment of methodological quality

The results of the assessment of the methodological quality of the included studies are shown in Figure 2. Only 41% of complete and 63% of incomplete SCI studies reported randomization of animals into groups (Carvalho et al., 2008; Engesser-Cesar et al., 2005,2007; Erschbamer et al., 2006; Fischer and Peduzzi, 2007; Fong et al., 2005; Goldshmit et al., 2008; Ichiyama et al., 2009; Kuerzi et al., 2010; Lankhorst et al., 2001; Lee et al., 2010; Liu et al., 2008; Lovely et al., 1986; Maier et al., 2009; Nothias et al., 2005; Park et al., 2010; Singh et al., 2011; Smith et al., 1982,2006; Tillakaratne et al., 2010; Ung et al., 2010; Van Meeteren et al., 2003). Blinded outcome measures were undertaken in 29% of complete and 54% of incomplete SCI studies (Carvalho et al., 2008; Engesser-Cesar et al., 2007; Erschbamer et al., 2006; Foret et al., 2010; Fouad et al., 2000; Ichiyama et al., 2009; Kubasak et al., 2008; Kuerzi et al., 2010; Lankhorst et al., 2001; Maier et al., 2009; Multon et al., 2003; Oh et al., 2009; Park et al., 2010; Singh et al., 2011; Smith et al., 2006; Tillakaratne et al., 2010; Timoszyk et al., 2005; Zhang et al., 2007). Histological analysis to confirm the extent of the lesion were performed in 76% of complete, but only 46% of incomplete SCI studies (Barriere et al., 2008; Boyce et al., 2007; De Leon and Acosta, 2006; Fong et al., 2005; Fouad et al., 2000;

TABLE 4. RESULTS OF THE STUDIES BY MEASURED VARIABLES

		Complete spinal cord injury		Incomplete spinal cord injury	
		Studies reporting positive effect (%)	Studies reporting negative effect (%)	Studies reporting positive effect (%)	Studies reporting negative effect (%)
Number of studies		11	6	19	5
Animal	Rat	6 (55)	5 (83)	15 (79)	5 (100)
	Mouse	1 (9)	1 (17)	3 (16)	0 (0)
	Cat	4 (36)	0 (0)	1 (5)	0 (0)
Age	Adult	9 (82)	4 (67)	11 (58)	4 (80)
	Neonate	2 (19)	1 (17)	0 (0)	1 (20)
	Young	1 (9)	0 (0)	1 (5)	0 (0)
	Aged	0 (0)	0 (0)	1 (5)	0 (0)
	Not reported	0 (0)	1 (17)	5 (26)	0 (0)
Methodological quality assessment	Randomization	3 (27)	4 (67)	12 (63)	3 (60)
	Blinded assessor	3 (27)	2 (33)	9 (47)	4 (80)
	Analysis of lesion extension	9 (82)	4 (67)	9 (47)	2 (40)
Type of exercise	Treadmill	3 (27)	0 (0)	7 (37)	1 (20)
	Body weight-supported treadmill	5 (45)	2 (33)	3 (16)	1 (20)
	Robotic assisted treadmill	3 (27)	3 (50)	0 (0)	0 (0)
	Voluntary wheel running	0 (0)	0 (0)	3 (16)	1 (20)
	Environmental enrichment	0 (0)	0 (0)	3 (16)	1 (20)
	Swimming	0 (0)	0 (0)	3 (16)	1 (20)
	Cycling	0 (0)	1 (17)	1 (5)	0 (0)
Initiation of training after surgery	1–5 days	5 (45)	2 (33)	3 (16)	2 (40)
	7 days	2 (19)	0 (0)	6 (31)	1 (20)
	8–15 days	1 (9)	0 (0)	5 (26)	0 (0)
	16–30 days	4 (36)	3 (50)	1 (5)	1 (20)
	< 30 days	0 (0)	1 (17)	2 (10)	0 (0)
Duration of training regimen	Not reported	0 (0)	0 (0)	2 (10)	1 (20)
	1–7 weeks	5 (45)	4 (67)	9 (47)	3 (60)
	8–12 weeks	5 (45)	0 (0)	10 (53)	2 (40)
	< 12 weeks	2 (19)	2 (33)	3 (16)	0 (0)

Kuerzi et al., 2010; Lankhorst et al., 2001; Lee et al., 2010; Liu et al., 2008; Lovely et al., 1986; Maier et al., 2009; Moshonkina et al., 2002, 2004; Multon et al., 2003; Nothias et al., 2005; Park et al., 2010; Petruska et al., 2007; Singh et al., 2011; Smith et al., 1982, 2009, 2006; Tillakaratne et al., 2010; Ung et al., 2010; Zhang et al., 2007). The three studies (7%; Barriere et al., 2008; Carvalho et al., 2008; Van Meeteren et al., 2003) that did not report the time point when the exercise regimen was initiated were all conducted in animals with incomplete SCI. Sample size calculations were not reported in any study.

Only 6 studies (15%) were considered to be of higher methodological quality according to our criteria (Kuerzi et al., 2010; Lankhorst et al., 2001; Maier et al., 2009; Park et al., 2010; Singh et al., 2011; Smith et al., 2006), and all used an incomplete SCI model. Training paradigms varied substantially, from shallow water swimming, EE, and staircase climbing, to BWSTT and treadmill training. All these studies reported at least one positive locomotor outcome. Locomotor recovery was assessed using at least one objective tool in four of these studies (Kuerzi et al., 2010; Lankhorst et al., 2001; Maier et al., 2009; Singh et al., 2011). The remaining two studies (Park et al., 2010; Smith et al., 2006) used only subjective methods (functional scales), but the assessors were blinded. Two studies with higher methodological quality used objective tools to assess the effect of training on free over-ground stepping performance (Lankhorst et al., 2001; Singh et al., 2011).

Of the 17 studies that were not considered higher quality but used objective assessment tools, 8 (47%) reported the use of at least two quality criteria. Six of these studies were randomized, and five used blinded assessments. Only three used both blinding and randomization (Timoszyk et al., 2005; Ichiyama et al., 2009; Engesser-Cesar et al., 2007). The remaining studies used randomization or blinded assessments in addition to histological analysis of the extent of the SCI lesion. Four of these studies reported a positive locomotor outcome.

Locomotor outcome

Exercise training was reported to improve some aspect of locomotor recovery in 30 of the 41 studies, and positive outcomes were more likely in studies employing incomplete injury models (Table 4).

For complete SCI studies, positive outcomes were observed in 55% of rat, 50% of mouse, and 100% of the cat studies. In rats, 66% of studies in younger animals had positive outcomes compared to 50% in older animals. Initiating exercise less than 16 days after injury had positive outcomes in 83% of studies, compared to 20% when exercise was delayed by more than 20 days. Only 40% of the studies were considered successful if training lasted less than 6 weeks, whereas 100% were successful if they used 6–9 weeks of training. Studies longer than 9 weeks were not successful, but training was initiated

late in these studies. Notably the cat studies, which were all successful, used long-duration (12–36 weeks) training programs. Regarding the type of training, treadmill and body weight-supported methods produced positive outcomes in both rats and cats (100% in rats and cats for treadmill training, and 75% for BWSTT in rats and 100% in cats). In contrast, robotic-assisted training was only successful in 25% of studies in rats, and cycling was not effective in rats.

For incomplete SCI studies, positive outcomes were observed in 100% of the cat and mouse studies, and in 75% of the rat studies. In rats, 80% of the studies in adults were positive, but only 50% of the studies of young animals had positive outcomes. Initiating exercise less than 5 days after injury had positive outcomes in 60% of studies, and success increased with longer delays (86% for 7–10 days; 100% for 14 days). The duration of the training program had no clear influence on the outcome; however, the majority of studies trained animals for more than 4 weeks. Most forms of training provided positive outcomes (treadmill 83%; BWSTT 75%; voluntary wheel running 67%; EE 100%; stair-climbing 100%), with the exception of swimming (25%). In cases for which the injury type and severity were clearly documented, positive outcomes were more likely to be achieved in compression (100%) and moderate contusion (88%) models. Studies using transection (50%) and severe contusion (33%) models were less likely to report positive outcomes.

The three studies that assessed over-ground stepping using objective methods reported a positive outcome in only a few of the over-ground test parameters (Van Meeteren et al., 2003; Lankhorst et al., 2001; Singh et al., 2011). Importantly, two of these studies were of higher methodological quality (Lankhorst et al., 2001; Singh et al., 2011).

Influence of bias on outcomes

Complete injury models. In complete injury models randomization was used in three rat, two cat, and two mouse studies. None of the randomized studies in rats had positive outcomes, whereas 78% of the rat studies that did not use randomization reported positive outcomes. Blinding was only employed in five rat studies, and 60% of these had positive outcomes; interestingly, positive outcomes were reported in only 50% of the non-blinded studies. Only one of the complete SCI studies (in rats) used both randomization and blinded outcome assessment, and this study had a negative outcome.

Incomplete injury models. In incomplete injury models randomization was employed in 12 rat and 3 mouse studies, but not in the cat study. Positive outcomes were observed with equal frequency (75%) in both randomized and non-randomized rat studies. Blinding was used in one mouse and 12 rat studies, but was not used in the cat study. In rats, positive outcomes were higher in the non-blinded (80%) compared to the blinded (69%) studies. Nine of the rat studies and one mouse study used both randomization and blinding. For these studies, 70% reported positive outcomes. Studies that used swimming alone as the training intervention had negative outcomes.

Discussion

This is the first systematic review to summarize the pre-clinical evidence on the use of exercise training to improve locomotor recovery following SCI. Our review suggests that

exercise training has the potential to improve animal performance in the trained motor task; however, there is little evidence that this benefit will translate to over-ground stepping (i.e., on a non-uniform substrate or environment). Most studies used female animals and applied incomplete lesions to low thoracic spinal cord segments. Regardless of species, positive locomotor outcomes were associated with commencement of training within a critical period of 1–2 weeks after SCI. The studies displayed considerable heterogeneity in training paradigms and the methods used to assess or quantify recovery. Methodological quality was considered high in only 15% of the surveyed studies; almost half of the studies did not use randomization, blinding, or histological characterization of the SCI lesion. There was a universal failure to justify group sizes using statistical power analysis. Below we briefly discuss the limitations of our review, our key findings in studies with positive outcomes, comment on their relevance for human SCI, and provide recommendations for improving the methodological quality of future animal studies that employ exercise as an intervention for SCI.

As with all systematic reviews, it is possible that some relevant studies were not identified. Our search strategy, which was limited to electronic databases and reference lists, does not account for unpublished studies. Because of the heterogeneity of the included studies, meta-analysis could not be performed. Importantly, it is notoriously difficult to publish negative results in animal research; therefore our findings may be subject to publication bias (Sena et al., 2007). For example, none of the published cat studies had negative outcomes. As only publications in the English language were included, this review may be subject to language bias.

The first experiments assessing the effect of exercise after SCI were undertaken over 30 years ago in cats. More recent work has used adult rats and only a few studies have used mice. Interestingly, females were used in the majority of the studies, possibly because of the increased risk of neurogenic pulmonary edema in male animals following SCI (Sedy et al., 2007). The overwhelming use of female animals for examining exercise-induced recovery after SCI contrasts with the dominant use of males in other preclinical work that examines recovery mechanisms associated with damage to the nervous system (e.g., pain and stroke; Mogil and Chanda, 2005). Given the well-known neuroprotective role of estrogen, it will be important for future SCI studies to test exercise interventions in male animals and examine gender effects (Hurn and Macrae, 2000).

Timing and length of training are features that appear to distinguish studies with positive or negative outcomes. Studies were more likely to report a positive outcome when exercise training was initiated within 16 days of injury (complete SCI), or between 7–10 days (incomplete SCI) after injury. Conversely, 72% of studies with negative results initiated training very early (within 5 days) after injury, or had a delay of more than 16 days. The outcome of studies using longer training periods, 8–12 weeks, also tended to be positive. Although more research is needed to understand the influence of these factors on recovery of function, our results suggest that exercise training initiated during the subacute phase of SCI (between 7 and 16 days), and with an extended training period (8–12 weeks), may be more effective in enhancing locomotor function. These are important considerations for future studies that seek to determine the cellular mechanisms underlying recovery from SCI.

Different exercise training strategies have been applied to improve locomotion following SCI. These strategies vary from forced exercise, such as treadmill training with or without partial body-weight support or robotic or manual assistance, and swimming or bicycling, to voluntary exercise via wheel running and exercise opportunities provided by EE. By necessity some form of assisted treadmill training was used in all the studies of complete SCI. For the studies using incomplete SCI lesions, most used forced training (e.g., treadmill and swimming) rather than voluntary activity. Regardless of lesion type, treadmill training was the most effective. From a mechanistic viewpoint the majority of exercise paradigms identified in this systematic review rely on proprioceptive and cutaneous inputs provided by contracting muscles to activate spinal circuitry and induce plasticity in spinal cord neurons (Goldshmit et al., 2008). This sensory feedback is thought to be important for weight support during training (Edgerton et al., 2004). Indeed, in this review swimming training, which lacks the limb-loading phase, was beneficial only when at least cutaneous feedback was provided (i.e., by inverted centrifuge tubes; Kuerzi et al., 2010; Smith et al., 2009, 2006).

We found that functional scales (e.g., the BBB scale) were the most common method used to assess recovery of locomotion. Although some of these scales are validated and reliable, they rely on observation and interpretation and are thus susceptible to bias. Therefore these scales should never be used unless those undertaking the observations are blinded to treatment group. Moreover, these scales may not be appropriate for all models of SCI. In this review for example, 10% of included studies used the BBB scale in non-contusion injury, a model for which this scale has not been validated. Of 28 studies using observational scales, only 13 (46%) used blinded observers. Of the 16 studies that used observational scales alone, only 3 showed a negative result (Erschbamer et al., 2006; Ung et al., 2010), which raises concerns regarding the validity of a number of studies with positive results. Less reliance on scales vulnerable to bias is desirable, and should be a focus of future studies. Also, assessment of a range of locomotor characteristics, including those obtained from kinematic assessments and an evaluation of over-ground stepping, would provide a much more comprehensive analysis of the type and quality of the locomotor improvement.

A wide variety of methods were used to induce SCI and this lack of consistency makes it difficult to directly compare results across studies. The contusion model is considered the closest to human injury (Metz et al., 2000), and animals with a severe contusion SCI were less likely to have a positive outcome. With this model, however, it is difficult to control the exact location and extent of the lesion (Rosenzweig and McDonald, 2004). Better analysis and reporting of lesion extent or size would overcome this problem, as well as ensure that any improvement in locomotor function resulted from the intervention rather than an undersized lesion. Surprisingly, only 46% of the incomplete SCI studies analyzed lesion extent, whereas the majority (76%) of studies using complete SCI performed histological analysis of the lesion. This was unexpected, as the evaluation of the size and extent of the lesion might be considered more variable and therefore more important to report in incomplete spinal cord injury than in the complete model.

All studies used a low thoracic lesion in their model of SCI. In fact, lesion location was one of the few consistencies across

the included studies. From a translational point of view it must be noted that a thoracic lesion is chosen for experimental convenience over clinical relevance of the injury model. The thoracic cord can be easily accessed for surgery and the injured animals may still retain bladder and bowel control. Most SCIs in humans, however, occur in the cervical region, and few preclinical studies have examined recovery of function in cervical SCI models (Anderson et al., 2005; Dai et al., 2011; Girgis et al., 2007; Norton, 2010; Sandrow-Feinberg et al., 2009).

The methodological quality of the included studies was often poor. Failure to randomize or conduct blinded outcome assessments was a major limitation. In rat studies that used complete transections, the majority of the non-randomized studies had positive results, while results from randomized studies were all negative. Thus allocation bias in these studies is a major confounder. In incomplete injury studies, a positive outcome was more likely in those studies that did not employ blinding. These findings corroborate previous work showing that failure to use randomization and blinded assessment increases the likelihood of finding positive results (Bebarta et al., 2003), and raises concerns about their validity (Kilkenny et al., 2009). Only six studies in this review were considered of higher quality. Four of these used an objective method to assess locomotion; however, only two evaluated the effect of training on free over-ground stepping. Importantly, the effect of training on this outcome was modest.

Many of the studies analyzed in the review (including some with negative outcomes) used small numbers of animals and sample size calculation was not reported. This suggests that many studies may have lacked statistical power. These design features, which are normally required in clinical trials, are not commonly employed in animal experiments (Bebarta et al., 2003), and may contribute to discrepancies between the effects of interventions in animals versus humans (Macleod et al., 2009). Therefore, the use of appropriate controls, randomization, blinding of assessments, use of objective assessment tools, and sample size calculations in future studies would ensure that decisions regarding possible translation are based on unbiased and reliable data (Sena et al., 2007).

Animal studies are often the necessary first step towards developing and evaluating an intervention for trials in humans, and systematic reviews have been shown to be important for translating the findings from animal studies to human trials (Horn et al., 2001; Macleod et al., 2005; van der Worp et al., 2007). The majority of studies examined in this review, including those of higher methodological quality, showed that exercise training is beneficial for improving some aspects of locomotion after SCI, but the impact on the key functional outcome of over-ground locomotion/stepping remains to be thoroughly investigated. The observation that there appears to be a critical period of 1–2 weeks after injury for initiating exercise training suggests that identifying a similar optimal time for initiating human exercise programs needs to be explored. In animals, this optimal time is clearly sooner, rather than later, after SCI. Also, studies employing a longer duration of training appear to be more effective. Thus longer-duration rehabilitation programs might be expected to result in better outcomes in humans. Together, these findings are in accord with a recent systematic review of human trials, showing that commencing gait training (over-ground or with body-weight support) in the subacute phase of SCI and employing intensive

training were more likely to improve walking velocity and walking independence (Wessels et al., 2010).

For humans one of the major outcomes required of an exercise program following SCI is an improvement in over-ground stepping. Randomized clinical trials comparing treadmill training with conventional over-ground training have shown only small or no differences between these two approaches in outcomes related to walking (Dietz et al., 1995, Dobkin et al., 2006, 2007; Field-Fote and Roach, 2011). It can be argued that over-ground training offers an environment more similar to functional walking than the treadmill. As all forms of motor learning are known to be task-specific, exercise may only improve performance in the trained activity, and may even reduce the capacity to perform other motor tasks (De Leon et al., 1998; Magnuson et al., 2009). In this review, we identified that over-ground stepping has rarely been assessed. Importantly, in the early studies in spinalized cats, on which subsequent clinical decisions were based, the role of the task-specific component of exercise training was not known. Although the best way to assess over-ground locomotion in animals is not yet clear, kinematic analysis or the Catwalk method would provide data on the dynamics of locomotion (e.g., inter- and intra-limb coordination) that is necessary for normal gait in animals. It must also be remembered that the bipedal gait of humans is markedly different from the quadrupedal gait of the species used in studies so far.

In summary, the results of this systematic review suggest that exercise training following SCI in animals improves performance in the trained motor task, but there is little evidence to indicate that it improves functional stepping/walking. Exercise interventions that include initiation of training 1–2 weeks after SCI, and that last at least 8 weeks, are more likely to have positive outcomes. Training requiring at least partial weight-bearing activity appears essential. In studies of incomplete SCI, contusion is the most frequently employed method of lesion induction, and capacity to recover appears to be dependent on injury severity. Inclusion of control groups, randomization of animals to group, blinding of assessments, assessment of lesion extent, and reporting of sample size calculations are necessary to minimize bias in future studies. The age and gender of the animals used should also be reported. A small and accepted battery of assessment methods, which are objective and can be routinely employed across laboratories, are needed to allow future meta-analyses of the effectiveness of exercise interventions on locomotor recovery. Finally, investigators should be encouraged to publish studies with negative outcomes.

Acknowledgments

This study was supported by National Health and Medical Research Council Project grant no. 628765.

Author Disclosure Statement

No competing financial interests exist.

References

Anderson, K.D., Gunawan, A., and Steward, O. (2005). Quantitative assessment of forelimb motor function after cervical spinal cord injury in rats: relationship to the corticospinal tract. *Exp. Neurol.* 194, 161–174.

- Barriere, G., Leblond, H., Provencher, J., and Rossignol, S. (2008). Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. *J. Neurosci.* 28, 3976–3987.
- Basso, D.M., Beattie, M.S., and Bresnahan, J.C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J. Neurotrauma* 12, 1–21.
- Bebarta, V., Luyten, D., and Heard, K. (2003). Emergency medicine animal research: does use of randomization and blinding affect the results? *Acad. Emerg. Med.* 10, 684–687.
- Boyce, V.S., Tumolo, M., Fischer, I., Murray, M., and Lemay, M.A. (2007). Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats. *J. Neurophysiol.* 98, 1988–1996.
- Bunge, R.P., Puckett, W.R., Becerra, J.I., Marcillo, A., and Quencer, R.M. (1993). Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic compression with extensive focal demyelination. *Adv. Neurol.* 59, 75–89.
- Carvalho, K.A.T., Cunha, R.C., Vialle, E.N., Osiecki, R., Moreira, G.H.G., Simeoni, R.B., Francisco, J.C., Guarita-Souza, L.C., Oliveira, L., Zocche, L., and Olandoski, M. (2008). Functional outcome of bone marrow stem cells (CD45(+)/CD34(-)) after cell therapy in acute spinal cord injury: in exercise training and in sedentary rats. *Transplant. Proc.* 40, 847–849.
- Dai, H., McAtee, M., Hockenbury, N., Das, P., and Bregman, B.S. (2011). Delayed rehabilitation with task-specific therapies improves forelimb function after a cervical spinal cord injury. *Restor. Neurol. Neurosci.* 29, 91–103.
- De Leon, R.D., and Acosta, C.N. (2006). Effect of robotic-assisted treadmill training and chronic quipazine treatment on hindlimb stepping in spinally transected rats. *J. Neurotrauma* 23, 1147–1163.
- De Leon, R.D., Hodgson, J.A., Roy, R.R., and Edgerton, V.R. (1998). Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J. Neurophysiol.* 80, 83–91.
- Dietz, V., Colombo, G., Jensen, L., and Baumgartner, L. (1995). Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* 37, 574–582.
- Dobkin, B., Apple, D., Barbeau, H., Basso, M., Behrman, A., Deforge, D., Ditunno, J., Dudley, G., Elashoff, R., Fugate, L., Harkema, S., Saulino, M., Scott, M., and Spinal Cord Injury Locomotor Trial G. (2006). Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. *Neurology* 66, 484–493.
- Dobkin, B., Barbeau, H., Deforge, D., Ditunno, J., Elashoff, R., Apple, D., Basso, M., Behrman, A., Harkema, S., Saulino, M., Scott, M., and Spinal Cord Injury Locomotor Trial G. (2007). The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: the multicenter randomized Spinal Cord Injury Locomotor Trial. *Neurorehabil. Neural Repair.* 21, 25–35.
- Downs, S.H., and Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J. Epidemiol. Community Health* 52, 377–384.
- Edgerton, V.R., Tillakaratne, N.J.K., Bigbee, A.J., de Leon, R.D., and Roy, R.R. (2004). Plasticity of the spinal neural circuitry after injury. *Annu. Rev. Neurosci.* 27, 145–167.
- Engesser-Cesar, C., Anderson, A.J., Basso, D.M., Edgerton, V.R., and Cotman, C.W. (2005). Voluntary wheel running improves recovery from a moderate spinal cord injury. *J. Neurotrauma* 22, 157–171.

- Engesser-Cesar, C., Ichiyama, R.M., Nefas, A.L., Hill, M.A., Edgerton, V.R., Cotman, C.W., and Anderson, A.J. (2007). Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. *Eur. J. Neurosci.* 25, 1931–1939.
- Erschbamer, M.K., Pham, T.M., Zwart, M.C., Baumans, V., and Olson, L. (2006). Neither environmental enrichment nor voluntary wheel running enhances recovery from incomplete spinal cord injury in rats. *Exp. Neurol.* 201, 154–164.
- Field-Fote, F.C. (2003). Quantification of functional behavior in humans and animals time for a paradigm shift. *J. Rehabil. Res. Dev.* 40: 19–24.
- Field-Fote, E.C., and Roach, K.E. (2011). Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: a randomized clinical trial. *Phys. Ther.* 91, 48–60.
- Fischer, F.R., and Peduzzi, J.D. (2007). Functional recovery in rats with chronic spinal cord injuries after exposure to an enriched environment. *J. Spinal Cord Med.* 30, 147–155.
- Fong, A.J., Cai, L.L., Ootoshi, C.K., Reinkensmeyer, D.J., Burdick, J.W., Roy, R.R., and Edgerton, V.R. (2005). Spinal cord-transected mice learn to step in response to quipazine treatment and robotic training. *J. Neurosci.* 25, 11738–11747.
- Foret, A., Quertainmont, R., Botman, O., Bouhy, D., Amabili, P., Brook, G., Schoenen, J., and Franzen, R. (2010). Stem cells in the adult rat spinal cord: plasticity after injury and treadmill training exercise. *J. Neurochem.* 112, 762–772.
- Forssberg, H., Grillner, S., and Halbertsma, J. (1980). The locomotion of the low spinal cat. I. Coordination within a hindlimb. *Acta. Physiol. Scand.* 108, 269–281.
- Fouad, K., Metz, G.A., Merkler, D., Dietz, V., and Schwab, M.E. (2000). Treadmill training in incomplete spinal cord injured rats. *Behav. Brain Res.* 115, 107–113.
- Girgis, J., Merrett, D., Kirkland, S., Metz, G.A.S., Verge, V., and Fouad, K. (2007). Reaching training in rats with spinal cord injury promotes plasticity and task specific recovery. *Brain* 130, 2993–3003.
- Goldshmit, Y., Lythgo, N., Galea, M.P., and Turnley, A.M. (2008). Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery. *J. Neurotrauma* 25, 449–465.
- Heng, C., and de Leon, R.D. (2009). Treadmill training enhances the recovery of normal stepping patterns in spinal cord contused rats. *Exp. Neurol.* 216, 139–147.
- Horn, J., de Haan, R.J., Vermeulen, M., Luiten, P.G., and Limburg, M. (2001). Nimodipine in animal model experiments of focal cerebral ischemia: a systematic review. *Stroke* 32, 2433–2438.
- Hurn, P.D., and Macrae, I.M. (2000). Estrogen as a neuroprotectant in stroke. *J. Cereb. Blood Flow Metab.* 20, 631–652.
- Ichiyama, R., Potuzak, M., Balak, M., Kalderon, N., and Edgerton, V.R. (2009). Enhanced motor function by training in spinal cord contused rats following radiation therapy. *PLoS One* 4, doi:10.1371/journal.pone.0006862.
- Kakulas, B.A. (1999). The applied neuropathology of human spinal cord injury. *Spinal Cord* 37, 79–88.
- Kilkenny, C., Parsons, N., Kadyzewski, E., Festing, M.F.W., Cuthill, I.C., Fry, D., Hutton, J., and Altman, D.G. (2009). Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS One.* 4, e7824. doi:10.1371/journal.pone.0007824.
- Kirshblum, S., Millis, S., McKinley, W., and Tulskey, D. (2004). Late neurologic recovery after traumatic spinal cord injury. *Arch Phys. Med. Rehab.* 85, 1811–1817.
- Kubasak, M.D., Jindrich, D.L., Zhong, H., Takeoka, A., McFarland, K.C., Munoz-Quiles, C., Roy, R.R., Edgerton, V.R., Ramon-Cueto, A., and Phelps, P.E. (2008). OEG implantation and step training enhance hindlimb-stepping ability in adult spinal transected rats. *Brain* 131, 264–276.
- Kuerzi, J., Brown, E.H., Shum-Siu, A., Siu, A., Burke, D., Morehouse, J., Smith, R.R., and Magnuson, D.S.K. (2010). Task-specificity vs. ceiling effect: step-training in shallow water after spinal cord injury. *Exp. Neurol.* 224, 178–187.
- Lankhorst, A.J., ter Laak, M.P., van Laar, T.J., van Meeteren, N.L., de Groot, J.C., Schrama, L.H., Hamers, F.P., and Gispén, W.H. (2001). Effects of enriched housing on functional recovery after spinal cord contusive injury in the adult rat. *J. Neurotrauma* 18, 203–215.
- Lee, Y.-S., Zdunowski, S., Edgerton, V.R., Roy, R.R., Zhong, H., Hsiao, I., and Lin, V.W. (2010). Improvement of gait patterns in step-trained, complete spinal cord-transected rats treated with a peripheral nerve graft and acidic fibroblast growth factor. *Exp. Neurol.* 224, 429–437.
- Liu, M., Bose, P., Walter, G.A., Thompson, F.J., and Vandenberg, K. (2008). A longitudinal study of skeletal muscle following spinal cord injury and locomotor training. *Spinal Cord* 46, 488–493.
- Lovely, R.G., Gregor, R.J., Roy, R.R., and Edgerton, V.R. (1986). Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. *Exp. Neurol.* 92, 421–435.
- Macleod, M.R., Ebrahim, S., and Roberts, I. (2005). Surveying the literature from animal experiments: systematic review and meta-analysis are important contributions. *BMJ* 331, 110.
- Macleod, M.R., Fisher, M., O'Collins, V., Sena, E.S., Dirnagl, U., Bath, P.M.W., Buchan, A., van der Worp, H.B., Traystman, R., Minematsu, K., Donnan, G.A., and Howells, D.W. (2009). Good laboratory practice: preventing introduction of bias at the bench. *Stroke* 40, e50–e52.
- Macrae, I. (2011). Preclinical stroke research—advantages and disadvantages of the most common rodent models of focal ischaemia. *Br. J. Pharmacol.* 164, 1062–1078.
- Magnuson, D.S.K., Smith, R.R., Brown, E.H., Enzmann, G., Angeli, C., Quesada, P.M., and Burke, D. (2009). Swimming as a model of task-specific locomotor retraining after spinal cord injury in the rat. *Neurorehabil. Neural Repair* 23, 535–545.
- Maier, I.C., Ichiyama, R.M., Courtine, G., Schnell, L., Lavrov, I., Edgerton, V.R., and Schwab, M.E. (2009). Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain* 132, 1426–1440.
- Metz, G.A., Curt, A., van de Meent, H., Klusman, I., Schwab, M.E., and Dietz, V. (2000). Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. *J. Neurotrauma* 17, 1–17.
- Mogil, J.S., and Chanda, M.L. (2005). The case for the inclusion of female subjects in basic science studies of pain. *Pain* 117, 1–5.
- Moshonkina, T., Avelev, V., Gerasimenko, Y., Mathur, R., and Bijlani, R.L. (2002). Treadmill training accelerates restoration of locomotion after complete spinal cord transection in the rat. *Indian J. Physiol. Pharmacol.* 46, 499–503.
- Moshonkina, T.R., Gilerovich, E.G., Fedorova, E.A., Avelev, V.D., Gerasimenko, Y.P., and Otellin, V.A. (2004). Morphofunctional basis for recovery of locomotor movements in rats with completely crossed spinal cord. *Bull. Exp. Biol. Med.* 138, 198–201.
- Multon, S., Franzen, R., Poirrier, A.-L., Scholtes, F., and Schoenen, J. (2003). The effect of treadmill training on motor

- recovery after a partial spinal cord compression-injury in the adult rat. *J. Neurotrauma* 20, 699–706.
- Norton, L. (2010). Spinal cord injury, Australia 2007–08. AIHW cat no. INJCAT 128. Injury Research and Statistics Series. Australian Institute of Health and Welfare.
- Nothias, J.M., Mitsui, T., Shumsky, J.S., Fischer, I., Antonacci, M.D., and Murray, M. (2005). Combined effects of neurotrophin secreting transplants, exercise, and serotonergic drug challenge improve function in spinal rats. *Neurorehabil. Neural Repair* 19, 296–312.
- Oh, M.J., Seo, T.B., Kwon, K.B., Yoon, S.J., Elzi, D.J., Kim, B.G., and Namgung, U. (2009). Axonal outgrowth and Erk1/2 activation by training after spinal cord injury in rats. *J. Neurotrauma* 26, 2071–2082.
- Park, K., Lee, Y., Park, S., Lee, S., Hong, Y., and Lee, S.K. (2010). Synergistic effect of melatonin on exercise-induced neuronal reconstruction and functional recovery in a spinal cord injury animal model. *J. Pineal Research* 48, 270–281.
- Petruska, J.C., Ichiyama, R.M., Jindrich, D.L., Crown, E.D., Tansey, K.E., Roy, R.R., Edgerton, V.R., and Mendell, L.M. (2007). Changes in motoneuron properties and synaptic inputs related to step training after spinal cord transection in rats. *J. Neurosci.* 27, 4460–4471.
- Robert, A.A., Ibrahim, A.L., Jadid, M.S., Bin Afif, S., Abdulmohsen, A.L., Sowayed, A., and Al-Mubarak, S. (2010). The effects of different rehabilitation strategies on the functional recovery of spinal cord injured rats: An experimental study. *Spine* 35, E1273–E1277.
- Rosenzweig, E.S., and McDonald, J.W. (2004). Rodent models for treatment of spinal cord injury: research trends and progress toward useful repair. *Curr. Opin. Neurol.* 17, 121–131.
- Sandrow-Feinberg, H.R., Izzi, J., Shumsky, J.S., Zhukareva, V., and Houle, J.D. (2009). Forced exercise as a rehabilitation strategy after unilateral cervical spinal cord contusion injury. *J. Neurotrauma* 26, 721–731.
- Sedy, J., Urdzikova, L., Likavcanova, K., Hejcl, A., Jendelova, P., and Sykova, E. (2007). A new model of severe neurogenic pulmonary edema in spinal cord injured rat. *Neurosci. Letters* 423, 167–171.
- Sena, E., van der Worp, H.B., Howells, D., and Macleod, M. (2007). How can we improve the pre-clinical development of drugs for stroke? *Trends Neurosci.* 30, 433–439.
- Siegenthaler, M.M., Berchtold, N.C., Cotman, C.W., and Keirstead, H.S. (2008). Voluntary running attenuates age-related deficits following SCI. *Exp. Neurol.* 210, 207–216.
- Sim, J., and Wright, C.C. (2005). The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys. Ther.* 85, 257–268.
- Singh, A., Murray, M., and Houle, J.D. (2011). A training paradigm to enhance motor recovery in contused rats: effects of staircase training. *Neurorehabil. Neural Repair* 25, 24–34.
- Smith, J.L., Smith, L.A., Zernicke, R.F., and Hoy, M. (1982). Locomotion in exercised and nonexercised cats cordotomized at two or twelve weeks of age. *Exp. Neurol.* 76, 393–413.
- Smith, R.R., Brown, E.H., Shum-Siu, A., Whelan, A., Burke, D.A., Benton, R.L., and Magnuson, D.S.K. (2009). Swim training initiated acutely after spinal cord injury is ineffective and induces extravasation in and around the epicenter. *J. Neurotrauma* 26, 1017–1027.
- Smith, R.R., Shum-Siu, A., Baltzley, R., Bunger, M., Baldini, A., Burke, D.A., and Magnuson, D.S.K. (2006). Effects of swimming on functional recovery after incomplete spinal cord injury in rats. *J. Neurotrauma* 23, 908–919.
- Stevens, J.E., Liu, M., Bose, P., O'Steen, W.A., Thompson, F.J., Anderson, D.K., and Vandenborne, K. (2006). Changes in soleus muscle function and fiber morphology with one week of locomotor training in spinal cord contusion injured rats. *J. Neurotrauma* 23, 1671–1681.
- Strauss, D.J., DeVivo, M.J., Paculdo, D.R., and Shavelle, R.M. (2006). Trends in life expectancy after spinal cord injury. *Arch. Phys. Med. Rehabil.* 87, 1079–1085.
- Tillakaratne, N.J.K., Guu, J.J., de Leon, R.D., Bigbee, A.J., London, N.J., Zhong, H., Ziegler, M.D., Joynes, R.L., Roy, R.R., and Edgerton, V.R. (2010). Functional recovery of stepping in rats after a complete neonatal spinal cord transection is not due to regrowth across the lesion site. *Neuroscience* 166, 23–33.
- Timoszyk, W.K., Nessler, J.A., Acosta, C., Roy, R.R., Edgerton, V.R., Reinkensmeyer, D.J., and de Leon, R. (2005). Hindlimb loading determines stepping quantity and quality following spinal cord transection. *Brain Res.* 1050, 180–189.
- Ung, R.V., Lapointe, N.P., Rouleau, P., and Guertin, P.A. (2010). Non-assisted treadmill training does not improve motor recovery and body composition in spinal cord-transected mice. *Spinal Cord* 48, 750–755.
- van der Worp, H.B., Sena, E.S., Donnan, G.A., Howells, D.W., and Macleod, M.R. (2007). Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain* 130, 3063–3074.
- Van Meeteren, N.L.U., Eggers, R., Lankhorst, A.J., Gispen, W.H., and Hamers, F.P.T. (2003). Locomotor recovery after spinal cord contusion injury in rats is improved by spontaneous exercise. *J. Neurotrauma* 20, 1029–1037.
- Vesterinen, H.M., Vesterinen, H.V., Egan, K., Deister, A., Schlattmann, P., Macleod, M.R., and Dirnagl, U. (2011). Systematic survey of the design, statistical analysis, and reporting of studies published in the 2008 volume of the *Journal of Cerebral Blood Flow and Metabolism*. *J. Cereb. Blood Flow. Metab.* 31, 1064–1072.
- Waters, R.L., Adkins, R.H., Yakura, I.S., and Sie, I. (1993). Motor and sensory recovery following complete tetraplegia. *Arch. Phys. Med. Rehabil.* 74, 242–247.
- Wessels, M., Lucas, C., Eriks, I., and de Groot, S. (2010). Body weight-supported gait training for restoration of walking in people with an incomplete spinal cord injury: a systematic review. *J. Rehabil. Med.* 42, 513–519.
- Zhang, Y., Ji, S.R., Wu, C.Y., Fan, X.H., Zhou, H.J., and Liu, G.L. (2007). Observation of locomotor functional recovery in adult complete spinal rats BSWTT using semiquantitative and qualitative methods. *Spinal Cord* 45, 496–501.

Address correspondence to author:
 Camila R. Battistuzzo, BPhysio
 Department of Physiotherapy
 The University of Melbourne
 200 Berkeley Street, 3010
 Melbourne, Victoria, Australia

E-mail: camilarb@student.unimelb.edu.au