

Rat Models of Traumatic Spinal Cord Injury to Assess Motor Recovery

Stephen M. Onifer, Alexander G. Rabchevsky, and Stephen W. Scheff

Abstract

Devastating motor, sensory, and autonomic dysfunctions render long-term personal hardships to the survivors of traumatic spinal cord injury (SCI). The suffering also extends to the survivors' families and friends, who endure emotional, physical, and financial burdens in providing for necessary surgeries, care, and rehabilitation. After the primary mechanical SCI, there is a complex secondary injury cascade that leads to the progressive death of otherwise potentially viable axons and cells and that impairs endogenous recovery processes. Investigations of possible cures and of ways to alleviate the hardships of traumatic SCI include those of interventions that attenuate or overcome the secondary injury cascade, enhance the endogenous repair mechanisms, regenerate axons, replace lost cells, and rehabilitate. These investigations have led to the creation of laboratory animal models of the different types of traumatic human SCI and components of the secondary injury cascade. However, no particular model completely addresses all aspects of traumatic SCI. In this article, we describe adult rat SCI models and the motor, and in some cases sensory and autonomic, deficits that each produces. Importantly, as researchers in this area move toward clinical trials to alleviate the hardships of traumatic SCI, there is a need for standardized small and large animal SCI models as well as quantitative behavioral and electrophysiological assessments of their outcomes so that investigators testing various interventions can directly compare their results and correlate them with the molecular, biochemical, and histological alterations.

Key Words: compression; contusion; demyelination; excitotoxicity; free radicals; inflammation; ischemia; laceration

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Introduction

Traumatic spinal cord injury (SCI)¹ in the United States happens to approximately 11,000 persons each year (Spinal Cord Injury Information Network, www.spinalcord.uab.edu). While the majority of these injuries occur to the cervical spinal cord, devastating motor, sensory, and autonomic dysfunctions below the injury render long-term hardships to the survivors of all levels of SCI—cervical, thoracic, lumbar, and sacral. The suffering also extends to survivors' families and friends, who endure emotional, physical, and financial burdens in providing for necessary surgeries, care, and rehabilitation.

Based on imaging and histology of injured human spinal cords, Bunge and colleagues classified each traumatic SCI as (1) a contusion evolving to cavity formation, (2) a massive compression, or (3) a laceration (Bunge et al. 1993, 1997). The most frequent contusion injuries are focal spinal cord compression and render both an intact glial limitans and pia surrounding varying extents of intact white and gray matter. Fluid-filled cavities or cysts evolve from the hemorrhage into the spinal cord parenchyma and where tissue has degenerated. In contrast, the glial limitans and pia are cut in massive compression and laceration injuries. Massive compression injuries occur over substantial lengths of the spinal cord and include maceration. Laceration injuries are focal and, similar to massive compression injuries, result in the formation over time of a connective tissue mass in the spinal cord.

After the primary mechanical SCI, there is a complex secondary injury cascade (Tator and Fehlings 1991) that leads to the progressive death of otherwise potentially viable axons and cells and that impairs endogenous recovery processes. Investigations of possible cures and ways to alleviate the hardships of traumatic SCI include those of interventions that attenuate or overcome the secondary injury cascade, enhance the endogenous repair mechanisms, regenerate axons, replace lost cells, and rehabilitate (Anderson et al. 2005a; Blight and Tuszynski 2006; Bradbury and McMahon 2006; Kleitman 2004; Rabchevsky and Smith 2001; Tator 2006; Thuret et al. 2006). These investigations

¹Abbreviations used in this article: BBB scale, Basso, Beattie, and Bresnahan open-field locomotor rating scale; EMG, electromyographic; IH, Infinite Horizon; MIER, magnetically evoked interenlargement response; OSU, Ohio State University; SCI, spinal cord injury; SSEP, somatosensory evoked potential; tcMMEP, transcranial magnetic motor evoked potential.

have led to the creation of laboratory animal models of traumatic human SCI.

In this article, we describe adult rat SCI models and the motor, and in some cases sensory and autonomic, deficits that each produces. Rats have been chosen to study traumatic SCI not only because they are readily available but also because the morphological, biochemical, and functional changes that occur after SCI are similar to those seen in humans (Fleming et al. 2006; McTigue et al. 2000; Metz et al. 2000; Norenberg et al. 2004). The rats are always anesthetized for the SCI and other surgical procedures, after which they require specialized veterinary care, the extent of which depends on both the location and severity of the injury (Santos-Benito et al. 2006). Female rats are preferable because of the relative ease of manual bladder emptying after SCI, resulting in less frequent urinary tract infections.

Assessments of Motor Deficits and Recovery in Adult Rats with SCI

A number of assessments of behavioral and electrophysiological function are available for adult rats (for reviews, see Basso 2004; Kesslak and Keirstead 2003; Nichols et al. 2005; Webb and Muir 2005). Importantly, most of these assessments evaluate the sensorimotor function rather than sensory or motor functions individually. Some are quantitative while others are not. We recommend the use of quantitative assessments even though they require training the rats to criterion. The completion of assessments before SCI helps in establishing baseline values for each rat. We, like others, also recommend a battery of assessments whenever appropriate. For information purposes, we briefly discuss commonly used quantitative and semiquantitative assessments for adult rat models of traumatic SCI. Detailed information about these assessments is available in the reviews indicated above and the references throughout this section.

Behavior

Because rats prehend food with their mouths then manipulate it with their forepaws, pellet retrieval tests are useful to assess reach, grasp, and retrieval behaviors after cervical SCI (Anderson et al. 2005b; Nash et al. 2002; Onifer et al. 1997b; Schrimsher and Reier 1992; Weidner et al. 2001). Importantly, it is necessary to train the rats used in post-SCI studies to prehend with their paws. Use of the "Staircase Test" platform and chamber (Montoya et al. 1991) enables cervical spinal cord-injured rats to avoid having to support themselves on three dysfunctional limbs while reaching for, grasping, and retrieving pellets (Onifer et al. 1997b). Investigators have examined forelimb and forepaw usage for support during spontaneous vertical exploration in a cylinder after cervical SCI (Liu et al. 1999b; Soblosky et al. 2001; Webb and Muir 2004). There are also studies of forelimb

grip strength in which the cervical-SCI rat is pulled away while grasping a bar (Anderson et al. 2005b; Onifer et al. 1997b). Other studies evaluate forelimb and hindlimb placing and footfalls while the rat walks or locomotes on a horizontal (Onifer et al. 2005; Pearse et al. 2005) or inclined (Li et al. 2003) grid, horizontal ladder (Soblosky et al. 2001; Webb and Muir 2003, 2004), beam (Jeffery and Blakemore 1997; Kim et al. 2001), or rope (Anderson et al. 2005b; Kim et al. 2001).

Many of the current models of SCI utilize the Basso, Beattie, and Bresnahan open-field locomotor rating scale (BBB scale¹; Basso et al. 1995) to assess functional outcome. The BBB scale has a range from zero (no hindlimb movements) to 21 (normal coordinate gait), using paw placement, joint movement, and truncal stability as important factors in determining the level of functional recovery. Scores in the 0 to 7 range focus primarily on hip, knee, and ankle joint movement, the 8 to 13 range keys in on paw placement and coordination, and scores of 14 to 21 rely heavily on trunk stability, tail position, and paw placement. Tarlov and Klinger (1954) developed one of the original open-field behavior tests for SCI and it involved a rather simplistic observational assessment of the animal's locomotor ability. A modified version of the Tarlov scale (Gale et al. 1985) consisting of six levels of motor movement (from 0 for no hindlimb movement to 5 for normal walking) appears extensively in the literature (Haghighi et al. 2000; Voda et al. 2005). Other analyses of locomotion include footprint analysis (Cheng et al. 1997; Kunkel-Bagden et al. 1993), the CatWalk-assisted gait analysis (Gensel et al. 2006; Hamers et al. 2001; Hendriks et al. 2006), and kinematics (Broton et al. 1996; Collazos-Castro et al. 2006). Several studies correlate these data to electromyographic (EMG¹) recordings made at the same time from electrodes implanted in limb muscles (Ballermann et al. 2006; Kaegi et al. 2002; Thota et al. 2005).

Numerous studies have used the inclined plane to evaluate functional outcome in rats after SCI (Rivlin and Tator 1977). The device consists of a hinged board raised and lowered to different angles. The object is for the rat to maintain itself on the board for 5 seconds as the angle is gradually increased at 5° intervals. The assigned score is the maximum angle of the plane that the rat can maintain for 5 seconds without sliding off onto a padded surface. Uninjured rats achieve scores of approximately 80°.

Electrophysiology

In both humans and animals with traumatic SCI, electrophysiology is a valuable tool for investigation of the neural substrates underlying deficits and functional recovery as revealed by behavioral testing. Terminal electrophysiology procedures can be useful in experimental animals (e.g., Massey et al. 2006), but it is much more advantageous to reproduce the procedure a number of times after SCI (Nashmi et al. 1997) and without anesthesia. The transcranial

nial magnetic motor evoked potential (tcMMEP¹) procedure involves noninvasive magnetic stimulation at the unanesthetized rat's skull and the recording of evoked potentials with EMG electrodes temporarily inserted into hindlimb muscles (Fishback et al. 1995; Linden et al. 1999). While the tcMMEP procedure assesses supraspinal axon conduction, magnetically evoked interenlargement (MIER¹) and somatosensory evoked potential (SSEP¹) procedures are effective for the evaluation of propriospinal and sensory spinal axon conduction, respectively, in unanesthetized rats. The MIER procedure involves noninvasive magnetic stimulation at the rat's hip or knee and the recording of evoked potentials with EMG electrodes temporarily inserted into forelimb and masseter muscles (Beaumont et al. 2006). The SSEP procedure involves electrical stimulation of the paws with electrodes temporarily inserted into them, and the recording of evoked potentials from electrodes previously implanted in the cranium over the somatosensory cortex (Onifer et al. 2005). All three procedures take only a few minutes and cause only slight pain and distress and so are done in unanesthetized rats.

Rat Models of Traumatic SCI

Weight Drop

The first well-documented animal experimentation of SCI was that described by Allen (1911), which used a weight-drop technique on dogs. The approach was quite simple: A laminectomy was performed on the anesthetized animal, exposing the dorsal (posterior) surface of the spinal cord. A given weight was dropped from a known height, down a vented guide tube positioned perpendicular to the laminectomy site. As the weight struck the exposed spinal cord, either directly or through an impounder plate that rested on the exposed cord (and may have diffused the injury over a wider area), the underlying tissue was subjected to a dynamic compression. Depending on the weight and the height, different amounts of force were applied to the cord and resulted in varying degrees of SCI. The shape and diameter of the impounder surface were important variables in the injury outcome (Gerber and Corrie 1979; Koozekanani et al. 1976) as was the resistance encountered during the "free fall" (Dohrmann and Panjabi 1976; Dohrmann et al. 1978).

While Allen's original work utilized dogs, a weight-drop technique was later adapted for the anesthetized rat (Panjabi and Wrathall 1988; Wrathall et al. 1985). Because of the difference in size of the impactor tip, the technique was initially thought to be too unreliable in the rodent (Khan and Griebel 1983), with consistency observed only at the high severity end of the spectrum. Important variables that made the Wrathall model work were stabilization of the spinal column before the injury and a strain gauge, used to measure force, mounted on a "C" ring attached to an impounder resting on the exposed spinal cord dura. Many of

the subsequent weight-drop models that were scaled down for producing cervical (Onifer et al. 1997b; Schrimsher and Reier 1992; Soblosky et al. 2001) or thoracic SCI in the rat offered limited information on the biomechanical properties of the injury, although they did allow for reproducibility and graded levels of functional outcome (Wrathall et al. 1985).

It is extremely important that an injury model have the capability of generating different degrees of injury severity and functional outcomes. If injuries are too moderate, spontaneous recovery occurs very rapidly and it is difficult to assess potential therapeutic outcomes. Injuries that are too severe result in extremely limited functional outcome and again can mask potentially useful therapeutic strategies. In an improvement over the Wrathall model, Khan and colleagues (1999) compared functional outcomes after injury in both rats and cats. While both species showed injury-related declines in behavioral and electrophysiological function, the time course of some spontaneous recovery was significantly shorter for the cats. As with earlier weight-drop models, little information concerning the biomechanical parameters of the SCI was available.

One of the most popular weight-drop models is the New York University (NYU)/MASCIS device. This rather sophisticated device was developed by Gruner (1992) and consists of dropping a 10-gram weight from 6.25, 12.5, 25, or 50 mm directly onto the exposed dorsal spinal cord dura. An electrical circuit determines cord surface before the injury, thus eliminating concerns about a preload injury, common with models that use an impounder plate. One of the major advantages to this model is the monitoring of injury parameters such as impact velocity and tissue displacement, providing a mechanism to eliminate injured animals that do not meet preestablished criteria. The device has greatly reduced the risk of multiple injuries because of drop-weight "bounce."

After the injury, all of the rats demonstrate a severe loss of locomotor activity. Basso and colleagues (Basso et al. 1996) used the BBB scale to describe graded locomotor outcomes following different thoracic injury severities with this device. With the exception of the 6.25-mm drop, all groups showed severe loss of locomotor movement in the hind legs. However, most of the groups showed spontaneous improvement over 3 to 4 weeks, after which locomotor ability reached a plateau. Investigators have reported graded motor deficits and recovery as found with grooming, paw preference, the CatWalk, and horizontal ladder tests, as well as kinematics and the BBB scale for rats following cervical or lumbar injury severities with this device (Collazos-Castro et al. 2005; Gensel et al. 2006; Magnuson et al. 2005).

Aneurysm Clip Compression

As mentioned above, several research groups considered the rat too small for the weight-drop method originally designed by Allen. To overcome this problem and to more closely model the ventral (anterior) compression normally observed

in the human clinical condition, investigators effected SCI through sustained compression with specially modified Kerr-Lougheed aneurysm clips (Rivlin and Tator 1978). This model entails exposing the rat spinal cord and applying the aneurysm clip with one blade under the ventral surface of the cord and the other over the dorsal surface. The clip, calibrated for a known compression force, is rapidly released and allowed to compress the cord for a predetermined amount of time (e.g., 60 seconds) before release and removal. In the initial study, this type of injury resulted in a quite dramatic and sustained deficit as measured with an inclined plane task. Some investigators have applied compression force-calibrated aneurysm clips vertically (laterally) and assessed behavior outcome (von Euler et al. 1997a). In both cases, results indicated a high correlation of all neurological outcomes (BBB scale, inclined plane, beam walk) to the compression force.

Calibrated Forceps Compression

As an alternative to the use of aneurysm clips that produce a very focal injury, Blight (1991) developed a moderate-severity injury technique in the guinea pig using modified forceps. This compression injury produced a considerably larger volume of tissue compression and displacement than the aneurysm clips, with the added advantage of a special spinal column stabilized support. A detailed study in the rat (Gruner et al. 1996) carefully monitored functional recovery using a modified Tarlov scale following a wide range of spinal cord compressions. The results demonstrated significant differences in the temporal pattern of the behavioral recovery when comparing the rat and the guinea pig. A delayed functional loss in the guinea pig was not observed in the rat, indicating possible significant species differences.

Contusion

In an attempt to gain more control over the injury severity and more closely monitor biomechanical properties, several laboratories began to experiment with controlled pneumatic compression models (Anderson 1982; Kearney et al. 1988). One such model that used rats reported no indications of changes in functional outcome (Narayana et al. 1999). A report by Noyes (1987) described a new electromechanical spinal cord impactor, now known as the Ohio State University (OSU¹) device. This model injures the spinal cord by means of a solenoid-controlled air cylinder mounted on a rigid frame with a tip that impacts the exposed dorsal spinal cord. The spinal column is firmly secured with no impounder plate on the exposed spinal cord. A companion study (Somerson and Stokes 1987) demonstrated the functional outcome following three different levels of injury using the Tarlov scale. Additional studies (Behrmann et al. 1992; Bresnahan et al. 1987) more carefully described functional recovery patterns in rats based on thoracic injury

severity in terms of tissue displacement and post-trauma recovery time using the Tarlov scale, inclined plane task, grid walking, and footprint analysis. As with the weight-drop studies, functional deficits appeared immediately following the trauma and showed spontaneous recovery that plateaued around 2 to 3 weeks following injury. Reports describe graded motor deficits and recovery found with forelimb grip strength, inclined plane, and grid-walk tests as well as footprint analysis and the BBB scale for rats following different cervical injury severities with this device (Pearse et al. 2005). Although the lack of commercial availability of the OSU device limits its experimental usage by other laboratories, this sophisticated model provides excellent control over the biomechanical aspects of the injury (i.e., velocity, depth of compression, force) that can effectively dictate the severity of the injury.

A relatively new, commercially available kinetic contusion device uses force rather than tissue displacement to injure the rat spinal cord (Scheff et al. 2003). Similar to the OSU device, this model requires exposure of the dorsal surface of the spinal cord and the rigid stabilization of the spinal column. One of the unique features of this computerized device, first produced by Infinite Horizon (IH¹), is the ability to inflict reproducible injuries without touching the spinal cord before the hit. Some experimenters believe that any device that touches the exposed spinal cord before a more severe compression could cause a priming injury and thus alter the intended injury outcome. The IH instrumentation senses the surface of the exposed spinal cord and continues to displace spinal tissue until it attains a predetermined force, at which point the impactor tip immediately withdraws. Initial behavioral and electrophysiological characterization studies used the BBB scale and the tcMMEP procedure to describe the loss and reacquisition of hind-limb function and motor axon conduction following a wide range of injury severities (Cao et al. 2005; Scheff et al. 2003). A subsequent paper, evaluating creatine as a neuroprotective agent, compared injury with the IH device to the NYU/MASCIS weight-drop model (Rabchevsky et al. 2003) and found that both models produced significant SCI and resulted in very similar functional recovery profiles. An advantage of the IH device is that it enables monitoring of various biomechanical parameters (i.e., velocity, force, tissue compression/displacement) and thus the early elimination from the study of animals that do not meet preset standards.

Laceration

Of the many traumatic SCI models, the most frequently used methods of injury include complete transection, focal myelotomy (incision), dorsal or lateral hemisection, lateral overhemisection, resection, or aspiration lesions that definitively sever spinal cord axons of passage. Although these types of laceration injuries are not typically seen clinically, they can effectively disconnect both ascending and descending axonal pathways at designated levels of SCI. This ap-

proach allows the study of mechanisms that govern the inhibition or successful regeneration of axons across or around laceration injuries as well as of the resulting functional deficits and potential recovery. Importantly, the severity and level of SCI entirely dictate the applicability of quantitative assessments for impaired motor, sensory, and/or autonomic functions.

One of the many syndromes that occur after both complete and incomplete human SCI at thoracic level 6 (T6) or above is a condition called autonomic dysreflexia (Karlsson 1999; Rabchevsky 2006). The most common trigger of this arduous hypertensive disorder is painful distension of the bladder or bowel, which elicits massive discharge of uninhibited sympathetic neurons in the injured spinal cord disconnected from brainstem control. The inflation of a balloon catheter in the distal colon of unanesthetized rats induces autonomic dysreflexia 2 weeks after complete transection of the cervical (C) or high thoracic spinal cord (Cameron et al. 2006; Chau et al. 2000; Krassioukov et al. 2002; Krassioukov and Weaver 1995; Osborn et al. 1989, 1990). While there is no perception of noxious stimuli, the rats experience autonomic cardiopathophysiology that stems from post-traumatic intraspinal plasticity (Rabchevsky 2006).

Unlike complete spinal cord transection injuries, the reproducibility of partial spinal cord laceration injuries is somewhat inconsistent. Such injuries include focal myelotomies at spinal level C4, with the subsequent use of a pellet retrieval test to examine the effects of damage to axon pathways in the dorsolateral and ventrolateral funiculi on forelimb reach, grasp, and retrieval behaviors (Schrimsher and Reier 1993). Unilateral SCI of distinct ascending and descending spinal pathways at similar levels is useful to investigate locomotor and sensory deficits as well as compensatory recovery using electromechanical assessments (Webb and Muir 2002, 2003, 2004). An alternative to unilateral SCI is more extensive dorsal-ventral and ventral-dorsal laceration SCI (Schucht et al. 2002). Among the behavioral tests utilized with these lesions are a forepaw usage task, the BBB scale, analyses of footslips during ladder or grid walking, quantitative kinetic measurements of ground reaction forces during locomotion, and Von Frey filament testing for tactile sensation. The recently created *VibraKnife*TM creates discrete myelotomies and dorsal hemisections of the midcervical spinal cord that elicit differential alterations in forelimb SSEPs and in upper extremity sensorimotor deficits (Onifer et al. 2005). After various discrete thoracic spinal cord hemisection injuries, use of the MIER procedure demonstrates differential alterations in ascending propriospinal axon pathway conduction that significantly correlate both with white matter sparing in the lateral funiculus and with hindlimb function assessed by the BBB scale (Beaumont et al. 2006).

Combinatorial injuries, consisting of a thoracic spinal cord dorsal hemisection and unilateral transection of a pyramidal tract, show that new intraspinal circuitry forms spontaneously in the injured spinal cord of adult rats

(Bareyre et al. 2004). In particular, transected hindlimb corticospinal tract axons sprouted into the cervical spinal cord gray matter to contact long propriospinal neurons that bridged the lesion and extended processes to lumbar motoneurons. This created a new intraspinal circuit relaying supraspinal input to its original spinal targets. Electrophysiological and behavioral testing, as well as retrograde transynaptic tract tracing that revealed progressive changes in cortical representations over time, confirmed functionality.

Complete resection injuries and discrete hemisection aspiration lesions of the thoracic spinal cord have also been used to test various “bridge” interface approaches by grafting neural tissues, alone or in combination with biosynthetic guidance channels, to promote successful graft survival and axonal regeneration in the injured adult rat spinal cord (Aguayo et al. 1981; Bregman et al. 1993; Cheng et al. 1996; David and Aguayo 1981; Iannotti et al. 2003; Jekman and Reier 1991; Reier et al. 1986; Xu et al. 1995, 1997, 1999). Outcome measures for such interventions include histological and axonal tract tracing methods to assess tissue integrity, cytoarchitecture and neural connectivity between graft and host, the combined behavior score, the modified Tarlov scale, and forelimb functional recovery tasks.

Chemical-mediated SCI

Procedures involving a variety of chemical-mediated injuries throughout the adult rat spinal cord can be useful to model specific aspects of the secondary injury cascade that occur after the initial traumatic SCI and to address questions about spinal cord circuitry. In contrast to the previously described models, chemical-mediated SCI is a targeted approach that may not include all the components of traumatic SCI. The clinical relevance of findings from experiments that evaluate treatments in these models must take this into account.

Vascular damage occurs after traumatic SCI and results in hemorrhage, reduced blood flow, and ischemia (poor oxygen and nutrient supply) (Casella et al. 2002; Fleming et al. 2006; Loy et al. 2002a; Norenberg et al. 2004; Tator and Fehlings 1991). There is evidence that ischemia causes spinal cord damage and then dysfunction in both humans and rats after occlusion of the descending thoracoabdominal aorta (for review, Black and Cambria 2006; Zhang et al. 2000). It is also possible to produce ischemia in the rat spinal cord by combining intravenous injection of the photosensitive dyes rose bengal (Watson et al. 1986) or erythrosin B (Cameron et al. 1990; Hao et al. 1991) with irradiation of the exposed vertebrae to produce vascular thrombosis. An alternative is to bathe the dura-covered spinal cord with rose bengal then use irradiation (Garcia-Alias et al. 2003; Verdu et al. 2003). Irradiation duration is an effective way to grade the ensuing motor behavioral and axon conduction deficits (Cameron et al. 1990; Garcia-Alias et al. 2003; Prado et al. 1987; Wiesenfeld-Hallin et al. 1993)

as well as white and gray matter pathology (Bunge et al. 1994; Hao et al. 1991; Olby and Blakemore 1996; Prado et al. 1987; Verdu et al. 2003; von Euler et al. 1997b; Watson et al. 1986) in these models of spinal cord ischemia. Intrathecal delivery (Hokfelt et al. 1989; Salzman et al. 1996; Westmark et al. 1995) and minimally traumatic spinal cord gray matter injection (Benton et al. 2005) of the vasoconstrictor endothelin can also produce ischemia that leads to locomotor deficits (Salzman et al. 1996). Spinal cord gray matter injection of the nonspecific nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester additionally causes neuron degeneration, possibly by vasoconstriction due to reduced levels of nitric oxide (Dora et al. 1998; Yeziarski et al. 1996).

Excitatory amino acid concentrations rapidly rise to toxic levels after traumatic SCI (Liu et al. 1991) and the resultant excitotoxicity plays a major role in white and gray matter pathology (for a review, Park et al. 2004). Models of excitotoxic rat oligodendrocyte and neuron death as well as of evoked potential conduction block involve administering glutamate, glutamate and aspartate, N-methyl-D-aspartate (NMDA), or kainate, an agonist of the AMPA/kainate glutamate receptor, through microdialysis fibers implanted in the spinal cord (Liu 1994; Liu et al. 1999a; Xu et al. 2004). Intrathecal delivery of kainate also results in both oligodendrocyte and neuron death (Nottingham and Springer 2003; Sun et al. 2006). Topical application of kainate or NMDA to the dorsal spinal cord causes excitotoxic neuron death (Ikonomidou et al. 1996), whereas microinjections of kainate or quisqualic acid, an agonist of the AMPA-metabotropic receptor, into gray matter produces spinal cord gray matter degeneration (Hadi et al. 2000; Magnuson et al. 1999; Nothias and Peschanski 1990; Onifer et al. 1997a; Pisharodi and Nauta 1985; Yeziarski et al. 1993). Combining spinal cord gray matter injection of kainate with the proinflammatory cytokine tumor necrosis factor- α , at doses where these chemicals are not toxic, also causes gray matter degeneration (Hermann et al. 2001). Importantly, discrete excitotoxicity in cervical (Pisharodi and Nauta 1985) or lower (but not mid-) thoracic and upper lumbar (Hadi et al. 2000; Magnuson et al. 1999) spinal gray matter selectively damages neurons critical to adult rat forelimb or hindlimb function, respectively, but not long-tract motor axon conduction. Hindlimb locomotor deficits can also result after intrathecal delivery of kainate at the lumbar spinal cord (Sun et al. 2006).

Free radical, peroxynitrite, and calpain-induced damage to lipids and proteins occurs after traumatic SCI (for a review, Hall 2001). Models of this effect include the administration of the herbicide paraquat, a superoxide generator (Liu et al. 1995), hydrogen peroxide and FeCl_2 (Liu 1993), or S- or 3-morpholinopyridone, peroxynitrite donors (Bao et al. 2003; Bao and Liu 2002) through microdialysis fibers in rat spinal cord gray matter to generate superoxide, hydroxyl radical, and peroxynitrite. These oxidants destroy neurons, block motor axon conduction, and impair open-field locomotion, hindlimb function, and inclined plane sta-

bility when generated in lumbar spinal cord gray matter (Bao and Liu 2002; Liu 1994).

Inflammation occurs after traumatic SCI and is a cause of secondary degeneration (Fleming et al. 2006; Keane et al. 2006). Microinjection of zymosan, a yeast particulate that activates resident microglia/macrophages, into the rat spinal cord white and gray matter causes inflammation that leads to a pathology similar to that seen after traumatic SCI and open-field locomotion dysfunction (Popovich et al. 2002). Phospholipase A_2 is a family of enzymes that generate precursors of inflammatory mediators involved in spinal cord inflammatory disease (Kalyvas and David 2004). Microinjection of phospholipase A_2 into the rat thoracic spinal cord ventrolateral white matter produces a dose-dependent hemorrhage, inflammatory cell infiltration, demyelination, gray and white matter degeneration, and deficits in open-field locomotion and motor axon conduction (Liu et al. 2006).

Oligodendrocyte degeneration and demyelination are hallmarks of traumatic SCI (Guest et al. 2005). Intraspinal microinjection of the following cause rat oligodendrocyte death and demyelination: lysolecithin or L- α -phosphatidyl choline (a major component of oxidized low-density lipoproteins that has inflammatory properties) alone and after x-irradiation to prevent remyelination (Blakemore and Patterson 1978); ethidium bromide (an intercalating gliotoxin) alone (Graca and Blakemore 1986; Yajima and Suzuki 1979) and after x-irradiation (Blakemore and Crang 1989); complement proteins plus antibodies to galactocerebroside alone and after x-irradiation (Keirstead and Blakemore 1997); and an antibody to myelin oligodendrocyte glycoprotein and complement (Perez-Bouza et al. 2005). Importantly, chemical-mediated oligodendrocyte degeneration and demyelination in discrete rat spinal cord axon pathways can produce hindlimb and motor axon conduction deficits. For example, ethidium bromide injected into the cervical spinal cord dorsal funiculus produces hindlimb deficits during beam walking (Jeffery and Blakemore 1997). Photon irradiation of the thoracic spinal cord after injection of ethidium bromide into either the ventral or ventrolateral funiculus impairs open-field locomotion (Loy et al. 2002b). Moreover, oligodendrocyte degeneration and demyelination of the ventral white matter, of both the ventrolateral and dorsolateral funiculus, and of both the ventrolateral and dorsal funiculus results in greater open-field locomotion dysfunction (Loy et al. 2002b,c). Motor axon conduction deficits through the ventrolateral funiculus also result from ethidium bromide injection into the thoracic spinal cord ventrolateral funiculus and photon irradiation (Loy et al. 2002c). Intrathecal administration of cholera toxin B-subunit conjugated to saporin at the lumbar cord causes oligodendrocyte death, demyelination, and locomotion deficits (Jasmin et al. 2000).

Syringomyelia, a clinical syndrome that can occur after traumatic SCI (Wirth et al. 2001), is characterized by fluid-filled cavity formation, then enlargement with delayed and then progressively deteriorating function (for a review, see Brodbelt and Stoodley 2003). Modeling of syringomyelia

entails injection of a suspension of kaolin into the rat cisterna magna to produce hydrocephalus (Kashiwaguchi et al. 1989), into the spinal cord subarachnoid space to create arachnoiditis (Tatara 1992), or into the spinal cord dorsal columns (Stoodley et al. 1999) and central gray matter (Milhorat et al. 1993) to occlude the central canal. Microinjection of quisqualic acid in the spinal cord gray matter alone (Schwartz et al. 1999) and combined with a kaolin suspension injection into the spinal cord subarachnoid space (Yang et al. 2001) is another way to produce cavities. These models result in inflammation, demyelination, neuron loss, and both gray and white matter degeneration (Lee et al. 2005; Milhorat et al. 1993; Tatara 1992; Yeziarski et al. 1993).

Conclusions

No particular animal model of traumatic human SCI completely addresses all of its aspects, although the use of multiple paradigms has greatly advanced our understanding of the pathophysiology following spinal cord trauma. Importantly, as investigators in this area move toward clinical trials, there is clearly a need for standardized small and large, especially nonhuman primate (Courtine et al. 2007), animal models of SCI to enable testing of various interventions and to directly compare results. Equally important is the need to standardize behavioral and electrophysiological outcome measures for the different injury models because inappropriate assessment of functional deficits and recovery adds to the complexity of correlating their results with molecular, biochemical, and histological alterations. Bringing these concepts to a consensus among neuroscientists who use different models and assessments, based on preference or availability, may prove to be one of the biggest challenges to overcome in the search for ways to cure traumatic SCI in humans and to alleviate its hardships.

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References

- Aguayo AJ, David S, Bray GM. 1981. Influences of the glial environment on the elongation of axons after injury: Transplantation studies in adult rodents. *J Exp Biol* 95:231-240.
- Allen A. 1911. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: A preliminary report. *JAMA* 37:878-880.
- Anderson DK, Beattie M, Blesch A, Bresnahan J, Bunge M, Dietrich D, Dietz V, Dobkin B, Fawcett J, Fehlings M, Fischer I, Grossman R, Guest J, Hagg T, Hall ED, Houle J, Kleitman N, McDonald J, Murray M, Privat A, Reier P, Steeves J, Steward O, Tetzlaff W, Tuszynski MH, Waxman SG, Whittemore S, Wolpaw J, Young W, Zheng B. 2005a. Recommended guidelines for studies of human subjects with spinal cord injury. *Spinal Cord* 43:453-458.
- Anderson KD, Gunawan A, Steward O. 2005b. Quantitative assessment of forelimb motor function after cervical spinal cord injury in rats: Relationship to the corticospinal tract. *Exp Neurol* 194:161-174.
- Anderson TE. 1982. A controlled pneumatic technique for experimental spinal cord contusion. *J Neurosci Methods* 6:327-333.
- Ballermann M, Tse AD, Misiaszek JE, Fouad K. 2006. Adaptations in the walking pattern of spinal cord injured rats. *J Neurotrauma* 23:897-907.
- Bao F, DeWitt DS, Prough DS, Liu D. 2003. Peroxynitrite generated in the rat spinal cord induces oxidation and nitration of proteins: Reduction by Mn (III) tetrakis (4-benzoic acid) porphyrin. *J Neurosci Res* 71:220-227.
- Bao F, Liu D. 2002. Peroxynitrite generated in the rat spinal cord induces neuron death and neurological deficits. *Neuroscience* 115:839-849.
- Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME. 2004. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat Neurosci* 7:269-277.
- Basso DM. 2004. Behavioral testing after spinal cord injury: Congruities, complexities, and controversies. *J Neurotrauma* 21:395-404.
- Basso DM, Beattie MS, Bresnahan JC. 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 12:1-21.
- Basso DM, Beattie MS, Bresnahan JC. 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol* 139:244-256.
- Beaumont E, Onifer SM, Reed WR, Magnuson DS. 2006. Magnetically evoked inter-enlargement response: An assessment of ascending propriospinal fibers following spinal cord injury. *Exp Neurol* 201:428-440.
- Behrmann DL, Bresnahan JC, Beattie MS, Shah BR. 1992. Spinal cord injury produced by consistent mechanical displacement of the cord in rats: Behavioral and histologic analysis. *J Neurotrauma* 9:197-217.
- Benton RL, Woock JP, Gozal E, Hetman M, Whittemore SR. 2005. Intra-spinal application of endothelin results in focal ischemic injury of spinal gray matter and restricts the differentiation of engrafted neural stem cells. *Neurochem Res* 30:809-823.
- Black JH 3rd, Cambria RP. 2006. Contemporary results of open surgical repair of descending thoracic aortic aneurysms. *Semin Vasc Surg* 19:11-17.
- Blakemore WF, Crang AJ. 1989. The relationship between type-1 astrocytes, Schwann cells and oligodendrocytes following transplantation of glial cell cultures into demyelinating lesions in the adult rat spinal cord. *J Neurocytol* 18:519-528.
- Blakemore WF, Patterson RC. 1978. Suppression of remyelination in the CNS by X-irradiation. *Acta Neuropathol (Berl)* 42:105-113.
- Blight AR. 1991. Morphometric analysis of a model of spinal cord injury in guinea pigs, with behavioral evidence of delayed secondary pathology. *J Neurol Sci* 103:156-171.
- Blight AR, Tuszynski MH. 2006. Clinical trials in spinal cord injury. *J Neurotrauma* 23:586-593.
- Bradbury EJ, McMahon SB. 2006. Spinal cord repair strategies: Why do they work? *Nature Reviews* 7:644-653.
- Bregman BS, Kunkel-Bagden E, Reier PJ, Dai HN, McAtee M, Gao D. 1993. Recovery of function after spinal cord injury: Mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. *Exp Neurol* 123:3-16.
- Bresnahan JC, Beattie MS, Todd FD, Noyes DH. 1987. A behavioral and anatomical analysis of spinal cord injury produced by a feedback controlled impaction device. *Exp Neurol* 95:548-570.
- Brodbeck AR, Stoodley MA. 2003. Post-traumatic syringomyelia: A review. *J Clin Neurosci* 10:401-408.
- Brotton JG, Nikolic Z, Suys S, Calancie B. 1996. Kinematic analysis of limb position during quadrupedal locomotion in rats. *J Neurotrauma* 13:409-416.
- Bunge MB, Holets VR, Bates ML, Clarke TS, Watson BD. 1994. Characterization of photochemically induced spinal cord injury in the rat by light and electron microscopy. *Exp Neurol* 127:76-93.

- Bunge RP, Puckett WR, Becerra JL, Marcillo A, Quencer RM. 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 cord compression with extensive focal demyelination. *Adv Neurol* 59: 75-89.
- Bunge RP, Puckett WR, Hiester ED. 1997. Observations on the pathology of several types of human spinal cord injury, with emphasis on the astrocyte response to penetrating injuries. *Adv Neurol* 72:305-315.
- Cameron AA, Smith GM, Randall DC, Brown DR, Rabchevsky AG. 2006. Genetic manipulation of intraspinal plasticity after spinal cord injury alters the severity of autonomic dysreflexia. *J Neurosci* 26:2923-2932.
- Cameron T, Prado R, Watson BD, Gonzalez-Carvajal M, Holets VR. 1990. Photochemically induced cystic lesion in the rat spinal cord. I. Behavioral and morphological analysis. *Exp Neurol* 109:214-223.
- Cao Q, Zhang YP, Iannotti C, DeVries WH, Xu XM, Shields CB, Whittemore SR. 2005. Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. *Exp Neurol* 191 Suppl 1:S3-S16.
- Casella GT, Marcillo A, Bunge MB, Wood PM. 2002. New vascular tissue rapidly replaces neural parenchyma and vessels destroyed by a contusion injury to the rat spinal cord. *Exp Neurol* 173:63-76.
- Chau D, Johns DG, Schramm LP. 2000. Ongoing and stimulus-evoked activity of sympathetically correlated neurons in the intermediate zone and dorsal horn of acutely spinalized rats. *J Neurophysiol* 83:2699-2707.
- Cheng H, Almstrom S, Gimenez-Llort L, Chang R, Ove Ogren S, Hoffer B, Olson L. 1997. Gait analysis of adult paraplegic rats after spinal cord repair. *Exp Neurol* 148:544-557.
- Cheng H, Cao Y, Olson L. 1996. Spinal cord repair in adult paraplegic rats: Partial restoration of hindlimb function. *Science* 273:510-513.
- Collazos-Castro JE, Lopez-Dolado E, Nieto-Sampedro M. 2006. Locomotor deficits and adaptive mechanisms after thoracic spinal cord contusion in the adult rat. *J Neurotrauma* 23:1-17.
- Collazos-Castro JE, Soto VM, Gutierrez-Davila M, Nieto-Sampedro M. 2005. Motoneuron loss associated with chronic locomotion impairments after spinal cord contusion in the rat. *J Neurotrauma* 22:544-558.
- Courtine G, Bunge MB, Fawcett JW, Grossman RG, Kaas JH, Lemon R, Maier I, Martin J, Nudo RJ, Ramon-Cueto A, Rouiller EM, Schnell L, Wannier T, Schwab ME, Edgerton VR. 2007. Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? *Nat Med* 13:561-566.
- David S, Aguayo AJ. 1981. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science* 214:931-933.
- Dohrmann GJ, Panjabi MM. 1976. "Standardized" spinal cord trauma: Biomechanical parameters and lesion volume. *Surg Neurol* 6:263-267.
- Dohrmann GJ, Panjabi MM, Banks D. 1978. Biomechanics of experimental spinal cord trauma. *J Neurosurg* 48:993-1001.
- Dora CD, Koch S, Sanchez A, Ruenes G, Liu S, Yezierski RP. 1998. Intraspinal injection of adenosine agonists protect against L-NAME induced neuronal loss in the rat. *J Neurotrauma* 15:473-483.
- Fishback AS, Shields CB, Linden RD, Zhang YP, Burke D. 1995. The effects of propofol on rat transcranial magnetic motor evoked potentials. *Neurosurgery* 37:969-974.
- Fleming JC, Norenberg MD, Ramsay DA, Dekaban GA, Marcillo AE, Saenz AD, Pasquale-Styles M, Dietrich WD, Weaver LC. 2006. The cellular inflammatory response in human spinal cords after injury. *Brain* 129:3249-3269.
- Gale K, Kerasidis H, Wrathall JR. 1985. Spinal cord contusion in the rat: Behavioral analysis of functional neurologic impairment. *Exp Neurol* 88:123-134.
- Garcia-Alias G, Verdu E, Fores J, Lopez-Vales R, Navarro X. 2003. Functional and electrophysiological characterization of photochemical graded spinal cord injury in the rat. *J Neurotrauma* 20:501-510.
- Gensel JC, Tovar CA, Hamers FP, Deibert RJ, Beattie MS, Bresnahan JC. 2006. Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. *J Neurotrauma* 23:36-54.
- Gerber AM, Corrie WS. 1979. Effect of impounder contact area on experimental spinal cord injury. *J Neurosurg* 51:539-542.
- Graca DL, Blakemore WF. 1986. Delayed remyelination in rat spinal cord following ethidium bromide injection. *Neuropathol Appl Neurobiol* 12:593-605.
- Gruner JA. 1992. A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma* 9:123-126; discussion 126-128.
- Gruner JA, Yee AK, Blight AR. 1996. Histological and functional evaluation of experimental spinal cord injury: Evidence of a stepwise response to graded compression. *Brain Res* 729:90-101.
- Guest JD, Hiester ED, Bunge RP. 2005. Demyelination and Schwann cell responses adjacent to injury epicenter cavities following chronic human spinal cord injury. *Exp Neurol* 192:384-393.
- Hadi B, Zhang YP, Burke DA, Shields CB, Magnuson DS. 2000. Lasting paraplegia caused by loss of lumbar spinal cord interneurons in rats: No direct correlation with motor neuron loss. *J Neurosurg* 93:266-275.
- Haghighi SS, Agrawal SK, Surdell D Jr, Plambeck R, Agrawal S, Johnson GC, Walker A. 2000. Effects of methylprednisolone and MK-801 on functional recovery after experimental chronic spinal cord injury. *Spinal Cord* 38:733-740.
- Hall ED. 2001. Pharmacological treatment of acute spinal cord injury: How do we build on past success? *J Spinal Cord Med* 24:142-146.
- Hamers FP, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispen WH. 2001. Automated quantitative gait analysis during overground locomotion in the rat: Its application to spinal cord contusion and transection injuries. *J Neurotrauma* 18:187-201.
- Hao JX, Xu XJ, Aldskogius H, Seiger A, Wiesenfeld-Hallin Z. 1991. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain* 45:175-185.
- Hendriks WT, Eggers R, Ruitenber MJ, Blits B, Hamers FP, Verhaagen J, Boe GJ. 2006. Profound differences in spontaneous long-term functional recovery after defined spinal tract lesions in the rat. *J Neurotrauma* 23:18-35.
- Hermann GE, Rogers RC, Bresnahan JC, Beattie MS. 2001. Tumor necrosis factor-alpha induces cFOS and strongly potentiates glutamate-mediated cell death in the rat spinal cord. *Neurobiol Dis* 8:590-599.
- Hokfelt T, Post C, Freedman J, Lundberg JM, Terenius L. 1989. Endothelin induces spinal lesions after intrathecal administration. *Acta Physiol Scand* 137:555-556.
- Iannotti C, Li H, Yan P, Lu X, Wirthlin L, Xu XM. 2003. Glial cell line-derived neurotrophic factor-enriched bridging transplants promote propriospinal axonal regeneration and enhance myelination after spinal cord injury. *Exp Neurol* 183:379-393.
- Ikonomidou C, Qin Y, Labruyere J, Olney JW. 1996. Motor neuron degeneration induced by excitotoxin agonists has features in common with those seen in the SOD-1 transgenic mouse model of amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 55:211-224.
- Jakeman LB, Reier PJ. 1991. Axonal projections between fetal spinal cord transplants and the adult rat spinal cord: A neuroanatomical tracing study of local interactions. *J Comp Neurol* 307:311-334.
- Jasmin L, Janni G, Moallem TM, Lappi DA, Ohara PT. 2000. Schwann cells are removed from the spinal cord after effecting recovery from paraplegia. *J Neurosci* 20:9215-9223.
- Jeffery ND, Blakemore WF. 1997. Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination. *Brain* 120 (Pt 1):27-37.
- Kaegi S, Schwab ME, Dietz V, Fouad K. 2002. Electromyographic activity associated with spontaneous functional recovery after spinal cord injury in rats. *Eur J Neurosci* 16:249-258.
- Kalyvas A, David S. 2004. Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. *Neuron* 41:323-335.
- Karlsson AK. 1999. Autonomic dysreflexia. *Spinal Cord* 37:383-391.
- Kashiwaguchi S, Masaki K, Ikata T. 1989. Experimental studies on permeability of tracers into the spinal cord. *Paraplegia* 27:372-381.
- Keane RW, Davis AR, Dietrich WD. 2006. Inflammatory and apoptotic signaling after spinal cord injury. *J Neurotrauma* 23:335-344.
- Kearney PA, Ridella SA, Viano DC, Anderson TE. 1988. Interaction of

- contact velocity and cord compression in determining the severity of spinal cord injury. *J Neurotrauma* 5:187-208.
- Keirstead HS, Blakemore WF. 1997. Identification of post-mitotic oligodendrocytes incapable of remyelination within the demyelinated adult spinal cord. *J Neuropathol Exp Neurol* 56:1191-1201.
- Kesslak JP, Keirstead HS. 2003. Assessment of behavior in animal models of spinal cord injury. *J Spinal Cord Med* 26:323-328.
- Khan M, Griebel R. 1983. Acute spinal cord injury in the rat: Comparison of three experimental techniques. *Can J Neurol Sci* 10:161-165.
- Khan T, Havey RM, Sayers ST, Patwardhan A, King WW. 1999. Animal models of spinal cord contusion injuries. *Lab Anim Sci* 49:161-172.
- Kim D, Schallert T, Liu Y, Browaruk T, Nayeri N, Tessler A, Fischer I, Murray M. 2001. Transplantation of genetically modified fibroblasts expressing BDNF in adult rats with a subtotal hemisection improves specific motor and sensory functions. *Neurorehabil Neural Repair* 15: 141-150.
- Kleitman N. 2004. Keeping promises: Translating basic research into new spinal cord injury therapies. *J Spinal Cord Med* 27:311-318.
- Koozekanani SH, Vise WM, Hashemi RM, McGhee RB. 1976. Possible mechanisms for observed pathophysiological variability in experimental spinal cord injury by the method of Allen. *J Neurosurg* 44:429-434.
- Krassioukov AV, Johns DG, Schramm LP. 2002. Sensitivity of sympathetically correlated spinal interneurons, renal sympathetic nerve activity, and arterial pressure to somatic and visceral stimuli after chronic spinal injury. *J Neurotrauma* 19:1521-1529.
- Krassioukov AV, Weaver LC. 1995. Episodic hypertension due to autonomic dysreflexia in acute and chronic spinal cord-injured rats. *Am J Physiol* 268:H2077-H2083.
- Kunkel-Bagden E, Dai HN, Bregman BS. 1993. Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. *Exp Neurol* 119:153-164.
- Lee GY, Jones NR, Mayrhofer G, Brown C, Cleland L. 2005. Origin of macrophages in a kaolin-induced model of rat syringomyelia: A study using radiation bone marrow chimeras. *Spine* 30:194-200.
- Li Y, Decherchi P, Raisman G. 2003. Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. *J Neurosci* 23:727-731.
- Linden RD, Zhang YP, Burke DA, Hunt MA, Harpring JE, Shields CB. 1999. Magnetic motor evoked potential monitoring in the rat. *J Neurosurg* 91:205-210.
- Liu D. 1993. Generation and detection of hydroxyl radical in vivo in rat spinal cord by microdialysis administration of Fenton's reagents and microdialysis sampling. *J Biochem Biophys Methods* 27:281-291.
- Liu D. 1994. An experimental model combining microdialysis with electrophysiology, histology, and neurochemistry for studying excitotoxicity in spinal cord injury: Effect of NMDA and kainate. *Mol Chem Neuropathol* 23:77-92.
- Liu D, Thangnipon W, McAdoo DJ. 1991. Excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord. *Brain Res* 547: 344-348.
- Liu D, Xu GY, Pan E, McAdoo DJ. 1999a. Neurotoxicity of glutamate at the concentration released upon spinal cord injury. *Neuroscience* 93: 1383-1389.
- Liu D, Yang J, Li L, McAdoo DJ. 1995. Paraquat—a superoxide generator—kills neurons in the rat spinal cord. *Free Radic Biol Med* 18:861-867.
- Liu NK, Zhang YP, Titsworth WL, Jiang X, Han S, Lu PH, Shields CB, Xu XM. 2006. A novel role of phospholipase A2 in mediating spinal cord secondary injury. *Ann Neurol* 59:606-619.
- Liu Y, Kim D, Himes BT, Chow SY, Schallert T, Murray M, Tessler A, Fischer I. 1999b. Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. *J Neurosci* 19:4370-4387.
- Loy DN, Crawford CH, Darnall JB, Burke DA, Onifer SM, Whittemore SR. 2002a. Temporal progression of angiogenesis and basal lamina deposition after contusive spinal cord injury in the adult rat. *J Comp Neurol* 445:308-324.
- Loy DN, Magnuson DS, Zhang YP, Onifer SM, Mills MD, Cao QL, Darnall JB, Fajardo LC, Burke DA, Whittemore SR. 2002b. Functional redundancy of ventral spinal locomotor pathways. *J Neurosci* 22:315-323.
- Loy DN, Talbott JF, Onifer SM, Mills MD, Burke DA, Dennison JB, Fajardo LC, Magnuson DS, Whittemore SR. 2002c. Both dorsal and ventral spinal cord pathways contribute to overground locomotion in the adult rat. *Exp Neurol* 177:575-580.
- Magnuson DS, Lovett R, Coffee C, Gray R, Han Y, Zhang YP, Burke DA. 2005. Functional consequences of lumbar spinal cord contusion injuries in the adult rat. *J Neurotrauma* 22:529-543.
- Magnuson DS, Trinder TC, Zhang YP, Burke D, Morassutti DJ, Shields CB. 1999. Comparing deficits following excitotoxic and contusion injuries in the thoracic and lumbar spinal cord of the adult rat. *Exp Neurol* 156:191-204.
- Massey JM, Hubscher CH, Wagoner MR, Decker JA, Aups J, Silver J, Onifer SM. 2006. Chondroitinase ABC digestion of the perineuronal net promotes functional collateral sprouting in the cuneate nucleus after cervical spinal cord injury. *J Neurosci* 26:4406-4414.
- McTigue DM, Popovich PG, Jakeman LB, Stokes BT. 2000. Strategies for spinal cord injury repair. *Prog Brain Res* 128:3-8.
- Metz GA, Curt A, van de Meent H, Klusman I, Schwab ME, 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.
- Milhorat TH, Nobandegani F, Miller JJ, Rao C. 1993. Noncommunicating syringomyelia following occlusion of central canal in rats: Experimental model and histological findings. *J Neurosurg* 78:274-279.
- Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. 1991. The "staircase test": A measure of independent forelimb reaching and grasping abilities in rats. *J Neurosci Methods* 36:219-228.
- Narayana P, Abbe R, Liu SJ, Johnston D. 1999. Does loss of gray- and white-matter contrast in injured spinal cord signify secondary injury? In vivo longitudinal MRI studies. *Magn Reson Med* 41:315-320.
- Nash HH, Borke RC, Anders JJ. 2002. Ensheathing cells and methylprednisolone promote axonal regeneration and functional recovery in the lesioned adult rat spinal cord. *J Neurosci* 22:7111-120.
- Nashmi R, Imamura H, Tator CH, Fehlings MG. 1997. Serial recording of somatosensory and myoelectric motor evoked potentials: Role in assessing functional recovery after graded spinal cord injury in the rat. *J Neurotrauma* 14:151-159.
- Nichols CM, Myckatyn TM, Rickman SR, Fox IK, Hadlock T, Mackinnon SE. 2005. Choosing the correct functional assay: A comprehensive assessment of functional tests in the rat. *Behav Brain Res* 163:143-158.
- Norenberg MD, Smith J, Marcillo A. 2004. The pathology of human spinal cord injury: Defining the problems. *J Neurotrauma* 21:429-440.
- Nothias F, Peschanski M. 1990. Homotypic fetal transplants into an experimental model of spinal cord neurodegeneration. *J Comp Neurol* 301:520-534.
- Nottingham SA, Springer JE. 2003. Temporal and spatial distribution of activated caspase-3 after subdural kainic acid infusions in rat spinal cord. *J Comp Neurol* 464:463-471.
- Noyes DH. 1987. Electromechanical impactor for producing experimental spinal cord injury in animals. *Med Biol Eng Comput* 25:335-340.
- Olby NJ, Blakemore WF. 1996. Reconstruction of the glial environment of a photochemically induced lesion in the rat spinal cord by transplantation of mixed glial cells. *J Neurocytol* 25:481-498.
- Onifer SM, Cannon AB, Whittemore SR. 1997a. Altered differentiation of CNS neural progenitor cells after transplantation into the injured adult rat spinal cord. *Cell Transplant* 6:327-338.
- Onifer SM, Rodriguez JF, Santiago DI, Benitez JC, Kim DT, Brunschwig J-P, Pacheco JT, Perrone JV, Llorente O, Martinez-Arizala A. 1997b. Cervical spinal cord injury in the adult rat: Assessment of forelimb dysfunction. *Restor Neurol Neurosci* 11:211-223.
- Onifer SM, Zhang YP, Burke DA, Brooks DL, Decker JA, McClure NJ, Floyd AR, Hall J, Proffitt BL, Shields CB, Magnuson DS. 2005. Adult rat forelimb dysfunction after dorsal cervical spinal cord injury. *Exp Neurol* 192:25-38.
- Osborn JW, Taylor RF, Schramm LP. 1989. Determinants of arterial pressure after chronic spinal transection in rats. *Am J Physiol* 256:R666-R673.

- Osborn JW, Taylor RF, Schramm LP. 1990. Chronic cervical spinal cord injury and autonomic hyperreflexia in rats. *Am J Physiol* 258:R169-R174.
- Panjabi MM, Wrathall JR. 1988. Biomechanical analysis of experimental spinal cord injury and functional loss. *Spine* 13:1365-1370.
- Park E, Velumian AA, Fehlings MG. 2004. The role of excitotoxicity in secondary mechanisms of spinal cord injury: A review with an emphasis on the implications for white matter degeneration. *J Neurotrauma* 21:754-774.
- Pearse DD, Lo TP, Jr., Cho KS, Lynch MP, Garg MS, Marcillo AE, Sanchez AR, Cruz Y, Dietrich WD. 2005. Histopathological and behavioral characterization of a novel cervical spinal cord displacement contusion injury in the rat. *J Neurotrauma* 22:680-702.
- Perez-Bouza A, Glaser T, Brustle O. 2005. ES cell-derived glial precursors contribute to remyelination in acutely demyelinated spinal cord lesions. *Brain Pathol* 15:208-216.
- Pisharodi M, Nauta HJ. 1985. An animal model for neuron-specific spinal cord lesions by the microinjection of N-methylaspartate, kainic acid, and quisqualic acid. *Appl Neurophysiol* 48:226-233.
- Popovich PG, Guan Z, McGaughy V, Fisher L, Hickey WF, Basso DM. 2002. The neuropathological and behavioral consequences of intraspinal microglial/macrophage activation. *J Neuropathol Exp Neurol* 61: 623-633.
- Prado R, Dietrich WD, Watson BD, Ginsberg MD, Green BA. 1987. Photochemically induced graded spinal cord infarction: Behavioral, electrophysiological, and morphological correlates. *J Neurosurg* 67:745-753.
- Rabchevsky AG. 2006. Segmental organization of spinal reflexes mediating autonomic dysreflexia after spinal cord injury. *Prog Brain Res* 152:265-274.
- Rabchevsky AG, Smith GM. 2001. Therapeutic interventions following mammalian spinal cord injury. *Arch Neurol* 58:721-726.
- Rabchevsky AG, Sullivan PG, Fugaccia I, Scheff SW. 2003. Creatine diet supplement for spinal cord injury: Influences on functional recovery and tissue sparing in rats. *J Neurotrauma* 20:659-669.
- Reier PJ, Bregman BS, Wujek JR. 1986. Intraspinal transplantation of embryonic spinal cord tissue in neonatal and adult rats. *J Comp Neurol* 247:275-296.
- Rivlin AS, Tator CH. 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. *J Neurosurg* 47: 577-581.
- Rivlin AS, Tator CH. 1978. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. *Surg Neurol* 10:38-43.
- Salzman SK, Acosta R, Beck G, Madden J, Boxer B, Ohlstein EH. 1996. Spinal endothelin content is elevated after moderate local trauma in the rat to levels associated with locomotor dysfunction after intrathecal injection. *J Neurotrauma* 13:93-101.
- Santos-Benito FF, Muñoz-Quiles C, Ramón-Cueto A. 2006. Long-term care of paraplegic laboratory mammals. *J Neurotrauma* 23:521-536.
- Scheff SW, Rabchevsky AG, Fugaccia I, Main JA, Lumpp JE Jr. 2003. Experimental modeling of spinal cord injury: Characterization of a force-defined injury device. *J Neurotrauma* 20:179-193.
- Schrimsher GW, Reier PJ. 1992. Forelimb motor performance following cervical spinal cord contusion injury in the rat. *Exp Neurol* 117:287-298.
- Schrimsher GW, Reier PJ. 1993. Forelimb motor performance following dorsal column, dorsolateral funiculi, or ventrolateral funiculi lesions of the cervical spinal cord in the rat. *Exp Neurol* 120:264-276.
- Schucht P, Raineteau O, Schwab ME, Fouad K. 2002. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. *Exp Neurol* 176:143-153.
- Schwartz ED, Yezierski RP, Pattany PM, Quencer RM, Weaver RG. 1999. Diffusion-weighted MR imaging in a rat model of syringomyelia after excitotoxic spinal cord injury. *Am J Neuroradiol* 20:1422-1428.
- Soblosky JS, Song JH, Dinh DH. 2001. Graded unilateral cervical spinal cord injury in the rat: Evaluation of forelimb recovery and histological effects. *Behav Brain Res* 119:1-13.
- Somerson SK, Stokes BT. 1987. Functional analysis of an electromechanical spinal cord injury device. *Exp Neurol* 96:82-96.
- Spinal Cord Injury Information Network. 2006. <http://www.spinalcord.uab.edu/>.
- Stoodley MA, Gutschmidt B, Jones NR. 1999. Cerebrospinal fluid flow in an animal model of noncommunicating syringomyelia. *Neurosurgery* 44:1065-1075; discussion 1075-1076.
- Sun H, Kawahara Y, Ito K, Kanazawa I, Kwak S. 2006. Slow and selective death of spinal motor neurons in vivo by intrathecal infusion of kainic acid: Implications for AMPA receptor-mediated excitotoxicity in ALS. *J Neurochem* 98:782-791.
- Tarlov IM, Klinger H. 1954. Spinal cord compression studies. II. Time limits for recovery after acute compression in dogs. *AMA Arch Neurol Psychiatry* 71:271-290.
- Tatara N. 1992. Experimental syringomyelia in rabbits and rats after localized spinal arachnoiditis. *No To Shinkei* 44:1115-1125.
- Tator CH. 2006. Review of treatment trials in human spinal cord injury: Issues, difficulties, and recommendations. *Neurosurgery* 59:957-982; discussion 982-987.
- Tator CH, Fehlings MG. 1991. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* 75:15-26.
- Thota AK, Watson SC, Knapp E, Thompson B, Jung R. 2005. Neuromechanical control of locomotion in the rat. *J Neurotrauma* 22:442-465.
- Thuret S, Moon LD, Gage FH. 2006. Therapeutic interventions after spinal cord injury. *Nature Reviews* 7:628-643.
- Verdu E, Garcia-Alias G, Fores J, Vela JM, Cuadras J, Lopez-Vales R, Navarro X. 2003. Morphological characterization of photochemically graded spinal cord injury in the rat. *J Neurotrauma* 20:483-499.
- Voda J, Yamaji T, Gold BG. 2005. Neuroimmunophilin ligands improve functional recovery and increase axonal growth after spinal cord hemisection in rats. *J Neurotrauma* 22:1150-1161.
- von Euler M, Seiger A, Sundstrom E. 1997a. Clip compression injury in the spinal cord: A correlative study of neurological and morphological alterations. *Exp Neurol* 145:502-510.
- von Euler M, Sundstrom E, Seiger A. 1997b. Morphological characterization of the evolving rat spinal cord injury after photochemically induced ischemia. *Acta Neuropathol (Berl)* 94:232-239.
- Watson BD, Prado R, Dietrich WD, Ginsberg MD, Green BA. 1986. Photochemically induced spinal cord injury in the rat. *Brain Res* 367:296-300.
- Webb AA, Muir GD. 2002. Compensatory locomotor adjustments of rats with cervical or thoracic spinal cord hemisections. *J Neurotrauma* 19: 239-256.
- Webb AA, Muir GD. 2003. Unilateral dorsal column and rubrospinal tract injuries affect overground locomotion in the unrestrained rat. *Eur J Neurosci* 18:412-422.
- Webb AA, Muir GD. 2004. Course of motor recovery following ventrolateral spinal cord injury in the rat. *Behav Brain Res* 155:55-65.
- Webb AA, Muir GD. 2005. Sensorimotor behaviour following incomplete cervical spinal cord injury in the rat. *Behav Brain Res* 165:147-159.
- Weidner N, Ner A, Salimi N, Tuszyński MH. 2001. Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. *Proc Natl Acad Sci U S A* 98:3513-3518.
- Westmark R, Noble LJ, Fukuda K, Aihara N, McKenzie AL. 1995. Intrathecal administration of endothelin-1 in the rat: Impact on spinal cord blood flow and the blood-spinal cord barrier. *Neurosci Lett* 192:173-176.
- Wiesenfeld-Hallin Z, Hao JX, Xu XJ, Aldskogius H, Seiger A. 1993. Genetic factors influence the development of mechanical hypersensitivity, motor deficits and morphological damage after transient spinal cord ischemia in the rat. *Pain* 55:235-241.
- Wirth ED 3rd, Reier PJ, Fessler RG, Thompson FJ, Uthman B, Behrman A, Beard J, Vierck CJ, Anderson DK. 2001. Feasibility and safety of neural tissue transplantation in patients with syringomyelia. *J Neurotrauma* 18:911-929.
- Wrathall JR, Pettegrew RK, Harvey F. 1985. Spinal cord contusion in the

- rat: Production of graded, reproducible, injury groups. *Exp Neurol* 88:108-122.
- Xu GY, Hughes MG, Ye Z, Hulsebosch CE, McAdoo DJ. 2004. Concentrations of glutamate released following spinal cord injury kill oligodendrocytes in the spinal cord. *Exp Neurol* 187:329-336.
- Xu XM, Chen A, Guenard V, Kleitman N, Bunge MB. 1997. Bridging Schwann cell transplants promote axonal regeneration from both the rostral and caudal stumps of transected adult rat spinal cord. *J Neurocytol* 26:1-16.
- Xu XM, Guenard V, Kleitman N, Bunge MB. 1995. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. *J Comp Neurol* 351:145-160.
- Xu XM, Zhang SX, Li H, Aebischer P, Bunge MB. 1999. Regrowth of axons into the distal spinal cord through a Schwann-cell-seeded minichannel implanted into hemisectioned adult rat spinal cord. *Eur J Neurosci* 11:1723-1740.
- Yajima K, Suzuki K. 1979. Demyelination and remyelination in the rat central nervous system following ethidium bromide injection. *Lab Invest* 41:385-392.
- Yang L, Jones NR, Stoodley MA, Blumbergs PC, Brown CJ. 2001. Excitotoxic model of post-traumatic syringomyelia in the rat. *Spine* 26:1842-1849.
- Yeziarski RP, Liu S, Ruenes GL, Busto R, Dietrich WD. 1996. Neuronal damage following intraspinal injection of a nitric oxide synthase inhibitor in the rat. *J Cereb Blood Flow Metab* 16:996-1004.
- Yeziarski RP, Santana M, Park SH, Madsen PW. 1993. Neuronal degeneration and spinal cavitation following intraspinal injections of quisqualic acid in the rat. *J Neurotrauma* 10:445-456.
- Zhang P, Abraham VS, Kraft KR, Rabchevsky AG, Scheff SW, Swain JA. 2000. Hyperthermic preconditioning protects against spinal cord ischemic injury. *Ann Thorac Surg* 70:1490-1495.