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# Assessing Forelimb Function after Unilateral Cervical Spinal Cord Injury: Novel Forelimb Tasks Predict Lesion Severity and Recovery

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## Abstract

Cervical spinal cord injury (cSCI) can cause devastating neurological deficits, including impairment or loss of upper limb and hand function. Recently there has been increasing interest in cervical spinal cord injury models because the majority of spinal cord injuries are at cervical levels. Here we examined spontaneous functional recovery of adult rats with either laminectomy or lateral hemisection of the cervical spinal cord at C3–C4. Behavioral tests were carried out, including the forelimb locomotor scale (FLS), a postural instability test (PIT), a pasta-handling test that has been used to assess forepaw digit function and latency to eat, forelimb use during vertical-lateral wall exploration in a cylindrical enclosure, and vibrissae-elicited forelimb placing tests. In addition, a forelimb step-alternation test was developed to assess functional recovery at 12 weeks post-injury. All tests detected cSCI-induced deficits relative to laminectomy. Interestingly, the severity of deficits in the forelimb step-alternation test was associated with more extensive spinal damage, greater impairment, and less recovery in the FLS and other tests. For the pasta-handling test we found that rats with a milder cervical injury (alternators) were more likely to use both forepaws together compared to rats with a more severe injury (non-alternators). In addition, using the PIT, we detected enhanced function of the good limb, suggesting that neural plasticity on the unaffected side of the spinal cord may have occurred to compensate for deficits in the impaired forelimb. These outcome measures should be useful for investigating neural events associated with cSCI, and for developing novel treatment strategies.

**Key words:** cervical spinal cord injury; limb step-alternation test; novel behavioral analyses; pasta-eating test

## Introduction

Injury to the spinal cord, especially at cervical levels (cSCI), can lead to devastating impairments, and yet to date, there is no reliable clinical treatment. In humans, cSCI, including complete and incomplete tetraplegia, represents about 62% of all spinal cord injuries (http://www.spinalcord.uab.edu). This type of injury can cause severe impairments, including use of the upper extremities. Regaining partial or full function of the arm and/or hand could make significant improvements in patient quality of life, and is considered to be a priority for patients with cervical cord injuries (Anderson, 2004). Recently, there has been increasing interest in developing cSCI models in rodents; hence, having sensitive and reliable methods to evaluate forelimb motor functions is of potential importance.

High cervical unilateral hemisection injuries in humans result in the Brown-Sequard syndrome (Brown-Sequard, 1868), in which one-half of the spinal cord is completely injured, and limbs on the contralesional side have virtually intact motor function, but are usually overused (Tattersall and Turner, 2000). Remarkably, the limbs on the contralesional side are insensitive to pain and temperature due to the interruption in the crossed spinothalamic tract, while the limbs of the ipsilesional side retain their perception of pain and temperature (Herr and Barrett, 1987). This type of modification is also seen after a complete hemisection in experimental rodent models of cSCI, and the associated adaptations to the sensorimotor cortex have recently been described (Ghosh et al., 2009). These adaptations include corticospinal axon sprouting across the midline, and new and altered sensory cortex representation areas.

A number of behavioral tests to assess forelimb function after cSCI in rodents have been developed. The forelimb locomotor scale (FLS) was described by Cao and colleagues

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and used with a lateral funicular lesion at the C3–C4 level (Cao et al., 2008). It is an open-field locomotor test, and a modification of the Basso-Beattie-Bresnahan (BBB) scale developed previously (Basso et al., 1995), with scores ranging from 0 (no function) to 17 (no deficit). Martinez and colleagues also used a general locomotor test to evaluate forelimb and hindlimb function after varying degrees of injury to the cervical spinal cord (Martinez et al., 2009). More recently, another forelimb locomotor assessment scale (FLAS) was used to evaluate forelimb function after bilateral contusion injury of the cervical spinal cord (Anderson et al., 2009). In addition to general locomotor assessment during open-field locomotion, tests have been developed to determine forelimb function in a task-specific manner. These tests include the grip strength meter test (Anderson et al., 2004,2005,2007), the food pellet reaching task (Ballermann et al., 2001b; Whishaw and Pellis, 1990), the cylinder forelimb usage test (Dai et al., 2009,2011; Gharbawie et al., 2004; Liu et al., 1999; Schallert et al., 2000), the grooming task (Gensel et al., 2006), the staircase test (Montoya et al., 1991), the horizontal ladder beam test (Metz and Whishaw, 2002; Soblosky et al., 2001), the forelimb inhibitory function during swimming task (Kim et al., 2001), and the sticker removal test (Schallert et al., 1982,2000; Schrimsher and Reier, 1992).

In this study, we sought to improve the range of behavioral tests that can be used to detect deficits after cSCI at levels 3 and 4 (C3–C4). Notably, we have developed a behavioral test (forelimb step-alternation test) that may prove to be reliable, sensitive, useful, and informative regarding injury extent and location after cSCI. Remarkably, the forelimb step-alternation test predicts the extent of deficits of alternators and non-alternators, and this was confirmed by results from other behavioral tests. Moreover, we detected enhanced function of the good limb, suggesting that plasticity may have occurred in the non-targeted contralateral side of the spinal cord, or in brain areas connected to this region, to compensate for ipsilateral impairments.

## Methods

### *Spinal cord surgery and recovery*

All animal procedures were done in accordance with National Institutes of Health (NIH) guidelines and were approved by the internal IACUC at The University of Texas at Austin. A rat lateral cSCI hemisection model was chosen for these studies. Adult female rats (Sprague-Dawley; Harlan, Indianapolis, IN), 6–8 weeks old, were anesthetized with isoflurane (3% to induce with 2% maintenance). There were a total of 31 animals used for this study: 9 sham, 15 lesioned alternators, and 7 lesioned non-alternators.

A dorsal laminectomy was performed at C3–C4 to expose the underlying spinal cord. In lesioned animals, the dura was broken using iridectomy scissors and fine forceps. A lateral hemisection was made using micro-scissors at cervical level C3–C4, removing a segment of the spinal cord about 2.5–3 mm in length. The lesion was extended ventrally using gentle aspiration. The wounds were closed in layers (muscle and overlying skin) using 5-0 absorbable sutures. The animals were then allowed to recover on a warming pad.

Pain associated with the surgery was managed with buprenorphine (0.03 mg/kg; Reckitt and Colman, Richmond, VA) administered subcutaneously immediately after surgery, and every 12 h for 2 days. The antibiotic cefazolin (5 mg/kg; Novopharm, Toronto, Ontario, Canada) was administered subcutaneously daily for 5 days starting 1 day before surgery, and repeated if signs of infection were detected. Ringer's solution (4 mL) was injected subcutaneously on the day of surgery and at later stages if the animals showed signs of dehydration. This type of injury to the cervical spinal cord at C3–C4 resulted in animals with limited or no use of their forelimb (or hindlimb immediately after injury) on the injured side. However, we have observed that the rats were able to access food and water readily. All animals were monitored three times daily for the first 5 days. Softened food (normal kibble soaked in water) was made available to them for 5 days post-injury. The animals were weighed each day and if they lost more than 10% of their pre-surgery body weight, dietary supplements such as DietGel (ClearH$_2$O, Portland, MA) or Nutri-Cal (EVSCO; Petco Animal Supplies, Austin, TX) were used to supplement their diet. This type of injury did not affect bladder function. Therefore routine bladder expressions were not necessary. However, voluntary voiding was monitored for the first 3 days.

### *Behavioral tests*

A total of 2–3 weeks of handling and pre-testing was performed to acclimate the rats to being handled and to ensure that they exhibited normal behavior prior to surgery.

#### Forelimb locomotor scale.

Rats were filmed in a rectangular acrylic glass arena measuring 30×90×120 cm. Forelimb function was evaluated based on the FLS created to describe common deficits observed in rats with cervical injury (Sandrow et al., 2008). The 17-point scale describes deficits observed with body-weight support, forelimb range of motion during stepping, and paw position on stepping liftoff and landing. Each rat was scored live while recording a 2.5-min video of their motion within the arena. Each rat was independently rated by two experienced observers who were blinded to treatment group. The rats were examined 1 week prior to surgery, at 3 days post-surgery, and weekly thereafter.

#### Postural instability test (PIT).

Chronically-injured rats were examined at 12–16 weeks after cSCI. The rats were held in an almost vertical position with only their forelimbs in contact with the tabletop, in a "wheelbarrow" type position, while their hindlimbs were allowed to hang free. The tabletop was covered with sandpaper to prevent slipping, bracing, or dragging of the forelimbs during the test. The experimenter lined the rats' noses up with the zero line as observed from above. Each rat was moved by the experimenter to shift the rat's center of gravity forward over the forelimbs, stimulating the rat to take a step to regain balance. The new position of the nose upon stepping was measured as the distance needed to trigger a step. Each forelimb was tested independently by lightly restraining one forelimb during testing and holding it back gently. Five trials on each forelimb were performed.

#### Forelimb step-alternation test.

We have developed this new behavioral test, which is a variation on the PIT, which yields information regarding injury extent and location after cSCI. This test involves both forelimbs touching the tabletop initially in a comfortable stance, as determined by allowing the forelimbs to reach the table while the rat was held in an upright "wheelbarrow" position. Using this test, we determined whether the rats were alternators (alternate the use of their forelimbs, as normal rats do) or non-alternators (do not alternate the use of their forelimbs). While the experimenter moved the rat forward, first step paw preference was noted (left or right), and the rats were observed for their ability to alternate steps while being moved forward over their forepaws. If rats showed this ability, step-alternation was again tested after a 5-sec wait. The rats were moved forward, shifting their center of gravity until one step was taken, after which the experimenter held them in that position for 5 sec before continuing to move them forward over their forepaws. Some rats were observed to step with their alternate forelimb, whereas others used the same forelimb as was used for the initial step. The forelimb step-alternation test was performed five times per week on each rat. Chronically-injured rats were examined at 12–16 weeks after cSCI.

Vibrissae-elicited forelimb placing test. A vibrissae-elicited forelimb placing test was used to determine forelimb placing asymmetry, modified from a previous version (Schallert et al., 2000). The rats were held by their torsos, allowing their forelimbs and hindlimbs to hang free. The tail also was required to swing free (i.e., the score was not counted if the tail was used for support by wrapping around or touching the experimenter's arm). When instances of struggling stopped and muscle relaxation was achieved, the vibrissae on one side, and then the other, were stimulated by brushing them along a tabletop for 10 trials on each side. A score of 0–4 for each trial was given based on the targeted forelimb's placing response (ipsilateral versus contralateral). Intact animals typically place either forelimb on the tabletop and receive a score of 4 because of the accuracy of placement. A score of 0 was given when no response was seen. A score of 1 was given if the animal's limb showed movement, a score of 2 was given if the animal touched the table but not the top of the table, and a score of 3 was given if the animal touched the tabletop with non-normal paw position. Average scores were calculated based on treatment group and lesion size. The rats were examined 1 week prior to surgery, at 3 days post-surgery, and weekly thereafter.

Vibrissae-elicited cross-midline forelimb placing. The vibrissae-elicited cross-midline forelimb placing test was used to verify that any limb use impairment observed in the vibrissae-elicited same-side forelimb placing test reflected a deficit in motor function and not a lack of adequate sensory input from the ipsilesional vibrissae. Similarly to the same-side forelimb placing test, the animals were held by the torso while the forelimbs and hindlimbs were allowed to hang free, and the experimenter facilitated muscle relaxation. The rats were turned sideways, orienting the vibrissae perpendicular to the tabletop. The downward limb was gently restrained to prevent a same-side forelimb placing response while the vibrissae were brushed against the tabletop for 10 trials. Again the rats were scored on the 0–4 scale described above, and averages of the scores were determined based on treatment group and lesion size. Four variants of the vibrissae-elicited response test were performed, with ipsilesional and contralesional testing of same-side and cross-midline placing. As previously noted, visual information does not change scores, as no effect was observed when the rats' eyes were occluded by the experimenter during testing (Woodlee et al., 2005). The rats were examined 1 week prior to surgery, at 3 days post-surgery, and weekly thereafter.

Cylinder paw-use preference test. The rats were rated live while being filmed in a transparent cylinder (20 cm diameter × 30 cm height) for 20 steps, but at most up to 2 min (Schallert et al., 2000). A mirror was placed behind the cylinder at an angle to allow the rater to view the rats when they were on the opposite side of the cylinder from the camera. Three behaviors were scored during vertical exploration: (1) use of the left forelimb alone for weight bearing, (2) use of the right forelimb alone for weight bearing, and (3) use of both forelimbs simultaneously or rapidly alternating for weight bearing. In rare cases when the experimenters were unable to distinguish which limbs were used, simultaneously or independently, the movement was not scored. Behaviors were scored as percent use of each forelimb to total limb use, where half of the simultaneous use was calculated for each limb (100 * [independent + 1/2 simultaneous]/total). The rats were examined 1 week prior to surgery, at 3 days post-surgery, and weekly thereafter.

Pasta handling and eating. Rats were observed and timed while eating 3.7-cm strands of dry pasta (Allred et al., 2008; Tennant et al., 2010). Paw preference and time to eat the entire pasta strand were noted during a pasta-eating session. The test was administered at approximately the same time each testing day. Two weeks prior to injury rats were given the same type of pasta to prepare them for testing. The rats were observed and timed beginning 1 week prior to injury to observe improvement due to training. Rats were used for the experiment if their pasta-eating time was consistent for at least 3 days. Baseline measures were taken the day prior to injury. The animals were tested with three pasta pieces weekly following injury. Some rats ate pasta using only one paw, others used one paw above the other to guide the pasta into their mouths, and others used two paws at the same height.

### *Tissue harvest and lesion verification*

At the end of the experimental period, the animals were deeply anesthetized using an overdose of ketamine/xylazine. Trans-cardiac perfusion was performed using ice-cold PBS (pH 7.4, ~200 mL) followed by cold 4% paraformaldehyde (~300 mL). The spinal cords were removed, post-fixed in the same paraformaldehyde solution overnight at 4°C, and treated with 30% sucrose solution prior to freezing. Sections were obtained using a cryostat at 18 $\mu$m, thaw-mounted onto gelatin-coated glass slides, and stored at −80°C. Standard Nissl staining was performed on cross-sectioned tissue of each spinal cord at 500-$\mu$m intervals (~5 mm long cervical segment) to determine the extent of the lesion as well as the location. In all cases, the right half of the cervical spinal cord was removed completely. However, in some cases the dorsal portion of the intact side was affected by the lesion, resulting in a more severe bilateral lesion. To quantify this, we developed a scoring system that reflects the level of damage to the intended intact side of the spinal cord (0 = no damage, 1 = moderately affected, 2 = highly affected).

### Corticospinal tract tracing and analysis

To label the descending corticospinal tract (CST), 10% biodextran amine (BDA, 10,000 MW; Invitrogen, Carlsbad, CA) anterograde tracer was injected into the somatosensory cortex. Multiple microinjections, as described previously (Anderson et al., 2007), were made into the somatosensory cortex 1.5 and 2.5 mm lateral from the midline, 1 mm anterior to the bregma, at the bregma, and 1 and 2 mm posterior to the bregma at a depth of 1 mm from the cortical surface. Using a pulled micropipette with an outer diameter of 50–100 $\mu$m, attached to a 10-$\mu$L Hamilton microsyringe, 250 nL of 10% BDA was injected into each site on the contralesional cortex, and a total of eight injection sites were used for each animal.

Animals ($n = 13$; 3 sham, 5 lesioned alternators, 5 lesioned non-alternators) received BDA injections at 8 weeks post-injury into the cortex contralateral to the spinal cord section at C3–C4. This was used to determine the damage to the CST on the ipsilesional side. Animals were anesthetized as described above. The hair on the scalp was shaved, cleaned, and cut using a scalpel. First the location of bregma was identified, and using aseptic technique, small holes were drilled in the skull using a dental drill at the appropriate locations. After each injection, flow-back of dye was prevented by waiting 3 min before retracting the glass pipette. After completion of all eight injections, the scalp was sutured and cleaned. The animals were allowed to recover completely from anesthesia on a warming pad before returning them to their home cage. Pain associated with the tracer injection surgery was alleviated using carprofen (5 mg/kg) for at least 2 days. Behavioral tests were not performed for 3 weeks post-BDA injections. The animals were allowed to survive for at least 3 weeks before perfusion.

Tissue sections from all spinal cords with BDA injections were examined at 200-$\mu$m intervals. We detected BDA-labeled axons by washing the samples 3 times with 0.3% Triton-X 100 in PBS for 5 min each, then incubating the samples with streptavidin Cy3 (1:500; Invitrogen) in buffer containing 0.1% Triton-X 100 overnight at 4°C. The samples were washed thoroughly with PBS the next day and were counter-stained using 4′,6-diamidino-2-phenylindole (DAPI, 1:1000; Invitrogen) to label all nuclei.

We next examined the spared CST after the lateral hemisection injury to the cervical cord. Cy3-labeled sections at the cervical cord posterior to the cut were examined. The labeled axons within the cross-sectioned spinal cord were counted in two sections (200 $\mu$m apart) caudal to the lesion.

### Statistical analysis

All behavioral data were analyzed by two-way analysis of variance (ANOVA) between groups (lesioned and non-lesioned) and time after injury, with the exception of the data obtained from the limb alternation task and the pasta-eating test. *Post-hoc* analyses were performed when appropriate using the Mann-Whitney *U* test (FLS), or the Bonferroni test (PIT, vibrissae-induced placing, and cylinder preference test). All descriptive statistics were reported as mean ± standard error of the mean (SEM).

Histological and anatomical data were also analyzed using a one-way ANOVA (lesion status for the number of BDA-labeled axons, and alternation status for average lesion extent score). In addition, Spearman's rank-correlation test was used to determine correlation between anatomical score (CST and DC damage score) and behavior (FLS and forelimb placing) of all lesioned animals. All statistical analyses were performed using SPSS software (PASW Statistics 18.0.0.).

## Results

### Forelimb locomotor scale

To determine the general ability of the animals to use their forelimbs, we used a previously described FLS 1 week prior to surgery, 3 days post-surgery, and weekly thereafter for 12 weeks (Cao et al., 2008; Sandrow et al., 2008). The resulting FLS scores indicated that there was a significant effect of lesion [$F_{(1,1)} = 16.61$, $p < 0.002$] and of time [$F_{(1,7)} = 6.71$, $p < 0.0001$]. In addition, there was a significant lesion × time interaction [$F_{(1,7)} = 6.78$, $p < 0.0001$]. In *post-hoc* analysis, animals with cervical SCI had significant impairment at 3 days post-injury ($p < 0.0001$) compared to animals with sham surgery (Fig. 1). SCI animals showed rapid improvement in their FLS score in the next 2 weeks and up to 8 weeks post-injury, at which point they had recovered significantly from 3 days post-injury ($p < 0.0001$; Fig. 1).

### Postural instability test

Unilateral impairments of a limb can cause changes not only to the impaired limb, but also to the non-impaired limb. To determine the function of the forelimbs after unilateral hemisection of the cervical spinal cord, we performed the postural instability test (PIT, Fig. 2), as previously described (Woodlee et al., 2008). There was a significant effect of lesion status [$F_{(1,1)} = 8.17$, $p < 0.01$], and in the ipsilesional side (right forelimb), injured animals had significantly larger forelimb displacement (distance to regain center of gravity) compared to animals with sham surgery (6.00 ± 0.24 cm versus

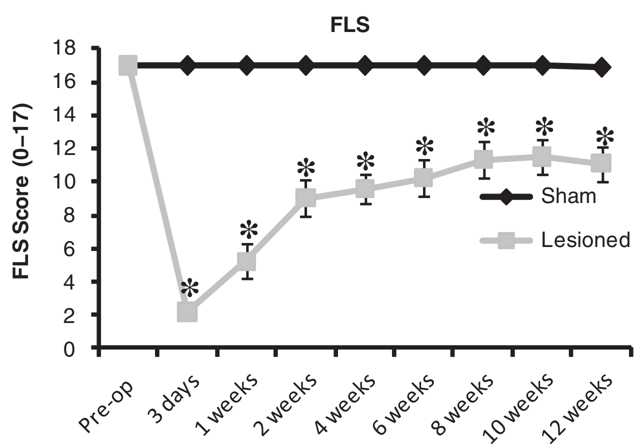


**FIG. 1.** Spontaneous recovery after lateral hemisection of the cervical spinal cord as assessed using an arena test (forelimb locomotor scale, FLS). Lesioned animals showed significant impairments of their forelimb use at 3 days post-injury and at all other time points examined (*$p < 0.01$) compared to animals with sham surgery. At 2 weeks, lesioned animals showed significant improvement from 3 days post-injury ($p < 0.0001$), indicative of spontaneous recovery. No significant additional improvements were observed after 2 weeks.


**FIG. 2.** Chronic forelimb behavioral assessment using the postural instability test (PIT). As expected, the ipsilesional forelimb in animals with cervical spinal cord injury (SCI) had significantly larger displacement distance than animals with sham surgery (6 cm versus 8 cm; $*p<00.0001$). In addition, the displacement distance on the contralesional forelimb was significantly smaller in lesioned animals compared to sham-operated animals (6 cm versus 4 cm; $*p<0.0001$). This may suggest enhanced function of the contralesional limb during PIT in lesioned animals.

$8.00 \pm 0.10$ cm, $p<0.0001$). In addition, the displacement distance on the contralesional forelimb was significantly smaller in lesioned animals compared to sham-operated animals ($6.00 \pm 0.25$ cm for sham versus $4.00 \pm 0.10$ cm for cSCI, $p<0.0001$). This result suggests that there was an enhancement of function in the uninjured, contralesional limb (i.e., more effective postural adjustment by the good limb to regain center of gravity).

### *Forelimb step-alternation test*

Using this test, we determined that only 50% of the lesioned animals were able to alternate the use of their forelimbs when held in a wheelbarrow posture and allowed to step with the forelimbs in response to weight shift (Fig. 3; see behavioral video at http://homepage.psy.utexas.edu/HomePage/Group/SchallertLAB/alt.v.nonalt_mpg.mpg). Next we determined whether forelimb step-alternation was affected if a delay was introduced between the first and second step in alternators. To test this, a delay (5 sec) was introduced by holding the animal still after the first step before moving the animal forward for the second step. We found that of the 50% of the animals that were alternating the use of their limbs, only 50% (25% of the total lesioned group) were able to alternate the use of their limb after a 5-sec delay (Fig. 3).

After discovering that there was a sub-group of lesioned animals with a more severe limitation (alternator versus non-alternator), we re-analyzed our FLS data. There was again an effect of lesion status, which includes sham, lesioned alternators, and lesioned non-alternators [$F_{(1,2)}=35.30$, $p<0.0001$]. In addition, there was an effect of time [$F_{(1,7)}=19.01$, $p<0.0001$]. Alternators were significantly less impaired compared to the non-alternators at 1 week post-injury (alternators' average FLS score = 5.5, non-alternators' average FLS score = 2.5; $p<0.05$; Fig. 4). This means that lesioned animals that could alternate improved significantly more than non-alternators from 1 week up to 12 weeks post-injury. In addition, we re-analyzed the PIT data, and determined that non-alternators were unable to use their ipsilesional forelimb (Table 1).

### *Vibrissae-elicited forelimb placing*

Vibrissae-elicited forelimb placing was administered to assess sensory-induced motor (forelimb) response. There was a significant effect of lesion [$F_{(1,2)}=23.76$, $p<0.0001$], and the injured rats had very limited vibrissae-elicited forelimb responses on the ipsilesional side, while the response was intact on the contralesional forelimb compared to the sham surgery group (Fig. 5A). In addition, lesioned alternators scored higher ($1.05 \pm 0.15$) than the non-alternators ($0.52 \pm 0.071$), although not significantly ($p=0.21$).

A vibrissae-elicited cross-midline placing test was administered to determine whether sensory activation on one side can induce motor response in the opposite limb (Fig. 5B). In combination with vibrissae-elicited forelimb placing, this

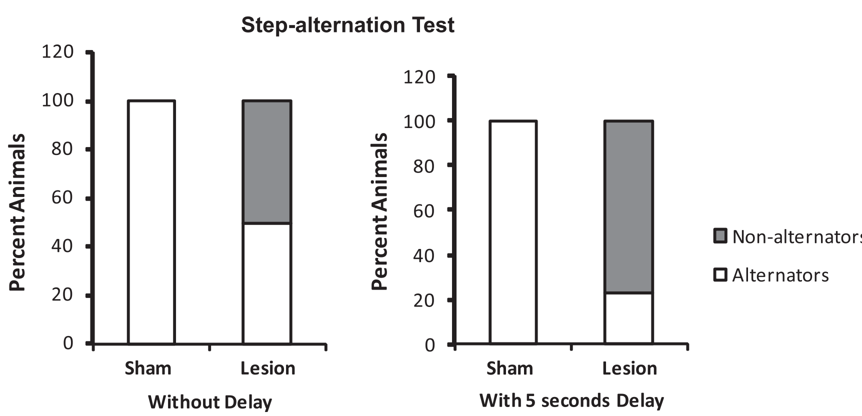


**FIG. 3.** Chronic assessment of forelimb function using a novel forelimb step-alternation test revealed two different groups of animals with different lesion severity. Animals showed a difference in behavior during forward stepping using both paws. Only 50% of the lesioned animals alternated paws while stepping (alternators; 5 out of 10 lesioned rats), whereas the others showed a lack of step-alternation by taking two contralesional steps in a row (non-alternators; 5 out of 10 lesioned rats). More animals displayed this tendency to take multiple contralesional steps if a 5-sec delay was introduced between steps (8 out of 10 lesioned rats).

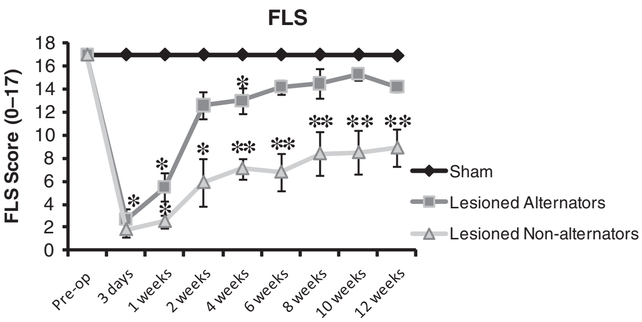


**FIG. 4.** The forelimb step-alternation test can separate lesioned animals into groups predicting their spontaneous recovery profiles. By separating results based on step-alternation, a significant difference in groups appears using data obtained from the forelimb locomotor scale (FLS). The FLS showed, starting from 1 week, that alternators recovered significantly more than non-alternators (*$p < 0.05$ for 1 and 2 weeks, **$p < 0.001$ for all following weeks). Alternators scored significantly different from sham animals through the first week (**$p < 0.001$), and at the fourth week (*$p < 0.05$), but no significant difference was seen between the sham and alternator groups following the fourth week.

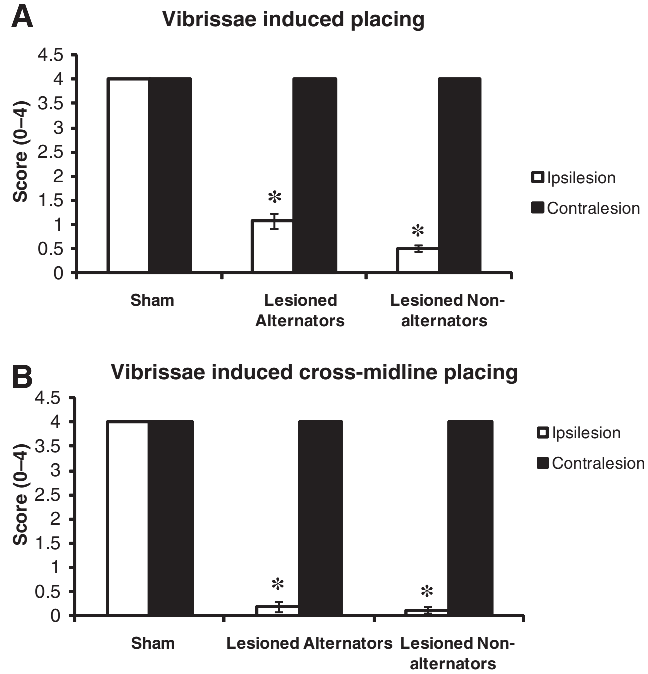


**FIG. 5.** Forelimb sensory-induced motor response assessment. (**A**) Vibrissae-induced forelimb placing showed unimpaired function of the contralesional limb in both lesioned groups, and showed a significant difference between lesioned and sham animals when using the ipsilesional forelimb. (**B**) Vibrissae-induced cross-midline placing revealed impairment and no difference in both lesioned groups (alternators and non-alternators) on the ipsilesional side, and no reduction of function on the contralesional side in response to ipsilateral vibrissae stimulation. This revealed an intact sensory input from the ipsilesional vibrissae (*$p < 0.05$ for comparison of the ipsilesional placing score between sham and either lesioned alternators or non-alternators).

test can determine whether the impairment is sensory alone or primarily motor. We found that there was a significant reduction in the response of the ipsilesional forelimb in cSCI rats when the contralesional vibrissae were activated ($0.16 \pm 0.06$; $p < 0.0001$), whereas sham animals readily placed either forelimb in response to stimulation of either set of vibrissae. Most importantly, the contralesional forelimb response was intact when the ipsilesional vibrissae were activated. Therefore, our data indicate that the cSCI animals had sufficiently intact sensory activation of the ipsilesional vibrissae and an impaired motor response in the ipsilateral forelimb.

### *Pasta-handling and eating test*

This test was designed to test the skilled use of the forelimbs while eating a piece of pasta. This test has been used previously to detect deficits in skilled use of forelimbs in animal models of unilateral stroke and Parkinson's disease (Allred et al., 2008). At 12 weeks post-injury, all sham animals ate pasta using both paws during the test period. In the lesioned group, more of the animals in the alternator group (milder injury; 10 out of 15) were able to use their injured forelimb compared to the animals in the non-alternator group (severely injured group; 1 out of 7; Fig. 6). It is important to note that all three groups of animals (sham, lesioned alternators, and lesioned non-alternators) took a similar amount of time ($6.77 \pm 0.62$ sec; $n = 11$) to eat a standard size piece of pasta (Fig. 6).

Table 1. Postural Instability Test (PIT) after Cervical Spinal Cord Injury in Rats

| *Lesion status* | *Ipsilesion side (cm)* | *Contralesion side (cm)* |
|---|---|---|
| Sham | 6.00 | 5.73 |
| Lesioned alternators | 8.14 | 4.30 |
| Lesioned non-alternators | —[a] | 3.82 |

[a]These animals did not use their ipsilesion forelimb to step.

### *Cylinder paw-preference test*

This test was used to determine the animals' forelimb preference during normal vertical exploratory behavior. The cylinder test has been used in animals with unilateral cSCI (Dai et al., 2009; Lynskey et al., 2006; Plunet et al., 2008; Schallert et al., 2000; Tobias et al., 2005), as well as in other experimental models for deficits of the CNS (e.g., ischemic and Parkinson's disease models; Schallert et al., 2000). As expected, there was a significant difference in the animals' ability to use their ipsilesional paw during the test, and there was a significant effect of cSCI [$F_{(1,2)} = 149.35$, $p < 0.0001$], as well as of time [$F_{(1,2)} = 211.64$, $p < 0.0001$]. In the lesioned group, alternators increased the use of their injured limb during vertical exploration between 2 weeks and 12 weeks after injury (Fig. 7). However, only two lesioned alternators were observed using the injured limb independently during vertical exploration in the cylinder (right; $p < 0.03$ for alternators at 2 weeks compared to 12 weeks). No improvement in forelimb use was observed in the lesioned non-alternating group throughout the study.

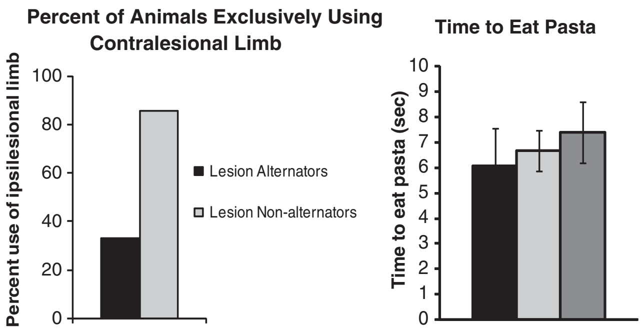


**FIG. 6.** Forelimb assessment using the pasta handling and eating test showed significant impairments in lesioned non-alternators. The animals were given a standard piece of pasta. Paw use and time to eat pasta were recorded. The results indicated that a low number of alternators (33%) exclusively used their contralesional forelimb compared to non-alternators (86%). In addition, animals in all three groups (sham, lesioned alternators, and lesioned non-alternators) took a similar amount of time to eat the pasta ( $p < 0.05$).

### *Lesion size analysis*

First, we determined the damage to the ipsilesional CST using BDA tracer injections into the somatosensory cortex opposite the lesioned side. We examined the spinal cords distal to the lesion site (C6–C7) and found that there were significantly fewer labeled CST axons in all lesioned animals compared to sham animals [$F_{(1,1)} = 28.25$, $p < 0.001$] in the ipsilesion spinal cord (Fig. 8). In addition, in the lesioned group, there were significantly fewer BDA-labeled CST axons in non-alternating animals compared to alternators [$F_{(1,2)} = 16.86$, $p < 0.02$; Fig. 8].

To generally determine the extent of injury, we also examined the Nissl-stained sections from all spinal cords at 200-$\mu$m intervals starting at a location proximal to the lesion site. There was a significant level of damage to the spinal cords of all lesioned animals, and all sham animals had no detectable damage to the spinal cord. In the lesioned animals, the damage spanned C3 through C4, and most of the ipsilesional cord tissue was removed (Fig. 9). Moreover, some lesioned animals exhibited damage to the contralesional cord tissue. To determine the number of animals with this larger form of lesion, we devised a scale of 0–2 (0 = no damage, 1 = moderately affected, 2 = highly affected), and assessed specific areas within the contralesional cord tissue. Interestingly, among the lesioned animals, non-alternators had significantly more damage to the CST [$F_{(1,1)} = 5.56$, $p < 0.05$], and in the dorsal column [DC; $F_{(1,1)} = 19.2$, $p < 0.003$] of the contralesional tissue compared to the alternators (Fig. 9). Moreover, there was a significant correlation between damage to the contralateral DC (DC score) and FLS scores starting at week 2 (rs = 0.824; $p < 0.032$), and only increased with time after lesion. At 12 weeks post-lesion, there was a significant correlation between damage to contralateral DC and FLS scores (rs = 0.933; $p < 0.001$). There was no correlation detected between DC damage scores and placing scores of lesioned animals. When we examined the correlation between damage to the contralateral CST and FLS scores, we only detected a significant correlation at week 6 (rs = 0.824; $p < 0.032$) and week 12 (rs = 0.824; $p < 0.032$). No significant correlation was detected between CST scores and placing scores of lesioned animals.

## Discussion

Accurate behavioral assessment is an important part of developing SCI repair strategies. Arena tests are user-friendly and widely used. However, they are not sufficient to detect certain deficits or recovery of limb function optimally after injury to the spinal cord. Therefore, considerable efforts have been made to design behavioral tests to fully assess function of both forelimbs and hindlimbs in animal models of spinal cord injury. Here we describe a forelimb step-alternation test to determine the function of forelimbs after cSCI. This test is

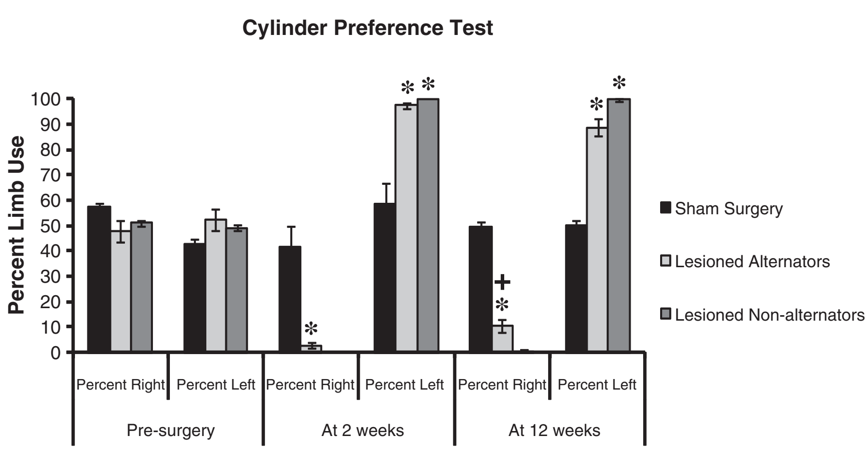


**FIG. 7.** Forelimb assessment using the cylinder paw-preference test showed significant impairment of the ipsilesional forelimb after cervical spinal cord injury. Differential recovery was observed in lesioned alternators compared to lesioned non-alternators. Before surgery, all animals showed ~ 50% use of their individual forelimbs (either left or right) during the vertical exploration in the cylinder. At 2 weeks post-injury, all sham-operated animals continued to use their forelimbs equally, whereas the lesioned animals (both alternators and non-alternators) showed only the use of their contralesional forelimb (left). At 12 weeks post-injury, 2 animals in the alternator group exhibited limited use of their ipsilesional forelimb (right; $p < 0.03$ for alternators at 2 weeks compared to 12 weeks), whereas the non-alternators continued to use their contralesional forelimb (left) exclusively (*$p < 0.05$ for comparison of scores of the same groups pre-surgery at 2 weeks or 12 weeks post-surgery; $^{+}p < 0.05$ between alternators at 2 weeks compared to alternators at 12 weeks post-surgery).

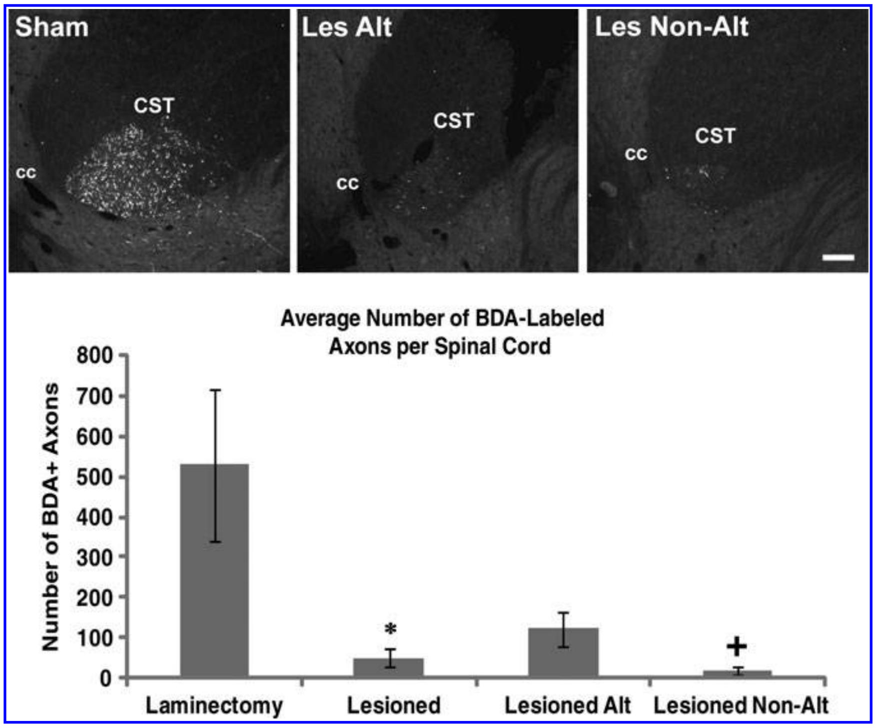


**FIG. 8.** Tract tracing of the ipsilesional corticospinal tract (CST) revealed that the forelimb step-alternation test can predict lesion severity. To determine the damage to the CST in the ipsilesional cord, biodextran amine (BDA) was injected into the contralesion somatosensory cortex. Detection of BDA-labeled CST axons at spinal cord levels distal to the lesion site (C6–C7) indicated that there were significantly fewer labeled axons in all lesioned animals (*$p < 0.0005$). In addition, lesioned non-alternators (Les Non-Alt, Lesioned Non-Alt) had fewer spared BDA-labeled CST axons compared to lesioned alternators (Les Alt, Lesioned Alt; $^{+}p < 0.02$; CC, corpus callosum; scale bar = 100$\mu$m).

designed to be simple, easy to administer, reliable, and sensitive. We used a number of other behavioral tests to assess forelimb function after cSCI. We found that outcome from the forelimb step-alternation test can predict the extent of the lesion and the level of behavioral impairment as early as 2 weeks after injury. Moreover, we found that more animals displayed a tendency to take multiple contralesional steps if a 5-sec delay was introduced between steps (8 out of 10 lesioned rats) during the step-alternation test. Time-limited cross-midline influences from the step-activated contralesional spinal cord to the ipsilesional spinal cord may have contributed to this effect.

We also introduced the use of the PIT and the pasta-eating test with cSCI. Using the PIT, we found lesion-induced changes in the ipsilesional forelimb as well as the contralesional forelimb, suggesting an enhancement of function in the uninjured contralesional limb (i.e., enhancement of the good limb). Using the pasta-eating test, we found that fewer animals (14% ipsilesional forelimb use) in the non-alternator group were able to include use of their ipsilesional forelimb compared to the animals in the alternator group (67% ipsilesional forelimb use).

### *Plasticity of the intact pathways after cervical SCI*

Most spinal cord injuries in humans are incomplete injuries. Plasticity exhibited by the remaining pathways is of interest to neuroscientists and as a potential target for therapy. Although the idea of spontaneous recovery after incomplete cSCI is not new (Fuller et al., 2008; Schrimsher and Reier, 1992; Weidner et al., 2001), many studies have focused on the possibility that intraspinal plasticity may play a major role. Recently, a number of articles have provided clues about potential anatomical, as well as functional, changes mediating this form of plasticity in the cortex (Ghosh et al., 2009,2010; Martinez et al., 2010). Specifically, Ghosh and colleagues found that after incomplete injury to the cervical spinal cord at C3–C4, there was significant sprouting of corticospinal axons from the intact cortex across the midline, innervating the spinal segments below the injury in both cervical and lumbar segments (Ghosh et al., 2009). These changes are thought to be responsible for the behavioral adaptation observed in the animals.

In the current study, BDA labeling of the descending CST in the ipsilesional cord, which is an important fiber system for voluntary skilled movements, showed that alternators retain significantly higher numbers of ipsilesional CST fibers compared to non-alternators. Since we labeled only the ipsilesional CST fibers, these fibers are not likely to represent the plasticity of the CST fibers from the intact cortex. Instead, it suggests that non-alternators may have a more complete removal of their CST than alternators. In addition, non-alternators also sustained injury to the contralesional cord tissue (CST and DC).

### *Enhanced function of the good limb after hemisection injury to the spinal cord*

In this study, a lateral hemisection of the cervical spinal cord was performed, producing a lesion that affects (1) the

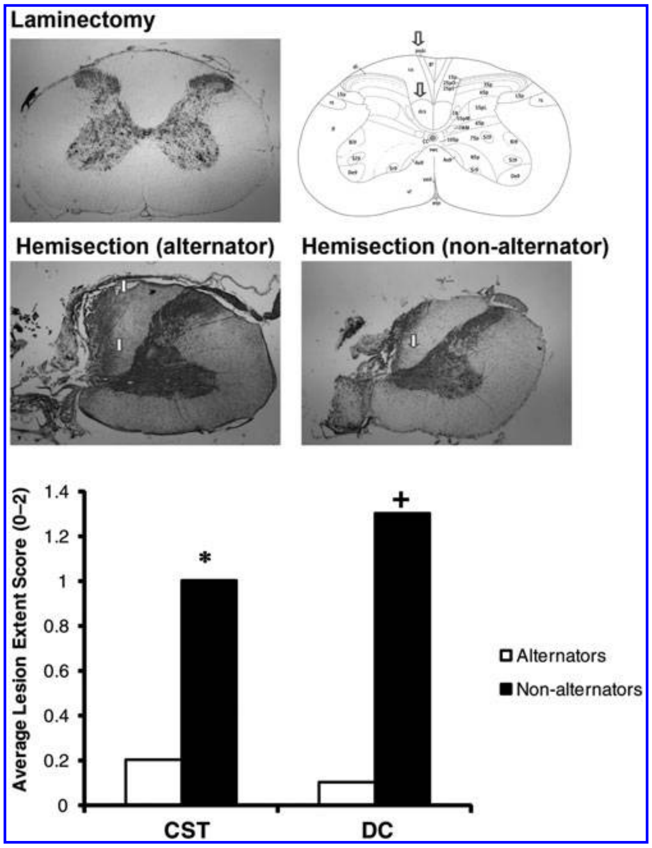


**FIG. 9.** Analysis of lesion size and location. Nissl-stained sections of the cervical spinal cord at C3 and C4 were evaluated at 200-$\mu$m intervals. All sham-operated animals showed intact spinal cords, and all lesioned animals showed significant damage on the right side of the spinal cord, with all grey and white matter removed. Some lesioned animals showed damage to the contralesional side. Semi-quantitative analysis indicated that non-alternators had significantly more damage to the dorsal column (DC, $^{*}p < 0.002$) and the corticospinal tract (CST, $^{+}p < 0.05$) compared to lesioned alternators. Arrows indicate DC (dorsal arrow) and CST (ventral arrow).

dorsal column and the long tract dorsal CST, (2) the dorsolateral funiculus including the rubrospinal tract and post-synaptic afferent pathways, and (3) the ventrolateral tracts including the reticulospinal and vestibulospinal pathways and post-synaptic afferent projections on the ipsilesion side.

Using the PIT, we found that there was a significant enhancement of the good forelimb in all lesioned animals. There are a number of possibilities for what may be contributing to this enhanced function of the good limb. There is ample evidence of intact brain areas taking over for injured tissue. There is also a theory that the changes in the unaffected cortex or striatum are linked to improved function in the unaffected limb (Schallert et al., 2003). In addition, PIT data also suggest that interlimb coordination may be partially responsible. These findings may have significant bearing on the design of future therapeutic interventions. Note, however, that constraining the use of the non-impaired limb to train the impaired limb may sometimes have long-term deleterious effects on the function of the non-impaired limb (Bury and Jones, 2004; Kleim et al., 2003; Kozlowski et al., 1996).

### Useful features of the forelimb step-alternation test

Hemisected cSCI rats showed impairment in the asymmetry of inter-limb coordination of their forelimbs. Here we showed that the forelimb step-alternation test was sensitive enough to detect differences in lesion severity using the impairments in inter-limb coordination (i.e., limb alternation). Using this test, we separated the lesioned animals into two groups of animals: alternators and non-alternators. Histological and anatomical data from this study suggest that these two groups had different extents of injury to the CST and DC in the contralateral cord and chronic spontaneous recovery profiles. We suggest therefore that the forelimb step-alternation test can provide a method of segregating more severely lesioned animals from less severe ones after lateral hemisection of the cervical spinal cord. This ability to segregate animals based on lesion severity prior to sacrificing the animals is useful in the assessment of future therapeutic interventions. This will allow the experimenter to separate lesioned animals into two distinct groups with different recovery profiles. Moreover, it would be interesting to compare forelimb step-alternation and PIT outcomes with published hemisection cSCI stepping data, and to examine the effects of exercise and related interventions (Ying et al., 2008). In addition, the role of spinal learning in limb use outcome after SCI and recovery would also be of great interest (Grau et al., 2006).

### Useful features of the pasta handling and eating test

Although the forelimb step-alternation test can assess the function of the two proximal joints of the forelimb, the pasta eating test was designed to assess the functioning of all three joints of the forelimb during a complex motor task. Pasta handling/eating tests have been used in animal models of stroke and Parkinson's disease (Allred et al., 2008; Ballermann et al., 2001b; Metz et al., 2001; Tennant et al., 2010), and also after DC lesions (Ballermann et al., 2001a). To our knowledge, the use of a pasta handling test in rodents with cSCI to assess their motor function has not previously been described in the literature. In this report, we analyzed paw use and time to eat pasta. The injury paradigm we employed was a one-sided lesion that was severe enough to abolish most of the paw function of the ipsilesional forelimb in almost all lesioned animals. In the current study, we were surprised to find that all lesioned animals (alternators and non-alternators) were indistinguishable from sham animals in the time that it took for them to eat a piece of pasta. These data are potentially important because rats with unilateral degeneration of the striatum in models of stroke or Parkinson's disease show reliably longer latencies to eat a strand of pasta compared to sham animals, even when both forelimbs are used (Allred et al., 2008). It is possible that rats with unilateral striatal cell loss have oro-motor deficits in swallowing, biting, or tongue function (Kane et al., 2011) that are not observed after unilateral cSCI, but that contribute substantially to the speed of pasta eating. Perhaps the data suggest enhanced function of the contralesional forelimb for pasta handling in cSCI rats. In summary, the pasta-eating test may prove to be useful for evaluating efficiency of forepaw use and behavioral adaptations during complex limb functions.

## Conclusions

We described novel behavioral outcomes after unilateral cSCI. Data from a forelimb step-alternation test predicted damage severity and recovery profiles. Use of this forelimb step-alternation test may provide a tool to separate animals according to injury severity. In addition, we described the use of the dry pasta handling test and postural instability test (PIT) to assess forelimb function after cSCI. The results indicated that both tests are appropriate for use in rodents after cSCI. Using the PIT, and perhaps also the pasta-handling test, we found what appears to be significant enhancement in the function of the contralesion forelimb. This finding suggests that future therapeutic strategies for unilateral cervical spinal cord injury should include assessment of function not just of the ipsilesional forelimb, but also the contralesional forelimb.

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## Author Disclosure Statement

No competing financial interests exist.

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