Spinal Cord (2014) 52, 529–535

www.nature.com/sc

ORIGINAL ARTICLE

Translation of the rat thoracic contusion model; part 2 — forward versus backward locomotion testing

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Study design: Experimental animal study.
Objectives: Locomotion analyses in rat spinal cord contusion injury (SCI) models are widely used for the evaluation of recovery of supraspinal locomotor control. However, many commonly used locomotion tests are inadequate to test for spinal cord integrity as they assess motor function that can be highly mediated through below-level propriospinal pattern-generating circuitry, independently of below-level perception. Here we report a behavioral motor test that is more sensitive for spinal cord integrity, even 6 weeks after injury: the backward locomotion rotating rod.
Setting: University of California - San Diego.
Methods: A modified rotating rod test was run in reverse. The rod diameter was increased and thin rubber lining was added. As a reference, we included commonly used motor tests: BBB score, catwalk gait analysis, motor-evoked potentials, single frame analyses, a forward rotating rod test and the 55° inclined ladder test.
Results: Unlike commonly used motor tests, the backward locomotion rotating rod test significantly discriminates between both sham-operated (falling latency: 20.4 s s.d. ± 4.5) vs mild SCI animals, and mild vs moderate SCI animals (differences between each group at acute, subacute and chronic phases: $\geqslant$6 s, $P \leqslant 0.01$). Moderate SCI animals were practically unable to make even slight backward hindpaw movements. The backward locomotion ability in the chronic phase correlates best with BBB locomotor scores from the acute phase.
Conclusion: Our data show that backward locomotion is a highly sensitive and quick test to discriminate between sham, mild and moderate SCI, even after 6 weeks. Backward locomotion testing may improve the translational value of experimental results for the clinic.
Spinal Cord (2014) **52,** 529–535; doi:10.1038/sc.2014.73; published online 13 May 2014

INTRODUCTION

The use of animal models in spinal cord injury (SCI) research is complicated due to difficulties in sensitive and specific quantification of locomotor outcome, even though the availability of behavioral outcomes is one of the most important arguments for using *in vivo* models. Locomotor outcomes are often assumed to correlate well with SCI regeneration (long-tract integrity), but this is a feature that might be poorly present in widely used animal models. An impressive amount of intrinsic spinal pattern generator circuitry is present in the rat lumbar spinal cord, which almost autonomously recovers basic locomotion after SCI.[1] In the acute phase (that is, up to 1 week post injury) locomotion deficits are strikingly present, even for mildly injured animals. However, in the subacute phase (about 3 weeks post injury) and chronic phase (from 6 weeks) most mildly injured thoracic contusion SCI rats have spontaneously recovered to a state in which they are indistinguishable from sham-operated animals, although only half of the spinal cord is spared.[2] Furthermore, also moderate SCI animals generally show only few mild deficits after the subacute phase, and even severely injured SCI animals, which are reported to have even $<$10% spared spinal cord tissue at the lesion site, regain weight-supported stepping.[2] In addition, recovery of below-level sensory perception seems barely required, if at all, for regaining basic quadruped locomotion (see the accompanying manuscript and Hofstetter *et al.*[3]). Hence, if current behavioral tests are associated with such difficulties in the detection of even fairly large disruptions in spinal cord parenchyma, how reliable are these tests for measuring any therapeutic effects? Moreover, are these effects then actually valuable for human patients?

Agreed, the animal spinal cord below the level of injury is since long known for its ability to implement/recover much basic motor functionality intrinsically by strengthening pre-existing propriospinal circuitries and, in particular, the central pattern generator.[1,4] This notion is further supported by the fact that even after full spinal cord transection some recovery in hindlimb function can still be noted, that is, when transection was performed after a preceding contusive SCI.[2] However, locomotion recovery through plasticity of the intrinsic pattern generator-driven spinal cord circuitry can be expected to be of much less relevance in the human condition. This is due to the higher degree of instability of the basic posture during human bipedalism that would logically require more advanced supraspinal modulation. In this perspective, it is not surprising that, in contrast to quadrupeds, (I) adequate below-level sensory perception after SCI in humans has

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Received 6 August 2013; revised 6 April 2014; accepted 8 April 2014; published online 13 May 2014

proved to be a good predictor for locomotor recovery,[5] and (II) for a human it takes relatively long to learn how to walk as a child when compared with rats in the early stage of their lives.[6] Hence, basic locomotor tests in SCI models not only seem to lack desirable sensitivity for SCI injury but they also seem not to be sufficiently specific for the human SCI condition.

Subsequently, we focused on motor tests that theoretically might be more sensitive for spinal cord integrity through the requirement of conscious supraspinal control (that is, requiring more advanced sensorimotor modulation). Hence, the 55° inclined ladder climbing test and the rotating rod test were included and tested for their sensitivity to detect deficits after mild and moderate thoracic spinal cord contusion injury in rats, widely used SCI models with reproducible results. It is known from their use in other SCI models that more severely injured animals barely recover function, if at all, as assessed with these tests, which is suggestive of a relatively high requirement of supraspinal motor control.[7–10] In order to further increase the requirement of supraspinal motor control on locomotion testing and decrease the impact of basic intrinsic pattern generator-based spinal locomotion circuitry, the backward locomotion rotating rod test was developed. Backward locomotion is hypothesized to be more specific for supraspinal, voluntary and conscious locomotion as brainstem stimulation in decerebrated quadrupeds was only able to elicit forward, but not backward, walking patterns.[11] As a reference, severe SCI animals were included, myogenic motor-evoked potentials (MMEPs) to transcranial electrical motor cortex stimulation were measured, and locomotion ability was scored with the widely used Basso, Beattie, Bresnahan (BBB)-locomotor scoring method.[2,12] To objectify some of the key, but arguably subjective, locomotor features of the BBB score, the single frame analysis and catwalk gait analysis were used.[7,13]

MATERIALS AND METHODS

Animals and surgeries

All animal studies were approved by the University of California San Diego institutional animal care and use committee. Twenty-two 12–week- old female Sprague–Dawley rats were anesthetized with isoflurane (5% induction, 2% maintenance; room air), placed on a Stereotaxic frame (Stoelting, Cat# 51600 with Cat# 51695, Wood Dale, IL, USA) and maintained at a core temperature of $37 \pm 0.3\,^{\circ}C$ using a heating blanket. A Th9 laminectomy was performed using a dental drill. The MASCIS/NYU apparatus (WM Keck Center for Collaborative Neuroscience, Rutgers University, Piscataway, NJ, USA) was used for SCI. The laminectomy site was filled with mineral oil in which the tip of a small thermocouple (Physitemp, Cat# IT-14, Clifton, NJ, USA) was submerged and used to manually keep the spinal cord at $37 \pm 0.3\,^{\circ}C$ with warmth from surgical lights (Fiber-Lite, Cat# MI-150 & BGG1823M, Dolan-Jenner, Boxborough, MA, USA). Next, oil and lamps were removed and the rod dropped using a height of 6.25 (mild SCI, $n=7$), 12.5 (moderate SCI, $n=5$) or 25 mm (severe SCI, $n=5$). Five sham-operated animals were included. Surgeries were performed in a mixed-group manner over 2 days.

Post-surgical care

Animals were housed socially (with max 3 animals per cage and in a mixed-group manner) at a regular 12/12 h light/dark cycle, on corn cob bedding and with *ad libitum* access to water and food pellets (Cat# Teklad 2014, Harlan, San-Diego, CA, USA). Buprenorphine ($0.05\,mg\,kg^{-1}$, s.c., Reckitt Benckiser, Richmond, VA, USA), 5 ml of Lactated ringer, and $10\,mg\,kg^{-1}$ of Cefazolin (Novaplus/Sandoz, Holzkirchen, Germany) was given after surgery. Bladders were emptied manually twice daily. Sulfamethoxazole and Trimethoprim USP (200 mg & 40 mg per 250 ml drinking water, Hi-Tech Pharmacal, Amityville, NY, USA) were given for at least 10–14 days or until autonomous bladder voiding occurred. Any ill-appearing animals received additional days of above-mentioned antibiotic treatments.

Behavioral testing

At least 15 minutes before all behavioral testing, cages were placed in the testing room ($20.5 \pm 1\,^{\circ}C$) with background music and the required lighting for testing. If multiple runs or trials per test were required, subsequent trials were only performed after the entire group had finished a full trial. Group designation was not known by the experimenters doing behavioral testing. SvG performed all behavioral testing while ML was involved in CatWalk, inclined ladder and single frame motion analysis. Moreover, ML and SN were involved in BBB scoring. OP was involved in MMEP-recordings and analyses.

Open-field locomotor testing

Locomotion recovery after SCI was monitored using a rating scale based on the BBB open-field locomotor rating scale (0–21), as described before.[2,12] Testing was done in the morning at day −7, 2, 7, 21 and 42 post-injury, once per animal, and in a fluorescent-lit room.

Gait analysis

The CatWalk apparatus (7.1, Noldus, The Netherlands) was used to quantify gait parameters by semi-automated footprint analyses during runway crossings.[13] In this system, a glass walkway ($109 \times 15 \times 0.6$ cm L × W × H) was illuminated along the long edge, and the illuminated footprints were video recorded from underneath the elevated walkway. The room was darkened to obtain an optimal contrast between the paws and the surroundings. Animals were trained to cross the walkway smoothly, without hesitations. For this purpose, animals had always direct access to their home cage and a treat (Certified Supreme Mini-Treats, Cat# F05472-1, Frenchtown, NJ, USA) at the end of the run. These incentives served to promote run completion and were also used during single frame and ladder testing. Training was performed in the afternoon at days −6 through −2, 5, 6, 19, 20, 40 and 41 post injury. Testing was done at days −7, 7, 21, 42 post injury. Per session, each animal completed (at least) three runs. Three uninterruptedly runs in a constant pace were used for analysis per time point.

Single frame hind limb motion analysis

Bilateral video captures allowed analysis of the foot-stepping angle (FSA) and the rump-height index (RHI), as described previously.[7] The FSA is the angle at which the hind paw is placed on the ground. The RHI was defined as the highest point of the base of the tail during a run. VirtualDub 1.9.11 (http://www.virtualdub.org) and Screen Ruler V1.0.1a (http://www.caveworks.net) were used for analyses. Training and testing sessions were performed under fluorescent lighting and in the afternoon, after every CatWalk training/testing session. Per session, each animal completed three runs.

Integration of objective measures (Catwalk, FSA and RHI) into BBB-locomotor score

BBB-scores allow some experimenter subjectivity; hence, we tried to objectify some of the mid-range items of the BBB-score, which are regarded as key items in the recovery of SCI. First, animals were grouped in BBB-scores $\leqslant 7$, 8, 9, 10 or $\geqslant 11$, using the single frame analysis. An FSA of $<90°$ defines plantar stepping and when found in more than 33% of the steps it was considered frequent. A RHI in the range of those from consistently coordinated walking animals defined weight support.[7] Second, frequency of forepaw-hindpaw coordination ('no', 'occassional', 'frequent' or 'consistent') was objectively assessed using the Catwalk gait analysis by counting the coordinated runs (0, 1, 2 or 3, respectively).[13]

Inclined ladder test

The 55° inclined ladder test was performed using an inclined ladder with 20 120 mm-wide rungs (Ø6.35 mm) spaced at equal intervals (60 mm) and having 150 mm-high side walls, as previously described.[7] The correct placing of a hind paw and sustained position until its next forward move was counted over the 18 center rungs during smooth runs. Training and testing sessions were performed in the afternoon after every session for the single frame hind limb motion analysis, in a fluorescent-lit room. Per session, each animal completed three runs.

Myogenic motor-evoked potentials

MMEPs in the gastrocnemius muscle were measured after being elicited by transcranial electrical stimulation of the motor cortex, as described before.[14] Animals were anesthetized using Ketamine (80 mg kg^{-1} i.p, Ketaset, Fort Dodge Animal Health, Overland Park, KS, USA). MMEPs were elicited by transcranial electrical stimulation (with a pulse duration of 1 ms at 7 mA using a DS3 constant current isolated stimulator (Digitimer, Welwyn Garden City, UK)) of the motor cortex using two percutaneously placed 30 G stainless steel stimulation electrodes. MMEPs were measured until three to five highest (stable) recorded potentials were similar. MMEPs were measured at day 43 post-injury.

Rotating rod and the backward locomotion test

A rotating rod apparatus (Rota-Rod, Stoelting, Cat# 52790, Wood Dale, IL, USA) was modified and used to assess motor performance (diameter increased from 9.5 cm to 12.75 cm and lined with 1.5 mm thick rubber). Rats were placed on the elevated reversed rotating rod, which was started at 4 r.p.m., linearly accelerated to 40 r.p.m. over 60 s, with a cutoff of 150 s. Maximum falling latency out of three trials was recorded. Timing started after hindpaw movement was observed. Rats were given a minimum of 30 min between trials. Two hand-held small transparent acrylic plates (400 × 50 mm; see Figure 1) and treats (as above) prevented rats from changing direction on the rotating rod and to motivate the animals for rotating rod testing. Testing sessions were performed in the morning after BBB-score-recording sessions on days 7, 21 and 42 post-injury, in a fluorescent-lit room. For each animal, three trials were recorded per session. A trial was defined by a persistent attempt of locomotion using the forepaws until falling. No training sessions were performed.

Statistical analyses

Results were analyzed using analysis of variance (ANOVA) (one-way, or two-way group × time repeated measures, using a fixed-effect model), with a Bonferroni *post hoc* test for multiple comparisons (GraphPad Prism, La Jolla, CA, USA). Unequal variances were not observed. Results were analyzed as two-tailed and expressed with s.e.m., unless specified otherwise. A *P*-value of 0.05 was considered significant.

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research.

RESULTS

Typical BBB-scores were observed after graded SCI, but these failed to differentiate mild injury from sham in the subacute and chronic phase

All SCI severities caused a significant reduction in BBB scores, as measured at 3 days post injury and compared with sham-operated animals (Figure 2a solid lines; Bonferroni: $P<0.001$). Next, a partial or complete recovery in BBB scores followed. Notably, differences between sham and mildly injured animals merely differed in the very acute phase (Bonferroni: $P<0.001$). Differences between moderately and severely injured animals only became marginally notable after 3 weeks post injury (Bonferroni: $P<0.05$). These BBB-scores are similar to the ones reported originally, using the same SCI model.[2,12] In these studies, most mild SCI animals also recovered to a BBB score of $\geqslant 20$, although a $>50\%$ white matter loss was reported at the level of injury.

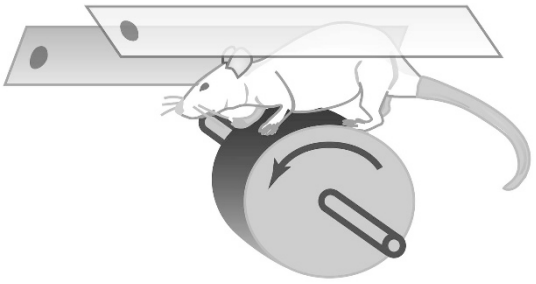

Figure 1 Backward locomotion rotating rod testing. A rotating rod apparatus was slightly modified by increasing the rod diameter to 12.75 cm and lining it with 1.5 mm thick rubber. Two hand-held small transparent acrylic plates (400 × 50 mm) and treats prevented rats from changing direction on the rod and motivated the rats to execute the test.

Catwalk, FSA and RHI barely improve sensitivity for detection of SCI deficits compared with the BBB-locomotor score

To minimize subjectivity as a source of bias for the BBB-score, we implemented objective measures (that is, the FSA, RHI and the Catwalk regularity index) into the BBB-locomotor scores. This resulted in only slight, non-significant, changes from the original BBB scores (compare solid and dotted lines in Figure 1a; repeated measures ANOVA per injury severity: $P\geqslant 0.63$). Individually, forepaw-hindpaw coordination, as assessed by the number of fully coordinated walkway crossings during gait analysis (coordinated crossing: a regularity index of 100%),[13] was not significantly affected in mild SCI animal when compared with sham-operated animals (Figure 1b; repeated measures ANOVA: $P=0.31$). Coordination in moderate and severe SCI animals differed significantly from each other at 3 and 6 weeks post injury (Bonferroni: $P<0.05$). Also, from the subacute phase, RHIs only show significant disabilities for the severe SCI animals, when comparing with sham animals (Figure 2c; Bonferroni: $P\leqslant 0.001$). Last, Foot-Stepping Angles were significantly increased for severe SCI animals in the acute and subacute phase (Figure 2d; Bonferroni *post hoc*: $P<0.05$) and for moderate SCI animals only in the acute phase (Bonferroni *post hoc*: $P<0.05$), when compared with sham animals. Nonetheless, in the acute phase, FSAs, as well as RHIs, did show significant different disabilities between moderate and severe SCI animals (Bonferroni: $P<0.05$), while BBB-scores did not.

The rotating rod, 55° inclined ladder test and MMEPs did not improve sensitivity for the detection of SCI deficits, but showed identical on/off patterns for supraspinal locomotor control

The patterns for subacute and chronic motor deficits are identical as measured by the rotating rod (with forward locomotion), 55° inclined ladder test and MMEPs. Again, no significant differences between sham and mild SCI were detected for the rotating rod test and MMEPs (Figures 3a, c and e; ANOVA: $P\geqslant 0.41$). Notably, the 55° inclined ladder test did show significant differences between sham-operated and mild SCI animals up to the subacute phase (Figure 3d; Bonferroni: $P<0.05$). Even in the chronic phase, the 55° inclined ladder climbing and MMEPs were significantly and fully reduced in both moderate and severe SCI animals, when compared with sham and mild SCI animals (Bonferroni: $P<0.05$). Nonetheless, even severe SCI animals showed constant stepping-like hindpaw movements during ladder climbing test tests, although these movements remained largely dysfunctional. In the rotating rod test, any hindlimb movement soon contributed to improvement in outcome, which was also largely due to stomach dragging and forepaw compensation.

The backward locomotion test showed the highest sensitivity for SCI-induced locomotion disability

Significant, acute, subacute and chronic decreases in locomotion function on the backward locomotion test were measured for all SCI severities, when compared with sham-operated animals, which specifically includes mild SCI (Figure 3b; Bonferroni *post hoc*: $P\leqslant 0.01$).

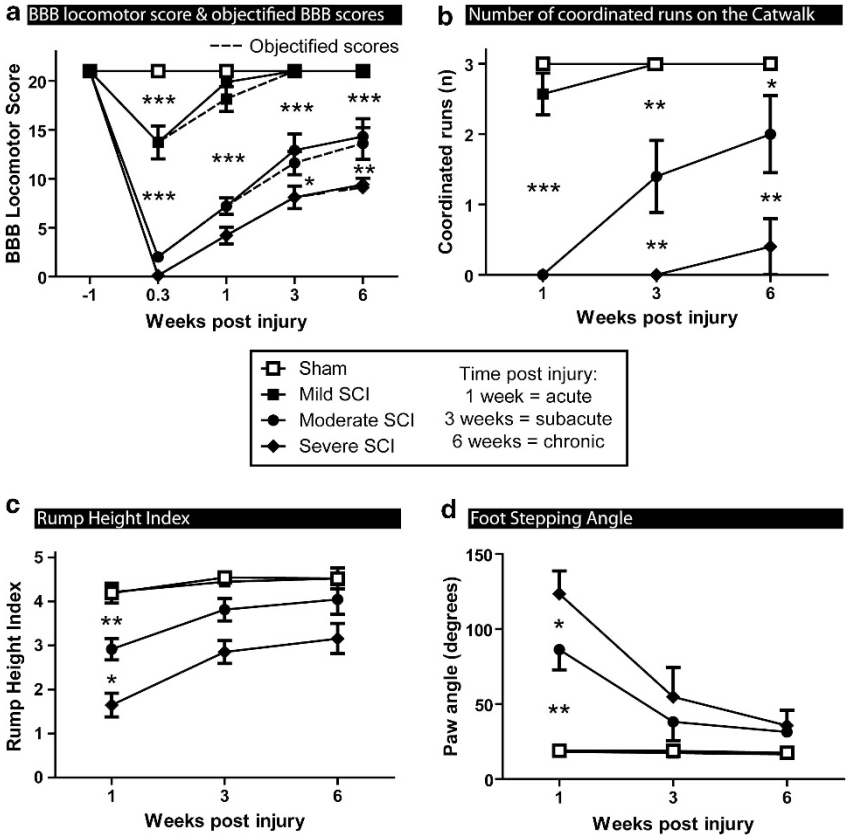

Figure 2 Results from locomotion tests characterized by low dependence on supraspinal locomotion control. Note that sham-operated and mild SCI rats are indistinguishable after the acute phase post-SCI. (**a**) open-field locomotion scores as assessed with the BBB locomotor scale (solid lines), and the BBB locomotor scale objectified for coordination, plantar stepping and weight-support (using the Catwalk, Foot Stepping Angles and Rump Height Index, respectively; interrupted lines). Note that even severe SCI animals regain weight support (scores of $\geqslant 9$). All four groups differed significantly from each other (repeated measures ANOVA: $P \leqslant 0.02$), but sham-operated and mild SCI only differed significantly at 3 days post injury (Bonferroni: $P \leqslant 0.001$). (**b**) The number of coordinated runs as assessed by the Catwalk gait analysis. Only moderate and severe SCI animals differed significantly from the other groups (repeated measures ANOVA: $P < 0.05$). (**c**, **d**) Shown are the Rump Height Indices (**c**) and Foot-Stepping Angles (**d**) as measured in the single frame analysis test. Again, only moderate and severe SCI animals differed significantly from sham-operated animals (ANOVA: $P \leqslant 0.03$) . *,** and *** correspond to *P*-values of <0.05, <0.01 and <0.001, respectively.

Note that significant differences between sham, mild and moderate SCI animals existed in all phases. Such sensitivity for differentiating sham, mild and moderate SCI was only observed for the backward locomotion test (see Table 1). The ability to perform backward hindpaw movements was strikingly reduced in animals with moderate and severe SCI, as barely any movement in the hindlimbs was noted in these animals during testing. Some of the moderate SCI animals, but all of the severe SCI animals, were unable to make even slight hindlimb movements when attempting backward walking, even at 6 weeks post injury, which was in sharp contrast to their forepaw movements.

DISCUSSION

This study shows that, unlike regular and widely used post-SCI hindpaw locomotor tests such as the BBB-score, the backward locomotion test is sensitive enough to allow reliable discrimination between sham-operated vs mild SCI animals in both the acute and chronic post-SCI phase. Moreover, it also allows the discrimination of mild vs moderate SCI animals in all post-SCI phases. Surprisingly, moderate SCI animals were nearly unable to perform any backward hindpaw movements, even at weeks after full weight support and forward locomotion had recovered. Hence, backward locomotion assessment is not only the first test sensitive enough to detect distinct and chronic locomotor differences between sham, mild and moderate SCI animals, but also provides a wide continuous scale, from sham to moderate injury, which is likely to be of great use for translational therapy evaluations.

For several reasons, the above-mentioned high sensitivity of backward locomotor testing for SCI can be hypothesized to be caused by backward locomotion highly depending on supraspinally and cortically coordinated sensorimotor control.

First, the lumbar spinal circuitries required for backward locomotion patterns likely remain intact, as locomotion patterns in both directions can be generated solely by similar spinal cord stimulation.[11] Although these locomotion patterns are rather basic and require externally provided full weight- and balance-support, they do indicate that segmental spinal circuitry required for conscious backward and forward locomotion is likely very similar or even identical. This suggests that the difference between backward and forward locomotion after SCI is not so much because of differences in lumbar spinal circuitry but rather because of differences in the requirement of supraspinal control.

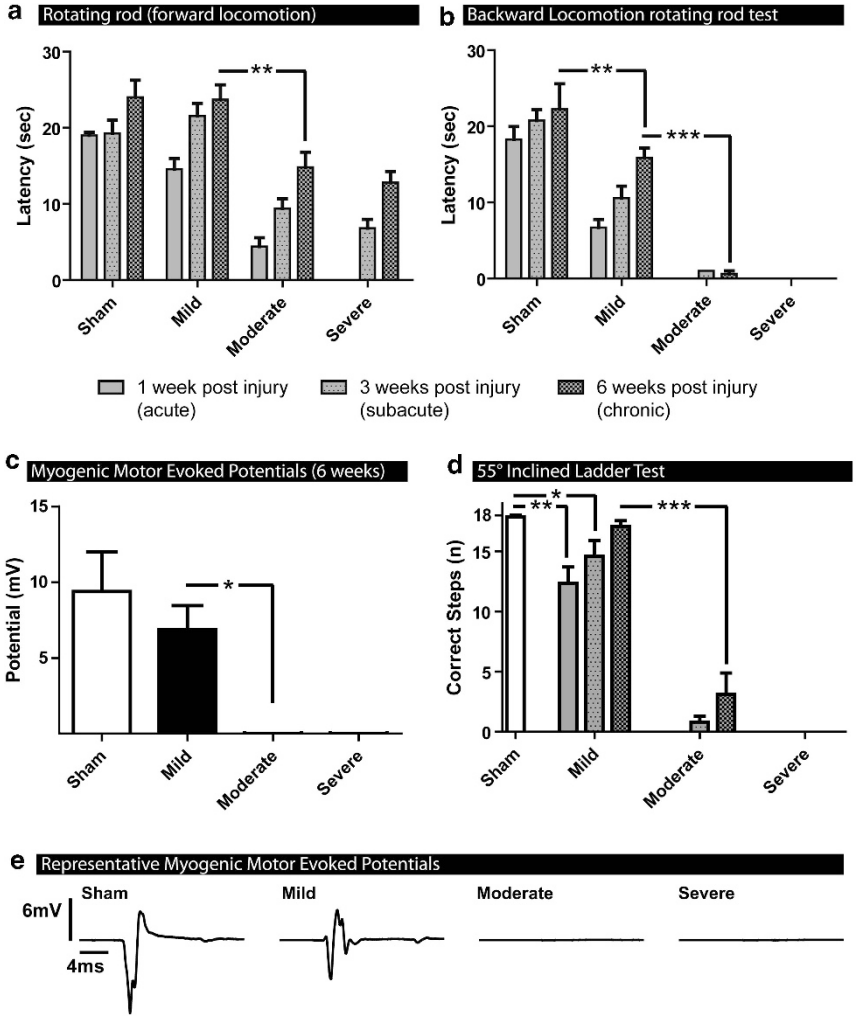

Figure 3 Results from MMEP assessments, ladder climbing and rotating rod tests, which depend more strongly on supraspinal locomotion control. Note the lack of differences between sham-operated and mild SCI animals in all but one (the backward locomotion rotating rod test) motor test in the chronic stage post injury. (**a**) Falling latencies during rotating rod testing with forward locomotion. Only differences between mild and moderate SCI animals, not sham and mild, or moderate and severe SCI animals, were significant (repeated measures ANOVA) at all three time points (Bonferroni *post hoc*: $P<0.01$). (**b**) Falling latencies during backward locomotion rotating rod testing. The backward locomotion rotating rod test was the only test that showed significant differences between sham-operated, mild and moderate SCI animals on every time point (Bonferroni *post hoc*: $P\leqslant 0.01$). (**c**) Myogenic motor-evoked potentials measured in the *M.* gastrocnemius after transcranial electric stimulation at 6 weeks post injury. A statistically significant drop in evoked potentials was only observed from mild to moderate SCI (one-way ANOVA: $P<0.05$). (**d**) Number of correct steps/paw placements while climbing the rungs of a 55° inclined ladder. Differences at 6 weeks post injury were only significant between mild and moderate SCI animals (Bonferroni: $P<0.001$). (**e**) Representative myogenic motor-evoked potentials measured in the gastrocnemius muscle (i.e. below injury level) after transcranial electric stimulation of the motor cortex at 6 weeks post injury (see panel **c** for quantification). *,** and *** correspond to *P*-values of <0.05, <0.01 and <0.001, respectively, and are only displayed at the latest time point on which two consecutive injury severity groups showed significant differences.

Second, supraspinal, or even cortical, sensorimotor control for backward locomotion is also suggested by the observation that the chronic backward locomotion deficits correlated best with the deficits in MMEPs. These MMEPs were motor cortex evoked and obtained in the chronic phase. Interestingly, MMEPs in rats are typically reported to not correlate with basic locomotion tests;[15] however, MMEP studies in human SCI patients found high sensitivities of MMEPs for spinal integrity and functional outcome.[16–18] Hence, the backward locomotion test seems to correlate with long tract function, which has translational value for locomotion function in humans.

Third, motor deficits recorded with the backward locomotion rotating rod test showed better correlation with the acute, subacute and chronic deficits in below-level sensory perception observed in the hindpaws of SCI animals[3] (see also the accompanying manuscript). In humans, lower extremity sensory function is also a good predictor for ambulation outcome after traumatic SCI.[5] This observation has already raised questions on the translational value of basic rat SCI locomotion scores considering that even numb rats have been reported to walk adequately after SCI.[3] Basic locomotor tests, like the BBB-score, rely heavily on intrinsic lumbar spinal sensorimotor reflexes for basic locomotion.[19] This lumbar spinal autonomy is thus very likely to hamper the translational value of results acquired in rat SCI models. Obviously, one can improve rat SCI locomotion assessments using tests that depend more on below-level perception and supraspinal sensorimotor control. This property does seem to be present in the backward locomotion test, as it shows a good correlation with

Table 1 Sensitivity of motor tests for detecting differences in SCI severity

Test	*Timing post injury*	*Sensitivity for difference in SCI severity*		
		Sham vs mild	*Mild vs moderate*	*Moderate vs severe*
BBB	Subacute	−	(++)[a]	+
	Chronic	−	(++)[a]	++
Objectified-BBB	Subacute	−	(++)[a]	+
	Chronic	−	(++)[a]	+
Catwalk Regularity Index	Subacute	−	(++)[a]	N/A[b]
	Chronic	−	(+)[a]	N/A[b]
Catwalk Coordinated runs	Subacute	−	(++)[a]	+[b]
	Chronic	−	(+/++)[a]	++[b]
single frame FSA	Subacute	−	(+/−)[a]	−
	Chronic	−	(+/−)[a]	−
single frame RHI	Subacute	−	(+/−)[a]	+/−
	Chronic	−	(+/−)[a]	+/−
Inclined ladder	Subacute	+	++	+/−
	Chronic	−	(++)[a]	+/−
Rotating rod	Subacute	−	(++)[a]	−[c]
	Chronic	−	(++)[a]	−[c]
Backward Rotating rod	Subacute	++	++	−[d]
	Chronic	+/++	++	−[d]
MMEPs	Chronic	+/−	+	−

−, absent; +/−, poor; +, adequate; ++, good. The only motor test with good to adequate sensitivity to detect subacute and chronic differences between sham vs mild *and* mild vs moderate SCI animals is the backward locomotion rotating rod test.
[a]The detected difference in motor function between mild and moderate SCI is virtually identical to the difference between sham and moderate SCI. Discrimination between mild and moderate SCI is not reliable in these tests.
[b]Severe SCI often causes poor paw clearing, rendering reliable Catwalk analysis impossible. In these cases, number of coordinated Catwalk runs is '0'.
[c]Here, the totally absent sensitivity to differentiate these SCI severities might have been biased by the presence of stomach dragging in the more severely injured group.
[d]Although falling latencies of both SCI severities are similar, different phenotypes of backward locomotion deficits can still be observed; all animals with severe SCI never show any backward hindpaw movement, although in most animals with a moderate SCI a slight backward hindpaw movement can occasionally be observed during testing.

below-level sensory *perception*. The absence of sensory perception is also shown by the 55° inclined ladder test, as mild SCI animals could normally detect and step on the rungs, while the sensory-impaired moderate SCI animals could not. Moreover, moderate and severe SCI animals still showed pronounced, but largely ineffective, intrinsic spinal hindlimb motor activity during ladder climbing. The appearance of this hindlimb activity is in line with reports showing rat spinal cord (forward) locomotor autonomy by propriospinal circuits as the major factor in rat SCI recovery.[1,20]

Fourth, among all locomotion tests performed in this study, the motor deficits recorded with the backward locomotion rotating rod test showed best correlation with the basic locomotion scores (for example, BBB) in the acute SCI phase (but not with scores in later phases). In the acute phase, basic locomotion is likely still insufficiently compensated by intrinsic pattern generator driven circuitry.[1]

Furthermore, it needs to be underlined that the backward locomotion even showed to be more sensitive to detect SCI functional deficits than the regularity index, obtained by the Catwalk apparatus. The regularity index is popular to evaluate locomotion recovery after rodent SCI because of its presumed high sensitivity for across-lesion regeneration and low dependence on intrinsic spinal cord-mediated recovery.[13] However, locomotion assessment with the Catwalk is complex and expensive, as it requires advanced equipment, training and is labor-intensive. The backward locomotion rotating rod test is far quicker, simpler and even seems to be more sensitive for changes in spinal cord integrity.

Although the extent of intrinsic spinal locomotion in quadrupeds is interesting by itself, its value is much less evident in humans.[21,22] Thus, the intrinsic spinal locomotion in quadrupeds may mainly prove to be an important hurdle for clinically translatable assessment of SCI and spinal cord regeneration in rats and other quadrupeds. Further investigation, using selective spinal cord lesions, will be required to demonstrate which major descending and/or ascending tracts are required for rat backward locomotor ability, and whether reconnectivity in any of these tracts indeed corresponds with subsequent recovery.

In conclusion, backward locomotion testing can be used as a suitable behavioral readout to discriminate between sham, mild and moderate locomotor effects of experimental SCI research.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank C. Santucci for her great technical help.

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