Evaluation of five tests for the assessment of functional recovery following C4 or T7 dorsal column transection in the rat

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Abstract

The dorsal column lesion model of spinal cord injury targets sensory fibres which originate from the dorsal root ganglia and ascend in the dorsal funiculus. It has the advantages that fibres can be specifically traced from the sciatic nerve, verifiably complete lesions can be performed of the labelled component, and it can be used to study sprouting in the central nervous system from the conditioning lesion effect. However, functional deficits from this type of lesion are mild making assessment of experimental treatment-induced functional recovery difficult. Here, we compared a number of functional tests for their suitability to reliably measure recovery of function after dorsal column injury. We have examined the tape removal test, the rope crossing test, CatWalk™XT gait analysis, the horizontal ladder crossing test, and we introduce a new test, named the inclined rolling ladder. We compared animals with dorsal column injuries at C4 or T7 level to sham-operated animals for a duration of eight weeks. In most tests we were able to detect a significant deficit over the course of the experiment, although in most tests function recovered within 2-6 weeks. Cervical lesions showed more robust deficits than thoracic lesions. The horizontal ladder test showed the most statistically robust deficits. However the inclined rolling ladder showed promise as the functional deficits appeared to persist for 7 weeks and may prove useful to monitor the efficacy of treatments for this lesion model.

Introduction

Lesions of the dorsal funiculus of the spinal cord sever the ascending sensory fibres that originate in the dorsal root ganglia and terminate in the brainstem, carrying proprioceptive, nociceptive and tactile sensory information. They also damage the dorsal component of the motor corticospinal tract. The dorsal column lesion model is useful because transganglionic labelling from the sciatic nerve allows visualization of a specific set of ascending fibres, and by examining brainstems it can be verified that the labelled fibre population was completely lesioned, obviating the risk of spared fibres confounding experiments. Furthermore, it is a useful model to study the potential for neuron-intrinsic factors to promote axon regeneration, because significant sprouting in the injured CNS can be observed if the sensory neurons are induced to regenerate following a peripheral nerve lesion (the conditioning lesion effect) (Chong et al., 1999; Neumann and Woolf, 1999; Oudega et al., 1994; Richardson and Issa, 1984; Richardson and Verge, 1987). Gene expression in these neurons can be manipulated e.g. by the use of AAV vectors (Bareyre et al., 2011; Parikh et al., 2011).

While this model is well suited for studying axon sprouting or regeneration in the CNS anatomically, in any spinal cord injury model, it is also important to quantify the effects on recovery of function resulting from an experimental intervention. A suitable functional test should show a detectable and sustained
functional deficit in lesioned animals compared to sham-lesioned animals, if functional improvements resulting from experimental treatments are to be detectable after injury. In full transections of the spinal cord and severe contusion lesions, animals have readily detectable, and often severe functional deficits but require intensive care including manual voiding of the bladder if the sympathetic or parasympathetic connectivity of the upper lumbar segments and mid-sacral segments are severed (Shefchyk, 2002). In contrast, a dorsal column (DC) transaction lesion results in only minimal deficits.

The sensory afferents ascending in the DC carry information to the brainstem comprising pain, temperature, proprioception and fine touch (including vibration). While tests are available for sensation of temperature and pain these generally stimulate paw withdrawal via local spinal cord circuitry. A DC lesion leaves this circuitry largely intact so such tests will still illicit a withdrawal response that is independent of higher sensorimotor control, making nociceptive testing impractical for this type of lesion.

The use of functional tests with transection lesions of the DC has until now been fairly limited, probably because a transection lesion of the DC results in mild functional deficits that are transient and difficult to detect. Functional deficits were detected after a crush lesion of the DC (Bradbury et al., 2002). However, following a DC transection lesion Kanagal and Muir found only transient deficits of the fore paws and no deficit in hind paw function (Kanagal and Muir, 2007). In a later study they show again deficits of the fore paws and only small and transient deficits in hind paw function after DC transection injury (Kanagal and Muir, 2008).

We selected four previously described tests for comparison based on their effectiveness in previously published studies. These were the adhesive tape removal test (Schallert et al., 1982), horizontal ladder test (Bregman et al., 1993), rope crossing test (Kim et al., 2001) and CatWalk™XT gait analysis (Hamers et al., 2006; Hamers et al., 2001). All of these tests in regards to a DC lesion except the adhesive tape test are based on the assumption that a decline in proprioception and possibly subtle motor deficits resulting from the lesion will result in less coordinated stepping over an uneven surface and an altered gait on an even surface. The sticky tape removal test assumes that an animal with a lesion affecting fine touch sensation will take longer to notice and remove a small piece of tape placed on the rear paw.

A DC lesion also impairs discrimination of texture (Ballermann et al., 2000; Vierck, Jr. and Cooper, 1998). Ballermann and colleagues describe a reaching task which exploits the deficit in fine touch and discrimination of texture (Ballermann et al., 2000). This test involves the animal reaching for and being required to discriminate between a food item and a similarly shaped but roughly textured non-food item, a testing paradigm which requires food restriction and is specific to the forepaws. We designed an adapted gridwalk test, named the inclined rolling ladder, to test both tactile discrimination and proprioceptive feedback simultaneously. This consists of ladder rungs on a 45 degrees angle that have an immobile heavily textured half and a smooth, freely rolling half. We
hypothesised that after training, animals with intact sensation of texture would be able to discriminate between rough fixed and smooth rolling bars, and either choose the textured sections or adapt their stepping action when they detected a smooth (and thus rolling) step. This may result in a detectable increase in slip rate in DC-lesioned animals. Compounding the loss of texture discrimination would be the loss of proprioception resulting in more errors in lesioned animals. To determine the optimal lesion level to produce functional deficit, animals received a C4 or T7 complete bilateral dorsal column transection or a sham laminectomy at the same level and underwent the described tests for a duration of 8 weeks.

**Materials and Methods**

**Experimental animals and surgical procedures**

In this study, a total of 24 Fischer rats (180–250 g; Harlan, Horst, the Netherlands) were used. Animals were housed under standard conditions with food and water ad libitum, and a 12-hour: 12-hour light/dark cycle. All experimental procedures and postoperative care were carried out with approval from the local animal experimentation ethical committee. Animals were anesthetized using isoflurane (2-2.5%). Following an incision along the dorsal midline, a laminectomy at C4 or T7 was performed to expose the spinal cord and the dura mater was opened. To minimize compression damage of the spinal cord we first inserted a 30G needle at 1mm (cervical level) or 0.6mm (thoracic level) lateral to the midline on either side to a depth of 1.6mm (cervical level) or 1.4mm (thoracic level). The resulting hole was then enlarged by inserting a 27G needle to the same depth. Finally the tips of a pair of microscissors were inserted in the same holes to the same depth and then closed. Sham-operated animals received only the laminectomy. The muscles overlying the spinal cord were loosely sutured together with a 5-0 suture and the wound closed. Animals were allowed to recover at 37 °C and received postoperative analgesia (Temgesic 0.03 ml/100 g body weight s.c.; Schering-Plough, Maarssen, the Netherlands), and to survive for eight weeks after injury.

Several functional tests (described below) were performed before injury as a baseline measurement and two or three days and seven days after injury, followed by weekly measurements. Three days before perfusion, the animals were re-anesthetized and the left sciatic nerve was exposed. Animals received an injection of cholera toxin subunit B (103B, List Laboratories Inc., Campbell, CA) into the sciatic nerve to transganglionically label ascending dorsal column axons in the spinal cord. Animals were injected with a lethal dose of pentobarbital and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer. Brain stems and spinal cords were post-fixed for 3–4 hours and transferred to 30% sucrose in phosphate-buffered saline, and were frozen in Tissue-Tek OCT (4583; Sakura Finetek Holland) the following day.
**Immunohistochemistry**

Brain stems were cut 50 μm thick on a cryostat and placed into PBS in two series. Free floating sections were incubated with 0.3% H2O2 and then blocked using TBS with 2% horse serum. Sections were incubated for 72 hours with goat anti-CTB (1:80,000; 703, List Laboratories Inc., Campbell, CA), followed by biotinylated horse anti-goat (1:300; Vector Labs), ABC reagent (1:200; Vector Labs), then washed in TBS and developed using diaminobenzidine with nickel ammonium sulphate. Sections were then mounted onto Superfrost Plus slides (Menzel-Gläser), dehydrated and embedded with Entellan. Spinal cords were cut 20 μm thick on a cryostat onto Superfrost Plus slides (Menzel-Gläser), and stained with the same procedure.

**CatWalk™XT gait analysis**

The CatWalk™XT gait analysis system (Hamers et al., 2006; Hamers et al., 2001) is a platform locomotor test in which animals cross a 7cm wide walkway with a glass floor (131 cm) located in a darkened room. The glass floor is illuminated from the side, which is scattered where a paw touches the glass. Three runs per animal were recorded by a high-speed camera that captures the paw prints from below. All four paws were automatically labelled using the Catwalk software and checked afterwards by a blinded experimenter for gait analysis. For each animal the base of support, stride length, swing time, print width, mean pixel intensity and maximum contact area for the hind paws were measured and automatically calculated using the CatWalk software package. Chocolate pellets were given to reward the animals after each successful run.

**Rope-crossing test**

The rope-crossing test adapted from Kim and colleagues was performed to evaluate sensorimotor function of the animals before and after a DC lesion (Kim et al., 2001). A 1.25-meter long rope with a diameter of 4.0 cm was set up between two platforms. The animal was required to traverse the rope three times and the number of slips and steps with the left hind paw were live-scored by two blinded observers. The number of slips was divided over the total number of steps for each run and averaged for three runs to calculate the mean error ratio. Chocolate pellets were given to reward the animals after each successful run.

**Inclined rolling ladder test**

The inclined rolling ladder is a 34 cm long ladder set at a 45 degrees angle with 9 adjustable rungs with half smooth (rolling) and half rough (and fixed) rungs (see figure 1), enabling simultaneous testing of tactile sense and proprioceptive control. Animals are required to walk up the ladder to the platform. Chocolate pellets were given to reward the animals after each successful run. In order to prevent a learning effect in animals the orientations of the ladder rungs were adjusted
randomly for each time point. Three runs per animal were video recorded and analysed by two independent blinded observers. Successful steps, slips and from which section of the rung these occurred were scored. The number of successful steps on smooth rungs was divided over the total number of steps for each run and averaged for three runs to calculate the mean ratio for successful smooth steps/total steps. To calculate the mean error ratio for slips/total smooth steps the number of slips was divided over the total number of steps on smooth rungs for each run and averaged for three runs.

**Figure 1** Schematic diagram of the inclined rolling ladder test. The inclined rolling ladder test consists of ladder rungs on a 45 degrees angle that have an immobile heavily textured half and a smooth, freely rolling half. Animals are required to walk up the ladder to the platform. After training, animals with intact sensation of texture should be able to discriminate between rough, fixed and smooth, rolling rungs, and either choose the textured sections or adapt their stepping action when they detected a smooth (and thus rolling) rung. We hypothesized that after DC injury animals cannot discriminate between smooth rolling rungs and fixed rough rungs, leading to an increase in errors.

**Horizontal ladder test**

The horizontal ladder test adapted from Bregman’s gridwalk test (Bregman et al., 1993) is a 0.9 meter long horizontal ladder with a diameter of 15.5 cm. The rungs of the ladder are adjustable with a possible gap of 3.5- 5.0cm and were randomly adjusted for each time point to prevent a learning effect. Two independent observers live-scored left and right rear slips and total left rear steps which was multiplied by two to determine total steps. The total number of slips was divided over the total number of steps for each run and averaged for three runs to calculate the mean error ratio. Chocolate pellets were given to reward the animals after each successful run.
Tape removal test
Sensory function was tested using the tape removal test, adopted from Schallert (Schallert et al., 1982). A piece of tape (Kip Hochkrepp, #803, Bocholt, Germany) of 15 x 15 millimetres was affixed to the palm of the left hind paw. The time until the animal had detected the tape was measured in three individual trials to calculate the mean sensing time. Trials in which the animals did not detect the tape were stopped after 180 seconds.

Statistical analysis
The rope test, the horizontal ladder test and the inclined rolling ladder test were all analysed as follows. Because we scored individual steps as either a successful step or a slip, the data follows a binomial distribution. The whole time-courses of lesioned vs sham were compared using binomial generalized linear mixed models (GLMM), with lesioned/sham and operated/baseline as fixed factors, animal as a random factor and time as a covariate. In the rope and horizontal ladder tests an observation-level random effect was included to account for overdispersion (Elston et al., 2001). Models were fitted in R (R Core Team, 2013) using package lme4 (Bates et al., 2013) p-values were calculated by parametric bootstrap using R package pbkrtest (Halekoh and Højsgaard, 2013). We calculated p-values for the interaction of lesioned/sham with time and the unconditional main effect of lesioned/sham. Per-time-point effects were calculated as the interaction of lesioned/sham with operated/baseline using a binomial GLMM treating baseline and the specific time point only.

For the CatWalk™XT parameters and the tape removal test, the time-courses of lesioned vs sham were compared using linear mixed models, with lesioned/sham and operated/baseline as fixed factors, animal as a random factor and time as a covariate. Models were fitted using lme4 and p values were calculated with function KRmodcomp in package pbkrtest. As before we calculated p-values for the interaction of lesioned/sham with time and the unconditional main effect of lesioned/sham. For the tape test we used the log of the withdrawal time as the data were expected to follow an exponential distribution. Per time point comparisons were made using unpaired t-tests.

Results

Lesion completeness
Animals received a dorsal column transection at T7 or C4 level or sham surgery. To check for lesion completeness, sensory afferents were transganglionically traced from the left sciatic nerve and the dorsal column nuclei in the brain stem checked
Figure 2 Histological evaluation of lesion completeness. Animals received a DC transection at T7 or C4 level or sham surgery and survived eight weeks after which sensory axons were labelled with CTB. Sections were processed for immunohistochemistry for CTB using DAB-nickle. (A) Section of the brain stem of an animal that received a sham surgery. The higher magnification image shows that many CTB-positive axons are visible in the nucleus gracilis of the brain stem. (B) Section of the brain stem of a lesioned animal. Bar = 500µm. The higher magnification image shows that no CTB-positive fibres were detected in the brain stem. Bar = 50µm.

Figure 3 Histological evaluation of the spinal cord after injury. Animals received a DC transection at T7 or C4 level or sham surgery and survived eight weeks after which sensory axons were labelled with CTB. Sections were processed for immunohistochemistry for CTB using DAB-nickle. (A) Section of a spinal cord from an animal that received a cervical DC injury. (B) Section of a spinal cord from an animal that received a thoracic DC injury. (C) Section of a spinal cord from a control animal that received a sham surgery. Bar = 100µm.
for spared fibres by histological analysis. We observed CTB-labelling in the brain stem of one animal in the group that received a thoracic lesion, indicating that sparing of axons occurred. This animal was excluded from further analysis. As expected, positive staining in the brain stem was observed in all sham animals (figure 2). In spinal cord sections we observed CTB-positive axons up to, but not crossing the lesion site in animals that received a cervical and thoracic dorsal column transection (figure 3).

**Tape removal test**

Tape removal tests were performed in order to evaluate touch perception from the left hind paw. The time until the animal notices a piece of adhesive tape placed on the palm of the left hind paw was measured by a blinded observer. We observed an overall significant increase in the time it takes to sense and find

![Figure 4 Tape removal test.](image)

The tape removal test was performed in order to evaluate sensory dysfunction of the left hind paw up to eight weeks after cervical and thoracic DC injury. We observed overall deficits in the ability to sense the adhesive tape on the left hind paw in both (A) C4 and (B) T7 injured animals compared to the sham groups (linear mixed model, †p=0.02 for interaction of C4 lesioned with time, *p=0.01 for C4 lesion main effect; †††p=0.0002 for interaction of T7 lesioned with time, T7 lesion main effect was n.s.). *p<0.05 for single time-point comparison (t-test); error bars are SEM, n=6 animals per group except n=5 for T7 lesion.

![Figure 5 Rope crossing test.](image)

Dysfunction of the walking pattern was assessed using the rope crossing test for eight weeks after cervical and thoracic DC injury. In animals that received either a (A) C4 or T7 (B) DC injury we observed an overall significant increase in the error rate compared to the sham groups (binomial generalized linear mixed model, †p=0.026 for interaction of C4 lesioned with time, **p=0.004 for C4 lesion main effect; *p= 0.03 for T7 lesion main effect, interaction of T7 lesioned with time was n.s.). *p<0.05, **p<0.01 for single time-point comparison (binomial generalized linear model); error bars are SEM, n=6 animals per group except n=5 for T7 lesion.
the adhesive tape for both the C4 and T7 injured animals (linear mixed model, p=0.02 for interaction of C4 lesioned with time, p=0.01 for C4 lesion main effect; p=0.0002 for interaction of T7 lesioned with time, T7 lesion main effect was n.s.) compared to the sham groups over the complete time period of 8 weeks (figure 4).

**Rope crossing test**

In this test the rats were allowed to cross a rope between two platforms. The number of steps and errors made with the left hind paw was scored by two blinded observers up to eight weeks after injury. In animals that received either a cervical C4 or thoracic T7 lesion we observed an overall significant increase in the error rates (binomial generalized linear mixed model, p=0.026 for interaction of C4 lesioned with time, p=0.004 for C4 lesion main effect; p= 0.03 for T7 lesion main effect, interaction of T7 lesioned with time was n.s.) compared to the sham animals (figure 5). The initial deficit at two days after cervical injury was less apparent for the remaining period.

**Horizontal ladder test**

Animals were allowed to cross a horizontal ladder with rung gaps of randomized length to assess dysfunction. The number of steps and errors made with the left hind paw was scored by two blinded observers up to eight weeks after injury. For both C4 and T7 lesioned animals we observed an overall increase in the error rates (binomial generalized linear mixed model, p=0.0003 for interaction of C4 lesioned with time, p=0.00035 for C4 lesion main effect; p= 0.04 for T7 lesioned interaction with time, p=0.025 for T7 lesion main effect) compared to the controls (figure 6). There was a clear deficit observed at early time points after injury, which was less apparent for the remaining period.

Figure 6 **Horizontal ladder test.** Animals were allowed to cross a horizontal grid with gaps of randomized size to assess dysfunction during eight weeks after cervical or thoracic DC lesion. For both (A) C4 and (B) T7 lesioned animals we observed an overall increase in the error rates compared to sham controls (binomial generalized linear mixed model, ††††p=0.0003 for interaction of C4 lesioned with time, ***p=0.00035 for C4 lesion main effect; †p=0.04 for T7 lesioned interaction with time, *p=0.025 for T7 lesion main effect). *p<0.05, **p<0.01, ***p<0.001 for single time-point comparison (binomial generalized linear model); error bars are SEM, n=6 animals per group except n=5 for T7 lesion.
A number of gait parameters were assessed using the CatWalk™XT system. In C4 lesioned (figure 7A) and T7 lesioned (figure 7B) animals we observed an overall slight increase in the base of support of the hind paws (linear mixed model, p=8.57E-07 for interaction of C4 lesioned with time, p=0.00144 for C4 lesion main effect) and for (B) animals that received a thoracic lesion (linear mixed model, **p=0.00944 for T7 lesion main effect, linear mixed model, ††p=0.00391 for C4 lesion mixed with time, **p=0.00042 for interaction of C4 lesioned with time). An increase in hind paw print width was observed in C4 lesioned animals (linear mixed model, ***p=0.00042 for C4 lesion main effect, interaction of C4 lesioned with time was n.s.). An increase in swing time of the hind paws was seen in C4 lesioned animals (linear mixed model, **p=0.00620 for C4 lesion main effect, interaction of C4 lesioned with time was n.s.). No differences were observed for hind paw prints were observed for animals that received a thoracic lesion. An increase in swing time of the hind paws was seen in C4 lesioned animals (linear mixed model, **p=0.00620 for C4 lesion main effect, interaction of C4 lesioned with time was n.s.). There was an overall significant increase (linear mixed model, **p=0.00620 for C4 lesion main effect, interaction of C4 lesioned with time was n.s.) in swing time for the hind paws of C4 animals (figure 7E), which was not observed in T7 lesioned animals (figure 7F) and may be related to observations of unusual walking behaviour characterized by large movements of the hind paws. This deficit was observed for the whole time period of eight weeks. We observed an overall difference in stride length of the hind paws of C4 injured animals (linear mixed model, **p=0.00133 for interaction of C4 lesioned with time, C4 lesion main effect was n.s.), however, this seems to be caused by the 5 week data point where a smaller stride length was measured for the sham animals (figure 7G). No differences were seen in animals that received a T7 lesion (figure 7H). No differences were found in the mean pixel intensity of the hind paws in both C4 and T7 injured animals (linear mixed model, **p=0.00996 for T7 lesion main effect, interaction of T7 lesioned with time was n.s.), while interestingly, the maximum contact area of the hind paws was significantly decreased in T7 injured (linear mixed model, **p=0.00996 for T7 lesion main effect, interaction of T7 lesioned with time was n.s.), but not C4 injured animals (figures 7K; 7L).
Inclined rolling ladder test

Functional deficit after dorsal column injury was also measured in the newly developed inclined rolling ladder test. We observed an overall significant decrease in sensorimotor control on smooth steps in both C4 (figure 8A) and T7 (figure 8B) lesioned animals compared to controls (binomial generalized linear mixed model, p=0.002 for C4 lesion main effect; p= 0.002 for T7 lesion main effect, interaction of C4/T7 lesioned with time was n.s.). The overall error rates were also significantly increased in both C4 (figure 8C) and T7 (figure 8D) injured animals (binomial generalized linear mixed model, p=0.03 for interaction of C4 lesioned with time, p=0.01 for C4 lesion main effect; p=0.03 for interaction of T7 lesioned with time p=0.02 for T7 lesion main effect). Interestingly, these deficits were detectable up to seven weeks in animals that received a cervical DC transection injury.

Figure 8 Inclined rolling ladder test. Animals were challenged on the inclined rolling ladder test to assess dysfunction after cervical or thoracic DC injury. (A-B) An overall significant decrease in sensorimotor control on smooth rungs was observed for animals that received a cervical or thoracic DC transection (binomial generalized linear mixed model, **p=0.002 for C4 lesion main effect; **p= 0.002 for T7 lesion main effect, interaction of C4/T7 lesioned with time was n.s.). *p<0.05, **p<0.01, ***p<0.001 for single time-point comparison (binomial generalized linear model); error bars are SEM, n=6 animals per group except n=5 for T7 lesion. (C-D) A significant increase in the overall error rates was seen in both C4 and T7 injured animals (binomial generalized linear mixed model, †p=0.03 for interaction of C4 lesioned with time, *p=0.01 for C4 lesion main effect; †p=0.03 for interaction of T7 lesioned with time *p=0.02 for T7 lesion main effect). * p < 0.05, ** p < 0.01 for single time-point comparison (binomial generalized linear model); error bars are SEM, n=6 animals per group except n=5 for T7 lesion.
Discussion
In this study we have compared several tests to measure recovery of function after cervical and thoracic DC injury in rats. A DC lesion spares other sensory spinal pathways, notably the spinothalamic pathway, so much sensory information still reaches the brain. For this reason it is necessary to use tests which target the modalities carried in the ascending DC. Although a complete DC lesion performed as we describe also unavoidably severs the corticospinal tracts resulting in minor motor deficits, in this study we focussed on tests targeting deficits in proprioception (horizontal ladder test, CatWalk™XT gait analysis, rope crossing test) and/or tactile sense (adhesive tape removal test) and developed the inclined rolling ladder test to assess loss of proprioception and tactile discrimination simultaneously.

We found indeed that in the majority of tests, lesions produced significant deficits over the time-course. In many of the tests, large deficits were visible at 2 days but these quickly diminished. It is well known that rodents show considerable adaptive recovery powers following spinal cord injury, and this is in part due to plasticity and remodelling that takes place in the spinal cord after lesion (Hendriks et al., 2006; Jeffery and Blakemore, 1999; Weidner et al., 2001; Bareyre et al., 2004). We note that in the CatWalk™XT, the inclined rolling ladder and (marginally) in the horizontal ladder test, more robust effects were seen from the cervical level lesion than thoracic lesion. Both of these lesions should block sensory information from the hind limbs to the brainstem in the dorsal column equally well. The corticospinal tract innervation of the lumbar spinal cord should also be similarly compromised. The differences may therefore reflect a greater capacity to remodel after the thoracic lesion, perhaps because alternate pathways that may be used to relay this information are more readily available. For experimental studies a cervical lesion would appear to be preferable.

We present here an alternative method to assess recovery of sensory function after DC transection injury. The inclined rolling ladder test combines both testing of proprioception and tactile discrimination and using this test we were able to show a consistent deficiency over a period of seven weeks compared to sham controls. This indicates that the inclined rolling ladder test shows potential for DC injury experiments with treatments aimed at promoting repair of the ascending dorsal columns. Of the two outcome measures tested, the measure of slips versus total steps appears to show the most robust and long-lasting deficits. This outcome did show variability from week to week, which was probably dependent on the exact arrangement of bars used in that week. The sensitivity of the test may also be improved by making the ladder longer or increasing the number of test runs performed, to increase the amount of data collected.

Furthermore, several other tests showed functional deficits albeit with shorter recovery periods, and CatWalk™XT gait analysis revealed that hind paw swing time appears to be significantly increased over the entire 8 week period making this the test with the longest window for treatment effects to be measured.
The adhesive tape removal test is based on the assumption that impairment of touch sensory input will lead to an increase in the time to notice the sticky tape on the hind paw. We observed an overall deficiency but this was not consistent over time. This test has been used successfully to measure impairment of touch after DC crush lesion (Bradbury et al., 2002). Onifer and colleagues have used the tape test for assessment of fore limb function after dorsal hemisection and similarly to our finding they show an initial large deficiency that returns to control levels at three weeks post injury (Onifer et al., 2005). The type of tape used in this test could lead to differences in sensitivity. We used a ridged paper type of tape which was described to be effective for testing the front paws of rats (Albertsmeier et al., 2007). Others used sewed adhesives for bandages and state that these are the best [reviewed in (Bouet et al., 2009)]. In our experience the ridged paper adhesive was superior to several other smooth paper or synthetic adhesives.

The rope crossing test and the horizontal ladder test were included based on the hypothesis that deficits in proprioceptive feedback mechanisms would lead to more stepping errors. For both tests we indeed observed a significant increase in the error rates. However, in the rope test these effects disappeared shortly after the initial increase in errors. Bradbury and colleagues (Bradbury et al., 2002) were able to measure consistent functional deficits in animals with a DC lesion with a similar horizontal ladder test for up to six weeks. We also found a clear deficit in this test although it appeared to recover somewhat more quickly.

We tested several parameters in the CatWalk™XT gait analysis. In cervical lesions, we found an increase in hind paw print width and an increase in hind paw base of support clearly detectable until 3-4 weeks. These findings are consistent with those of Bradbury et al., who found an increase in base of support and stride width at 6 weeks after DC crush injury (Bradbury et al., 2002). In their footprint analysis stride length was also decreased which we were unable to reproduce. This may be due to a difference in lesion severity, as we performed DC transection lesions, while Bradbury and colleagues performed DC crush lesions which may have resulted in a more severe deficiency in sensorimotor function. Another reason could be the strain used in the test since it has been shown that different rat strains may display differences in stride length and other locomotion parameters (Koopmans et al., 2007). Furthermore, in cervical lesions, we found a consistent increase in hind paw swing time which did not recover over the whole time course, making this a promising measure of function. This may directly reflect lack of proprioceptive function as it appears to be related to exaggerated circular hind limb stepping movements (‘windmilling’).

We also assessed the hind paw parameters ‘mean pixel intensity’ and ‘maximum contact area’ intended to detect changes in weight bearing and paw placement. The ‘mean pixel intensity’ parameter has been used by others to assess mechanical allodynia in neuropathic pain models (Vrinten and Hamers, 2003; Gabriel et al., 2009; Gabriel et al., 2007), however we observed no change in this parameter after thoracic or cervical DC transection injury. Interestingly, only the ‘maximum contact area’ parameter did respond consistently for longer
periods to thoracic lesions but not cervical, in contrast to the other tests. After a complete dorsal column transection no differences were found between injured and non-injured animals on a gridwalk test and in base of support, stride length and rotation angle in a platform locomotion task, while consistent deficits in these tests were found after a dorsal hemisection (Grill et al., 1997).

In a number of studies in which a cervical DC transection lesion was performed, several deficits of the fore paws were found (Chan et al., 2005; Kanagal and Muir, 2007; Kanagal and Muir, 2008). Kanagal and Muir found no deficit of the hind paws on a horizontal ladder test and in stance duration, stride length and stride duration on a platform locomotor task during eight weeks after a cervical DC transection or a deeper DC lesion that also fully transects the corticospinal tract (Kanagal and Muir, 2007). In accordance with our results, a comparison of cervical and thoracic DC transections has shown that cervical DC injury leads to larger deficits than thoracic injury (Kanagal and Muir, 2008). Both cervical and thoracic lesions resulted in transient deficits in a horizontal ladder test that was performed at two time points (at 2 weeks and 6 weeks after injury). Again, stance duration, stride length and stride duration of the on a platform locomotor task were unaffected after both levels of injury. These studies and our results indicate that transection lesions of the DC lead to mild and transient deficits of the hind paws.

In conclusion, we show here that although a number of classical functional tests can be used to measure an overall decline in sensorimotor function after DC transection injury, spontaneous recovery occurs within several weeks. Cervical lesions appear to produce clearer and longer lasting deficits than thoracic lesions. We identify a number of useful tests, including the newly developed inclined rolling ladder that allow reliable measurements of recovery of sensory function for longer periods of time.

References


