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# Adaptation of tape removal test for measurement of sensitivity in perineal area of rat

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Abbreviations:

BBB test - Basso Beattie and Bresnahan test, D - day, L - lumbar vertebrae, PFA - paraformaldehyde, SCI - spinal cord injury, T - thoracic vertebrae, W - week



## Abstract

Regeneration after spinal cord injury is a goal of many studies. Although the most obvious target is to recover motor function, restoration of sensation can also improve the quality of life after spinal cord injury. For many patients, recovery of sensation in the perineal and genital area is a high priority. Currently there is no experimental test in rodents for measuring changes in sensation in the perineal and genital area after spinal cord injury. The aim of our study was to develop a behavioural test for measuring the sensitivity of the perineal and genital area in rats. We have modified the tape removal test used routinely to test sensorimotor deficits after stroke and spinal cord injury to test the perineal area with several variations. A small piece of tape (approximately 1 cm<sup>2</sup>) was attached to the perineal area. Time to first contact and to the removal of the tape was measured. Each rat was trained for 5 consecutive days and then tested weekly. We compared different rat strains (Wistar, Sprague-Dawley, Long-Evans and Lewis), both genders, shaving and non-shaving and different types of tape. We found that the test was suitable for all tested strains, however, Lewis rats achieved the lowest contact times, but this difference was significant only for the first few days of learning the task. There were no significant differences between gender and different types of tape or shaving. After training the animals underwent dorsal column lesion at T10 and were tested at day 3, 8, 14 and 21. The test detected a sensory deficit, the average time across all animals to sense the stimulus increased from 1'32 up to 3'20. There was a strong relationship between lesion size and tape detection time, and only lesions that extended laterally to the dorsal root entry zone produced significant sensory deficits. Other standard behavioural tests (BBB, von Frey, ladder and Plantar test) were performed in the same animals. There was a correlation between lesion size and deficit for the ladder and BBB tests, but not for the von Frey and Plantar tests. We conclude that the tape removal test is suitable for testing perineal sensation in rats, can be used in different strains and is appropriate for monitoring changes in sensation after spinal cord injury.

Keywords: sensation, spinal cord injury, perineum, functional outcome, behavioural tests, sensory regeneration.

## Introduction

Spinal cord injury (SCI) is a serious state that affects about 250 000 – 500 000 people worldwide every year (<https://www.who.int/en/news-room/fact-sheets/detail/spinal-cord-injury>). The main causes are road traffic crashes, falls or violence. To date, no standard therapy for the regeneration of axons in the severed spinal cord of humans exists.

Research in this area is usually focused on motor recovery, but restoration of sensory function would also improve the life of patients. Moreover, sensory and motor recovery are closely linked, with motor function being dependent on sensory inputs to the cord. Many descending motor projections to the cord terminate on interneurons that also receive sensory inputs (Levine et al., 2014). In respect of sensory recovery, high priorities for human patients are restoration of sensation to the hands, and restoration of perineal and genital sensation (Anderson, 2004). A standard experimental model of spinal cord injury in rodents is the dorsal column lesion. This lesion disrupts ascending sensory fibres from the dorsal root ganglia carrying tactile information, discriminatory touch, vibration and proprioception (Sengul and Watson, 2015). There are several behavioural tests for assessment of sensation in the limbs but none as yet for the perineal area.

The tape removal test (also called sticky tape test or adhesive removal test) was developed by Schallert et al. (1982) to measure sensorimotor asymmetries after nigrostriatal damage in rats. It is based on attachment of a small piece of adhesive paper to various parts of body (snout, forepaws, hindpaws). The time taken to sense the stimulus and to remove the tape is measured.

Since its development (Schallert et al., 1982), this test has been used in many studies and with various modifications. In most studies the tape is usually attached to the forepaws (Aehling et al., 2018; Albertsmeier et al., 2007; Schallert et al., 2000). However, as tested by Schallert et al. (1982) it can be also used in other body parts including the snout (Fleming et al., 2013; Wang et al., 2016) or hindlimbs (Arakawa et al., 2014; Tsytsarev et al., 2017). The test has also been adapted to several other species beside rats, such as mice (Arakawa et al., 2014; Bouet et al., 2009; Fleming et al., 2013), dogs (Quaranta et al., 2004) or non-human primates (Annett et al., 1994).

The tape removal test, in the original setting, measures sensorimotor deficits (Schallert et al., 1982). The sensory part is the time to first contact with the tape and motor part the time to tape removal. An important feature of the test is that animals must be aware of the presence of the tape, indicating that sensory information has reached the brain and been processed to provide a motor output.

The test was originally used to lateralise sensorimotor deficits and detect asymmetry in rats with nigrostriatal damage (Schallert et al., 1982). Later, it has been adapted for different central nervous system injuries or disorders. Most commonly it is used in brain injury models such as stroke (Duricki et al., 2016; Zhang et al., 2000), cardiac arrest (Aehling et al., 2018; Albertsmeier et al., 2007) or Parkinson's disease models (Fleming et al., 2013; Schallert et al., 2000). However, it has also been used in several models of spinal cord injury (Fagoie et al., 2016; Onifer et al., 2005; Schallert et al., 2000).

Our goal was to adapt the test for testing sensation in perineal area. Restoration of sexual function, which involves perineal and genital sensation is a high priority for spinal injury patients. However, in this area, there is currently no animal model for sensory restoration and a specific test is therefore needed. The aim of our study was to develop a behavioural test for measuring the ability of

rats to sense touch in the perineal and genital area and to compare it with other conventional tests used for testing locomotion and limb sensitivity in normal animals and after spinal cord injury.

## Materials and Methods

### Animals

A total of 60 adult rats were used in the experiment; we performed the test in four different strains: Wistar (n=24), Sprague Dawley (n=19), Long Evans (n=9) and Lewis (n=8). The possible gender differences were tested in Wistar rats, therefore we used 8 female Wistar rats. The weight was dependent of the strain and it varied from 225-424 grams in males and 218-253 grams in females. Animals were housed in pairs under standard conditions in 12-hour light/dark cycle with food and water *ad libitum*.

All experiments were performed in accordance with the European Communities Council Directive on September 22, 2010 (2010/63/EU) regarding the use of animals in research and were approved by the ethics committee of the Institute of Experimental Medicine, Czech Academy of Sciences.

### Tape removal test

#### *Preparation of perineum*

Animals were anesthetized with 2% Isoflurane and 0.3 l/min airflow. The rat perineal area was shaved with a razor blade 1 day before testing (Figure 1A, D). In groups where we tested the effect of hair removal, we tested animals treated with depilatory cream (Strep Opilca) (Figure 1C) or did not shave at all (Figure 1B).

For the tape removal test, each rat was trained over 5 consecutive days (D1-5) and then tested weekly (W1-7). Lesioned animals were tested at day 3, 8, 14 and 21 post surgery. Animals were placed in the testing room at least 30 minutes before the test to adapt. The rat was then removed from its home cage and placed into an empty testing cage and acclimatized there for 5 minutes. A small piece of tape (approximately 1 cm<sup>2</sup>) was attached to perineal area on the left side (Figure 1A). Time to first touch (contact time) and to remove the tape was measured. If the rat did not remove the tape by 5 minutes the tape was removed and a time of 5 min was recorded. This session was repeated 4 times for each animal with a 3 min pause between each trial. (video in supplement)

Three different types of tape were used: textile (SPOFAPLAST 3M), plastic (certoplast PVC tape) and paper (TimeMed Labeling Systems, Inc.) (Figure 1E).

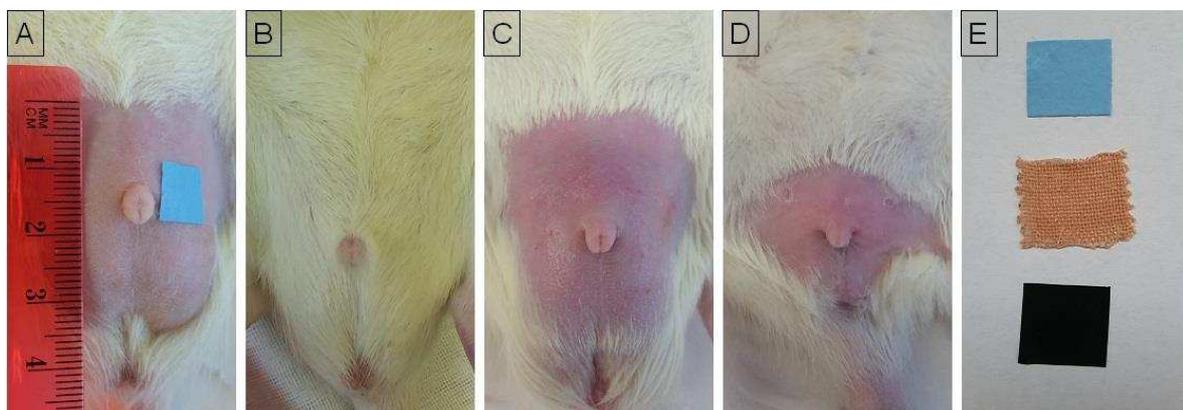


Figure 1. Photos of rat perineum, A: placement and size of the tape, male-shaved, B: male-unshaved, C: male-depilatory cream, D: female-shaved, E: paper, textile and plastic tape.

### Lesion/spinal cord injury

A dorsal column lesion was performed as described previously (Bradbury et al., 1999). Animals were anesthetized with 2% Isoflurane and 0.3 l/min airflow. The back was shaved and a skin incision was made between the T7 and L1 vertebrae. A laminectomy was performed on the T10 vertebra. The dorsal columns were cut with fine-tipped forceps (approximately 1 mm deep). Muscles and skin were sutured.

### BBB

Motor function was evaluated using Basso Beattie and Bresnahan (BBB) 21-point locomotor scale (Basso et al., 1995). Rats were placed into open-field and scored according to the criteria including hindlimb movement, weight support and forelimb-hindlimb coordination. Animals were tested from 2 and 3 weeks after injury.

### Ladder

Sensory-motor coordination was tested on the horizontal ladder walking test. Each rat was tested 2 times before the lesion to get a baseline score and then in the second and third week after the lesion. Animals were placed in the testing room at least 30 minutes before the test to adapt. The rat was then removed from its home cage and placed onto a horizontal ladder in the MotoRater (TSE Systems). They acclimatized there for 5 minutes and were returned to their home cage. Rats were then recorded with a high-speed camera (CamRecord CL600x2) walking along 155 cm long, 10 cm wide horizontal ladder with unevenly spaced rungs. Each step was given a score according to a 0-6 foot fault scoring system (Metz and Whishaw, 2009). The average score for each step from three trials was calculated.

### Plantar (Hargreaves) test

Changes in thermal sensation after SCI were measured by the Ugo Basile Plantar Heat test apparatus (Comerio VA, Italy). Each rat was tested 2 times before the lesion to get a baseline latency time and then second and third week after the lesion. Animals were placed in the testing room at least 30 minutes before the test to adapt. Rats were then removed from their home cage and placed into a plexiglass box on a glass surface and acclimatized there for 5 minutes. An infrared light beam was then applied to the plantar surface of the hind paw. The light and timer were activated simultaneously. When the rat withdrew its paw, the time was recorded. The infrared stimulus turned off automatically after 30 s. Five trials were performed for each hind paw of each animal with at least 3 min pause between individual trials. Of the 5 values the lowest and the highest were deleted and three rest were averaged.

### Von Frey

In the lesioned animals touch sensitivity was measured with the electronic von Frey test (IITC Inc., Life Science Instruments, Woodland Hills, CA, USA) as described previously (Ferrier et al., 2016; Martinov et al., 2013). Each rat was measured 2 times before the lesion to get a baseline withdrawal threshold and then 7, 13 and 20 days after the lesion. Animals were placed in the testing room at least 30 minutes before the test to adapt. The rat was then removed from its home cage and placed into a plexiglass box on a mesh floor stand (IITC Inc., Life Science Instruments, Woodland Hills, CA, USA) and acclimatized there for 15 minutes. The perineal area was then stimulated by slowly raising the probe with rigid tip to touch the shaved perineal area. Pressure was increased until nociceptive response or until the rat lifted up so it was not possible to go further with the tip. The value was recorded and measurement repeated until 5 values were measured for each rat. Of the 5 values the lowest and the highest were deleted and three rest were averaged.

### Perfusion

Animals were anesthetized with Isoflurane and injected with 1 ml Chloral hydrate per 100 g intraperitoneally. Animals were transcardially perfused with approximately 300 ml of PB and next with the same volume of 4% paraformaldehyde (PFA). Spine was cut off and stored in 4% PFA.

### Histology

Spinal cords were removed from spine and an approximately 2 cm long piece containing the lesion was cut out. It was embedded in paraffin and cut to 14  $\mu$ m cross sections. They were then labelled using Luxol blue/cresyl violet staining. Images were taken with Axiocam (Zeiss) microscope.

### Lesion size quantification

Lesions were reconstructed from the histological sections from all rats and lesion size was measured using ImageJ. The percentage of preserved area on the left side of the lesion was then correlated with behavioural deficit as described in the results section.

### Statistical analysis

All data are presented as mean  $\pm$  SEM. The GraphPad Prism 5 software was used to analyse the data. To find differences we performed two-way repeated measures ANOVA. In case ANOVA found a difference, it was followed by Bonferroni post hoc test to compare individual groups. If the P value was less than 0.05, the difference was considered statistically significant.

Results of behavioural tests of animals that underwent a dorsal column lesion were evaluated in two ways. In the first study we assessed relationship between lesion size and functional outcome. The percentage of preserved area in the lesion was correlated with the results of behavioural tests (tape removal, BBB, von Frey, ladder and plantar) and linear regression line was calculated. The result of individual behavioural test was calculated as a difference of animal performance before and after SCI.

Rats were then divided to three groups according to lesion size – small, medium, big. Differences between individual groups and within groups in time were evaluated using two-way repeated measures ANOVA in GraphPad Prism 5 software.

## Results

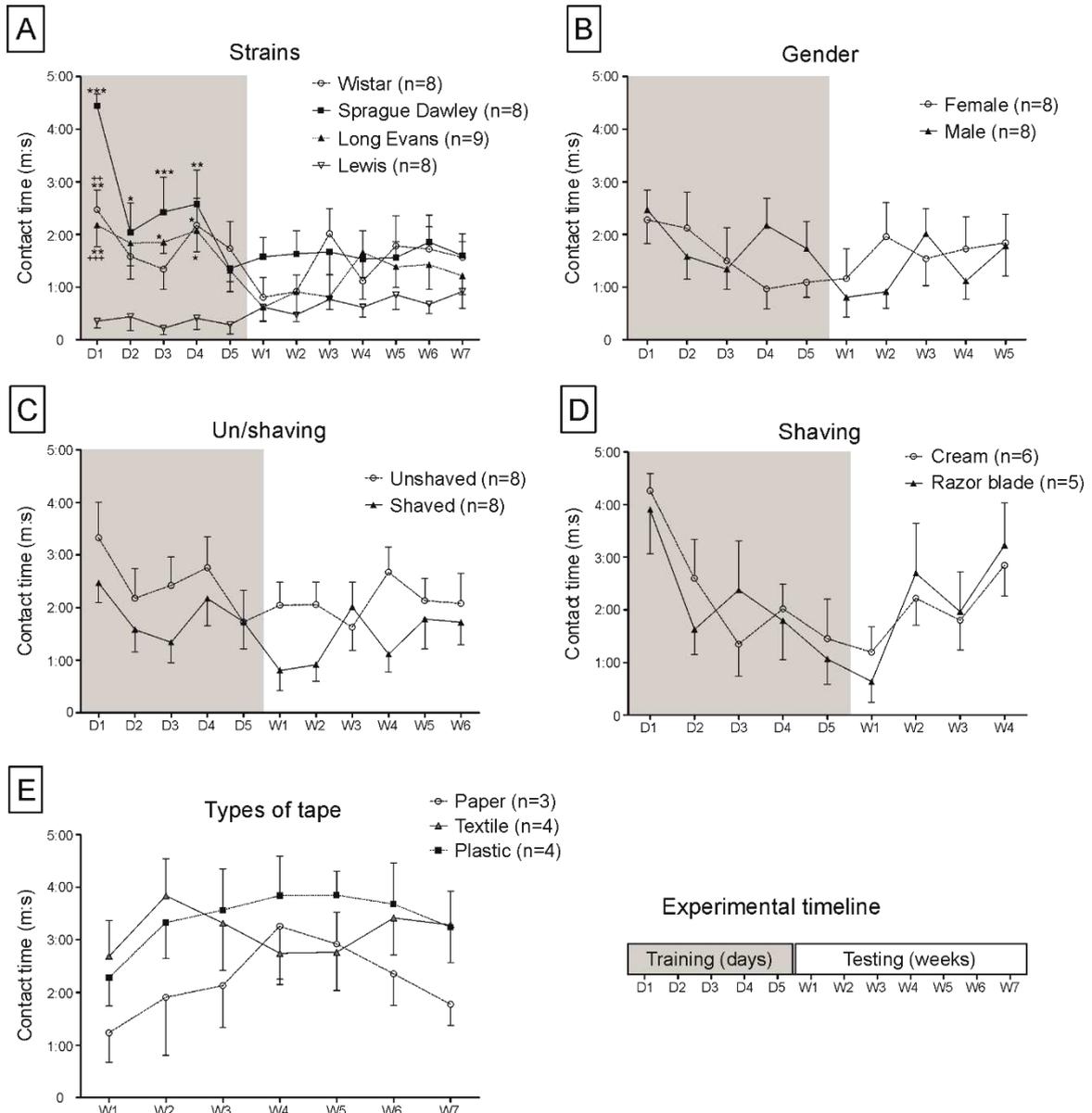


Figure 2. Comparison of different settings for tape removal test. A. Comparison of different rat strains. B. Comparison of female and male rats. C. Comparison of unshaved and shaved rats. D. Comparison of rats shaved with razor blade and depilatory cream. E. Comparison of different tapes. Data are presented as the group means  $\pm$  SEM. Statistical analysis was performed using two-way repeated measures ANOVA followed by Bonferroni post hoc test. \* indicates significant difference from Lewis strain. + indicates significant difference from Sprague Dawley strain. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

Tape removal test can be learned by different strains.

We evaluated different rat strains to see if the test is universal and can be used for commonly used strains (Figure 2A). Eight to nine animals tested from each rat strain were shaved and tested with the tape removal task. During the first week all rats were tested daily (D1-5) to learn the task (video in supplement). Then they were tested once a week for 7 weeks (W1-7). There was a high variability in performing the task during first two days and most of the rats needed several days to learn it, except for Lewis rats which removed the tape quickly already in the first trial. Therefore, there are significant differences between Lewis and the other strains in first 4 days. Later there were no significant differences although the Lewis rats were still the fastest in contacting the tape.

However, all strains were able to learn the task during first week and their further performance was stable.

#### Gender

We compared the performance in the tape removal test in both genders (Figure 2B). Eight female and eight male Wistar rats were used in this comparison. During the first week both groups were tested daily to learn the task. Then they were tested once a week for 5 weeks. There were no significant differences between male and female rats.

#### Perineum exposure

The animals were shaved with a standard razor blade, but we also tested different ways of preparing the perineum.

We investigated whether the test can be performed without shaving. Eight animals were not shaved and were compared to 8 shaved rats (Figure 2C). Otherwise the design of the experiment remained the same, i.e. during the first week all rats were tested daily (D1-5) to learn the task. Then they were tested once a week for 7 weeks (W1-7). In this case the unshaved animals showed a non-significant trend to slower time to contact the tape.

In the next experiment, we tested if the razor blade can be changed for depilatory cream (Figure 2D). One group of rats was shaved with a razor blade (n=5), while the other one was treated with depilatory cream (n=6). There were no differences in contact time in the tape removal test, however, sometimes the skin was visibly red and irritated after using the cream (Figure 1C).

#### Types of tape

The same group of animals used for the shaving experiment (razor blade versus depilatory cream) was then shaved with a razor blade and tested with different types of tape (Figure 2E). Rats were divided in 3 groups. Each group was tested with a different tape (textile, plastic and paper). Since these animals had already learned the task, they were tested only weekly for 7 weeks. The animals tested with paper tape showed better performance but this difference was not significant because of the low number of animals in groups.

#### Lesion

Thirty-three animals from different rat strains (from the first task) underwent a T10 dorsal column lesion.

The tape removal test was compared with additional sensory or motor tests before and after the surgery. Since the lesions varied in size, we divided the rats according to the extent of the damaged areas into three groups, small, medium and big lesion (Figure 3).

In the tape removal test, averaging all the animals together, the time rats took to first contact the tape increased at 3 days after SCI from 1:26 to 3:18 and this increase persisted for the next 5 days (3:25 at day 8 after the injury). Fourteen and 21 days after SCI the latency slowly decreased (2:25 and 2:10 resp.). Because the size of the lesions varied between animals, we divided them into three groups, big, medium, small. In the big and medium groups, the contact time remained higher when compared with healthy animals before lesioning (1:26; Figure 4A). In contrast, no significant change in contact time was observed in animals with small lesions at any time point after SCI. In the big lesion group, the tape contact time after injury was on average close to 5 minutes. This does not indicate that most animals failed to remove the tape and were therefore scored as 5 minutes. Only three animals failed to remove the tape, and it does not appear from our data that the number of animals failing to remove the tape at 5 minutes is a useful outcome measure.

The von Frey test is frequently used to measure touch sensitivity. In all three groups of rats the withdrawal threshold increased after SCI compared to baseline threshold. However, we were unable to detect any differences between big, medium and small lesions and the increase in withdrawal threshold was significant in comparison with animals before lesioning (Figure 4B).

For testing response to thermal stimuli, the plantar test was performed. No significant change after spinal injury was detected in any of the lesion size groups (Figure 4E).

Possible motor dysfunction was assessed by the BBB open field test. A decrease in the BBB score was detected in all lesioned animals and was dependent on the size of the lesion. Rats with big lesions dropped their score down to  $12.41 \pm 1.45$ , which was significantly lower than values in animals with medium size lesion ( $16.25 \pm 0.5$ ). The lowest deficit was recorded in animals with small lesion, where the average value was  $18.9 (\pm 0.66)$ , and the decrease was significant only 14 days after SCI, when compared with healthy animals before lesioning (Figure 4B).

As a sensory-motor coordination test, we applied the horizontal ladder walking test (Figure 4C). A large decrease in foot fault score was detected in animals with big lesions which was maintained to 23 days. Animals with medium and small lesions scored similarly  $5.03 \pm 0.24$  and  $5.45 \pm 0.11$  resp. at day 17 after the lesion and there was no statistical difference between these two groups. Although the scores of these two groups remained similar ( $4.99 \pm 0.23$  and  $5.63 \pm 0.10$ ) at 23 days after the lesion, only the scores from animals with medium lesions were statistically significant from animals before lesioning.

Because there were clear differences between the deficits depending on lesion size, we performed plots of lesion size against the pre- and post-lesion changes in the behavioural test results with a point for each individual animal (Figure 5). In the tape removal test, there was a strong linear correlation between preserved tissue area and the results of the test ( $p < 0.001$ ) and the BBB test and horizontal ladder walking test also showed significant changes correlated with lesion size. For the von Frey and plantar tests there was no significant linear correlation with lesion size.

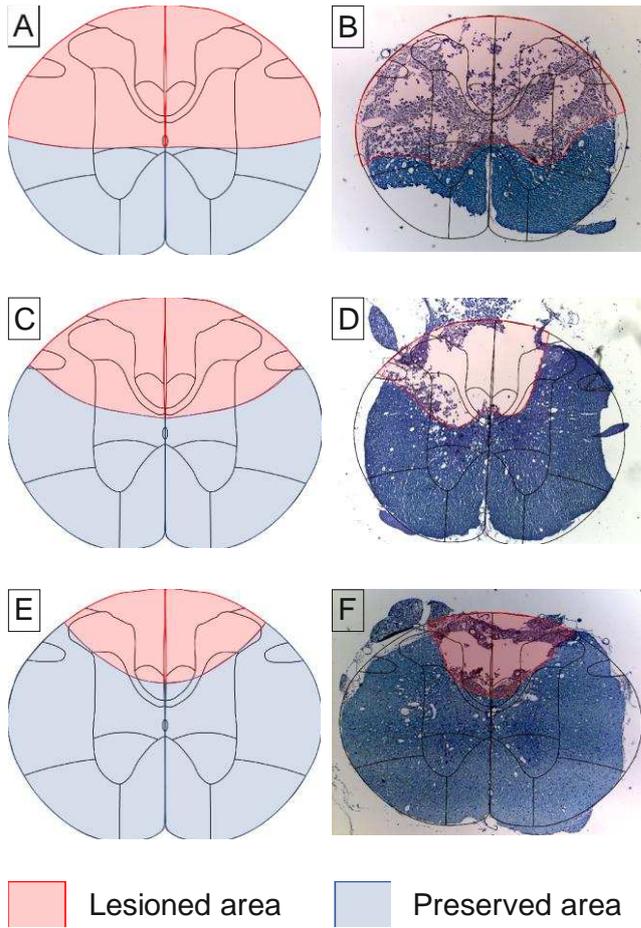


Figure 3. Distribution of rats into groups according to the lesion size. A. Schematic representation of big lesion. B. An example of big lesion. C. Schematic representation of medium lesion. D. An example of medium lesion. E. Schematic representation of small lesion. F. An example of small lesion.

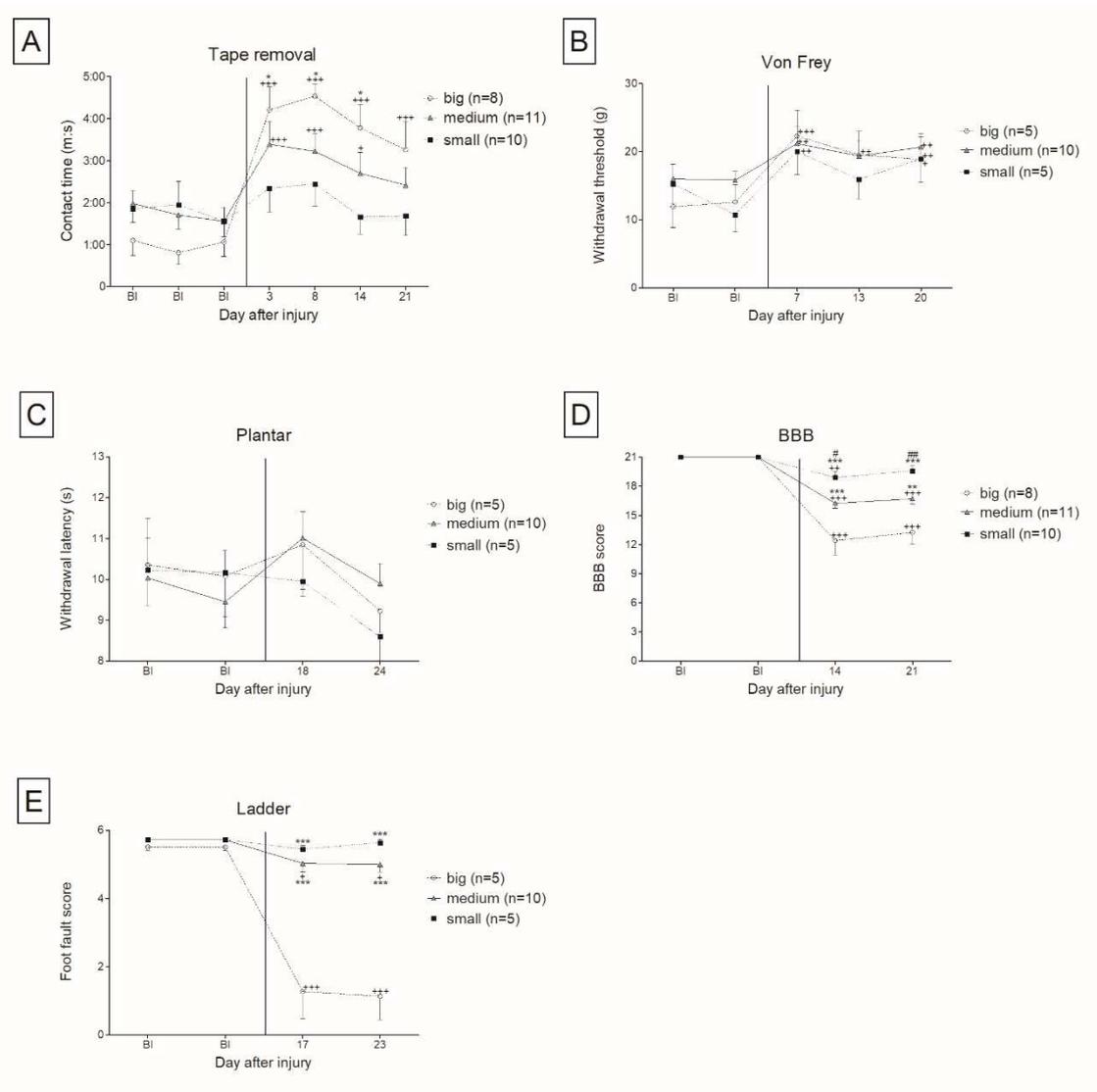


Figure 4. Results of behavioural tests of rats divided into three groups by the lesion size. A. tape removal test B. von Frey test C. plantar test D. BBB test E. horizontal ladder waking test. BI = before injury. The vertical line in the graphs represents time of the lesion. Data are presented as the group means  $\pm$  SEM. Statistical analysis was performed using two-way repeated measures ANOVA followed by Bonferroni post hoc test. \* indicates significant difference to small group. # indicates significant difference to medium group. + indicates significant difference compared to value before SCI (average of 2(3) values before lesion). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

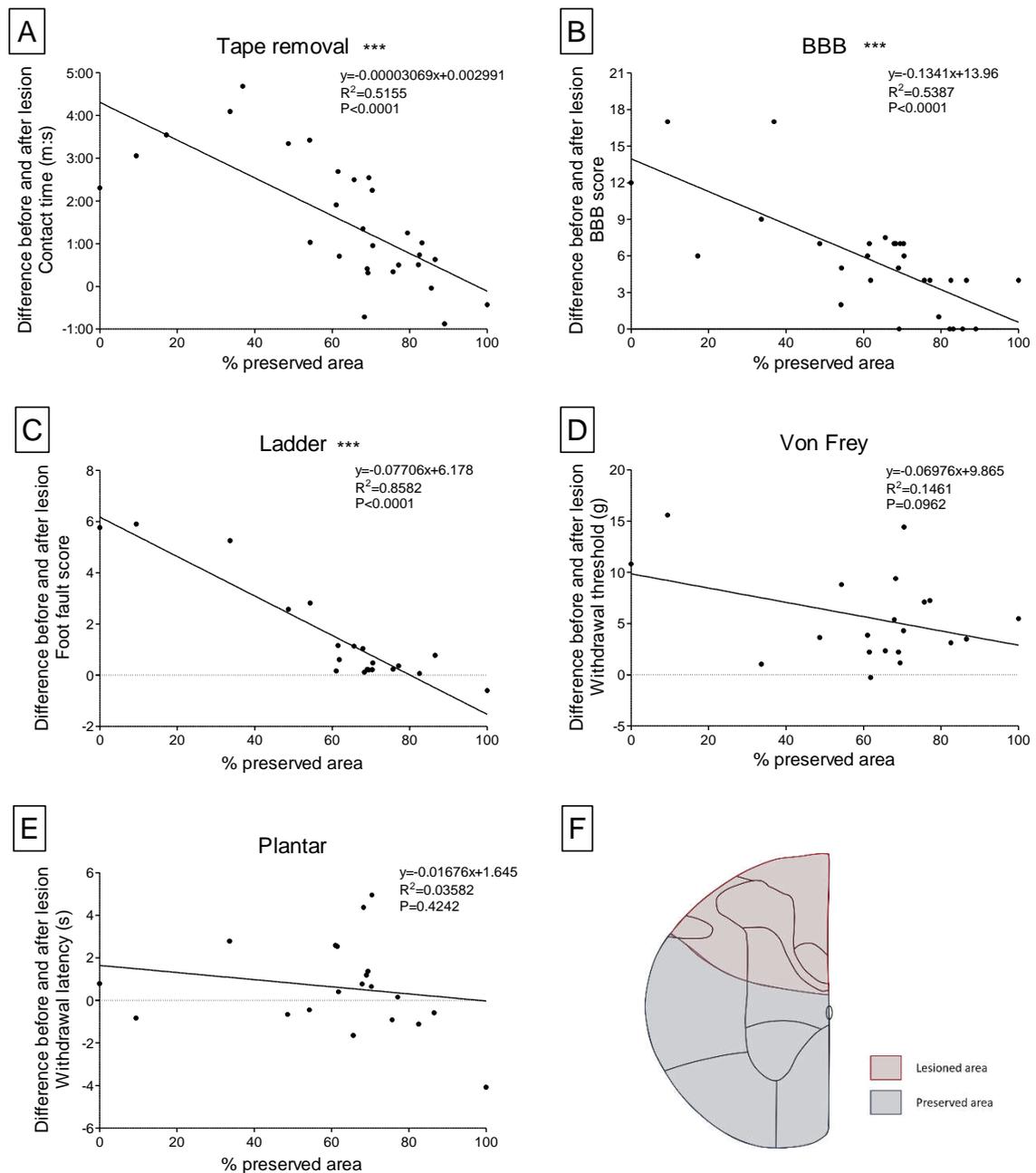


Figure 5. Correlation between behavioural test results and percentage of preserved area on the left side of spinal cord in the center of lesion. A. tape removal test B. BBB C. ladder D. von Frey E. plantar F. Schematic representation of lesion on T10 cross section. Damaged region is highlighted in red. Percentage of preserved tissue on the left side was calculated.

## Discussion

Spinal cord injury is a complex event resulting in motor as well as sensory deficits. Tests have been developed for the quantification of functional deficits and recovery of function after cervical or thoracic lesions and for various motor and sensory behaviours. A priority for many patients is restoration of sexual function including genital sensation. We therefore decided to find out if a quantitative test for perineal sensation can be achieved by modification of the tape removal test.

We measured only the first part of the test, i.e. sensing the stimuli. We began by measuring both the time taken for animals to make first contact with the tape and the time for tape removal. We found that removal time was affected by the overall size and weight of the animal while contact time was largely independent of animal size. We therefore used contact time for the rest of the study.

In order to produce a deficit in sensory pathways we lesioned the dorsal columns, which carry the majority of sensory axons that either travel directly to the medulla, or synapse in relay interneurons in nearby segments. We used dorsal column lesion, since dorsal lesions are most often used in spinal cord injury experiments in rats. We expect to test the L6, S1 and partly S2 dermatome (Takahashi and Nakajima, 1996), and the tested area corresponds to the peripheral distribution of the sensory portion of the pudendal nerve (Pastelín et al., 2008).

Shaving the perineum under a quick anaesthetic was straightforward. Following this, animals easily tolerated placement of a 1cm square of sticky tape. Animals did not usually respond to the tape immediately, but usually bent their head down to lick or bite the tape within 2 minutes. We used a five-day training period, during which the detection time decreased, to stabilise at 1-2 minutes. Rat strains differ in their response to sensory stimuli, so we examined four common strains to determine if there is any strain which is not able to learn and perform the task. We found differences in the learning curve of the strains and variability among individual animals, however, after one week of learning, all animals were able to perform the test. To our surprise, a faster response and less variability was observed in Lewis rats, which are in general considered as slow and lazy animals.

We also compared razor blade shaved and depilated animals with no difference, except for skin irritation, which occurred in some depilated animals. On the other hand, the fur grew faster after shaving and it is necessary to shave animals once a week in long term experiments, while depilation lasts for 2 weeks. Unshaved animals were able to perform the task with comparable results; however, the tape does not often stick with the whole surface to the fur and it is more difficult to assess the time of the contact and tape removal. This test was also not dependent on gender and the size of the animals, since all animals were able to perform the task regardless their weight (range from 225-424 grams). We tested different types of tapes finding that the most suitable tape was a paper tape. It did not irritate the skin of the animal, stuck well and the rats could remove it easily.

In all trained animals we performed a dorsal column lesion, destroying the dorsal columns of the spinal cord, targeting sensory fibres originating from the dorsal root ganglia and ascending in the dorsal funiculus. From the sensory ascending pathways the gracile and cuneate pathways are the most important. Axons from DRG enter ipsilateral dorsal column and divide into ascending and descending branches. The pathways have somatotopic organization and convey information about sensation of discriminatory touch, deep pressure, proprioception, sense of position of joints, stereognosis and vibration. We believe that the transection of this pathways leads to sensory loss measured by tape removal test as was also described by Attwell et al. (2018) and Bieler et al. (2018). A subpopulation of DRG neurons synapses are localized on dorsal horn neurons, which axons also project to the gracile and cuneate nuclei and form post-synaptic dorsal column pathway. They respond to innocuous mechanical and noxious peripheral stimuli. This is considered as major afferent pathway for visceral nociception. Dorsal column lesion also reduces nociceptive behavioural responses to visceral stimulation (Houghton et al., 1997). The spinothalamic tract conveys nociception, temperature and non-discriminative (crude) touch and pressure information to the somatosensory region of the thalamus. It is composed of an anterior and lateral pathway. The anterior spinothalamic tract transmits crude touch and pressure sensation. The lateral spinothalamic

tract transmits pain and temperature. Both tracts are not affected in the case of dorsal column lesion. The main function of the spinothalamic tract is to move the rat away from noxious stimuli by carrying pain and temperature information from the skin to the thalamus and then to primary sensory cortex cooperating with the motor cortex. We assume that this mechanism is less in function when using the tape removal test. The recognition and removal of the tape requires light and discriminatory touch sensation. Because of the extensive branching of ascending crude touch fibers, this type of touch is unlikely to be abolished by partial lesion in the spinal cord.

The post-lesion deficit in tape removal was dependent on the size of the lesion, with deeper lesions extending further laterally producing larger deficits regardless of the strain. For a stable measurable deficit, the lesion needs to extend as far laterally as the dorsal root entry zones. At the same time as testing perineal tape removal, we also used standard sensory and motor tests. Of these the two tests measuring motor deficits (BBB and horizontal ladder walking) showed a clear relation between lesion size and deficit, confirming that these tests examine sensory-motor function. The two tests focusing on sensory deficits, primarily developed for thermal and mechanical hyperalgesia (Plantar test and Von Frey test) did not show any correlation with the damaged area. Some deficit after lesioning (independent of the size of the lesion) was detected in Von Frey test, no differences were measured by the Plantar test. This is most likely due to fact that local circuitry below the lesion remained intact and the local circuitry that lies behind these reflexive withdrawal circuits remained functional; also nociceptive stimuli may ascend through ventral spinothalamic tract which is not part of dorsal columns. Animals with large thoracic lesions often show signs of allodynia in the hindpaws, depending on strain and lesion size (Lindsey et al., 2000; Mills et al., 2001). None of our animals with big lesion showed hypersensitivity in tape test or von Frey test.

In dorsal column lesion model only rats with the big lesions were affected by the urinary bladder retention. We had to manually express their bladder for about 14 days. The rats with the medium and small lesions did not exhibit urinary tract complications. Since the micturition involves several mechanisms that cooperates on different levels of the central nervous system; we can assume that the destruction of the spinal dorsal funiculus at T10 does not lead to urinary retention, unless the larger area is affected. Deficits in bladder function following spinal cord injury vary depending on the level of the injury (David and Steward, 2010). David et al. compared how the level of the thoracic spinal cord lesion affects urinary complication after SCI in rats. They compared moderate contusions at level T1, T4 and T9 and they found little deficit in rats with lesion at T1 and T4, while in the lesions at level T9 urinary retention developed. Using retrograde staining they found that the extent of bladder impairment is related to the degree of spared projections from the pontine micturition centre. Their results indicate that lesions at T1 vs. lower levels differentially spare descending projections that are critical for bladder function, even though other pathways are affected to a similar degree.

The pathways involved in urine storage and voiding are organized at different levels of the central nervous system. Affecting ascending pathways coming from the urinary bladder is only one part of the very complex process of the urinary complications in the spinal cord injured rats. The innervation of the low urinary tract is derived from three sets of peripheral nerves: sacral parasympathetic, thoracolumbar sympathetic and sacral somatic nerves. The micturition process is organized as reflex and voluntary on-off switching circuits that maintain a reciprocal relationship between the urinary bladder and urethral outlet (Beckel and de Groat, 2019). The sacral spinal cord plays important role in the bladder-sphincter control. The C fibres enters the sacral spinal cord and terminates at Rexed's laminae I,II. and V. and the information from bladder discomfort transmitted by C fibres is then relayed to the pontine nuclei, thalamus and hypothalamus. The A $\delta$  fibers conveying information about filling the bladder terminates at the same laminae but on separate cell groups – the lateral

collateral pathway of the Lissauer's tract. At the thoracic level where our lesion is performed the bundles of this tract are located around the lateral edge of the dorsal horn and through dorsolateral funiculus and we expect that are destroyed especially in big lesions.

The perineal tape removal test measured a clear functional deficit in perineal sensation for the three-week post-lesion duration of our experiments. However, the size of the deficit and its duration were dependent on lesion size. The classic tape removal test for limb sensation has been used in various studies of dorsal spinal cord injury. In one study for hindlimb sensation after a T7 thoracic injury the deficit was small and only significant at some time points (Fagoie et al., 2016). However after cervical lesions a stable deficit in forepaw tape removal to 8 and 12 weeks was seen in several studies (Agudo et al., 2008; Bradbury et al., 2002; Fagoie et al., 2016; Lu et al., 2005; Moreno-Flores et al., 2006).

We conclude that the modified tape removal test is suitable for testing sensation in the area of the perineum and gives similar results across strains and for both genders. Deficits in the perineal sensation after T10 dorsal column lesions can be detected for at least 3 weeks after lesioning and correlate with the size of the lesion. The test is therefore suitable for testing treatments for sensory restoration in the genital region in dorsal spinal cord injuries of sufficient size.

Declaration of interest

Authors have nothing to declare.

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Supplementary material

Video: <https://youtu.be/fqtn51dwIDE>

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