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Comparison of five different rat models of peripheral nerve injury

Tom Dowdall, Ian Robinson, Theo F. Meert*

Johnson and Johnson Pharmaceutical Research and Development, CNS Pain and Alzheimer, Turnhoutseweg 30, B-2340 Beerse, Belgium
School of Clinical and Laboratory Sciences, Faculty of Medical Sciences, Medical School, University of Newcastle-Upon-Tyne, NE2 4HH

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Abstract

Described here is a comparison of five peripheral sciatic nerve injury models in rats which all result in various degrees of neuropathic pain symptoms. They are the chronic constriction injury (CCI), the spinal nerve ligation (SNL), the partial sciatic ligation (PSL), the tibial and sural transection (TST), and the complete sciatic transection (CST) model. Behavioral testing was performed on these models over a 56-day period under strict experimental conditions to minimise variability between the surgical models and to allow for an accurate evaluation of the sensory deficits produced by each model. Overall, all five models of neuropathic pain produced signs of allodynia and hyperalgesia to various stimuli. However, the duration and magnitude of the evoked responses varied considerably between the different models.

Keywords: Chronic constriction injury; Spinal nerve ligation; Partial nerve ligation; Tibial; Sural; Sciatic transection; Sensory testing; Neuropathic pain symptoms

1. Introduction

There has been some degree of success in treating neuropathic pain symptoms by using medications, such as gabapentin, and more recently pregabalin, as short-term, first-line treatments (Sindrup and Jensen, 1999; Dworkin et al., 2003). However, pharmacotherapy for neuropathic pain has generally had limited success with little response to commonly used pain reducing drugs, such as NSAIDS and opiates (Woolf and Mannion, 1999; Przewlocki and Przewlocki, 2001). Consequently, there is still a considerable need to explore novel treatment modalities. The evaluation of neuropathic pain in humans is complex, because only stimuli that do not produce irreversible harm can be used in these subjects. It can also be difficult to recruit the significant numbers of patients needed for a clinical trial. Therefore, there is a need to use animal models of peripheral nerve injury to broaden our knowledge of the mechanisms behind neuropathic pain. Such models should result in reproducible sensory deficits (allodynia, hyperalgesia, and spontaneous pain) over a sustained period of time that can be evaluated by sensory testing. By establishing the degree of mechanical, chemical, and temperature-induced allodynia and hyperalgesia present, several physiophathological conditions observed in humans can be modeled allowing the evaluation of pharmacotherapies.

Several animal models using mechanical peripheral nerve injury are currently described. The first and simplest of the models was developed by Wall et al. (1979) and involved complete transection (CT) of the sciatic nerve at midthigh level. This resulted in autotomy, which Wall et al. used to quantify the degree of neuropathic pain. However, due to animal health care issues, this model is rarely used. A well-established model is Bennett and Xie's (1988) Chronic Constriction Injury (CCI) model. In rodents, four catgut ligatures are loosely tied around the sciatic nerve proximal to the sciatic trifurcation. This constriction of the nerve leads to intraneural oedema, a focal ischemia, and an axonal (Wallerian) degeneration. As a consequence, this model results in chemical and heat-evoked hyperalgesia, as well as cold and mechanical allodynia, and some symptoms of spontaneous pain which lasts for a period of more than 2

* Corresponding author. Tel.: +32 14 60 32 14; fax: +32 14 60 59 44.
E-mail address: tmeert@prdbe.jnj.com (T.F. Meert).

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months (Bennett and Xie, 1988; Attal et al., 1990). Seltzer et al. (1990) developed the partial sciatic nerve ligation (PSL) model by tightly ligating 1/3 to 1/2 of the sciatic nerve with a single ligature. This ligation induces mechanical allodynia, heat-evoked hyperalgesia, and spontaneous pain, which are present for up to 7 months. The spinal nerve ligation (SNL) model was developed by Kim and Chung (1992) by tightly ligating the L_5 and L_6 spinal nerves close to their respective ganglia. Mechanical and heat-evoked hyperalgesia together with spontaneous pain lasting at least 4 months were observed (Choi et al., 1994). A recently developed animal neuropathic pain model is described by Lee et al. (2000). They transected different combinations of the three branches of the sciatic nerve (tibial, sural, and common peroneal) to investigate which combination produced the most robust and stable degrees of allodynia and hyperalgesia. The authors reported that transecting the tibial and sural nerves resulted in the largest amount of mechanical allodynia, chemical hypperreactivity, and spontaneous pain.

Much research has been performed on the described models of neuropathic pain (Kingery and Vallin, 1989; Kim et al., 1997; Jasmin et al., 1998; Begon et al., 2000; Martin et al., 2003). However, it is very difficult to compare the general outcomes of these various models as the testing has been performed by different laboratories often with small differences in the surgical and analytical techniques used. Several studies have also indicated that small changes in parameters [e.g., housing, bedding, diet, surgery techniques, suture material (for ligations), and evaluation methods] can affect the outcomes of the surgical interventions with regard to allodynia and hyperalgesia for different stimuli (Shir et al., 1998; Field et al., 1999; Chesler et al., 2002; Cunha et al., 2004; Robinson et al., 2004; Robinson and Meert, in press). Even the order of testing of the various parameters should be controlled. As such, a detailed comparison between the different models is difficult to obtain from the literature, and a head-to-head comparison would be a useful addition to the studies currently reported. There has not been a comprehensive study performed, which includes all the models, over a sustained period of time, involving a broad range of sensory testing. Therefore, this report aims to compare the five previously mentioned peripheral nerve injury models over a period of 56 days under strict conditions to standardise the surgical and test procedures. The testing stimuli chosen were cold plate, hot plate, neutral plate, the Von Frey test, the pinprick test, and the acetone spray test. These tests include most of the range of stimuli often employed in the various models applied by different research groups.

2. Materials and methods

Guidelines for animal research by the International Association for the Study of Pain were followed (Zimmerman, 1983), and the study was approved by the institutional ethical committee.

2.1. Experimental animals and production of neuropathy

Male Sprague–Dawley rats (Harlan, Germany), weighing between 240 and 325 g at time of surgery were used. They were singly housed in individually ventilated cages on sawdust with food and water available ad libitum. The environment was maintained at 22±0.5 °C with a 12-h light/dark cycle. All tests were performed during the light phase (08:00–16:00).

Nerve or sham surgery was performed on all rats under gas anaesthesia with a mixture of isoflurane (5% for induction and 3% for maintenance) and oxygen. Surgery was always performed on the left hind leg.

Tibial and sural transection (TST; n=14): The methods of Lee et al. (2000) were followed. Briefly, skin of the lateral surface of the left thigh was incised and a cut made directly through the biceps femoris muscle to expose the sciatic nerve and its three terminal branches (the sural, common peroneal, and tibial nerves). Once exposed, the tibial and sural nerves were cut at 2 mm distal to the trifurcation, and a 2-mm section of nerve was removed to prevent the two nerves rejoining. The common peroneal nerve was left intact, and no contact was made with it.

Chronic constriction injury (CCI; n=10): Surgery was performed according to the methods of Bennett and Xie (1988). The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue, and four ligatures (5.0 Ethicon chromic catgut) were tied loosely around it with about 1-mm spacing. Great care was taken to tie the ligatures, such that the diameter of the nerve was seen to be just barely constricted.

Complete sciatic transection (CST; n=14): The methods of Dib-Hajj et al. (1998) were applied. The sciatic nerve was exposed at the upper point of the thigh. The sciatic nerve was then tightly ligated with 5-0 Ethicon chromic catgut and was then transected at the level of the pyriform tendon.

Spinal nerve ligation (SNL; n=10; as described by Kim and Chung, 1992): An incision was made into the back of the animal exposing the L_5 and L_6 spinal nerves. These nerves were isolated from the surrounding tissue and tightly ligated with a 5-0 Mersilk ligature.

Partial nerve ligation (PSL; n=14): The methods of Seltzer et al. (1990) were followed. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. The nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. An 8-0 Ethilon (Polyamide 6) suture was inserted into the nerve and tightly ligated so that the dorsal 1/3–1/2 of the nerve thickness was trapped in the ligature.

For all neuropathic surgery, the muscle and the skin were closed in two layers with the use of Vicryl 6/0 for the muscle

and Mersilk 5/0 for the skin. Sham controls were performed for each surgery by exposing the nerves without inducing any lesion or ligation.

All surgical procedures were carried out under normal sterile conditions and were performed by the same person. A schematic representation of the different surgical procedures is given in Fig. 1.

Three groups of subjects were randomly allocated to one of the following experimental groups:

- Naïve rats: no surgery was carried out
- Sham rats: the rats sciatic nerve was exposed but left unaffected
- Operational rats: the full operation was carried out as described above.

2.2. Behavioural tests

Six behavioural tests aiming to validate different types of neuropathic pain symptoms were carried out. Some of these tests monitor a complex range of factors.

Behavioral tests were conducted for all rats 3 days prior to surgery, and 1, 3, 7, 9, 11, 14, 16, 21, 28, 35, 42, 49, 56 days postoperatively. The order of testing was fixed, taking into account the degree of distress of each test on the following one. The order was therefore defined as follows: control plate, hot plate, Von Frey, pinprick, acetone, and cold plate. All animals were tested on each test day with the different stimuli in the same sequence. When moved to a novel environment, the animal was left for 30 min undisturbed to habituate to the testing environment.

2.2.1. Neutral plate test

A neutral plate was used to evaluate spontaneous paw lifting over time, which could be used as a measurement of spontaneous pain and as a base line to compare the hot and cold plate results against. This control plate consisted of a transparent plastic box (30×30 cm), which the animal was placed in, on an acrylic plate at room temperature (Lee et al., 2000). The duration of lifting excluding lifting, which is part of normal locomotion, was calculated over a period of 5 min for both the left and right paw. The lifting of the left paw is a combination of ongoing pain and the animal adjusting its weight bearing.

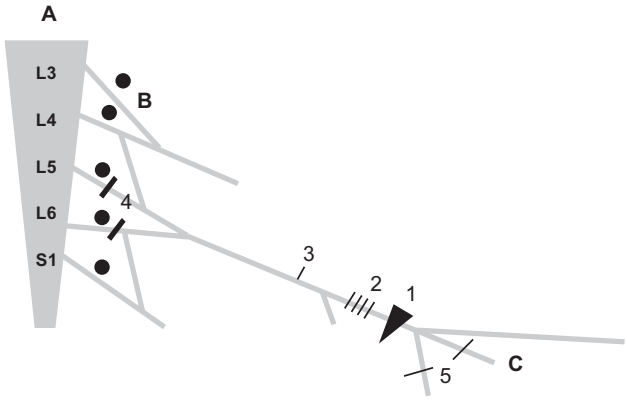

Fig. 1. Schematic representation of the spinal cord (A), the exiting spinal nerves (B), and the sciatic nerve (C) with the specific anatomical locations of the nerve injury applicated in different models of neuropathic pain in rodent: (1) complete sciatic transection (CST; Wall et al. 1979); (2) chronic constriction injury (CCI; Bennett and Xie, 1988); (3) partial sciatic nerve ligation (Seltzer et al., 1990); (4) spinal nerve ligation (SNL; Kim and Chung, 1992); (5) tibial and sural transection (TST; Lee et al., 2000).

2.2.2. Hot plate

The animals were placed on a metal hot plate (45±0.5 °C) of dimensions 30×30 cm for an evaluation period of 5 min. The total duration each animal lifted both the left and right hind paw was measured. Lifting for normal locomotion was excluded. The lifting of the left paw can be explained by a combination of factors including thermal allodynia and the animal adjusting its weight onto the uninjured paw.

2.2.3. Von Frey testing

The pressure algometer (Somedic Sales, Hörby, Sweden, with a probe of 1-mm diameter) was used to test mechanical threshold as performed by Moller et al. (1998). Rats were placed inside acrylic cages on top of a wire mesh grid, which allowed access to the paws. For testing, a probe was applied to the midplantar left and right hind paws, avoiding the less sensitive footpads. The order in which the paws are tested may affect the results, and the left paw was always tested first to minimize variability. The probe was applied in the same way as Von Frey hairs, and the pressure was gradually increased. The stimulus was continued until the paw was withdrawn or slowly elevated such that the force leveled off or decreased. In this manner, a maximally accepted force was determined. The application was repeated three times on each paw with a minimum interval of 10 s between testing. An average of three measurements was used as the final readout.

2.2.4. The pinprick test

A pinprick test was performed (Erichsen and Blackburn-Munro, 2002) using a bent needle (at 90° to the syringe) attached to a syringe to measure mechanical hyperalgesia. The animals were placed inside acrylic cages on top of a wire mesh grid, which allowed access to the paws. The lateral plantar surface of both the hind paws was touched with the point of the needle with sufficient intensity to produce a reflex withdrawal response in normal unoperated animals, but at an intensity which was insufficient to penetrate the skin. The duration of the paw withdrawal was recorded with a stopwatch. A cutoff time of 20 s was applied to long withdrawals often seen for the left hind paw. The most sensitive area of the paw was tested for each type of injury. This area varied for each type of neuropathic injury. The areas tested for each animal were as follows: PST and CST, the medial side of the plantar surface of the paw (the medial side is defined as the furthest point from the centre without leaving the plantar surface of the foot); for

SNL and PSL, the centre of the paw was used; for CCI, the rear of the paw was tested. Any lifting observed by the right hind paw upon stimulation was also noted.

2.2.5. The acetone test

A slightly modified method of De la Calle et al. (2002) was used for the determination of the reactivity to a cold chemical stimulus. The rat was placed in acrylic cages on top of a wire mesh grid, which allowed access to the paws, and acetone was applied to the plantar surface of the hind paw. To do this, 100 μl of acetone was sprayed onto the plantar surface of the rat's hind leg from below the grid with an Eppendorf multistepper pipette with a Combitip holding 2.5 ml. The time spent with the leg withdrawn from floor during the 60 s following exposure to acetone was recorded. Both hind legs were tested in each animal, starting with the unoperated right leg, with an interval of 5–10 min between each test. A minimal value of 0.5 s was assigned to convey a fast or brisk reaction, while 0 was assigned if there was no reaction at all. This acetone test has been described as composing of cold, chemical, and possibly mechanical stimulation.

2.2.6. The cold plate test

Cold allodynia was tested using a cold metal plate (Julabo MH cooler) following the Vissers et al. (2003) method. The animals were placed on a cold plate (−0.5±0.5 °C) inside a transparent plastic box (30×30 cm). For an evaluation period of 5 min, the cumulative duration of time each animal was noted to lift each hind paw was calculated. However, the foot lifts associated with body repositioning or locomotion were not included. The cold plate, as well as measuring cold allodynia, also includes changes in body repositioning, which needs to be taken into the interpretation of this stimulus.

2.3. Data analysis

Due to the fact that most of the models show obvious deformity of the hind limb, data could not be collected in a blind way. Data are expressed as the mean±standard error of the mean (S.E.M.). Differences between treatment groups were evaluated against the left hind paw of the corresponding controls and baseline values using the Mann–Whitney *U*-test (two tailed) with a correction for repeated measures. *P* values of less than 0.05 were taken as significant.

3. Results

Apart from an initial drop in body weight after surgery, there was no significant difference between the weights of operated and nonoperated animals throughout the experiment. Mean average body weight at the point of possible surgery and after habituation to the lab environment was 300±25 g and was 400±85 g at the cessation of the study. No differences were seen between groups.

The amount of animals which had to be subjected to euthanasia because of severe autotomy varied with the different surgical models. For the TST and the PSL, no animals experienced autotomy severe enough to be removed from the experiment. For the SNL and CCI models, one animal in each operated group had to be euthanized. The CST model produced the highest amounts of autotomy, and by the final day of the experiment, nine of the operated rats had been removed from testing.

3.1. Neutral plate

The left paw of the control groups on the neutral plate showed lifting durations ranging from 0 to 0.13±0.13s, with no significant differences between the groups. In the five sham groups, the left paw varies from 0 to 0.84 s. These results are comparable for all five sham groups. The right hind paws of the operated, sham, and control groups remained unresponsive throughout the experiment for all the groups. On the neutral plate, no significant lifting was seen over the entire 56-day observation period with the TST model. The operated animals all showed very little lifting behaviour (Fig. 2A). Lifting behaviour was clearly elevated in the CCI model compared to the sham group (Fig. 2B). The behaviour began on day 1 and was significantly different from the control and baseline data. A peak value of 31.52±13.63 s was reached on day 9. After this point, the duration of lifting decreased to a level of approximately 10 s and stayed at this level until the end of the experiment. The CST model showed very low durations of lifting on the neutral plate over the entire observation period (Fig. 2C), although there were some statistically significant differences compared to controls. The maximum value obtained was 12.57±9.94 s on day 42. The PSL model produced very little lifting on the neutral plate (Fig. 2D). There was a brief period of lifting observed on days 1 and 7, but after this, the lifting behaviour stopped. The SNL model showed some lifting from day 1 onwards (Fig. 2E). A peak value of 9.90±4.67 s was recorded on day 16. After day 21 until the final day of testing, limited lifting was observed.

3.2. Hot plate

In all five control groups on the hot plate, the values for the left hind paw ranged from 0 to 1.42±0.65 s, with no significant difference seen between the groups. The left hind paw values for the sham groups range from 0 to 11.70±2.20 s, with little difference seen between the groups. The values for the right hind paws of the control, sham, and operated groups are all comparable to the left hind paw controls.

The hot plate produced a small amount of lifting behaviour in the TST model, which was statistically significant compared to the control and baseline. However, this only reached a maximum value of 5.02±2.72 s over the entire 5-min observation period (Fig. 3A). The CCI model produced a significant amount lifting of the operated leg on

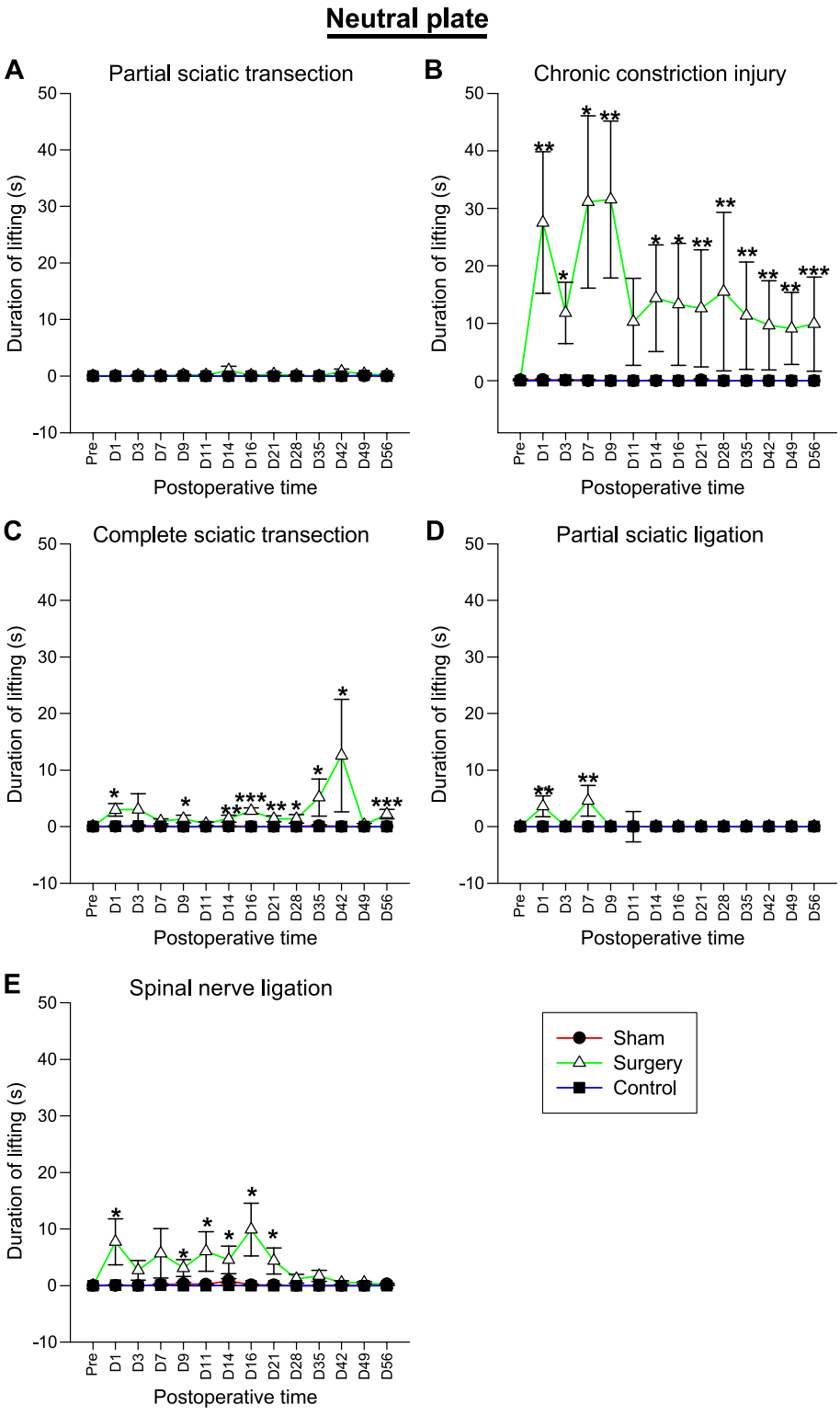

Fig. 2. The duration of lifting of the left hind paw on the neutral plate over a period of 56 days. Given are mean (±S.E.M.) values of control, sham operated, and surgery groups before and at several time points after a surgery. The Mann–Whitney *U*-test (two-tailed) was used to analyse for statistical differences against controls: *p<0.05; **p<0.01; ***p<0.001.

the hot plate over the 56 days of the trial (Fig. 3B). This was first noted on day 1 and continued to be significant until the final day of testing. A peak lifting duration of 36.34±14.97 s was observed on day 9. A limited amount of lifting was observed with the CST model (Fig. 3C) throughout the entire testing period. Lifting behaviour began day 1 before reaching a plateau level, which was present until the end of testing. A maximum lifting duration of 6.10±2.70 s was

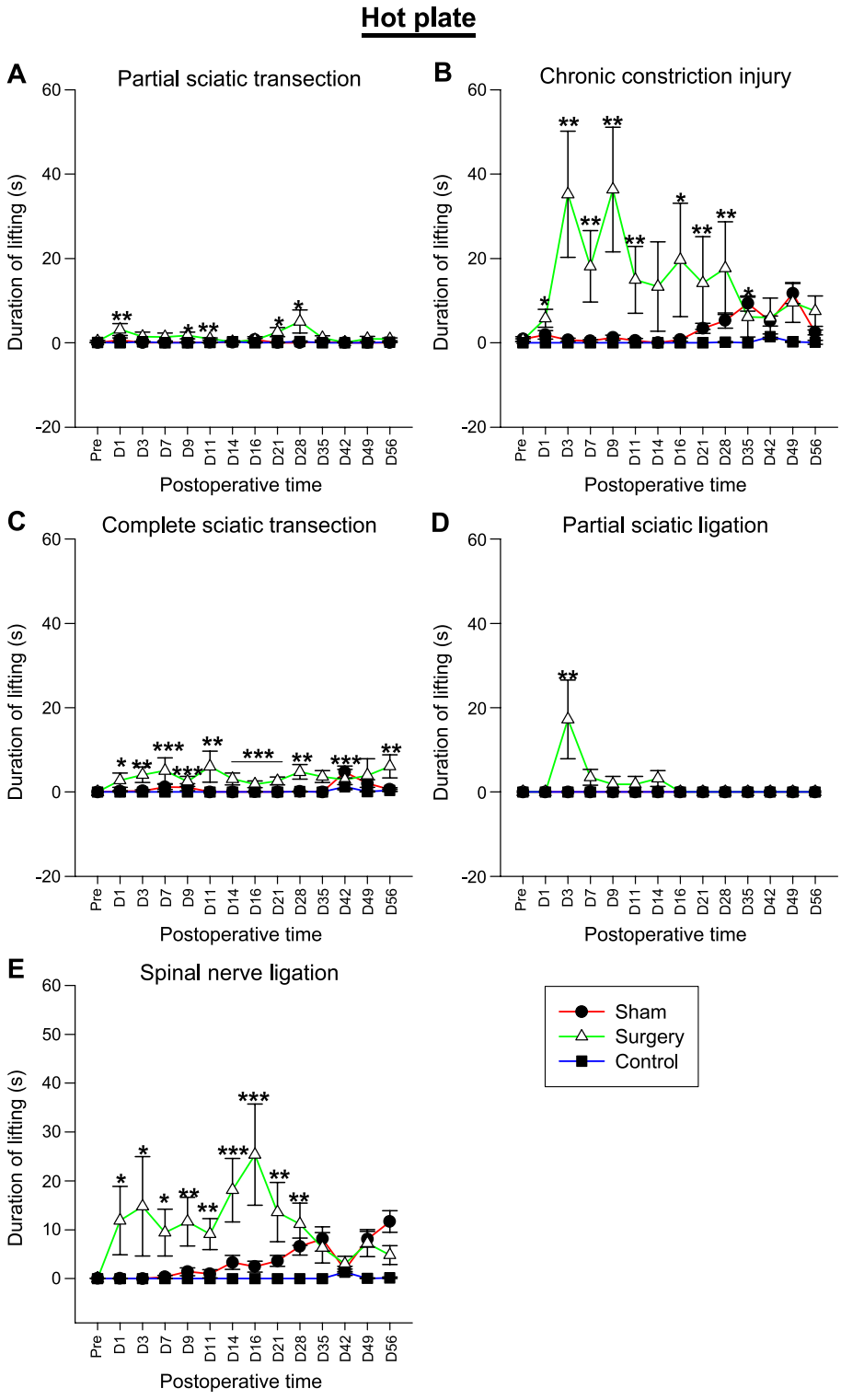

Fig. 3. The duration of lifting of the left hind paw on the neutral plate over a period of 56 days. Given are mean (±S.E.M.) values of control, sham operated, and surgery groups before and at several time points after a possible surgery. The Mann–Whitney *U*-test (two-tailed) was used to assess for statistical differences against controls: **p*<0.05; ***p*<0.01; ****p*<0.001.

observed on the final day of testing. The PSL model produced minimal lifting behaviour on the neutral plate with a brief period of lifting on day 3 followed by an absence of lifting until the end of the experiment (Fig. 3D). There was some significant lifting shown on the operated leg in the SNL model with the hot plate (Fig. 3E). A peak of 18.11±6.47 s was observed on day 16. After this point, the average lifting decreased rapidly until the operated

group displayed similar durations of lifting as the sham group.

3.3. Von Frey

The left hind paw mechanical threshold values remained relatively constant throughout the experiment for the control groups in all five models. The prevalue was 62±2 g for all the models, and this ranged from 48.73±3.82 to 78.10±6.34 g over the course of the experiment. In the five sham groups, the left hind paw threshold was comparable throughout the experiment. A prevalue of 61.00±2.00 g was observed in all the sham groups. Throughout the experiment, the mechanical threshold of the corresponding paw of the sham groups ranged from 46.94±5.04 to 87.73±4.34 g. The right hind paw for all groups remained constant at baseline level for the entirety of the experiment.

The TST showed a significant difference between the operated group and the control and sham groups in the Von Frey test. The mean value dropped from a prevalue of 60.23±3.70 to 21.25±1.94 g at 1 day postoperatively in the operated group. Over the following 56 days, the average mechanical thresholds varied between 16.09±1.37 and 27.09±3.76 g. For the duration of this period, the values were significantly different from baseline and control (Fig. 4A). The CCI model showed a marked difference between the nerve injury group and the control and sham groups (see Fig. 4B). This behaviour was present immediately after surgery (on day 1). The mean threshold value dropped from 61.58±5.14 to 12.43±2.17 g and remained at this level until day 49. From day 49 onwards, a slight increase was observed in the mechanical threshold. The CCI group was significantly different from the baseline and control figures from day 1 onwards. Von Frey testing in the CST model showed a clearly reduced resistance on the operated leg (Fig. 4C). This was significantly different from the sham and control groups and from the baseline figures. The CST groups mechanical threshold dropped from a prevalue of 59.40±3.94 to 18.73±1.97 g on day 1 and remained around this level until day 56, the final day of testing. The mechanical threshold decreased significantly in the PSL model from day 1 postoperatively (Fig. 4D). The operated group showed prevalues of 57.63±4.53 g. Following surgery, the resistance decreased rapidly and reached a minimum level of 24.27±5.46 g on day 28. After this, the resistance rapidly increased until it reached a value that was not significantly different from baseline or control on days 49 and 56. The SNL model showed a dip in mechanical threshold immediately after surgery when tested with the Von Frey probe (Fig. 4E). In the SNL operated group, the average prevalue was 59.21±3.77 g. After surgery, this decreased to 24.01±3.77 g and reached the least resistance on day 16 with 10.16±1.08 g. After this point, the resistance of the operated leg to the Von Frey probe gradually increased up to 40.74±5.05 on day 56. This was still significantly different from the baseline and control values.

3.4. Pinprick

In all five control groups, the duration of lifting after pinprick stimulation varied from 0 to 0.79±0.39 s. There were no significant differences between the control groups. The sham groups were comparable throughout the study. The mean duration of lifting varied from 0 to 0.76±0.47 s for all five groups. The right hind paw of the sham, control, and operated groups all remained comparable throughout the experiment.

The pinprick (Fig. 5A) test produced a significant amount of lifting in the TST model when compared to the sham and control groups. This started 1 day after surgery and continued until the end of the observation period on day 56. A peak value of 9.92±2.09 s was observed on day 21. Between days 7 and 21, a plateau of approximately 9 s was observed. In the CCI model, a significant amount of lifting was observed when stimulated with the pinprick (Fig. 5B). This was initially observed on day 9 and remained statistically significant until the final day of testing. A peak value of 11.74±2.61 s was observed on the final day of testing. The CST model showed increased lifting with the pinprick test (Fig. 5C). Significant lifting was first observed on day 3 and reached a peak of 12.08±4.31 s on day 42. There was still a significant difference on the final day of investigation. The PSL model produced significant lifting from day 1 onwards with the pinprick test (Fig. 5D). A peak of 15.32±1.38 s was reached on day 11. The results obtained continued to be significantly different from control and baseline until day 56. The SNL model produced significant lifting behaviour throughout the trial when stimulated with the pinprick (Fig. 5E). This started on day 1 and reached a peak of 11.22±2.50 s on day 28. After this point, there was a slight decline in the duration of lifting. The duration of lifting recorded was still significantly different from control and baseline on the final day of testing.

3.5. Acetone spray

The duration of lifting of the left hind paw after acetone challenge ranged from 0 to 4.21±1.99 s in all five control groups. No significant difference between the groups was observed. In all five sham groups, the duration of lifting was comparable. The duration of lifting of the left paw varies from 0 to 0.84 s. There is no significant difference among the surgical groups. The right hind paw of the operated, sham, and control groups remained unresponsive throughout the experiment for all the groups. The acetone test (Fig. 6A) produced a significant increase in lifting in the TST model compared with sham and control groups. Lifting after stimulation with the acetone spray was observed on

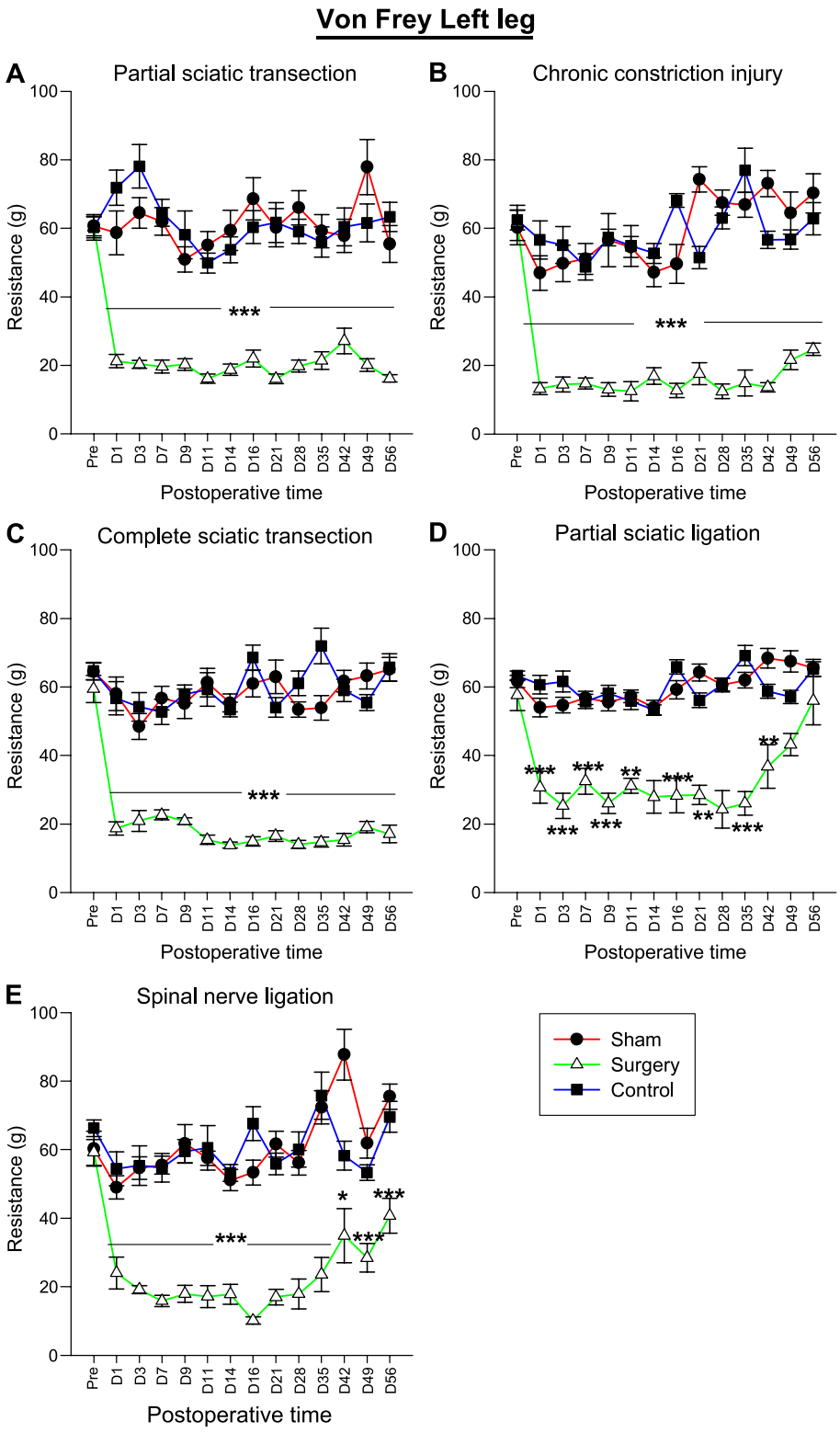

Fig. 4. The resistance of the left hind paw in grams over a period of 56 days. Given are mean (±S.E.M.) values of control, sham operated, and surgery groups before and at several time points after a possible surgery. The Mann–Whitney *U*-test (two-tailed) was used to assess for statistical differences against controls: **p*<0.05; ***p*<0.01; ****p*<0.001.

day 1 and continued until the end of the experiment. A peak of 31.29±4.84 s was seen on day 35. The CCI model showed significant lifting when stimulated with acetone (Fig. 6B). This started on day 1 and continued to a peak of 52.04±2.94 s on day 3. After this time, a plateau was observed until day 11 when lifting decreased slightly. The

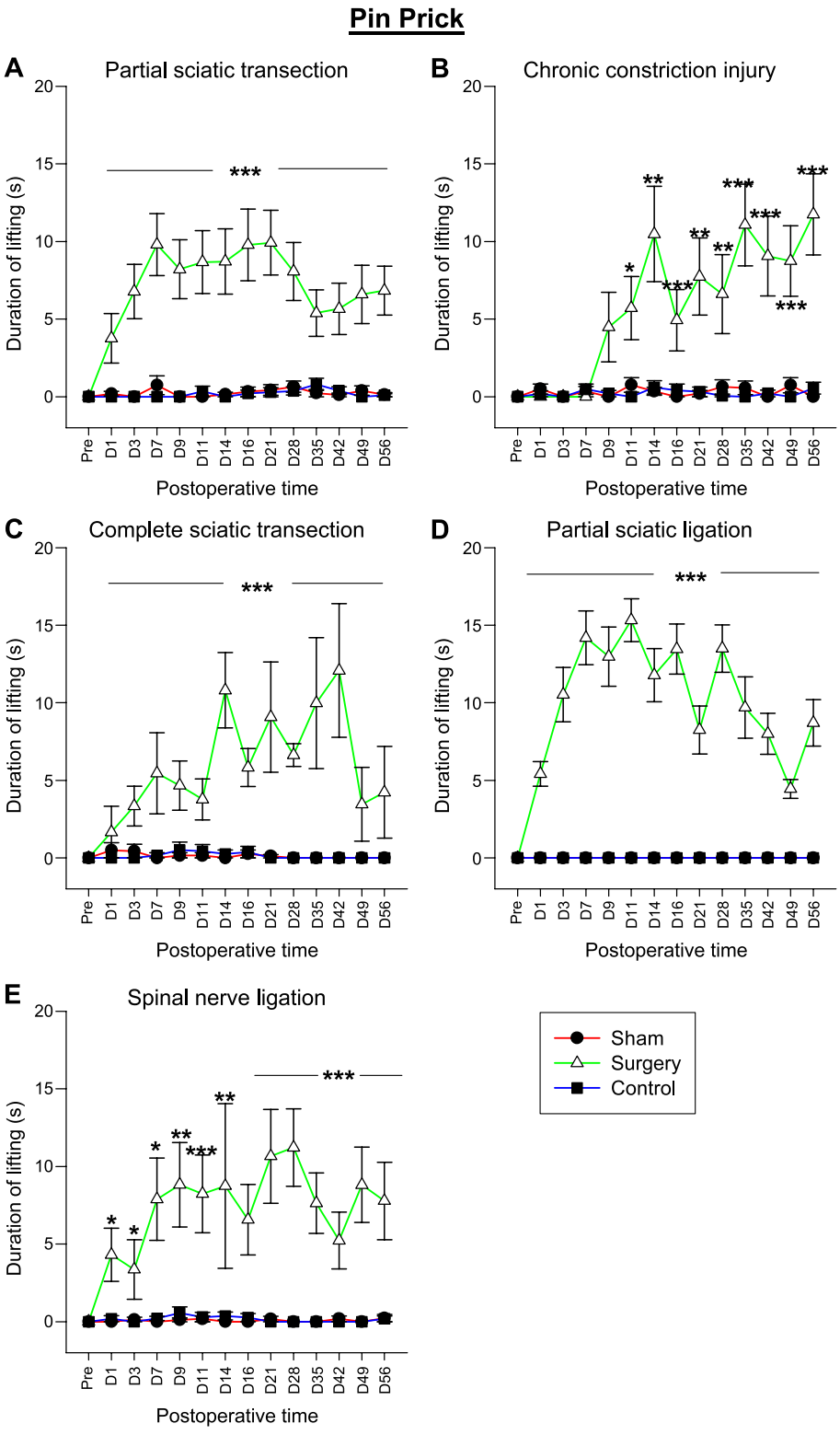

Fig. 5. The duration of lifting of the left hind paw after stimulation with a pinprick over a period of 56 days. Given are mean (±S.E.M.) values of control, sham operated, and surgery groups before and at several time points after a possible surgery. The Mann–Whitney *U*-test (two-tailed) was used to analyse for statistical differences against controls: **p*<0.05; ***p*<0.01; ****p*<0.001.

duration of lifting still remained statistically significant until day 56. The CST model showed increased lifting with the acetone test (Fig. 6C). This started at day 1 and reached a peak at day 7 of 41.34±4.9 s. After this, a plateau was seen until day 35, when the duration of lifting decreased. Acetone-induced lifting was observed until the end of the

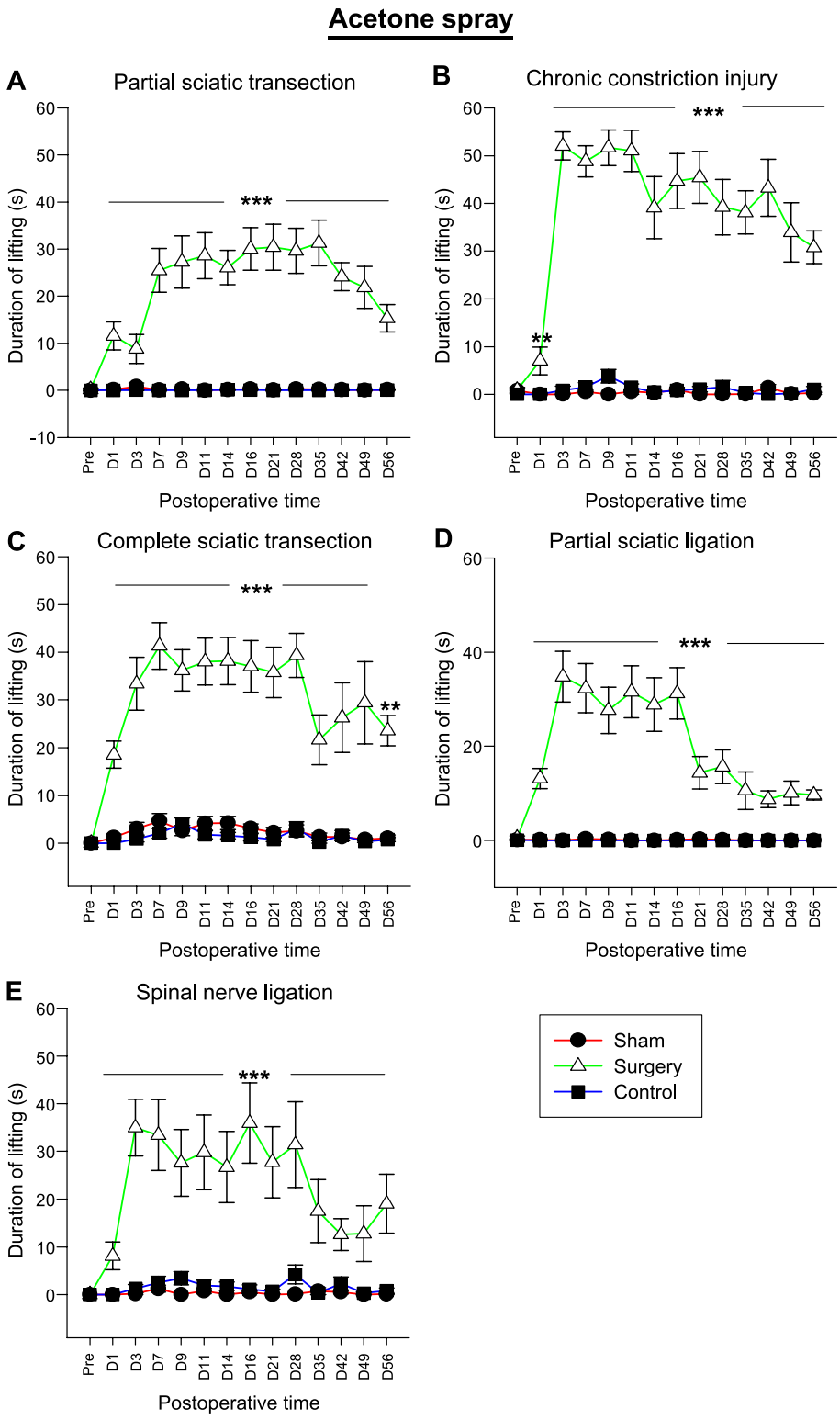

Fig. 6. The duration of lifting of the left hind paw after stimulation with an acetone spray over a period of 56 days. Given are mean (±S.E.M.) values of control, sham operated, and surgery groups before and at several time points after a possible surgery. The Mann–Whitney *U*-test (two-tailed) was used to assess for statistical differences against controls: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

experiment. The acetone spray produced significant amounts of lifting behaviour over the 56-day trial with the PSL model. Lifting was first observed on day 1 and rapidly increased to 34.79±5.40 s on day 3 (Fig. 6D). A plateau was observed from day 3 to day 16. After this time point, the duration of lifting decreased but still remained significantly different from baseline and control values until the end of the experiment. Also, in the SNL model, acetone

significantly increased lifting (Fig. 6E). A rapid increase in lifting behaviour was seen when acetone was sprayed onto the operated foot from 1 day after surgery. A plateau was observed from day 3 to day 28 with a maximum lifting value of 35.92±8.41 s reached on day 16. After day 28, the duration of lifting decreased but was still significantly different from the control and baseline readings up to day 56.

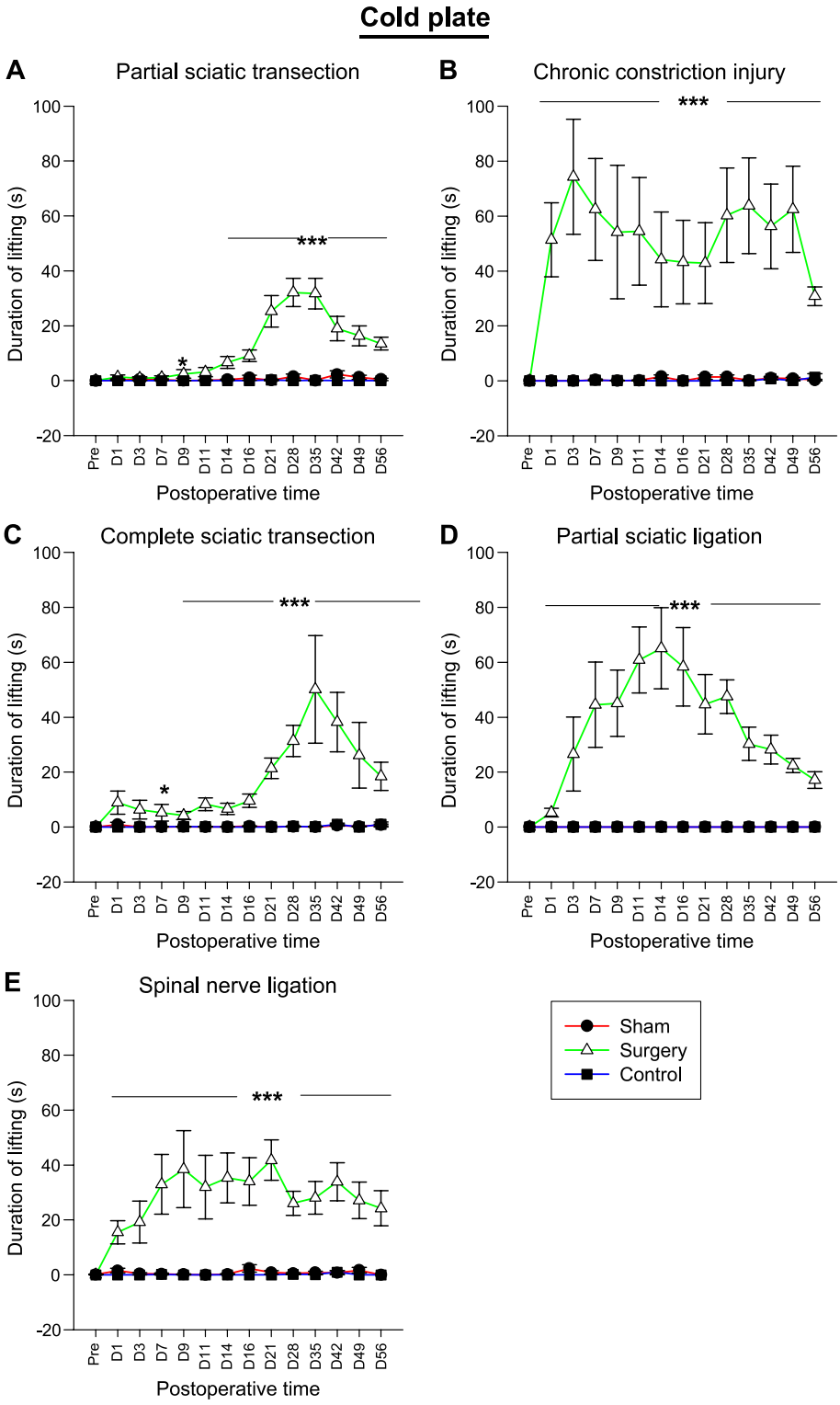

Fig. 7. The duration of lifting of the left hind paw on the cold plate over a period of 56 days. Given are mean (S.E.M.) values of control, sham operated, and Surgery groups before and at several time points after a possible surgery. The Mann–Whitney *U*-test (two-tailed) was used to assess for statistical differences against controls: *p<0.05; **p<0.01; ***p<0.001.

3.6. Cold plate

In all five control groups, the duration of lifting of the left hind paw varied from 0 to 1.29±1.29 s in the cold plate test. The control groups all had comparable lifting behaviour. There were no significant differences between the sham groups. The mean duration of lifting varied from 0 to 2.34±1.37 s for all five groups. The right leg of the sham, control, and operated groups all remained comparable throughout the experiment.

The cold plate showed increased lifting behaviour with the TST group. This was statistically significant compared to the control and sham groups. Significant lifting on the operated foot was first observed on day 11, and this continued, reaching a peak of 32.12±5.10 s on day 28. After this point, lifting gradually decreased. Significant lifting was observed until the last day of observation (Fig. 7A). With the CCI group, lifting of the operated hind paw was observed from day 1 onwards (Fig. 7B). A peak of 74.31±20.98 s was observed on day 3. The cumulative duration of lifting remained around this level until day 49.

The cold plate produced significant amounts of lifting with the CST model (Fig. 7C). This started on day 1 and reached a peak value of 50.10±19.61 s on day 35. Lifting decreased after this until day 56 but remained significantly different from control and baseline figures. The PSL model produced significant lifting in the cold plate test (Fig. 7D). Cold-induced lifting was observed 1 day postoperatively and climbed to a peak of 65.08±14.77 s on day 14. After this point, average lifting duration decreased gradually but was still at a significant level on day 56. Cold allodynia was seen in the SNL over the entire test period (Fig. 7E). Lifting was first observed on day 1 and climbed to a maximum on day 21 of 41.77±7.38 s. After this point, a plateau was observed until day 28, when the duration of lifting decreased. Lifting remained significantly different from the control group until the cessation of the study.

4. Discussion

A need exists for validated and easily reproducible animal models of neuropathic pain symptoms to accurately evaluate the analgesic potential of novel pharmacotherapies for the treatment of neuropathic pain. However, between laboratories, some variation in the experimental outcome is reported with animal models of neuropathic pain. Therefore, in this study, we have compared and described in detail five well-established models known to evoke neuropathic pain symptoms in rats. To validate these models, we chose a wide range of sensory tests to incorporate several stimuli, including neutral plate, hot plate, Von Frey, pinprick, acetone spray, and cold plate.

The models chosen were the complete sciatic transection (CST) model (Wall et al., 1979), the chronic constriction injury (CCI; Bennett and Xie, 1988), the partial sciatic ligation (PSL) developed by Seltzer et al. (1990), the spinal nerve ligation (SNL; Kim and Chung, 1992), and a transection of the tibial and sural branches of the sciatic nerve (Lee et al., 2000). These models were chosen as they have all been used in previous studies. They also all produce injury via damage to the peripheral nervous system at the sciatic nerve. It has been reported that internal and external factors can affect the development of neuropathic pain dramatically (Chesler et al., 2002). Internal factors include different species strains (Yoon et al., 1999; Shir et al., 2001a), gender (Perissin et al., 2003), and circadian rhythms (Folkard et al., 1976). External factors include diet (Shir et al., 2001b), social variables (Raber and Devor, 2002), housing (Brown and Grunberg, 1995; Robinson et al., 2004), and the suture material used for making nerve ligations (Robinson and Meert, in press). Variability may also exist between researchers. As these factors vary at different laboratories, there is a need to keep these variables as constant as possible to accurately compare the various peripheral nerve injury models. In this study, we minimised both the internal and external variables to allow for a more complete comparison. Previously, the models that were employed in this study have been examined over time scales that differed greatly from one another. For example, the initial PSL experiment (Seltzer et al., 1990) was run for a maximum of 53 days, whereas the CCI (Bennet and Xie, 1988) lasted for 140 days. A fixed time scale is therefore needed on which all the models can be fairly compared. To assess the progression of neuropathic symptoms within these models, they were observed over a sustained time period of 56 days. Furthermore, different stimuli were applied in previous studies. A standardised range of sensory tests chosen to include several types of stimuli was therefore used for each model. The tests chosen were neutral plate, hot plate, Von Frey, pinprick, acetone spray, and cold plate.

The most consistent observation found with every model was the rapid drop in mechanical threshold as measured using the Von Frey algometer. This decrease in resistance was found globally in all models, and the effect was always present from the first day after surgery. The largest difference observed between the operated animals and the control animals was found within the CCI group. For the four other models, the difference was still considerable. In the TST, CCI, and CST models, the decreased threshold remained constant throughout the experiment. For the PSL and the SNL models, the threshold was seen to increase back to preoperative values towards the end of the experiment. The sham and control groups for all the groups remained relatively constant at baseline levels throughout the experiment. Mechanical threshold decreases have been found in many other studies. Similar decreases in threshold were observed by Moller et al. (1998) and Tal and Bennett (1994) with the CCI model. A decreased mechanical threshold was also observed after SNL injury (Choi et al., 1994; Saade et al., 2003), in the PSL model (Seltzer et al., 1990; Mitchell et al., 1999), and the TST model (Lee et al., 2000). As far as

we are aware, no Von Frey testing has been published on the CST model.

The mechanical hypersensitivity displayed in the pinprick test was observed in all models when compared to the sham and control groups. Apart from a small delay in the CCI model, the mechanical hypersensitivity was present from the first until the final day of testing in all models. The response to the pinprick remained relatively stable over time. The sham, control, and right leg remained unresponsive to the pinprick within each group.

With regard to the mechanical hyperalgesia observed in our study, our findings are supported by several previous reports. A study by Benoliel et al. (2001) produced comparable CCI results to those obtained in this study, with an initial hypoalgesic stage, followed by hyperalgesia after 9 days. Jett et al. (1997) observed increased hyperalgesia after an SNL injury. This continued for a sustained period of time as seen in our study. The original PSL study by Seltzer et al. (1990) found comparable results to our study. They observed an initial increase in the mechanical hyperalgesia followed by a small decrease after 10 days. Increases in mechanical hyperalgesia after a transection of two branches of the sciatic nerve have been observed in other studies (Decosterd and Woolf, 2000; Erichsen and Blackburn-Munro, 2002). This correlates well with our study, as in both experiments, mechanical hyperalgesia was seen immediately after surgery and began to decrease after day 25. A thorough search of the available literature revealed no previous examples of pinprick testing within the CST model.

Lifting on the neutral plate indicates a combination of spontaneous pain and adjustment of weight bearing in the operated animals. Both these parameters play a role in producing spontaneous lifting behaviour. There were obvious differences in the duration of lifting between the models on the neutral plate. The TST model induced almost no lifting behaviour over the entire trial, while limited responses were also seen for the CST, PSL, and SNL models. A larger response was noted with the CCI model, although not all of the animals displayed spontaneous lifting after a CCI. Large durations of lifting were only observed for three animals. The right leg and sham and control groups remained relatively constant throughout the duration of this study. The original CCI developed by Bennett and Xie (1988) showed similar levels of spontaneous lifting on a thermally neutral plate. Seltzer et al. (1990) observed a cumulative duration of lifting of approximately 30 s with a very large standard error. This is very similar to our own observations. Choi et al. (1994) also examined the SNL model on a neutral plate of 30 °C over a 20-week period. The results obtained are similar to those described presently with an initial peak of approximately 6 s after surgery, followed by very low levels of lifting. Kim et al. (1997) have described lifting behaviour on the neutral plate of approximately 10-s duration, which was maintained for the 8-week testing period. This is slightly different from our observations, for which only a limited amount of lifting was observed in the first week. However, it should be noted that Kim et al. used a plate, which was at 30 °C, whereas our plate was at room temperature (22 °C), which may have been more comfortable for the animal. Lee et al. (2000) studied the TST model on a thermally neutral plate. and increased spontaneous lifting after 7 days, which continued until at least week 10. Again, there are differences in comparison to our findings, as we did not observe lifting behaviour on the neutral plate with this particular form of nerve injury. The anomaly between the two studies may be due to the surgical technique employed. Lee et al. ligated the sciatic branches before the transection, but we did not perform ligations in our investigation.

Lifting on the hot plate is due to a combination of thermal allodynia, spontaneous pain, and adjustment of weight bearing in the operated animals. All three of these factors play a role in producing the lifting behaviour observed on this plate. A comparable effect to the neutral plate was seen with the hot plate. A slightly larger response was observed with the SNL model. The other four models remained at similar levels to those observed on the neutral plate. The neutral and hot plate results were relatively similar, which is indicative of a lack of heat allodynia under these conditions. A more exaggerated response was seen on the cold plate in comparison to the neutral and hot plates. Any lifting observed on this plate is due to a combination of three factors: cold allodynia, spontaneous pain, and adjustment of the weight-bearing paw. Additionally, animals with a nerve injury will also experience some mechanical stimulation when they lower their injured foot to the floor. The largest amount of lifting was observed in the CCI model. The lifting began directly after surgery and was maintained until the end of testing. The results obtained with the SNL model are comparable to those for the CCI, but the magnitude of the response was slightly diminished. The TST, CST, and PSL groups all show a U-curve. The PSL group displayed lifting immediately after surgery, but after 2 weeks, this response started to dissipate. The TST and CST models both show a biphasic pattern with cold allodynia not present until about 2 weeks into the trial. The right leg and the sham and control groups remained at baseline levels throughout the trial. Similar results with the CCI model to the ones found in this study are reported by Lee et al. (1998) on the cold plate. However, in Lee's study, the lifting duration decreased to baseline levels by week 8. This was not found in our study and could be due to the different temperatures of the cold plate. In our study, the plate was set at 0 °C, whereas in Lee et al. study, the plate was controlled at 5 °C. Choi et al. (1994) tested the SNL animals on the cold plate and observed comparable results to our study. They observed lifting, which started on day 1 and continued to at least week 8 as seen with our results. Also, Kim et al. (1997) observed lifting with the PSL model on the cold plate. The maximal duration of lifting was similar to our results. However, in their study, the

magnitude remained constant from day 1 to week 8, but in our study, we experienced a U-shaped curve.

The exaggerated paw lifting observed after acetone application is due to the combined effects of cold allodynia, chemical stimuli, and even possibly mechanical stimulation. Chemical hyperreactivity to acetone was evident in all five models with a fast onset from day 1 onwards. A plateau phase was also observed in all five groups. In the TST, CST, PSL, and SNL groups, a marked reduction in the duration of paw withdrawal occurred towards the end of the experiment. The highest magnitude of chemical hyperreactivity was produced in the CCI model. There is a clear difference between the left and right leg and between each of the treatment groups (i.e., operated versus sham or control). The acetone response seen in this study has been replicated in other trials. Lee et al. (1998) observed the effect of acetone application in both the CCI and PSL models and noted a response very similar to that seen in the current experiment with both forms of nerve injury. With the SNL model, Choi et al. (1994) observed an initial increase in lifting after acetone application followed by a plateau phase until week 6. The response thereafter was diminished.

From our observations, it is possible to suggest which stimuli can optimally be used to assess the neuropathic pain symptoms evoked by each surgical model. The TST model produced clear-cut and stable responses to the Von Frey, pinprick, and acetone tests throughout the 56-day trial. Although TST animals displayed a considerable amount of lifting on the cold plate, the response was not sustained and decreased over time. Thus, the cold plate may not be a suitable sensory test for longer studies involving the TST nerve injury. The neutral and hot plate tests in this model did not show any significant lifting, and it may therefore be unnecessary to include these tests in future trials with the TST. The TST model has several advantages. The surgery is relatively rapid to perform, and the model possesses a high reproducibility, as the number of injured fibres remains the same each time. The CCI model produces significantly large responses within all the behavioural tests used. This is especially true of the Von Frey, pinprick, acetone, and cold plate. The hot plate and neutral plate response were generally limited to a few animals in the group. The CCI model produces the most sustained and greatest responses out of the five models compared. However, a skilled technician with experience of the surgical technique would be required as it is difficult to gauge or reproduce the exact tension for the ligation needed. Additionally, there are often animals which do not respond in the group. The CST model shows clear-cut responses on the pinprick, acetone, and cold plate tests. However, as with the TST model, the duration of lifting observed on the cold plate is relatively short. This surgical model also shows very limited responses to the hot and neutral plate. Unless the effects of a complete transection need to be examined, this model should be avoided for long-term studies due to the increased prevalence of autotomy as time progresses. Von Frey, pinprick, acetone, and cold plate all show significant responses with the PSL model. The neutral plate and the hot plate displayed only a limited response. The PSL model is not particularly suitable for long-term studies as the responses to the behavioural stimuli diminish after 3 to 5 weeks. This is probably due to a loss of tension in the ligation after this period. The diameter of the ligation also varies between operated animals, which makes this model difficult to standardise. The SNL model displayed definitive responses to Von Frey, pinprick, acetone, and cold plate, but limited responses with the neutral and hot plates. The SNL model also has a trend to decrease in response towards the end of the trial, although this is not as dramatic a decline as with the PSL model. The SNL model is the most difficult and invasive surgery to perform out of the five models evaluated. This increases the likelihood of nonresponders and increases the chances for tissue and muscle damage.

Conclusions regarding the sensory test employed in this study can also be made from the data presented here. The VF test shows clear-cut deficits in all five surgical models. The interanimal variability in this test is the smallest of all the sensory tests performed. This test can also be used to ascertain if the neuropathic surgery performed was successful, as a response is seen almost immediately after surgery. If a response is seen with the VF test, the animal can then be moved forward to further screening. The pinprick test also shows clear responses in all five sensory tests, although the time taken to reach the maximum duration of lifting varies significantly between models. Some variability in the magnitude of the response is observed over the time course of the surgical models with no steady plateau phase reached in the CCI, CST, SNL, or PSL models. Furthermore, there is a large interindividual variability between the subjects in the test. A further issue with the pinprick test is the inability to exactly standardise the force being applied to the paw with the pinprick. This may explain some of the variations seen in the models and could effect testing outcomes between individual researchers. The acetone spray tests produce clear chemical hyperreactivity in all five models shown with steady plateau phase reached rapidly in all models. This makes this test ideal for drug trials, as there is a wide window of time when testing can be performed. However, for the PSL and the SNL models especially, there is a rapid decline in reactivity after 3 to 4 weeks. The cold plate test produces lifting in all five models, but the amount of lifting varies greatly between the models. Only the CCI and SNL models reach a plateau stage, with the other three models showing a U-shaped curve. The cold plate test can be used satisfactorily with the CCI, PSL, and SNL models. The biphasic pattern shown with the TST and CST models suggests that the cold plate is not the best test to use for these two models. The neutral and hot plate test as performed here produce low amounts of lifting in all but the CCI model. Except for the use of screening for spontaneous pain, these tests should not be used in conjunction with these models.

In conclusion, with a few notable exceptions, it appears that all five models of peripheral neuropathic pain produce mechanical threshold decreases, mechanical hyperalgesia, chemical hyperreactivity, and cold allodynia. However, the magnitude and duration of responses on these four sensory clusters vary considerably depending on the surgical model used. Drug trials on the various models may help to further differentiate between the present models of peripheral nerve injury.

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