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# The effect of Spinal Cord Stimulation in mice with chronic neuropathic pain after partial ligation of the sciatic nerve

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ABSTRACT

The effect of Spinal Cord Stimulation (SCS) in chronic neuropathic pain is inversely related to the severity of mechanical allodynia and the underlying mechanisms are poorly understood. To understand these mechanisms further we aimed to develop a model of SCS in a neuropathic mouse. Further, the CatWalk analysis, which is claimed to be an improved test for mechanical allodynia and therapeutic intervention, was used to analyze the effect of SCS on mechanical allodynia. Male C57BL/6 mice ($N = 31$) underwent partial ligation of the sciatic nerve. After 14 days an electrode was implanted and the effect of SCS ($N = 22$) on mechanical allodynia was tested. Unligated mice ($N = 8$) also received SCS. Behavioral analysis was performed using von Frey filaments and the CatWalk system. The withdrawal threshold showed a significant decrease which remained over time. Changes in CatWalk parameters were observed after 2 days, but tended to diminish during the next 14 days. Thirty minutes of SCS resulted in a 100% response and return to pre-neuropathy levels of the withdrawal threshold. The effect of SCS on the withdrawal threshold was comparable for the most severe and milder allodynic animals. SCS did not affect any of the CatWalk parameters in all mice. In conclusion, we developed a model of SCS in a chronic neuropathic pain C57BL/6 mouse. The CatWalk gait analysis does not result in the detection of behavioral changes to SCS in mice with chronic neuropathic pain and control animals. This model allows future molecular-genetic studies on the mechanisms of SCS in chronic neuropathic pain.



## 1. Introduction

Despite the use of Spinal Cord Stimulation (SCS) in the treatment of patients with chronic neuropathic pain [18,19,23] understanding of the underlying mechanisms of its favorable effects is still relatively limited. In the experimental field, new insights have been obtained on mechanisms that could be responsible for the effect of SCS in chronic neuropathic pain models [6,24,28]. The most commonly used neuropathic pain model to study experimental SCS is the partial ligation of the sciatic nerve or Seltzer model [33]. The Seltzer ligation results in a fast and pronounced development of mechanical allodynia (as assessed with von Frey filaments) and thermal hyperalgesia in rats and this persists for more than 7 months [33].

In Seltzer injured rats, SCS results in a robust and fast increase in the von Frey withdrawal threshold of the injured limb which is still noticeable 60 min after cessation of SCS [34]. Similar to the clinical situation, 30–50% of rats with chronic neuropathic pain do not respond adequately to SCS [7,22,35]. Moreover, an inverse relationship of the degree of mechanical allodynia and the effect of SCS was shown [34]. From these experimental data, it was suggested that the selection and subdivision of patient groups similar to those defined in the rat model [34] may provide better pre-treatment prediction of possible therapeutic benefits of SCS. Indeed, after the analysis of various prognostic factors in 36 CRPS-I patients treated with SCS, the degree of brush-evoked allodynia was shown to be a prognostic factor of SCS treatment outcome (preliminary results by van Eijs).

To analyze the underlying mechanisms of SCS in experimental chronic neuropathic pain further it might be important to develop an SCS model in mice with chronic neuropathic pain. An SCS-mouse model might allow us to study the molecular-genetic mechanisms of neuropathic pain and SCS, using mice with specific gene modifications. In a pilot experiment we have already described the technical requirements and aspects to apply SCS in chronic neuropathic C57BL/6 mice [37]. It has been reported that C57BL/6 Seltzer injured mice develop mechanical allodynia [26] similar to that described in the Sprague–Dawley rats [28,33].

Whereas testing of mechanical allodynia using the von Frey withdrawal threshold is presently used as the gold standard, another method, the CatWalk gait analysis is additionally used in experimental pain research. The CatWalk is a fully automated objective gait analysis and was initially developed to analyze gait changes after spinal cord injury in rats [15]. In addition, the CatWalk has been suggested as a novel rapid and highly objective

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alternative to the von Frey method for measuring mechanical allodynia in rat models of chronic neuropathic pain [39] or acute inflammatory pain [12].

Therefore, the aim of this investigation was twofold: first to study the effect of SCS on mechanical allodynia in the Seltzer injured C57BL/6 mice using the von Frey assessment and second to implement the CatWalk gait analysis for detection of pain-related gait changes after SCS in mice with chronic neuropathic pain.

## 2. Methods

### 2.1. Animals

In this experiment a total of 39 male C57BL/6 mice (Charles River), weighing 20–25 g, were used. In 31 mice, the sciatic nerve was ligated and 8 mice served as controls, only receiving SCS. Housing was kept at a constant room temperature of 21 ± 2 °C and humidity (55 ± 15%) under a normal 12:12 h light/dark cycle. Water and food were available ad libitum. The well-being of the animals was monitored daily and documented by the responsible researcher.

### 2.2. General

Animal experiments were performed in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU) and were approved by the Committee for Experiments on Animals of Maastricht, The Netherlands. Surgery was performed under aseptic conditions in anesthetized animals. General anesthesia was induced by isoflurane inhalation (4% in a 1:1 mixture with air, flow 400 ml/min) in a Plexiglas induction chamber. Anesthesia was maintained with an univentor 400® injection system vaporizer (Zevenaar, Holland) with an open mask system (Fluovac, Harvard Apparatus) using 2% isoflurane in a 1:1 mixture with air flow of 250 ml/min. Loss of body temperature was prevented by placing the animal on an automated heating pad (37.5 ± 0.5 °C), (IDEE, University of Maastricht).

Animals that showed signs of extreme weight loss (>15% of body weight at delivery), cachectic behavior, or which did not groom, were euthanized.

### 2.3. Unilateral ligation of the sciatic nerve

A unilateral sciatic nerve ligation was performed similar to the approach described in rats by Seltzer et al. [33] and Malmberg and Basbaum [26]. In short, the left sciatic nerve was exposed by blunt dissection and carefully freed from surrounding connective tissue. Just distal to the posterior biceps semitendinosus, but proximal to the little fat pad that lies a few millimeters distal to this site, the ligation was performed. We used an 8/0 non-absorbable silk suture with a reverse cutting and 3/8 curve micro needle to ligate approximately 1/3–1/2 of the diameter of the left sciatic nerve. After the ligation, the wound was closed intracutaneously with a 5/0 silk suture. After surgery the mice were inspected daily, to assure that sutures were still in place.

### 2.4. Implantation of the Spinal Cord Stimulation (SCS) device

The procedure for implanting the electrodes in the mice was done under general anesthesia. Fourteen days post-operatively (DPO14) a laminectomy was performed at the level of T13. Using the 13th rib as an anatomical landmark to identify the processus spinosus of T13, a small hole was made in the centre of the lamina as rostrally as possible making sure not to penetrate the dura. After the introduction of the cathode into the epidural space, the cathode was glued to the lamina of T12 using hystoacryl glue (Histoacryl®, Braun) in order to prevent movement of the lead. The anode was placed subcutaneously, in the abdominal wall as low as possible. The two leads were then positioned subcutaneously, in a way that they could move freely without putting stress on the leads.

Next a small incision was made at the base of the skull of the mice to tunnel the bipolar connector including the leads subcutaneously to the incision site. The periosteum of the bone was carefully removed to ensure good adhesion of the connector. The connector was then firmly cemented to the base of the skull using bone cement (Paladur®, Kulzer).

Animals were allowed to recuperate after the surgery for 48 h, before being submitted to the SCS. For SCS we used a Grass S88 stimulator (Astro-med Grass Warwick, USA) fitted with a Grass SIU-5 stimulus isolator and a Grass constant current unit. The stimulus paradigm was as follows: first the motor threshold was assessed (frequency 2 Hz, pulse width 0.2 ms) and the actual stimulation was performed at 2/3 of the motor threshold (frequency 50 Hz, pulse width 0.2 ms). Spinal cord stimulation was maintained for 30 min. During SCS, the mice could move freely. No signs of discomfort were noted due to the stimulation. The settings used for SCS were similar to those used by others in rats [34] and mice [37].

To study the effect of SCS in control animals, electrodes were implanted into non-ligated mice. These animals were also allowed to recuperate after the surgery for 48 h, before being submitted to SCS.

### 2.5. Behavioral testing

#### 2.5.1. von Frey withdrawal threshold to mechanical stimuli

Before partially ligating the sciatic nerve each mouse was tested for the behavioral response to mechanical stimuli, using the von Frey test. This is the baseline withdrawal threshold (pre-operation threshold). The control animals were tested before receiving the SCS implant (baseline).

After the ligation of the sciatic nerve, the withdrawal threshold was tested on 2, 7 and 14 days post-operatively. Mice that did not show a decrease in withdrawal threshold of more than one von Frey filament compared to baseline were excluded and we qualified them as "non-allodynic". Testing was done in a set of Plexiglas cages, each measuring 6.5 × 7.5 × 8.0 cm, with a wire mesh floor. The animals were allowed to adapt for 60 min before testing. After acclimatization, the von Frey filaments were applied, in an order of increasing stiffness, through the wire mesh floor to the mid-plantar surface of the right or left hind paw until the filaments bent slightly. A positive withdrawal was scored when the animal showed a response (brisk withdrawal) to 3 of the 5 stimuli presented. The animals were tested using 10 von Frey monofilaments (North Coast Medical Inc., CA, USA) with logarithmically incremental stiffness (0.008, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, and 4.0 g).

Immediately before SCS the animals were tested for the presence of mechanical allodynia using the von Frey filaments (pre-SCS). Von Frey analysis was done at several time points after the initiation of SCS ($t$ = 15, 30, 45, 60, 75 and 90 min). Spinal cord stimulation was ceased after 30 min.

To study the effect of SCS related to the severity of mechanical allodynia, the allodynic mice were categorized based on their response to the various von Frey filaments just before SCS (pre-SCS) (Table 1).

#### 2.5.2. CatWalk gait analysis

To exclude a possible effect of the stimulation of the hind paws by the von Frey filaments on CatWalk parameters, CatWalk data collection was always done on the same day and before the von Frey testing.

**Table 1**
Effect of SCS on the withdrawal threshold of the different ''allodynic groups'' ($N = 22$). The first column depicts the absolute pre-nerve injury withdrawal threshold of the different ''allodynic groups'', defined by the degree of allodynia at pre-stimulation (Column 2). The third column depicts the absolute withdrawal threshold after 30 min of SCS. The last column depicts the effect of SCS expressed in percent of pre-nerve injury withdrawal threshold. No difference could be detected between the degree of allodynia and the response to SCS.

| | Pre-nerve injury withdrawal threshold (g) | Pre-stimulation withdrawal threshold (g) | Withdrawal threshold after 30 min SCS (g) | Percent of pre-nerve injury withdrawal threshold at SCS30 |
|---|---|---|---|---|
| | 1.0 ± 0.40 ($n = 2$) | 0.008 | 1.0 ± 0.00 | 119.05 ± 47.76 |
| | 0.9 ± 0.07 ($n = 6$) | 0.02 | 0.9 ± 0.07 | 104.44 ± 14.05 |
| | 1.0 ± 0.00 ($n = 2$) | 0.04 | 1.0 ± 0.20 | 120.00 ± 20.06 |
| | 1.0 ± 0.63 ($n = 4$) | 0.07 | 1.0 ± 0.12 | 133.33 ± 33.33 |
| | 0.9 ± 0.08 ($n = 5$) | 0.16 | 0.9 ± 0.13 | 108.00 ± 7.99 |
| | 1.5 ± 0.29 ($n = 3$) | 0.4 | 1.5 ± 0.33 | 90.48 ± 0.00 |
| Mean | 1.03 | 0.11 | 1.08 | 111.34 |
| SEM | 0.07 | 0.03 | 0.06 | 8.10 |

In general, mice cross the CatWalk runway easily and at a constant speed. CatWalk gait analysis was done on the same day as the von Frey test during the first 14 days of the experiment, or baseline for the control animals. During the SCS-protocol CatWalk data were obtained pre-SCS and after 30 min of stimulation and at 60 min after cessation of SCS. These time points were selected because a 15-min timeframe between von Frey assessments was too limited to allow us to collect appropriate CatWalk runs.

The CatWalk analysis has been described in detail [15,21]. In short, the CatWalk system consists of a glass walkway which contains light from a white fluorescent source. The light rays from this source are completely reflected internally. When an object touches the glass runway, the light is reflected downwards, where it is detected by a video camera (Sony 3CCD Color Video Camera; DXC-990/990P). This signal is then digitized, which allows analysis by the CatWalk program software [15]. With the CatWalk, a vast variety of static and dynamic gait parameters can be measured. Some of these parameters, including Mean intensity of paw placement, Stance duration and Swing duration of the hind paw, have been linked to mechanical allodynia and neuropathic pain [39]. Because the speed and consistency at which the animals cross the glass runway can affect various CatWalk parameters [21] a constant crossing time is a prerequisite for correct CatWalk analysis.

In the present study the following individual paw parameters were used:

*Mean intensity (expressed in arbitrary units, a.u.):* Mean intensity of the pixels forming the maximum area.
*Stance duration (expressed in seconds):* Time of contact of the paw with the glass floor.
*Swing duration (expressed in seconds):* Time that the paw is not in contact with the glass floor.

### 2.6. Statistical analysis

The data are presented as mean ± standard error of the mean (SEM). For statistical analysis of differences of the withdrawal thresholds and the CatWalk parameters over time we used the non-parametric Friedman test, followed by Dunn's post hoc test. The Wilcoxon test was used to compare the withdrawal thresholds and CatWalk parameters between DPO14 and pre-SCS. For analysis of differences in weight and crossing time the repeated measure analysis of variance (ANOVA) with Bonferroni correction was used. $p < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. General observations

The majority of the operated mice appeared healthy and well-groomed. None of the mice showed signs of autotomy after ligation of the left sciatic nerve. Paw gesture of the ipsilateral paw was slightly altered, but this did not interfere with the normal daily activities of the mice. Body weight decreased slightly after the partial ligation of the sciatic nerve but returned to pre-injury levels within 5 days.

A total of 29 of the 31 ligated animals could be used as they developed mechanical allodynia as assessed with von Frey filaments (2 animals were non-allodynic). For SCS 22 animals were used to study the effect on mechanical allodynia (2 animals were in poor health after implantation, in 3 animals the connector came loose from the cranium, and 2 animals did not show any reaction to the mechanical stimulation after implantation).

On the same 29 animals that were used for the von Frey analysis the CatWalk analysis of the gait during the development of mechanical allodynia was performed. To study the effect of SCS on gait we included 19 animals (3 additional animals were excluded compared to the von Frey analysis of SCS, because these animals were reluctant to cross the CatWalk runway.

A total of 7 of 8 control animals could be used for von Frey and CatWalk analyses (one animal was excluded because the connector came loose).

### 3.2. Withdrawal threshold to mechanical stimuli after partial nerve injury

A marked decrease in the withdrawal threshold of the ipsilateral paw at DPO2 compared to baseline was noted in Seltzer ligated animals ($n = 29$) (Fig. 1A). Before the Seltzer operation, the mean withdrawal threshold of the ipsilateral paw of these mice was 1.00 ± 0.05 g. Two days after the ligation a reduction was observed to 0.16 ± 0.03 g ($p < 0.001$), which remained stable up to DPO14. The withdrawal threshold of the contralateral paw at baseline (1.00 ± 0.06 g) was comparable to that of the ipsilateral paw (1.00 ± 0.05 g). The partial ligation of the left sciatic nerve did not result in alterations in withdrawal threshold of the contralateral paw (Fig. 1A).

### 3.3. Withdrawal threshold to mechanical stimuli during and after Spinal Cord Stimulation

A total of 29 allodynic animals were implanted with an SCS device at DPO14. Twenty-two animals received SCS. In this population of SCS responders ($n = 22$), the mean pre-operation withdrawal threshold (baseline) was 1.03 ± 0.07 g.

The mean pre-stimulation withdrawal threshold (pre-SCS) of the ipsilateral paw was 0.11 ± 0.03 g, which did not differ from the withdrawal threshold at DPO14 (pre-implantation) (0.15 ± 0.03 g, $p > 0.05$).

SCS increased the mean withdrawal threshold to 0.78 ± 0.08 g after 15 min of stimulation as compared to pre-SCS ($p < 0.001$)

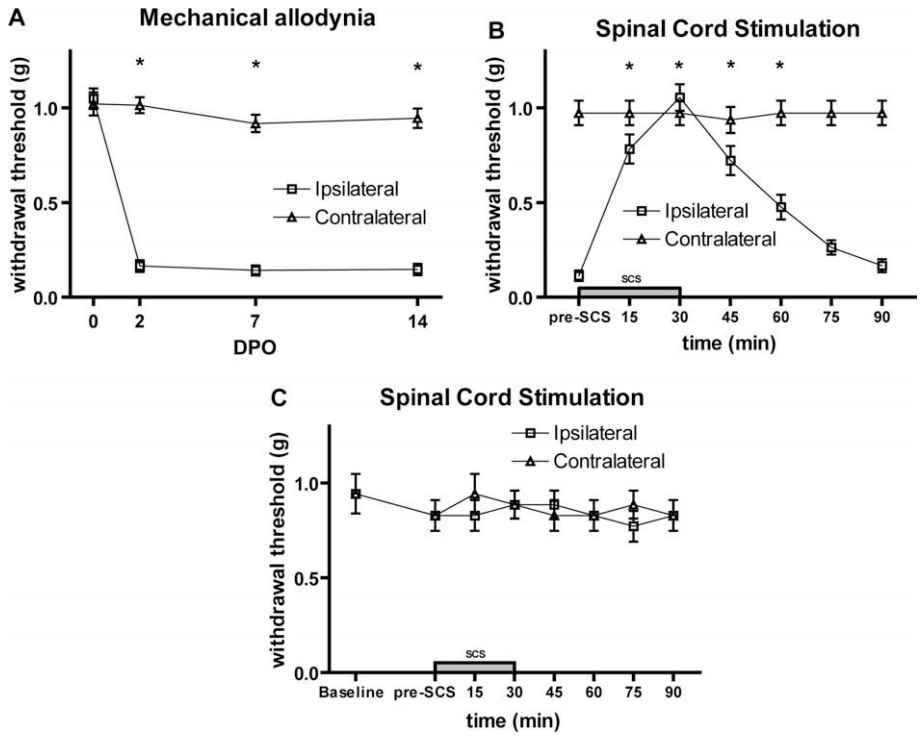


**Fig. 1.** Development of mechanical allodynia after partial ligation of the sciatic nerve. All data are represented as mean ± SEM. (A) Decrease in withdrawal threshold of the ipsilateral hind paw to mechanical (von Frey) stimulation following the Seltzer ligation ($N = 29$). No change in withdrawal threshold of the contralateral paw could be detected. (B) Spinal Cord Stimulation ($N = 22$) resulted in a rapid increase in the mean withdrawal threshold of the ipsilateral hind paw. After 15 min of stimulation withdrawal threshold reached 0.78 ± 0.08 g. Maximum stimulation effect to pre-neuropathy level was achieved after 30 min, which was statistically different from pre-SCS values ($p < 0.001$). Statistical significance ($p < 0.05$) compared to that at pre-SCS is indicated with asterisks. After cessation of SCS, withdrawal threshold gradually decreased to 0.26 ± 0.04 g at 75 min and was not significantly different from that at pre-SCS ($p > 0.05$). SCS did not affect the withdrawal threshold of the contralateral paw. (C) Effect of SCS on the withdrawal threshold in non-ligated animals ($n = 7$). No effect of SCS on the withdrawal threshold of both the ipsilateral and contralateral paws could be detected.

(Fig. 1B). Complete return to pre-neuropathy levels (1.08 ± 0.06 g, $p > 0.05$) of the withdrawal threshold was observed after 30 min of SCS (Fig. 1B).

Thirty minutes after its initiation, SCS was terminated. The mean withdrawal threshold decreased to 0.26 ± 0.04 g at 75 min and was not significantly different from that at pre-SCS ($p > 0.05$). During and after SCS no changes in withdrawal threshold of the contralateral paw were noted (Fig. 1B).

No effect on the withdrawal threshold of both the ipsilateral and contralateral hind paws after implantation and SCS could be observed in the control animals (Fig. 1C).

Based on their responses all allodynic animals were categorized to the various filaments after 16 days (pre-SCS) of chronic neuropathic pain (Table 1). The most "sensitive" animals responded to a filament of 0.008 g whereas the least "sensitive" animals responded to 0.4 g. Pre-nerve injury withdrawal threshold of the ipsilateral paw of all mice did not differ (Table 1). As can be deduced from our data, the degree of mechanical allodynia is not related to the response to SCS. The most severely allodynic animals responded as well to SCS as the less allodynic animals in the sense that all the animals returned to pre-operative withdrawal threshold values after 30 min of SCS (Table 1).

### 3.4. CatWalk gait analysis after partial nerve injury

First we performed a CatWalk gait analysis after a unilateral ligation of the sciatic nerve. With respect to a reliable comparison of gait parameters between animals with nerve ligation, two aspects are of high importance: body weight and speed of gait [21,41]. First, body weight may seriously confound interpretations about CatWalk parameters such as the Mean intensity parameter of gait. Body weight decreased after the operation to 21.81 ± 0.17 g compared to baseline (22.47 ± 0.16 g) (95% CI, 0.66 (0.34, 0.97)), but returned to pre-operation values within 5 days (22.23 ± 0.15 g) (95% CI, 0.23 (0.09, 0.55)). Second, the speed of gait may have a strong influence on gait parameters including print area, Stance duration and Swing duration. In the present experiment no correction for speed was needed because the CatWalk crossing time did not show significant differences in the ligated animals over time (baseline 1.46 ± 0.04 s, DPO2 1.46 ± 0.25 s, DPO7 1.44 ± 0.35 s, DPO14 1.37 ± 0.26 s, pre-SCS 1.38 ± 0.06 s, SCS30 1.43 ± 0.25 s and SCS90 1.43 ± 0.20 s) (ANOVA $p > 0.05$).

Partial ligation of the sciatic nerve did result in a significantly changed Intensity of the ipsilateral paw print. A decrease to 74.92 ± 6.64% at DPO2 (compared to baseline) ($p < 0.001$) was observed (Fig. 2A). Despite the fact that from DPO2 a gradual increase was noted, still a small but significant difference could be observed between the Intensity at baseline and DPO14 (84.31 ± 5.71%, $p < 0.05$). No changes in the Intensity of the contralateral paw were observed (Fig. 2B).

The Stance duration of the left hind paw in the Seltzer group significantly decreased to 55.14 ± 6.87% at DPO2 compared to baseline ($p < 0.001$). An increase in the Stance duration was noted over time and at DPO14 (80.64 ± 6.08%) no significant difference could be observed compared to baseline (Fig. 2A). No changes in the Stance duration of the contralateral paw could be detected (Fig. 2B).

A significant increase in the Swing duration of the ipsilateral paw was observed at DPO2 (116.39 ± 11.39%, $p < 0.001$) and DPO7 (120.90 ± 12.89%, $p < 0.01$) compared to baseline. At DPO14, the Swing duration (103.07 ± 8.45%) returned to baseline values (Fig. 2A). No changes in the Swing duration of the contralateral paw were observed (Fig. 2B).

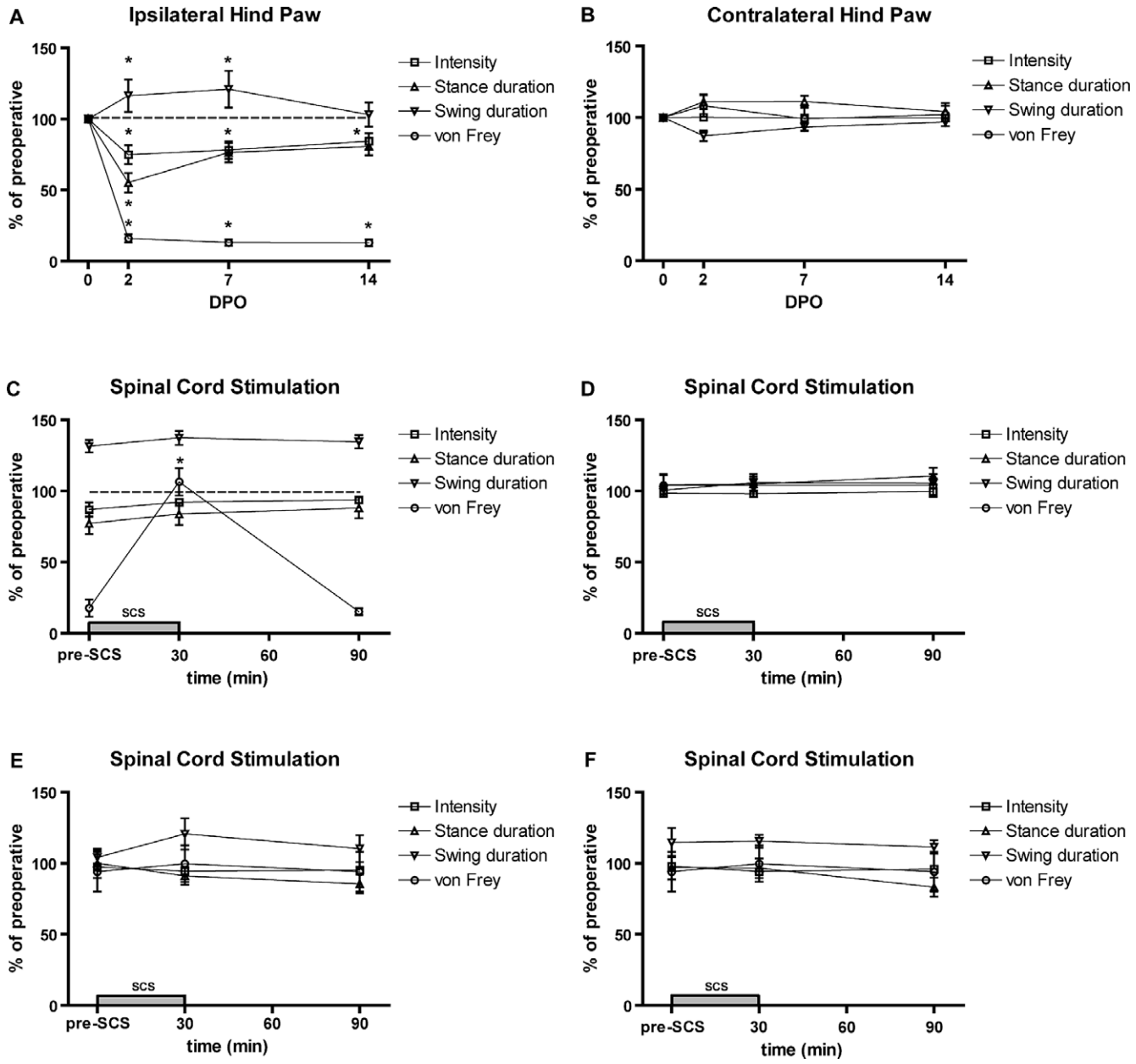


**Fig. 2.** Effect of partial ligation of the left sciatic nerve and SCS on gait using the CatWalk. All data are represented as percent of pre-operative (mean ± SEM). (A) Decrease of the Intensity to 74.92 ± 6.64% at DPO2 compared to baseline. After DPO2, the Intensity started to increase to 84.31 ± 5.71% of baseline at DPO14, but was still different from baseline. Reduction of the Stance duration to 55.14 ± 6.78% at DPO2 compared to baseline. Over time the Stance duration returned to baseline and at DPO14 no significant difference could be observed compared to baseline. A statistically significant increase in the Swing duration could be observed at DPO2 and DPO7, but it returned to baseline levels at DPO14. Withdrawal threshold of the ipsilateral paw decreased to 16.02 ± 2.88% of baseline values and remained unchanged at DPO14 (13.04 ± 2.43%). (B) No effect on Intensity, Stance duration, Swing duration or on responses to von Frey filaments was observed in the contralateral paw. (C) No effect of SCS on Intensity, Stance duration and Swing duration was observed. Thirty minutes of SCS completely returned withdrawal threshold to pre-neuropathy levels. Anti-allodynic effect of SCS lasted until $t = 75$ min. (D) No effect of SCS on the CatWalk parameters of the contralateral paw was noted. (E, F) SCS did not result in any changes in the CatWalk parameters or in withdrawal threshold (von Frey) in the non-ligated control animals ($n = 7$). Statistical significance ($p < 0.05$), compared to baseline is indicated with asterisks (A and B). Statistical significance ($p < 0.05$), compared to that at pre-SCS is indicated with asterisks (C and D).

### 3.5. CatWalk gait analysis after Spinal Cord Stimulation

To study the effect of the implantation procedure, the CatWalk parameters at pre-SCS and DPO14 were compared in the ligated animals. No effect could be detected for the Intensity and Stance duration. However an increase in Swing duration of the ipsilateral paw was noted (103.07 ± 8.45% at DPO14 compared to 131.46 ± 4.37% at pre-SCS, $p = 0.007$). The implantation did not affect the CatWalk parameters of the contralateral paw.

For CatWalk analysis after SCS, 19 animals were included. Three of the 22 animals did not cross the CatWalk appropriately. CatWalk crossing times of these remaining 19 mice at pre-SCS (1.38 ± 0.06 s) did not differ from the crossing times before implantation (1.37 ± 0.26 s, $p > 0.05$).

No effect on all CatWalk parameters studied could be observed after 30 min of SCS, for both the ipsilateral and contralateral hind paws. Intensity, Stance duration and Swing duration showed no changes compared to those at pre-SCS or 60 min after cessation of SCS (Fig. 2C and D).

In the control animals no changes in weight could be observed after the implantation compared to baseline (20.57 ± 0.20 g and 20.80 ± 0.21 g, respectively (95% CI, 0.23, (−0.148, 0.60)). Also the

crossing times did not differ over time (baseline 1.63 ± 0.08 s, pre-SCS 1.48 ± 0.10 s, SCS30 1.45 ± 0.05 s and SCS90 1.36 ± 0.05 s (ANOVA $p > 0.05$) and were not significantly different from those of the Seltzer animals.

Thirty minutes of SCS did not result in any changes in CatWalk parameters ($n = 7$) in either the ipsilateral or the contralateral hind paw (Fig. 2E and F). Even sixty minutes after the termination of SCS no changes in CatWalk parameters could be observed (Fig. 2E and F).

## 4. Discussion

The present study demonstrates the use of SCS as a treatment for mechanical allodynia in a mouse model of chronic neuropathic pain. Complete return to pre-neuropathy levels was observed after 30 min of SCS. The anti-allodynic effect remained up to 60 min after cessation of SCS. We also showed that the severity of mechanical allodynia 16 days after the Seltzer ligation is not related to the therapeutic response to SCS in C57BL/6 mice with chronic neuropathic pain. The CatWalk gait analysis did detect gait changes after the Seltzer ligation, but these changes became less pronounced over time. No changes in gait were detected after 30 min of SCS.

Gait analysis using the CatWalk was initially introduced to study functional deterioration and recovery after spinal cord injury [14,15,21] in rats. But it was also proposed to be a novel method to assess mechanical allodynia in a model of chronic neuropathic pain [39]. Nowadays the CatWalk is also being used in acute inflammatory pain models to detect development of mechanical allodynia [12] and behavior associated with inflammatory ankle-joint pain and therapeutic interventions [1]. In a carrageenan-induced monoarthritis model the effect of the analgesics Morphine and Rofecoxib on CatWalk gait parameters was examined. It was demonstrated that Morphine and Rofecoxib could partially reverse carrageenan-induced changes in CatWalk parameters [1]. On the other hand, Morphine and Ibuprofen were not able to reverse CatWalk changes in a chronic constriction injury model, whereas they were able to inhibit mechanical allodynia when the von Frey test was used [27]. Recently, the use of the CatWalk for assessment of mechanical allodynia in acute inflammatory pain was disputed. It was reported that at 2 weeks after carrageenan injection no changes in the CatWalk parameters Intensity, Stance phase and Swing phase could be detected, whereas mechanical allodynia (as measured with the von Frey test) was still present [11]. Furthermore, a discrepancy was shown between the onset of mechanical allodynia and gait changes after complete sciatic nerve resection [9]. A clear decrease in CatWalk gait parameters was observed immediately after resection of the sciatic nerve, but mechanical allodynia (measured with the von Frey test) was found to develop only after a delay of 2 weeks [9]. On the other hand, both the CatWalk parameter Print Area and von Frey values were decreased when full motor function recovery was observed in rats with sciatic nerve crush (using the De Medinacelli method) [38].

When measuring "chronic neuropathic pain" with the use of the CatWalk, one must appreciate the type of injury used for the development of the chronic pain state. Many different experimental models of peripheral mononeuropathy have been developed in rats, including chronic constriction injury of the sciatic nerve [3], partial ligation of the sciatic nerve [33] and ligation of the L5 root of the sciatic nerve [20]. To our knowledge, the CCI model, sciatic nerve crush model and the carrageenan rat model have been used to assess prolonged changes in gait using the CatWalk [11,38,39]. Because gait is a complex process, involving the function of both motor and sensory axons, damage to either of these can affect gait. Degeneration and axonal loss of all axon types were reported after chronic constriction of the sciatic nerve [2,5,40] and similar pathological changes were reported in the Seltzer type ligation [25]. These results suggested that both motor and sensory axons are equally lost following damage to the sciatic nerve. Therefore the behavioral changes as measured by the CatWalk resulting from damage to the sciatic nerve at mid-thigh level (involving both motor and sensory nerve fibers) are likely to be a compromise between pain and motor-related alterations.

Obviously we were not able to detect any effect of SCS on the CatWalk gait parameters. At the same time the animals did show a profound effect of SCS on the withdrawal threshold as measured with the von Frey filaments. This strongly suggests that, in this model of chronic neuropathic pain, the CatWalk gait analysis does not allow the investigation of the effect of SCS on mechanical allodynia.

An interesting observation is that in C57BL/6 mice a 100% response to SCS was observed and that no relation could be detected between the severity of mechanical allodynia and the effect of SCS.

Previously, it was shown that a subdivision of the Sprague–Dawley rats into 3 groups could be made based on the degree of mechanical allodynia in the Sprague–Dawley rats [34]. The most severely allodynic rats (withdrawal threshold 0.16–1.0 g) did not respond to SCS whereas the mildly allodynic rats (withdrawal threshold 8–25 g) showed a return to pre-injury withdrawal threshold after 30 min of SCS. In our mouse experiment even the most severely allodynic animals, with a reduction in withdrawal threshold to 0.8% of pre-injury values, responded optimally to SCS with a complete return to pre-injury withdrawal threshold. The difference in response to SCS after a peripheral nerve injury may be related to differential heritability of nociception. Compared to other mouse strains, the C57BL/6 is relatively resistant to mechanical allodynia [29,30]. It should be noted that in rats also genetic differences occur in sensitivity to mechanical allodynia [8]. Nevertheless, the findings in the Sprague–Dawley rats on the relation between efficacy of SCS and degree of mechanical allodynia have been shown to be comparable to those in patients with CRPS-I (preliminary results by van Eijs, 2008).

An explanation for the difference in efficacy of SCS between the Sprague–Dawley rats with chronic neuropathic pain and the C57BL/6 mice with chronic neuropathic pain could be of a technical nature. The effect of SCS and the way in which the dorsal column is exited is related to current density, fiber diameter and distance between the nerve fiber and the cathode [16]. The cathode used in mice ($2.25 \times 0.76 \times 0.025$ mm) is smaller than that used in rats ($3.00 \times 1.00 \times 0.025$ mm). With consistent electrode conductivity, current density is inversely proportional to the electrode size. Whereas the contact area of the stimulating electrode decreases for specific current intensity, the current density increases [16]. This increase in current density might result in the stimulation of more fibers located in the dorsal funiculus, eventually resulting in a better anti-allodynic effect in allodynic mice as compared to rats.

One of the proposed mechanisms underlying the effect of SCS in neuropathic pain is the facilitation of the GABAergic system [6]. Administration of a $GABA_b$ agonist (baclofen) potentiates the behavioral response to SCS in rats previously responding poorly to SCS. Loss of GABA and its synthesizing enzyme glutamate decarboxylase (GAD) from the dorsal horn has been reported following nerve injury in rats [4,10,17,31]. In contrast, an initial increase in GABA was detected and no changes in GAD were observed in mice with spared nerve injury [32]. This could be another explanation for the 100% response to SCS in mice.

Furthermore, it has been suggested recently that the anti-allodynic effect of electrical stimulation could be attributed to the down-regulation of NR1 phosphorylation in a rat model of visceral hypersensitivity [36]. The phosphorylation of the NMDA receptor has been suggested as an important component in the development of central sensitization and chronic pain [13,42,43]. One could speculate that SCS in chronic neuropathic pain has a more pronounced effect on the phosphorylation of the NMDA receptor and, if so, is more efficient in C57BL/6 mice than in the Sprague–Dawley rats.

## 5. Conclusions

We have shown that SCS in a mouse model of chronic neuropathic pain completely abolishes mechanical allodynia. Severity of mechanical allodynia is not a prediction for the efficacy of SCS in this mouse model. We have demonstrated that the CatWalk cannot be used in this model of chronic neuropathic pain to assess mechanical allodynia and the effect of SCS on mechanical allodynia.

The present SCS-mouse model opens new avenues for studies on the mechanisms underlying neuromodulation as a therapy in chronic neuropathic pain.

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