

Burst and Tonic Spinal Cord Stimulation in the Mechanical Conflict-Avoidance System: Cognitive-Motivational Aspects

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Background: Clinical research suggests that a novel spinal cord stimulation (SCS) waveform, known as Burst-SCS, specifically targets cognitive-motivational aspects of pain. The objective of the present study was to assess the cognitive-motivational aspects of Tonic- and Burst SCS-induced pain relief, by means of exit latency in the mechanical conflict-avoidance system (MCAS), in a rat model of chronic neuropathic pain.

Methods: Exit latency on the MCAS operant testing setup was evaluated at various probe heights for rats ($n = 26$) with chronic neuropathic pain induced by a partial sciatic nerve ligation (PSNL). Von Frey paw withdrawal analysis was performed to assess mechanical hypersensitivity. In a second experiment ($n = 12$), the behavioral effect of Tonic SCS or biphasic Burst SCS on both Von Frey analysis and MCAS exit latency was assessed.

Results: Burst SCS exit latencies differed significantly from Tonic SCS exit latencies at 4 mm probe height (3.8 vs. 5.8 sec, respectively; $p < 0.01$) and 5 mm probe height (3.2 vs. 5.4 sec respectively; $p < 0.05$). This difference was not detected with reflex-based Von Frey testing (Tonic-SCS vs. Burst-SCS at 30 min stimulation: $p = 0.73$, and at 60 min stimulation; $p = 0.42$).

Conclusions: Testing of MCAS exit latency allows for detection of cognitive-motivational pain relieving aspects induced by either Tonic- or Burst-SCS in treatment of chronic neuropathic rats. Our behavioral findings strongly suggest that Burst-SCS specifically affects, much more than Tonic-SCS, the processing of cognitive-motivational aspects of pain.

Keywords: Chronic neuropathic pain, mechanical conflict-avoidance test, mechanical hypersensitivity, peripheral nerve injury, spinal cord stimulation

Conflict of Interest: Bert Joosten is a member of the advisory committee for Boston Scientific Inc. Bert Joosten received travel fees to attend the advisory committee for Boston Scientific Inc. The remaining authors have no conflicts of interest to report.

INTRODUCTION

The preclinical Spinal Cord Stimulation (SCS) field calls for an operant testing method able to assess cognitive-motivational aspects of pain (1). This is becoming increasingly important now that recent electroencephalography findings suggest that Burst and Tonic SCS may have different supraspinal working mechanisms; Burst SCS is hypothesized to selectively modulate brain areas associated with the processing of attention-related cognitive-motivational aspects of pain (2,3). Meanwhile, the majority of preclinical SCS studies still rely on reflex-mediated Von Frey analysis, a technique unable to assess supraspinal cognitive-motivational aspects of pain (4–12). Recently, an operant testing method was introduced which assesses cognitive and motivational aspects of pain in animals: the mechanical conflict-avoidance system (MCAS) (13). With the MCAS the animal is placed in a brightly lit compartment which leads to a passage with a height-adjustable array of nociceptive probes. The animal needs to cross the nociceptive probes to enter the innately preferred dark area. The “lesser of two evils principle” forces the animal to choose between two opposing motivational drives: to stay in the aversive, yet non-noxious, brightly lit compartment, or, to cross the noxious probes, which is rewarded by the innately preferred dark compartment. In order to resolve this conflict, it is hypothesized that the animal

applies a “cost-benefit” analysis including the level of ongoing pain, the height of the probes, and the averseness of the light (= negative reinforcement) (14). In general, as ongoing pain intensity and/ or probe height increases, animals require more time to exit the light chamber. Latency to exit the light chamber (defined as time from light being turned on to having all four paws on the

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probe bed) has been shown to be a stimulus-dependent measure in the Coy-MCAS system. Chronic neuropathic pain, induced by chronic constriction of the sciatic nerve, has been shown to affect latency to exit the bright compartment in the MCAS (15). However, other neuropathic pain models remain to be validated in the MCAS-setup. Furthermore, it is of great interest to the preclinical SCS field to assess whether the MCAS can shed light on the supraspinal mechanisms of Burst and Tonic SCS by addressing the cognitive-motivational aspects of pain that are becoming increasingly important for the assessment of novel SCS waveforms (3,16).

Our first objective was to assess the effect of the partial sciatic nerve ligation (PSNL) rat model for chronic neuropathic pain on exit latency in the MCAS. As cognitive-motivational aspects cannot be detected by reflex-based Von Frey analysis, our second objective was to assess the cognitive-motivational aspects of Tonic- and Burst SCS-induced pain relief, by means of exit latency in the MCAS operant testing system. We hypothesized that both Tonic- and Burst SCS would reduce MCAS exit latency, which will suggest a role for cognitive-motivational aspects in SCS-induced pain relief. Furthermore, based on literature indicating that Burst SCS selectively modulates brain areas associated with the processing of attention-related cognitive-motivational aspects of pain, we hypothesized that Burst-SCS would have a stronger effect on MCAS exit latency, as compared to Tonic-SCS (2,3).

MATERIALS AND METHODS

Ethics Statement

All 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). The protocol was approved by the Animal Research Committee of the Maastricht University Medical Centre (DEC-protocol 2014-086).

Animals

All experiments were performed using male Sprague Dawley rats ($n = 38$), which were young-adult (5 weeks of age) at the start of the experiment (150–200 g). Animals were housed in groups of 2, in filter-top polycarbonate cages in a climate-controlled vivarium maintained under controlled temperature ($21^{\circ}\text{C} \pm 1^{\circ}\text{C}$), relative humidity ($55\% \pm 15\%$) and artificial lighting (12:12 hour light/dark cycle) with distilled water and rodent chow available ad libitum. The vivarium was equipped with a mobile radio, continuously producing background music at 45 decibels, in order to desensitize the animals for translocation and experimenter-related noise. All procedures were conducted between 09:00 and 16:00 hours.

Partial Sciatic Nerve Ligation

A unilateral ligation of the left sciatic nerve was performed as described by Seltzer et al. 1990 (17), and previously applied in our laboratory (9,10). In short, animals were anesthetized with 3%–5% isoflurane (Abbott Laboratories Ltd., Kent, UK). The left sciatic nerve was exposed by blunt dissection and carefully freed from surrounding connective tissue. For sham-PSNL animals, the sciatic nerve was left unaffected and the wound was closed with a 4/0 silk suture. For PSNL animals, distal to the posterior biceps semitendinosus, but proximal to the little fat pad that lies a few millimeters distal to this site, the sciatic nerve was partially ligated. An 8/0 non-absorbable silk suture was used to ligate approximately 1/3 of the diameter of the left sciatic nerve. After ligation, the wound was closed with a 4/0

silk suture. The presence of mechanical hypersensitivity was considered successful if at 13 days post-surgery, paw withdrawal thresholds (PWTs) to Von Frey stimuli ($^{10}\log [50\%]$) were decreased by 0.2 units compared with baseline (day 0) (9,10).

Assessment of Mechanical Hypersensitivity (von Frey Assay)

Assessment of PWTs was performed according to the standard protocol (7–10). Mechanical hypersensitivity based on PWT was assessed according to the “up-down method” (18). The 50% PWT was calculated after completion of a sequence of six consecutive responses. A cut-off value of 28.84 g was defined. For statistical analysis, 50% PWTs were logarithmically transformed to obtain a linear scale.

Mechanical Conflict-Avoidance System

Familiarization and Training

Familiarization and Training was conducted as described in detail by Harte et al. 2016 (13).

Testing Procedure

Rats underwent room acclimation for 30 min prior to the start of behavioral testing each day. Rats were placed individually, in random order, into the start-compartment with the light turned off and the exit door closed. Animals were acclimated to the dark for 15 sec, before the compartment light was turned on for the duration of the test. Twenty seconds after the light was turned on the exit door was opened. The latency to exit the light compartment was recorded by means of a stopwatch, starting from the time the exit door was opened until all four paws were placed upon the nociceptive probes. If the animal reached the dark compartment, the door was closed, and the rat was returned gently to its home cage after being rewarded with 20 sec of darkness. Failure to exit the light compartment within 20 sec after opening of the exit door was marked as “failed exit,” which resulted in the exit door being closed and the rat being returned to its home cage. Rats that successfully escaped the light compartment but failed to enter the dark compartment after 120 sec, were marked as failed cross, and were returned to their home cage until the next trial. The test procedure was repeated three times (trials) per test session (a minimum of 20 min between trials), with one test session per probe height, per day. It was decided to introduce the different probe heights in a non-randomized ascending order over the six test days (starting with 0.5 mm on test day 1, followed by 1 mm on test day 2, 2 mm on test day 3, 3 mm on test day 4, 4 mm on test day 5, and 5 mm on day 6). All test sessions were video-recorded with an ultra-wide angle glass lens camera. Recordings were started immediately after the animal was placed inside the start-compartment (with the light turned off), and were continued until the animal was returned to its home cage. After finalization of the entire experiment all recordings of exit latency were re-timed with a stopwatch and compared with the manually collected data acquired during the experiment.

Implantation of Spinal Cord Stimulation Device

The implantation of the SCS device was performed according to the standard protocol (4,6,7,10–12,19). In short, the spinal cord was exposed by a midline, lumbar incision, followed by laminectomy at level T13. During the full procedure, the dura was kept intact. A custom-made cylindrical 4-contact lead (0.72 mm diameter; Boston Scientific Neuromodulation, Valencia, CA, USA) was introduced into

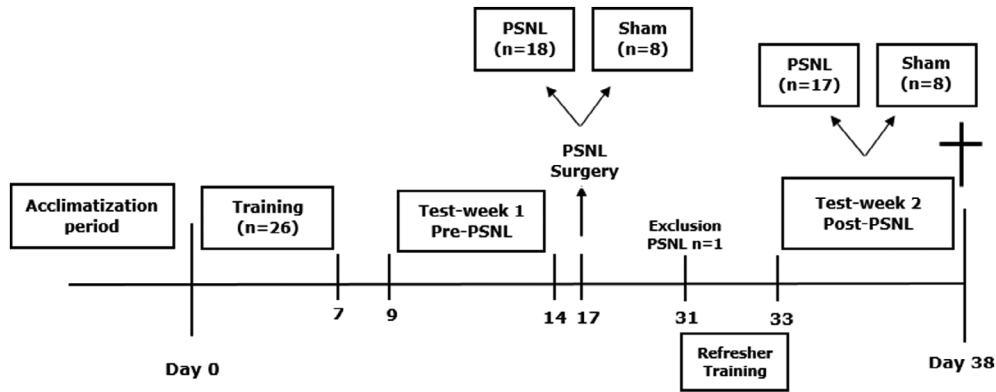


Figure 1. Timeline experiment 1.

the epidural space as previously was performed in Meuwissen et al. (6,7). The electrode was located caudally below the adjacent one or two lamina. Electrode configuration was set at alternating cathode and anode settings (rostral to caudal: + - + -). After implantation of the electrodes, the rats were given 2 days for recovery prior to the initiation of SCS.

Spinal Cord Stimulation

Tonic-SCS was performed according to the protocol described in Meuwissen et al. (6,7). The stimulator was set to deliver constant current biphasic stimulation, with a frequency of 50 Hz and a pulse width of 200 μ S at 66% of the Motor Threshold for Tonic SCS ($n = 5$) (7,10). For biphasic Burst SCS ($n = 5$) the stimulator was set to an interburst-frequency 40 Hz, a pulse width 1000 μ S, and five active biphasic spikes at 449 Hz intraburst frequency at 50% of the Motor Threshold (6,7). Animals were stimulated for 30 min. in the MCAS-set-up, with the light turned off and the door closed. Subsequently, stimulation was continued during the MCAS-testing session, which was performed according to the standard MCAS-testing protocol. The animals were randomized across experimental groups, and the investigator was blinded to the experimental condition during behavioral testing. SCS in the MCAS-system was performed with use of a custom-made experimental apparatus. The cables from the stimulator were guided to a swivel, which allowed 360° free movement. The cable was then further guided to the SCS connectors in the neck of the animal by means of a fender-tension spring system, which generated the appropriate amount of tension in order to prevent any slacking of the cables. The upper cover of the MCAS crossing area was removed to create access for the SCS-system. Removal of the upper cover was performed at the start of the experiment, before the training phase, to prevent distraction of the animal due to removal. Furthermore, animals underwent an additional training period of 3 days with the complete experimental apparatus before start of the SCS experiment, for the animals to become familiarized with the experimental apparatus before testing. To control for the effects of SCS in the MCAS-system, a sham-SCS group was included ($n = 2$).

Timeline of Experiments

After acclimatization to the vivarium, a 2-day familiarization period was initiated, followed by a 5-day training period (13). This was followed by a 2-day rest period, after which the baseline (pre-PSNL) test period was initiated, which consisted of six subsequent days of testing as described above. Subsequently, animals received

either PSNL ($n = 18$) or sham-PSNL surgery ($n = 8$). Following a 14-day observation period, during which animals underwent von Frey behavioral analysis in order to assess whether mechanical hypersensitivity was successfully induced, animals were subjected to a two-day "refresher" training period (PSNL [$n = 17$] and sham-PSNL [$n = 8$]). During these 2 days it was noted whether animals still displayed stable exit behavior. Finally, a 6-day, post-PSNL, testing period was initiated, identical to the baseline test period (PSNL [$n = 17$] and sham-PSNL [$n = 8$]) (Fig. 1). In a second experiment (Fig. 2), 12 animals received PSNL surgery and were subjected to the training- and testing period as described above. This period was followed by the implantation of the SCS-electrode, and a post-implantation (Pre-SCS) test week to assess possible implantation-related effects on the MCAS-outcome. Subsequently, two animals received Sham SCS, and the other animals ($n = 10$) were placed in the MCAS-system after which simultaneous MCAS-SCS testing was performed as described in section 2.7. Five animals received Burst SCS ($n = 5$), while the other five animals received Tonic SCS ($n = 5$).

Data Analysis

The PWTs to von Frey filaments are presented as mean \pm standard error of the mean (SEM). For statistical analysis, von Frey data were logarithmically transformed to obtain a linear scale to account for Weber's Law (20). For the analysis of differences in the withdrawal thresholds between groups, ipsilateral and contralateral PWTs were compared using paired-sampled *t*-tests. To account for skewness of data at higher probe heights MCAS exit latencies were logarithmically transformed. Effects of probe height on exit latencies were analyzed using repeated-measures analysis of variance (ANOVA). Probe height (six levels: 0, 1, 2, 3, 4, and 5 mm) was assigned as within-subjects factor and the experimental group (Sham-PSNL vs. PSNL or Pre-SCS vs. SCS) was assigned as between-subjects factor. If the assumption of sphericity was violated, the Greenhouse-Geisser correction was used to correct the degrees of freedom in subsequent univariate analyses. Multivariate analyses were used to test for differences between groups at specific probe heights. To assess within-group differences for the Sham-PSNL versus PSNL group, pre- versus post-surgery, and the Pre-SCS versus SCS group, paired-samples *t*-tests were performed. Furthermore, bivariate correlation between bodyweights, von Frey data, and MCAS exit-latencies were performed to identify possible causalities between the different outcome measures. All statistical analyses were performed with $\alpha = 0.05$ using IBM SPSS statistics 23.

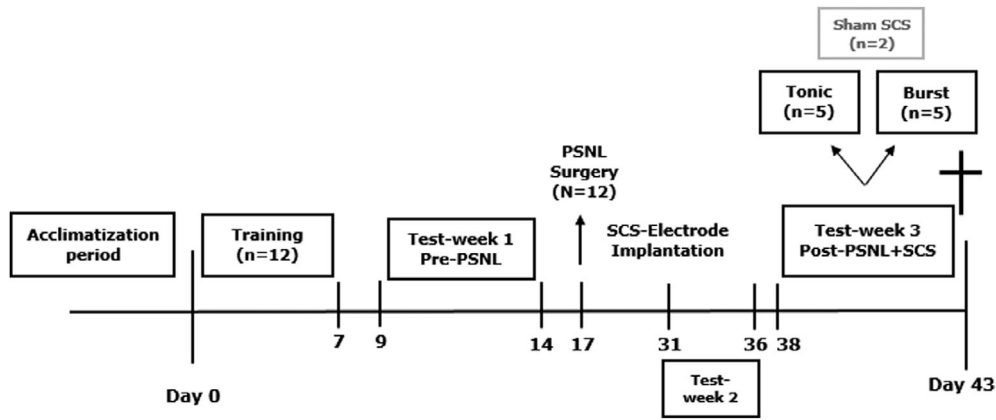


Figure 2. Timeline experiment 2.

RESULTS

Development of Mechanical Hypersensitivity (von Frey) in Chronic Neuropathic Rats

Pre-nerve injury, PWTs of the ipsilateral hind paws were comparable with the PWTs of the contralateral hind paws (ipsilateral 11.6 ± 1.5 g vs. contralateral 11.1 ± 1.3 g) in all animals (at day 16) in experiment 1 (Fig. 3a), and in experiment 2 (ipsilateral 10.9 ± 1.1 g vs. contralateral 11.4 ± 1.5 g) (Fig. 3b). Out of the 18 animals that received a unilateral PSNL in experiment 1, one animal did not develop mechanical hypersensitivity, and was excluded from the study (pre- and post-surgery). The remaining 17 animals qualified as hypersensitive to mechanical stimulation by von Frey filaments (ipsilateral average PWTs: 11.6 ± 1.5 g [pre-lesion] versus 1.6 ± 0.9 g [post-lesion]; $p = 0.0057$) (see Methods) and were selected for the PSNL-group (Fig. 3a). The ipsilateral hindpaw PWTs of the PSNL-group were significantly lower than the ipsilateral hindpaw PWTs of the sham-operated animals ($p = 0.012$). In the Sham-PSNL group, no significant difference between ipsilateral and contralateral PWTs was observed ($p = 0.27$), following the Sham-PSNL surgery, therefore, all eight animals were selected for the Sham-PSNL group. Of the 12 PSNL-animals in experiment 2, all 12 animals were qualified as hypersensitive due to increased response to mechanical stimulation by von Frey filaments (ipsilateral

average PWTs: 10.9 ± 1.1 g [pre-lesion] versus 1.3 ± 1.6 g [post-lesion]; $p = 0.0042$) (see Methods) and were selected for the SCS-group (Tonic or Burst SCS) (Fig. 3b).

Effect of SCS: von Frey Analysis

In animals of the SCS-group paw withdrawal thresholds (PWTs) of von Frey filaments was assessed before the start of SCS treatment (Tonic or Burst SCS) and at 30 and 60 mins after stimulation was turned on. No significant differences were observed in ipsilateral paw PWTs at baseline, pre-SCS, between groups ($p = 0.54$; Tonic-SCS 1.2 ± 0.5 g [n = 5] vs. Burst-SCS 1.3 ± 0.4 g [n = 5]). After 30 min of stimulation, PWTs of both the Burst-SCS group ($p = 0.0021$; 1.3 ± 0.4 g vs. 9.1 ± 1.1 g) and Tonic-SCS group ($p = 0.028$; 1.2 ± 0.5 g vs. 9.5 ± 1.5 g) significantly differed from baseline PWTs (Fig. 4). After 60 min of stimulation, PWTs of both the Burst-SCS group ($p = 0.0016$; 1.3 ± 0.4 g vs. 10.4 ± 1.3 g) and Tonic-SCS group ($p = 0.034$; 1.2 ± 0.5 g vs. 9.1 ± 1.4 g) significantly differed from baseline PWTs (Fig. 4). PWTs of the Burst-SCS group and Tonic-SCS group did not significantly differ at 30 min of stimulation ($p = 0.73$; Tonic-SCS 9.5 ± 1.5 g [n = 5] vs. Burst-SCS 9.1 ± 1.1 g [n = 5]) and 60 min of stimulation ($p = 0.42$; Tonic-SCS 9.1 ± 1.4 g [n = 5] vs. Burst-SCS 10.4 ± 1.3 g [n = 5]) (Fig. 4).

Development of Tactile Hypersensitivity

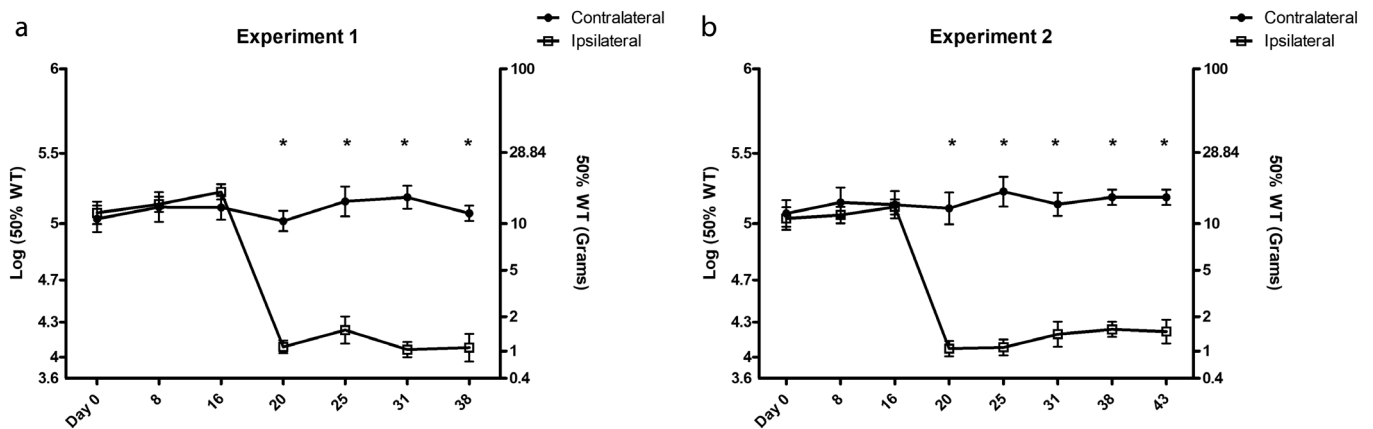


Figure 3. a. The effect of a Partial Sciatic Nerve Ligation (PSNL) (n = 17) on PWTs in experiment 1. After PSNL, the ipsilateral PWTs of the PSNL-group were significantly decreased at day 20, 25, 31, and 38, compared to the contralateral PWTs. b. The effect of a PSNL (n = 12) on PWTs in experiment 2. After PSNL, the ipsilateral PWTs were significantly decreased at day 20, 25, 31, 38, and 43 compared to contralateral PWTs.

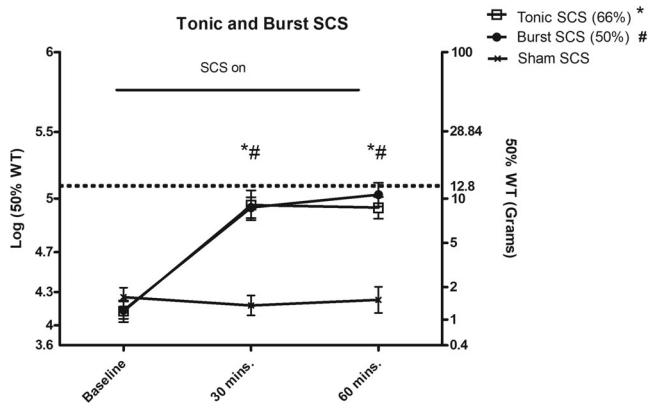


Figure 4. The effect of Tonic-SCS ($n = 5$) and Burst-SCS ($n = 5$) on PWTs based on sensitivity to von Frey filaments. PWTs were assessed at baseline (stimulation off) and 30 and 60 mins of SCS. The dotted line represents the average PWT baseline prior to sciatic nerve ligation (* $p < 0.05$ for Tonic SCS compared with pre-SCS baseline PWTs, # $p < 0.05$ for Burst SCS compared with pre-SCS baseline PWTs).

Mechanical Conflict-Avoidance Test in Chronic Neuropathic Rats

Exit Latency

Stimulus–response functions were obtained for PNL ($n = 17$) and sham-PNL rats ($n = 8$) in the MCAS both at pre-surgery (days 9–14) and at post-surgery (days 33–38). **Pre-surgery:** a significant main effect of probe height ($F_{3,264} = 21.971$; $p < 0.001$), but no significant interaction between probe height and group was found, suggesting that both PNL and Sham-PNL animals exhibited equal increase in exit latency as a function of probe height. **Post-surgery:** a significant main effect of probe height was noted ($F_{3,089} = 19.722$; $p < 0.001$) (Fig. 5). No significant interaction effect between probe height and group was observed. Tests of Between-subject effects approached significance ($F_1 = 3.427$; $p = 0.077$), suggesting that exit latencies were different for PNL- and sham-PNL animals, post-surgery. Furthermore, at 4 mm probe height, the exit latencies of the PNL group and sham-PNL group significantly differed (18.84 vs. 10.62 sec; $p = 0.038$), whereas exit latencies of the PNL group and sham-PNL group at 3 mm probe height closely approached significance (9.64 vs. 5.5 sec; $p = 0.053$).

Effect of SCS: MCAS Exit Latency

Post-PNL and Pre-SCS MCAS exit latencies did not significantly differ ($F_1 = 0.2717$; $p = 0.6069$). Thus, the implantation of the electrode itself did not have a significant effect on MCAS exit latency. Group differences in MCAS exit latency were assessed for the Pre-Tonic SCS group (the Tonic SCS group pre-stimulation) and Tonic SCS group ($n = 5$); a significant main effect of probe height ($F_5 = 21.92$; $p < 0.0001$), and a significant interaction between probe height and group ($F_5 = 3.255$; $p = 0.0147$) was noted (Fig. 6), which indicates that MCAS exit latencies were different pre-Tonic SCS and during Tonic SCS. This was confirmed by a significant between subjects effect, where the pre-Tonic SCS group and Tonic SCS group significantly differed overall in exit latencies ($F_1 = 22.42$; $p = 0.0015$). Furthermore, Pre-Tonic SCS and Tonic SCS groups significantly differed at 2 mm probe height (6.2 vs. 3.0 sec; $p < 0.05$), 3 mm probe height (7.8 vs. 3.4 sec; $p < 0.001$), and 4 mm probe height (10.0 vs. 5.8 sec; $p < 0.01$).

Group differences in MCAS exit latency were also assessed for the pre-Burst SCS (the Burst SCS group pre-stimulation) and Burst SCS group ($n = 5$); a significant main effect of probe height ($F_5 = 13.78$;

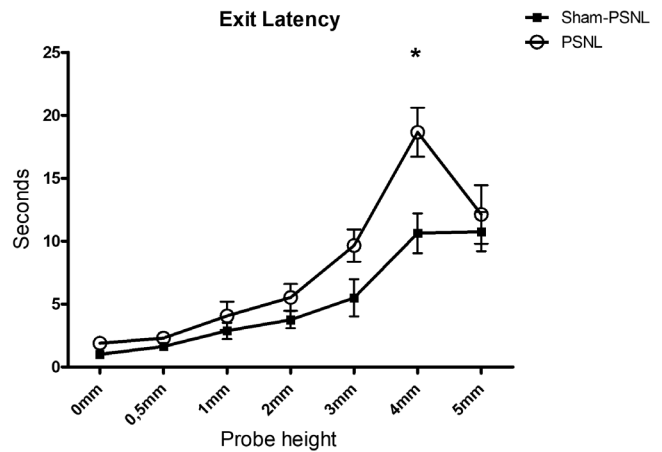


Figure 5. Stimulus response relationship of exit latency as a function of probe height between, PNL-rats ($n = 17$), and sham-PNL rats ($n = 8$). As probe height increases, exit latency also increases in both experimental groups ($p < 0.001$). Exit latencies of PNL rats were significantly greater than those of sham-PNL rats at 4 mm probe height (18.84 vs. 10.62 s; $p = 0.038$).

$p < 0.0001$), and a significant interaction between probe height and group ($F_5 = 7.797$; $p = 0.0001$) was noted (Fig. 7), which indicates that MCAS exit latencies differed between pre-Burst SCS and during Burst SCS. This was confirmed by a significant between subjects effect, where the pre-Burst SCS group and Burst SCS group significantly differed overall in exit latencies ($F_1 = 31.49$; $p = 0.0005$). Furthermore, pre-Burst SCS and Burst SCS groups significantly differed at 2 mm probe height (7.2 vs. 2.4 sec; $p < 0.01$), 3 mm probe height (9.0 vs. 2.8 sec; $p < 0.001$), 4 mm probe height (9.6 vs. 3.8; $p < 0.001$), and 5 mm probe height (10.8 vs. 3.2 sec; $p < 0.001$).

Area between the curve (ABC) analysis revealed a significant difference between the ABC for Pre-Burst and Burst and the ABC for Pre-Tonic and Tonic SCS (24.6 vs. 15.4 sec respectively; $p = 0.0225$) (Fig. 8). Furthermore, post-hoc analysis revealed a significant difference between the Tonic-SCS group and the Burst-SCS group at 4 mm probe height (5.8 vs. 3.8 sec respectively; $p < 0.01$) and 5 mm probe height (5.4 vs. 3.2 sec, respectively; $p < 0.05$) (Figs. 6 and 7). No significant differences were observed between the Pre-Sham-SCS group and the Sham-SCS group ($F_1 = 63.02$; $p = 0.481$; $n = 2$).

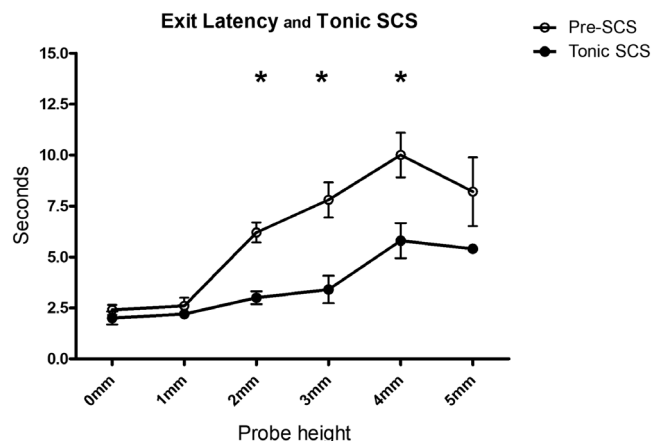


Figure 6. Stimulus response relationship of exit latency as a function of probe height between Pre-SCS group and Tonic SCS group ($n = 5$). Exit latencies of SCS rats were significantly lower than those of Pre-SCS group rats at 2 mm probe height (6.2 vs. 3.0 sec; $p < 0.05$), 3 mm probe height (7.8 vs. 3.4 sec; $p < 0.001$), 4 mm probe height (10.0 vs. 5.8 sec; $p < 0.01$), and 5 mm probe height (8.2 vs. 5.0 sec; $p < 0.05$).

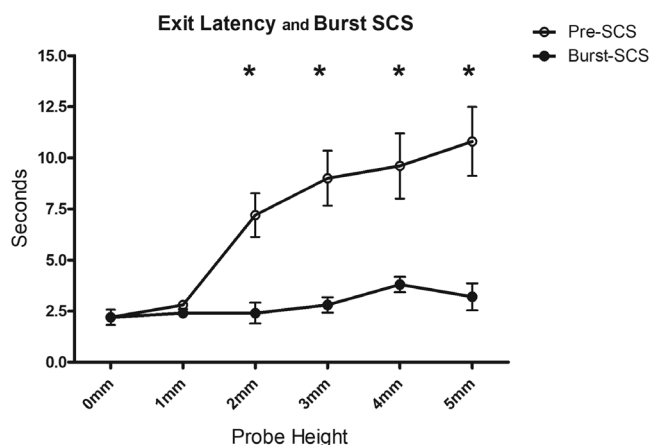


Figure 7. Stimulus response relationship of exit latency as a function of probe height between Pre-SCS group and Burst SCS group ($n = 5$). Exit latencies of SCS rats were significantly lower than those of Pre-SCS group rats at 2 mm probe height (7.2 vs. 2.4 sec; $p < 0.01$), 3 mm probe height (9.0 vs. 2.8 sec; $p < .001$), 4 mm probe height (9.6 vs. 3.8 sec; $p < 0.001$), and 5 mm probe height (10.8 vs. 3.2 sec; $p < 0.001$).

DISCUSSION

Our first objective was to assess the effect of the partial sciatic nerve ligation (PSNL) rat model for chronic neuropathic pain on exit latency in the MCAS. A significant difference at 4 mm probe height between exit latencies of neuropathic animals and sham-animals, demonstrates that the MCAS-setup is a valid operant testing method for the assessment of affective-motivational aspects of pain in neuropathic PSNL-rats. The second objective was to assess the cognitive-motivational aspects of Tonic- and Burst SCS-induced pain relief, by means of exit latency in the MCAS. This revealed significant differences in cognitive-motivational behavior for Burst-SCS and Tonic-SCS, and these differences could not be detected by reflex-based von Frey testing. Burst-SCS furthermore seems to specifically modulate, much more than Tonic-SCS, cognitive-motivational aspects of pain behavior.

MCAS Exit Latency

In this study, it was demonstrated how latency to exit from the light compartment in the MCAS was significantly increased in chronic neuropathic PSNL-rats, indicating a stimulus–response relationship. At 4 mm probe height PSNL-animals required significantly more time to exit the light compartment, compared with sham animals. In this respect, use of 4 mm probe height provides the most optimal window, allowing for discrimination between injured versus sham control animals and thus, for assessment of peripheral neuropathic pain. These findings are in line with those reported by Harte et al. (2016): here a similar significant difference in exit latency at 4 mm probe height was noted between naïve animals and CCI-animals (13). At 5 mm probe height, we observed a sharp drop in exit latency for PSNL-animals. Unfortunately, in the study performed by Harte et al. 2016, results from 5 mm probe height were not presented. Therefore, we were unable to compare data at 5 mm probe height with previous literature. Since we presented the probe heights in incremental order there is the possibility that the PSNL-animals went through a learning curve. Hence, at the fifth probe height, the “cost-benefit” analysis including the level of ongoing pain, the height of the probes, and the averseness of the light might have been overruled by a primal

instinct to carry through with the oncoming painful stimulus as quickly as possible. Another important difference between our findings and the findings of Harte and colleagues is that the animals in our study exhibit overall lower exit latencies. This could be related to the fact that Harte and colleagues performed their experiments in the inactive (light) phase of the rodent circadian rhythm, as compared to our experiments, which were performed during the active (dark) phase (21).

In 2008, Vierck and colleagues already addressed the relevance of operant testing, after reviewing several dissociations between reflex-based testing methods and operant testing methods. Interestingly, after closer examination of the aforementioned dissociations they discovered that operant testing more often resulted in a clinically concordant outcome (1,22). It is furthermore known that various experimental manipulations do not affect reflex and escape responses in a similar manner, including but not limited to, morphine, naloxone, stress, subcutaneous formalin injection, cutaneous application of mustard oil, and chronic constrictive nerve injury (22–27). Therefore, although reflex-based testing has contributed in many ways to the preclinical SCS field, it has become apparent that analysis of cognitive-motivational aspects of pain needs to be included. Thus, the MCAS may complement current behavioral testing methods (13). In the MCAS, for exit latency, the animal is forced to choose between two opposing motivational drives: 1) escape an aversive, yet non-noxious setting (light compartment) by subjecting itself to noxious stimuli (the nociceptive probes), or 2) avoid the noxious probes but remain in the aversive bright light compartment (13). This process requires a cost-benefit analysis, which requires input from cortical areas that process objective aspects of pain. In line with this, we made several observations of animals investigating the nociceptive probes with their forelimbs right before the exit process in the MCAS testing system. Thus, the fact that MCAS exit latency testing may recruit ascending pathways creates an opportunity to assess the involvement of supraspinal elements, for instance, as suggested with Tonic-SCS and in particular with Burst-SCS. From a broader perspective, including operant testing methods in preclinical assessment batteries could provide a valid measurement for the cognitive-motivational aspects of pain, which in its turn could prove to be an answer to the lack of translational progress in the pain field (1).

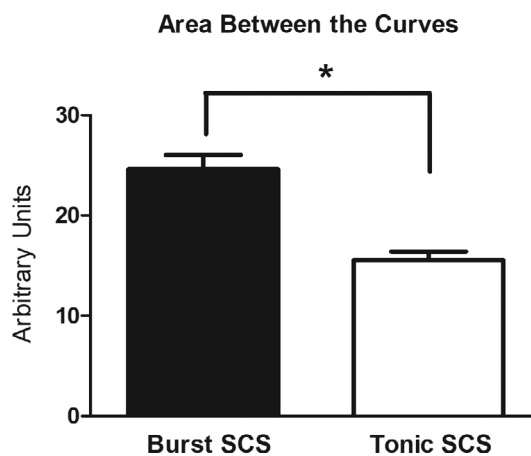


Figure 8. Area between the curves (ABC) for exit latency for the pre-tonic SCS group and tonic SCS group ($n = 5$) and for the pre-burst SCS group and burst SCS group ($n = 5$). ABC analysis revealed a significant difference between the ABC for pre-burst and burst and the ABC for pre-tonic and tonic SCS (24.6 vs. 15.4, respectively; $p = 0.0225$).

MCAS Exit Latency and SCS

When Tonic and Burst SCS were administered 30 min prior, and during MCAS-testing, a significant overall decrease in exit latency was observed for both Tonic and Burst SCS. Furthermore, at 4 mm probe height and 5 mm probe height the Tonic SCS group and the Burst SCS group significantly differed. The latter seems to suggest that Burst SCS has a more profound cognitive-motivational effect when the severity of the nociceptive stimulus is increased. Animals that received sham SCS showed no variations in exit latencies. In the clinical setting, questionnaires regarding quality of life and patients' preference tend to lean towards Burst SCS, as compared to other SCS-waveforms (28–30). However, these days, there is still no clear consensus in literature, neither preclinical (4,6,7) nor clinical (2,3,28–37), regarding the objective analgesic efficacy of Burst SCS (as measured on the VAS-scale), as compared to other SCS-waveforms. A recent clinical study by Kriek and colleagues has shown that the preferred stimulation setting is not solely driven by the amount of pain reduction, but is also influenced by which stimulation setting feels most comfortable and provides the best user-friendliness (35). Therefore, it is pivotal that we aim to elucidate the (supraspinal) mechanisms responsible for the level of comfort, the sensation that accompanies SCS-waveforms. As the MCAS can provide critical insight into cognitive-motivational processing of SCS, it could serve as a preclinical tool that allows for the optimization of supraspinal mechanisms of Burst-SCS. It is important to note that the difference between Tonic SCS and Burst SCS, as observed for the exit latencies, was not present in the reflex-based Von Frey analysis. This suggests that the MCAS allows for assessment of behavioral changes in pain that are not detected by reflex-based testing. Interestingly, this is in line with clinical observations that show a similar effect for Burst SCS and Tonic SCS on objective measures such as the VAS-scale (no clinically relevant difference) (30). Yet, a subset of patients expresses a preference for Burst SCS, over Tonic SCS, due to combination of improved psychological (cognitive-motivational) pain-aspects and the absence of unpleasant perceptions such as paresthesia (3,28,30,31,33–35,37). The supraspinal properties of Burst SCS were first discovered in 2013 when it was reported that Burst SCS could decrease the amount of attention patients paid to pain, in a statistically significant way, while Tonic SCS did not show a similar decrease (3). This was in 2016 corroborated by a source-localized EEG-study that demonstrated how Burst SCS was able to normalize the "pain supporting-suppressing balance" by having a greater effect on the dorsal anterior cingulate cortex, as compared to Tonic SCS (2). From the aforementioned studies it was hypothesized that Burst SCS had a specific supraspinal effect on areas associated with the medial pain system, a system associated with the affective components of the pain experience (3,38,39). Thus, it seems that Burst-SCS not only modulates the unpleasant sensory aspects of pain, but also affects the cognitive-motivational, mostly supraspinal, aspects of pain. Therefore, it is important that we now have a tool, the MCAS, which allows us to adequately assess these supraspinal aspects in a preclinical setting. A better understanding of the supraspinal aspects will allow further analyzing and optimizing of the emotional/motivational properties of the Burst-SCS-protocol. In conclusion, combined use of reflex-based von Frey analysis and the MCAS operant testing method provides us with the opportunity to work toward an optimal balance between sensory aspects and cognitive-motivational aspects of SCS-induced pain relief (40).

Limitations

Only male rats were used in order to prevent a potential bias associated with sex-related differences in neuropathic pain development

in rats (41), and to avoid a potential bias related to ovarian sex steroid-induced anti-nociception in female rats (42). Second, in contrast to Harte et al. 2016, we chose to present the various probe heights in incremental order, as previous studies have shown that initial exposure to extreme stimuli can result in large data variability, which can have a deterrent effect (43).

CONCLUSION

We conclude that the MCAS is a valid and reproducible method for the assessment of SCS-induced cognitive-motivational behavioral aspects of pain relief. Use of the MCAS operant testing method revealed significant differences in cognitive-motivational behavior for Burst SCS and Tonic SCS, and this difference could not be detected with reflex-based Von Frey testing. Our behavioral findings strongly suggest that Burst-SCS specifically affects, much more than Tonic-SCS, the processing of cognitive-motivational aspects of pain.

Authorship Statement

Koen P.V. Meuwissen performed the experiments, analyzed the data, and wrote the manuscript. Elbert A. J. Joosten edited the manuscript. Elbert A. J. Joosten and Maarten van Beek contributed to the design of the experiment.

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COMMENT

The Maastricht University group under Professor Joosten's leadership have shown that the Mechanical Conflict-Avoidance System is suitable behavioral system to demonstrate SCS induced cognitive-motivational aspects of pain relief. In this most elegant of behavioral pre-clinical work they have demonstrated significant differences in cognitive-motivational behaviors for tonic SCS versus Burst SCS that were not detected by vonFrey reflex paw withdrawal thresholds. This work strongly supports the theory that burst SCS affects pain perception pathways differently to that of tonic SCS. We are now in an era of new SCS waveform research and we have another pre-clinical tool with which to reveal their effects. I congratulate the team for their convincing research in this field.

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Comments not included in the Early View version of this paper.