

# Nonlinear Relation Between Burst Dorsal Root Ganglion Stimulation Amplitude and Behavioral Outcome in an Experimental Model of Painful Diabetic Peripheral Neuropathy

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**Background and objective:** Dorsal root ganglion stimulation (DRGS) has recently emerged as a neuromodulation modality in the treatment of chronic neuropathic pain. The objective of this study was to compare the efficacy of different Burst-DRGS amplitudes in an experimental model of painful diabetic peripheral neuropathy (PDPN).

**Methods:** Diabetes mellitus was induced in female Sprague–Dawley rats by intraperitoneal injection of streptozotocin (STZ,  $n = 28$ ). Animals were tested for mechanical hypersensitivity (von Frey paw withdrawal test) before, and four weeks after STZ injection. PDPN rats ( $n = 13$ ) were implanted with a unilateral bipolar electrode at the L5 DRG. Animals received Burst-DRGS at 0%, 10%, 33%, 50%, 66%, and 80% of motor threshold (MT) in a randomized crossover design on post-implantation days 2–7 ( $n = 9$ ). Mechanical hypersensitivity was assessed before stimulation onset, 15 and 30 min during stimulation, and 15 and 30 min after stimulation.

**Results:** Burst-DRGS at amplitudes of 33%, 50%, 66%, and 80% MT resulted in significant attenuation of STZ-induced mechanical hypersensitivity at 15 and 30 min during stimulation, as well as 15 min after cessation of stimulation. No effect on mechanical hypersensitivity was observed for Burst-DRGS at 0% MT and 10% MT. Optimal pain relief and highest responder rates were achieved with Burst-DRGS at 50–66% MT, with an estimated optimum at 52% MT.

**Conclusion:** Our findings indicate a nonlinear relationship between Burst-DRGS amplitude and behavioral outcome, with an estimated optimal amplitude of 52% MT. Further optimization and analysis of DRGS driven by insights into the underlying mechanisms related to the various stimulation paradigms is warranted.

**Keywords:** burst stimulation, dorsal root ganglion stimulation, neuromodulation, neuropathic pain, painful diabetic peripheral neuropathy, Von Frey

**Conflict of Interest:** Elbert A. J. Joosten is a consultant for Abbott Inc. Glenn Franken and Jacques Debets have no conflicts of interest to disclose.

## INTRODUCTION

Painful diabetic peripheral neuropathy (PDPN) is a frequent and disabling complication of diabetes mellitus (DM) (1–3). In PDPN, small fibers of the A<sub>δ</sub> and C type are damaged, leading to subsequent neuropathic pain in extremities and often starting in the lower limbs (4). As the effectiveness of pharmacological interventions in PDPN is limited (5), there is an urgent need for new non-pharmacological approaches. Spinal cord stimulation of the dorsal columns with conventional settings (Con-SCS) is an established last-resort treatment for PDPN patients who are refractory to pharmacological interventions. The effectiveness of Con-SCS in patients with PDPN has been demonstrated in two randomized clinical trials (RCTs) (6,7). However, despite considerable improvements with Con-SCS, Con-SCS has limitations with regard to effect size, responder rate, specificity, stability, and energy consumption (6–11). Recently, dorsal root ganglion stimulation (DRGS) was developed and expected to overcome some of the limitations observed with Con-SCS.

Since the implantation of the first fully implantable DRGS system in 2011 (9), DRGS has shown clinical success in groin pain,

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axial back pain (12), leg and foot pain,(9) complex regional pain syndrome (CRPS) (13), chest wall pain(14) and postamputation pain syndromes (15). Interestingly, a recent study by Eldabe and colleagues showed DRGS to be an effective and safe neuromodulation technique to improve painful symptoms in PDPN patients (16). However, despite considerable improvements in terms of pain scores using DRGS, many PDPN patients still experience unsatisfactory pain relief (16).

Besides changes in the anatomical target of stimulation, the introduction of novel stimulation waveforms has also aided to the field of spinal cord stimulation (SCS) in recent years. SCS paradigms that use bursting patterns (17) offer a paresthesia-free solution to conventional Con-SCS, and decrease pain intensity to a greater degree than Con-SCS in some studies (18,19). Nevertheless, only few studies have investigated the combination of both novel anatomical targeting such as DRGS, and the use of novel stimulation waveforms, such as burst stimulation.

In a previous experimental study, we showed that Burst-DRGS in a rat model of PDPN showed signs of a residual effect after cessation of stimulation, which was accompanied with higher responder rates when compared to conventional DRGS (Con-DRGS) (20). Although the results of this study already seem to favor Burst-DRGS over Con-DRGS, there is still room for improvement in terms of the optimal stimulation settings of Burst-DRGS. Previous experimental electrophysiological and behavioral studies suggest that Burst-SCS can be optimized by changing stimulation settings that are related to the amount of energy delivered to the system, such as amplitude and pulse width (21–23). This study aims to investigate the effect of stimulation intensity on the therapeutic efficacy or pain relief of Burst-DRGS in an experimental rat model of PDPN.

## METHODS

### Ethical 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 study was approved by the Central Authority for Scientific Procedures on Animals, The Netherlands (project license 2017-022).

### Animals

Experiments were conducted in female Sprague–Dawley rats (170–210 g at study onset;  $n = 28$ ). Animals were housed in pairs in polycarbonate cages placed in a controlled environment (temperature  $21 \pm 1^\circ\text{C}$ , humidity  $55 \pm 15\%$ ) in a reversed day/night cycle. Animals had ad libitum access to distilled water and food. Before onset of the experiments, the animals were habituated to the housing facility for one week without experimenter contact.

### Induction of DM

DM was induced by intraperitoneal injection of Streptozotocin (STZ; Sigma-Aldrich, Schnellendorf, Germany). Animals were weighed and fasted overnight, after which STZ was freshly dissolved in 0.9% NaCl to achieve a solution of 65 mg/mL. 65 mg/kg STZ was then injected in order to induce DM. One week following STZ injection, animals were tested for blood glucose levels via blood derived from the saphenous vein (Accu-Chek Aviva<sup>®</sup>, Roche Diagnostics GmbH, Mannheim, Germany). Rats with a blood glucose level of  $\geq 15$  mmol/L were considered diabetic (24). In case of

excessively high glucose values ( $>31.4$  mmol/L), one-third of a slow releasing insulin pellet (LinShin Canada, Inc.) was placed subcutaneously in the trunk of the animal.

### Assessment of Mechanical Hypersensitivity

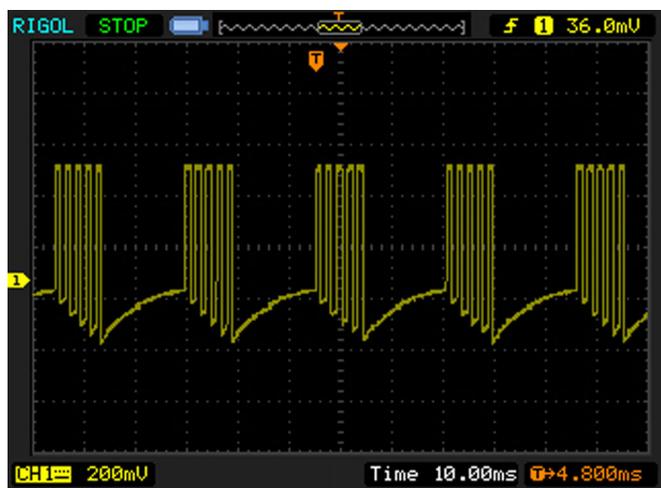
Mechanical hypersensitivity was assessed by determining the paw withdrawal threshold of the hind paw of the animals to calibrated Von Frey filaments. In short, animals were placed on an elevated mesh floor in a transparent box. Animals were allowed to acclimatize to the testing environment for 15 min. A series of calibrated Von Frey filaments (0.6, 1.2, 2.0, 3.6, 5.5, 8.5, 15.1, and 28.84 g) were then applied to the plantar surface of the hind paw for 5 sec using the “up-down” method (25). The 50% withdrawal threshold (WT) was calculated after completion of a sequence of six consecutive responses. A cutoff value of 28.84 g was defined to prevent tissue damage. Lastly, the 50% WT was multiplied by 10,000 and logarithmically transformed to account for Weber's law and obtain a linear scale (26).

Only animals displaying mechanical hypersensitivity at four weeks after STZ injection were implanted and treated with DRGS. Animals without mechanical hypersensitivity were excluded from the study. The presence of mechanical hypersensitivity was defined as a decrease of  $\geq 0.2$  unit in  $\log_{10}$  (10,000  $\times$  50% WT) when compared to pre-STZ values (27–29).

### DRGS

For DRGS, the lead fashioned out of two platinum-iridium wires (diameter 0.010 and 0.005 in.) custom made for experimental studies, was unilaterally implanted adjacent to the left L5 DRG as previously described (20,30). Via paravertebral incision the intervertebral foramen was exposed at the level of the L5 spinal nerve. The entry of the lead into the foramen was ensured by a blunt nerve hook gently probing inside the foramen. Then the lead was inserted into the foramen and secured to the transverse process of L6 using a small stainless steel wire and screw (diameter 0.86 mm, length 3.2 mm). Lastly, the lead was subcutaneously tunneled through the neck of the animal, after which the wounds were closed in layers. Animals were allowed to recover for two days before starting the stimulation protocol.

Animals received Burst-DRGS using an Abbott Inc. Proclaim implantable pulse generator (IPG) at 0%, 10%, 33%, 50%, 66%, and 80% of motor threshold (MT) at days 2–7 following implantation (one amplitude each day) using a randomized crossover design. Randomization was performed by an independent researcher using the website randomize.org. The experimenter was blinded for the applied amplitude. On stimulation days, animals were externally connected to the IPG and tested for MT after which the amplitude was set accordingly for each animal. The MT was determined using an interburst frequency of 10 Hz, intraburst frequency of 500 Hz, pulse width of 1000  $\mu\text{sec}$ , interpulse interval of 1000  $\mu\text{sec}$ , and a burst pulse count of 5. MT was defined as the current inducing contractions of the lower trunk or hind limb. Animals with an MT  $\geq 1$  mA were excluded from analysis. Settings for Burst-DRGS were as follows: monophasic stimulation with interburst frequency = 40 Hz, intraburst frequency = 500 Hz, pulse width = 1000  $\mu\text{sec}$ , interpulse interval = 1000  $\mu\text{sec}$ , burst pulse count = 5(17–19) (Fig. 1). Animals were not restrained during Burst-DRGS.



**Figure 1.** Oscilloscope recording of Burst-DRGS waveform. Settings for Burst-DRGS were as follows: monophasic stimulation with interburst frequency = 40 Hz, intraburst frequency = 500 Hz, pulse width = 1000  $\mu$ sec, interpulse interval = 1000  $\mu$ sec, burst pulse count = 5. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

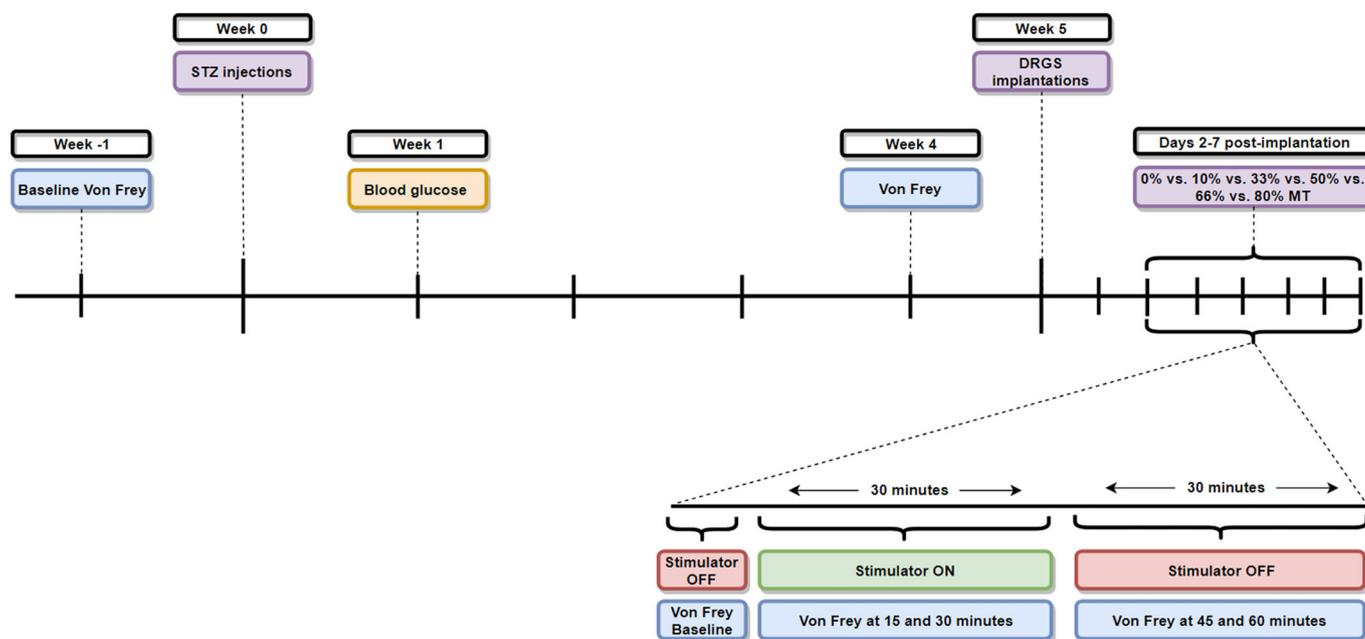
### Timeline of Experiments

Animals were tested for mechanical hypersensitivity (Von Frey) at baseline (week -1), after which animals were injected with STZ at week 0. Blood was taken from the animals at week 1 to confirm induction of DM (defined as blood glucose level  $\geq 15$  mmol/L<sup>24</sup>). Animals were again tested for mechanical hypersensitivity (Von Frey) at week 4, in order to select animals that developed PDPN (defined as  $\geq 0.2$  decrease in  $\log_{10}$  (10,000  $\times$  50% WT) on Von Frey test when compared to pre-STZ baseline<sup>(27–29)</sup>). Animals that developed PDPN were then implanted with a DRGS lead at week 5, and stimulated on days 2–7 following implantation using different amplitudes (0%, 10%, 33%, 50%, 66%, and 80% MT). Animals

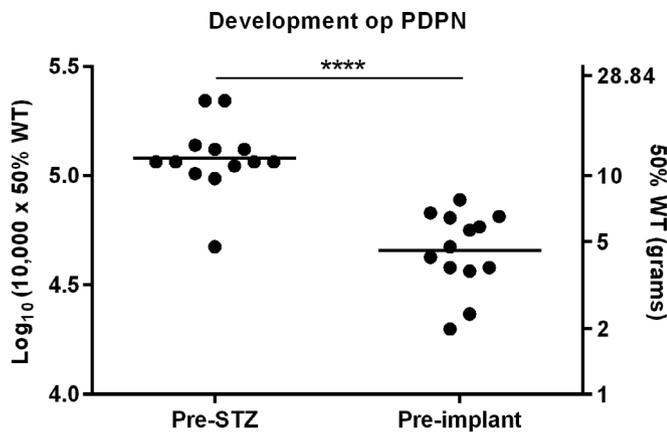
were each day tested for mechanical hypersensitivity on Von Frey just before Burst-DRGS onset (baseline), 15 and 30 min during Burst-DRGS, and 15 and 30 min after Burst-DRGS (45 and 60 min) (Fig. 2).

### Data Analysis

Values are presented as mean  $\pm$  standard error of the mean (SEM). For statistical analysis, Von Frey data were logarithmically transformed to account for Weber's Law and obtain a linear scale (26). For analysis of the effect of Burst-DRGS over time and intra-group changes in WT<sub>s</sub> over time, two-way repeated measures analysis of variance (ANOVA) was performed, followed by Dunnett's multiple comparison test. For between-groups analyses, a two-way ANOVA followed by Tukey's multiple comparisons test was used. For comparisons between pre-STZ WT<sub>s</sub> and preimplant WT<sub>s</sub>, a paired-samples *t*-test was used. For the analysis of MT<sub>s</sub> over time, a one-way repeated measures ANOVA with Dunnett's multiple comparisons test was used. For calculation of the amplitude-mechanical hypersensitivity relationship at each specific time point (baseline, 15 min, 30 min, 45 min, and 60 min), mean  $\log_{10}$  (10,000  $\times$  50% WT) values at each time point were expressed as a function of Burst-DRGS amplitude. Nonlinear regression was then performed for each time point, and the X-value corresponding to the vertex of the resulting parabola-shaped curve was defined as the optimal DRGS amplitude for that time point. For calculation of the overall optimal DRGS amplitude, area under the curve analysis was first performed for the effect of Burst-DRGS over time for each specific animal, at all tested Burst-DRGS amplitudes. The mean AUC values  $\pm$  SEM were then expressed as a function of Burst-DRGS amplitude, after which nonlinear regression was performed, and the X-value corresponding to the vertex of the parabola was identified as the overall optimal Burst-DRGS amplitude.



**Figure 2.** Study design. [Color figure can be viewed at [wileyonlinelibrary.com](#)]



**Figure 3.** Development of mechanical hypersensitivity after STZ injection of all implanted rats ( $n = 13$ ).  $****p < 0.0001$  compared to pre-STZ baseline.

## RESULTS

### Flowchart of Animals

Out of the 28 STZ-injected animals, 27 animals developed diabetes mellitus (96%; blood glucose level  $\geq 15$  mmol/L). Seven animals required insulin treatment (blood glucose level  $\geq 31.4$  mmol/L). Thirteen out of these 27 diabetic animals developed subsequent PDPN (48%;  $\geq 0.2$  decrease in  $\log_{10}$  [10,000  $\times$  50% WT] on Von Frey when compared to the pre-STZ injection baseline). All PDPN animals ( $n = 13$ ) were then implanted with a unilateral DRGS device at the L5 lumbar level. Two animals were withdrawn from the study due to excessively high MT ( $>1$  mA) and two animals were withdrawn due to not displaying neuropathic symptoms following implantation. In the end, nine of these 13 implanted animals successfully finished the six-day stimulation protocol.

### Development of STZ-Induced Mechanical Hypersensitivity

The mean  $\log_{10}$  (10,000  $\times$  50% WT) of the 13 implanted animals dropped from  $5.1 \pm 0.05$  g at pre-STZ-baseline to  $4.7 \pm 0.05$  g pre-implantation (four weeks after STZ injection) ( $p < 0.0001$ ) (Fig. 3).

### Intragroup Analyses: Effect of Burst-DRGS Over Time

Burst-DRGS significantly attenuated STZ-induced mechanical hypersensitivity over time ( $p < 0.0001$ , effect of factor time; two-way repeated measures ANOVA) (Fig. 4a). Burst-DRGS at 33% MT,

50% MT, 66% MT, and 80% MT significantly reduced mechanical hypersensitivity at 15 min, 30 min, and 45 min as compared to the corresponding baseline ( $p < 0.05$ ) (Table 1). As expected, no significant effects over time were observed for very low amplitudes of 0% MT and 10% MT ( $p > 0.05$ ) (Table 1).

### Intergroup Analyses: Effect Between Stimulation Amplitudes

A significant effect of amplitude was found ( $p < 0.01$ , effect of factor amplitude; two-way repeated measures ANOVA) (Fig. 4a). Significant differences were observed between Burst-DRGS at 33% MT, 50% MT, 66% MT, and 80% MT when compared to 10% MT and 0% MT ( $p < 0.05$ ) (Table 1). Additionally, Burst-DRGS at 50% MT was significantly more effective in normalizing STZ-induced mechanical hypersensitivity as compared to Burst-DRGS at 80% MT while the stimulator was turned on (15 and 30 min) ( $p < 0.05$ ) (Table 1). As expected, no significant effect of amplitude was observed in the contralateral hind paw ( $p = 0.69$ , effect of factor amplitude; two-way repeated measures ANOVA) (Fig. 4b).

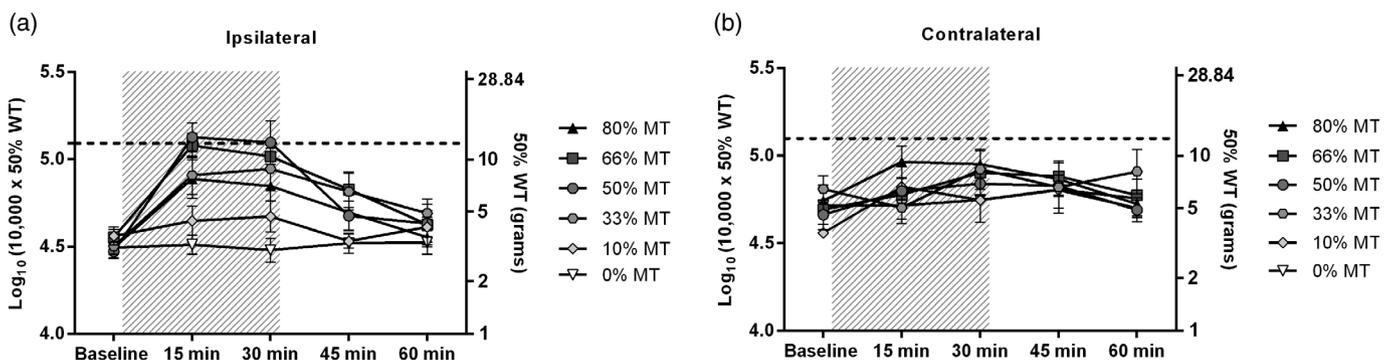
### Responder Rates

The percentage of animals responding to Burst-DRGS (responder defined as increase of the  $\log_{10}$  [10,000  $\times$  50% WT]  $\geq 0.2$  compared to prestimulation baseline) was highest in the 50% MT and 66% MT group at 15 min (100%), the 66% MT group at 30 min (89%), the 66% MT and 80% MT group at 45 min (56%), and the 50% MT group at 60 min (56%) (Table 2).

### Relationship Between Stimulation Amplitude and Mechanical Hypersensitivity

Calculation of the Optimal Burst-DRGS Amplitude at Each Specific Time Point

In order to investigate the relationship between amplitude and mechanical hypersensitivity,  $\log_{10}$  (10,000  $\times$  50% WT) responses were expressed as a function of Burst-DRGS amplitude. At prestimulation baseline (Fig. 5a), no substantial relationship was present between amplitude and mechanical hypersensitivity ( $R^2 < 0.001$ ). With the stimulator turned on (15 and 30 min),  $\log_{10}$  (10,000  $\times$  50% WT) values could be expressed by a polynomial function of the second order ( $y = c + bx - ax^2$ ) ( $R^2 = 0.42$  at 15 min,  $R^2 = 0.37$  at 30 min). The resulting plot was parabolic in shape, with the peak of the graph (Ymax) showing the estimated maximum  $\log_{10}$  (10,000  $\times$  50% WT) response and the corresponding amplitude (Xmax). The calculated optimal DRGS amplitudes (Xmax) at 15 and 30 min were 54% MT



**Figure 4.** A combined presentation of the effect of Burst-DRGS for different amplitudes. Dotted line = the mean pre-STZ baseline of all stimulated animals. Gray area = period of DRGS.

**Table 1.** Significance Values of Within and Between Stimulation Amplitude Comparisons.

	0% MT	10% MT	33% MT	50% MT	66% MT	80% MT
Baseline	4.49 ± 0.04	4.56 ± 0.05	4.50 ± 0.05	4.47 ± 0.04	4.55 ± 0.05	4.49 ± 0.06
15 min	4.51 ± 0.06	4.65 ± 0.08	4.91 ± 0.11 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.05	5.13 ± 0.08 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.0001 † <i>p</i> <0.05	5.08 ± 0.07 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.0001	4.89 ± 0.11 * <i>p</i> <0.0001 # <i>p</i> <0.001 § <i>p</i> <0.05
30 min	4.47 ± 0.07	4.67 ± 0.09	4.95 ± 0.12 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.05	5.10 ± 0.12 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.0001 † <i>p</i> <0.05	5.02 ± 0.08 * <i>p</i> <0.0001 # <i>p</i> <0.0001 § <i>p</i> <0.001	4.85 ± 0.09 * <i>p</i> <0.0001 # <i>p</i> <0.001
45 min	4.52 ± 0.06	4.53 ± 0.04	4.82 ± 0.11 * <i>p</i> <0.001 # <i>p</i> <0.01 § <i>p</i> <0.01	4.68 ± 0.08 * <i>p</i> <0.05	4.83 ± 0.10 * <i>p</i> <0.01 # <i>p</i> <0.01 § <i>p</i> <0.01	4.69 ± 0.10 * <i>p</i> <0.05
60 min	4.52 ± 0.07	4.61 ± 0.06	4.69 ± 0.08	4.63 ± 0.07	4.63 ± 0.11	4.55 ± 0.05

\**p* < 0.05 vs. baseline.  
#*p* < 0.05 vs. 0% MT.  
§*p* < 0.05 vs. 10% MT.  
†*p* < 0.05 vs. 80% MT.

and 51% MT, respectively (Fig. 5b,c). At 45 and 60 min (after the stimulator was turned off), this nonlinear relationship was less obvious ( $R^2 = 0.14$  at 45 min and  $R^2 = 0.05$  at 60 min). Optimal DRGS amplitudes were observed at an  $X_{max}$  of 54% MT and 64% MT for 45 min and 60 min, respectively (Fig. 5d,e).

#### Calculation of the Overall Optimal Burst-DRGS Amplitude

Also when area under the curves (AUC) from Figs. 4a and 6 were plotted, the nonlinear relationship between amplitude and mechanical hypersensitivity was clearly visible ( $R^2 = 0.35$ ), with an estimated optimal pain relieving effect of Burst-DRGS amplitude at  $X_{max} = 52\%$  MT (Fig. 6).

#### Motor Thresholds

Lastly, MTs remained stable throughout the experiment (day 1:  $0.12 \pm 0.008$  mA, day 2:  $0.14 \pm 0.01$  mA, day 3:  $0.18 \pm 0.03$  mA, day 4:  $0.18 \pm 0.02$  mA, day 5:  $0.15 \pm 0.02$  mA.  $p = 0.07$ ) (Fig. 7).

## DISCUSSION

The present study demonstrates that: 1) Burst-DRGS at 33%–80% MT is capable of attenuating STZ-induced mechanical hypersensitivity in rats not only during stimulation, but also 15 min after stimulation cessation. 2) There is a nonlinear relation

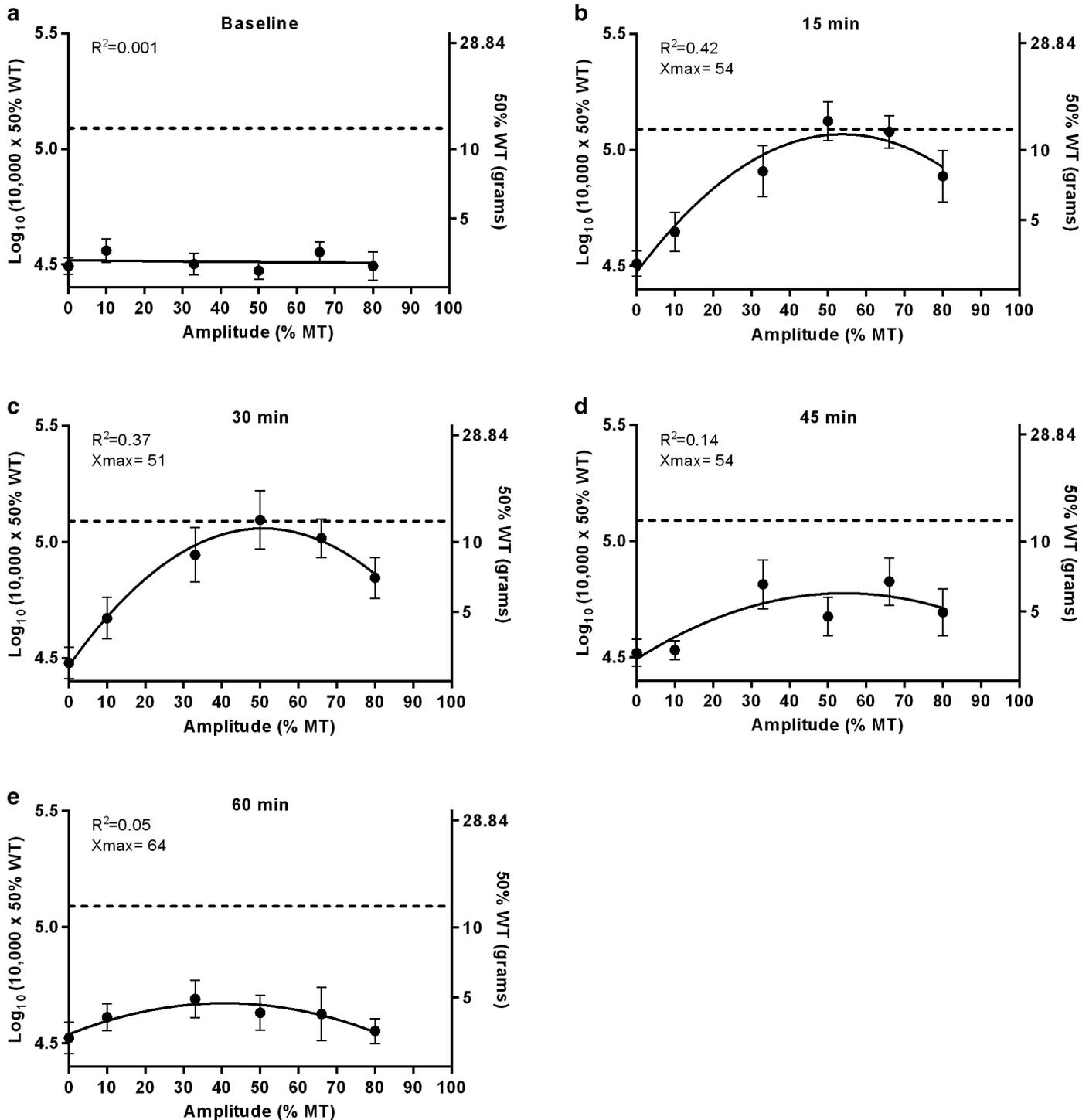
between Burst-DRGS amplitude and observed behavioral outcome with the best range of stimulation amplitude being 50%–66% MT, and an estimated optimal pain relieving effect at 52% MT.

Burst-DRGS at amplitudes of 33% MT, 50% MT, 66% MT, and 80% MT all significantly attenuated STZ-induced mechanical hypersensitivity *during* stimulation (15 min and 30 min) as well as 15 min *after* stimulation (45 min), after which mechanical hypersensitivity values returned to prestimulation baseline values (60 min). These behavioral effects validate an earlier study published by our group on Burst-DRGS at 66% MT, which indicated that Burst-DRGS is capable of treating STZ-induced mechanical hypersensitivity, with the advantage of a significant residual effect after turning off the stimulator (20). Along these lines, results from a recent randomized controlled trial found that Burst-SCS microdosing, a paradigm that relies on the introduction of stimulation-off phases in-between stimulation-on phases, is as effective as standard Burst-SCS, while having significantly lower battery consumption (31). Moreover, the analgesic efficacy of Burst-SCS microdosing was found to be equal as compared to standard Burst-SCS, as measured by rat fMRI brain responses following noxious stimulation (32). Together, these results strongly indicate a beneficial carryover effect of Burst stimulation, both when delivered at the dorsal column and at the DRG, and suggest that some form of plasticity is induced following each stimulation-on phase. It has recently been shown that the amplitude of spinal neuronal

**Table 2.** Responder Rate for Each Stimulation Amplitude.

	0% MT	10% MT	33% MT	50% MT	66% MT	80% MT
<b>15 min</b>	0/9 (0%)	4/9 (44%)	7/9 (78%)	9/9 (100%)	9/9 (100%)	6/9 (67%)
<b>30 min</b>	0/9 (0%)	3/9 (33%)	7/9 (78%)	7/9 (78%)	8/9 (89%)	6/9 (67%)
<b>45 min</b>	0/9 (0%)	1/9 (11%)	4/9 (44%)	4/9 (44%)	5/9 (56%)	5/9 (56%)
<b>60 min</b>	1/9 (11%)	3/9 (33%)	4/9 (44%)	5/9 (56%)	2/9 (22%)	2/9 (22%)

A responder to DRGS is defined as an animal with an increase of the  $\log_{10}(10,000 \times 50\% \text{ WT}) \geq 0.2$  compared to prestimulation.

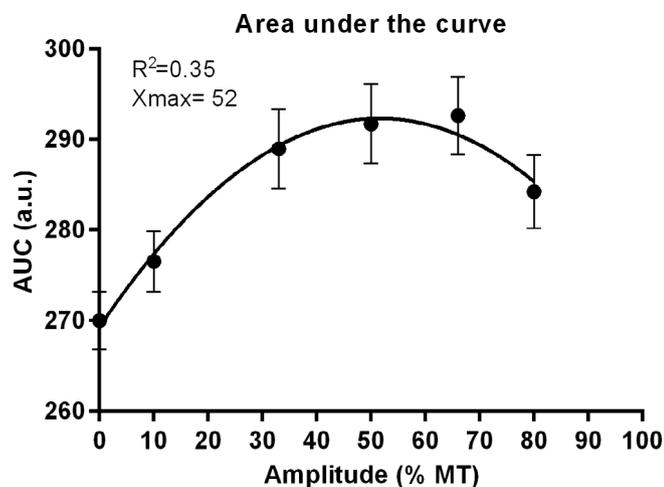


**Figure 5.** Plots resulting from nonlinear curve-fitting for the amplitude-mechanical hypersensitivity interaction for each time point. a. At baseline, no substantial relationship was present between amplitude and mechanical hypersensitivity. b,c. When the stimulator was turned on at 15 and 30 min, the curves describing the amplitude-mechanical hypersensitivity relationship were downward opening parabolas that can be described by the formula  $y = c + bx - ax^2$ . Optimal DRGS amplitudes were estimated at 51%–54% MT. d,e. When the stimulator was turned off at 45 and 60 min, the nonlinear relationship became less obvious. Here, the optimal DRGS amplitude was estimated at 54%–64% MT.

responses in rats can be potentiated for several minutes following a short burst of high-frequency tetanic pulses (555 Hz) (33). As Burst stimulation uses a similar intraburst frequency, it is reasonable to assume that Burst stimulation follows a similar mechanism, explaining the persistence of pain relief when the stimulator is off (stimulation-off phase). Furthermore, high-

frequency stimulation is known to induce long-term potentiation in lamina I of spinal projection neurons (34), and signs of short-term plasticity have been found in response to electrical stimulation, including those modulated by Burst stimulation (35).

Previous studies have indicated that Burst stimulation can be optimized by adjusting relevant stimulation parameters to

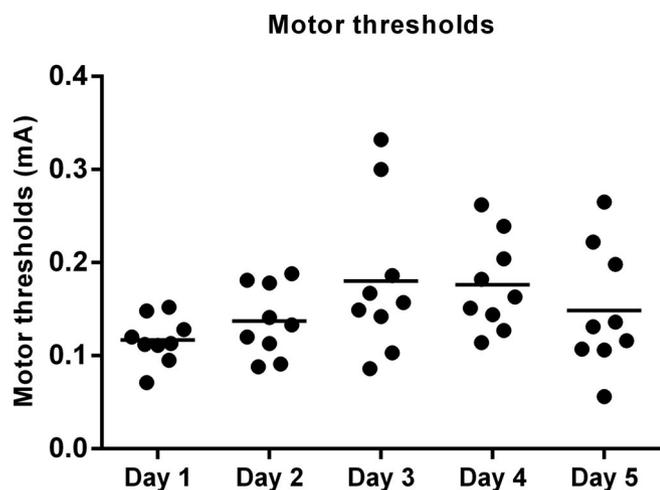


**Figure 6.** Plot resulting from nonlinear curve fitting for the amplitude-AUC interaction. AUCs were based on outcomes as depicted in Fig. 4a and were calculated for each animal individually before they were averaged. The resulting plot shows a clear nonlinear relationship between amplitude and AUC with an optimal DRGS amplitude at  $X_{max} = 52\%$  MT.

modulate the charge delivered to the spinal cord during stimulation, such as amplitude (22). In our study, there seems to be no linear correlation between Burst-DRGS amplitude and the observed behavioral outcome, as the highest amplitude (80% MT) did not result in optimal pain relief and highest responder rates. Significant differences were observed between 33% MT, 50% MT, and 66% MT (at 15 min, 30 min, and 45 min) when compared to 10% MT and 0% MT, and Burst-DRGS at 50% MT was significantly more effective in normalizing STZ-induced mechanical hypersensitivity as compared to Burst-DRGS at 80% MT while the stimulator was turned on (15 and 30 min). Moreover, Burst-DRGS at 50% MT and 66% MT (15 min) and Burst-DRGS at 66% MT (30 min) yielded the highest responder rates, seemingly favoring amplitudes of 50%–66% over the higher (80% MT) and lower (0%–33% MT) stimulation amplitudes. Lastly, the estimated optimal amplitude of DRGS based on nonlinear regression of AUCs was found to be 52% MT. Interestingly, previous work on high frequency SCS

suggests the sensation threshold, defined as the amplitude at which animals start to show signs of disturbance of their normal behavior, to be around 50% MT (36). The gradual decrease in therapy efficacy with stimulation amplitudes beyond this optimum might be explained by the fact that sensory, potential uncomfortable, sensations induced by the stimulation might have taken place. Interestingly, a recent study by Meuwissen et al. showed that Burst-SCS also followed this nonlinear course between amplitude and behavioral outcome (23). The optimal amplitude for Burst-SCS in that study was reported to be 50% MT, and the behavioral outcome was shown to decline rather rapidly when this optimal amplitude was surpassed (23). This is in line with the findings of Courtney et al. who demonstrated that the absolute therapeutic window of Burst-SCS in terms of amplitude is considerably smaller when compared to Con-SCS (37). In contrast, the Burst-DRGS paradigm as used in the present study demonstrated a larger optimal therapeutic window (50%–66% MT). Differences in terms of this optimal therapeutic window between our study and the study of Meuwissen and colleagues might be attributed to the experimental model used (PDPN vs. Seltzer lesion), the location of stimulation (spinal cord vs. DRG), the type of stimulation used (quadripolar vs. bipolar), and/or the Burst waveform used (biphasic with active recharge balance vs. monophasic with passive recharge balance) (23). Additionally, a study by Tang et al. found that while spinal neuronal responses to colorectal distension and pinch were reduced similarly using tonic SCS and Burst-SCS at 90% MT, Burst, but not tonic SCS significantly decreased the nociceptive somatic response after colorectal distension or pinch using lower amplitudes of 60% MT. (21) Combined, these data suggest that Burst stimulation is effective at lower amplitudes relative to the observed MT as compared to tonic stimulation. The combination of this relatively low optimal Burst stimulation amplitude, both in DRGS and SCS studies, together with the earlier described increased carryover effect of Burst stimulation, could have important implications for optimal stimulation delivery as well as battery life of the IPG in clinical practice.

To date, few studies have investigated the mechanisms underlying (Burst-)DRGS and the question remains how (Burst-)DRGS might affect the nociceptive afferents and firing of these afferents. The DRG consists of a unique pseudo unipolar design, in which the T-junction of the DRG can act as a selective filter for stimuli that is, action potentials traveling toward the spinal cord. (38) A study by Koopmeiners et al. reported DRGS to reduce the amplitude and/or amount of action potentials arising from the DRG, thereby inhibiting neuronal excitability (39). Interestingly, it was recently suggested that the outcome of dorsal column SCS is correlated to the active stimulation period or duty cycle (40), and that SCS can attenuate aberrant, hyperactive firing of pain transmission neurons (40,41). Along these lines, a stimulation mode with a high duty cycle, like Burst-SCS, but also Burst-DRGS, is more likely to counteract this aberrant firing of pain transmission neurons. The impact of the high duty cycle of Burst-DRGS may therefore preclude the strength–duration and charge–duration relationship from defining the relationship between single pulse parameters and neural activation thresholds. Besides these electrophysiological findings, DRGS has been linked to attenuation of brain areas that are considered to be part of the pain matrix like the contralateral thalamic VPL/VPM nuclei, and cortical S1 and S2 (42), and one can argue that also spinal mechanisms may underlie DRGS as modulating firing rates of DRG neurons by DRGS may also affect interneurons and GABAergic systems in the dorsal horn



**Figure 7.** Observed motor thresholds (MT) throughout the study period. MT was assessed using a pulse width of 1000  $\mu$ sec, and five pulses (500 Hz intraburst frequency) administered at an interburst frequency of 10 Hz.

as is the case in traditional SCS (43–45). However, more research is needed to fully understand the underlying mechanisms of DRGS and its relation to specific waveforms, like Burst-DRGS and/or individual DRGS-stimulation parameters, like amplitude.

Limitations of this study include the timeframe of the used stimulation protocol. In clinical practice, patients typically receive DRGS for a longer period of time as compared to the short-term protocol used in this study. Second, it should be stressed that using the MT for determining the desired stimulation amplitude in rodents may have several shortcomings. In preclinical studies, the MT often replaces the perception threshold (PT) as used in humans, as it is a quick, easy to use, and objective outcome measure based on visible contractions of the lower trunk and/or hind limbs. In contrast, the PT is relatively difficult to observe in rodents, as the experimenter has to “determine” whether or not an animal perceives the stimulation or not, which can lead to subjectivity. The PT has been estimated to be approximately 30%–50% MT in rats (36,46), and a study by Koyama et al. found that this PT/MT ratio varies across rats (46). The latter might interfere with direct translation of our results to the clinic, as there might have been differences in the amount of stimulation relative to the PT (% PT) delivered to the system across animals. However, it is worth noting that the MTs in the study of Koyama and colleagues were measured under anesthesia, which might significantly influence the (variation between) observed MTs in their study (46). We are convinced that future studies should try to objectively establish the PT in rodent models that would then allow for an even more precise translation of findings to the human situation. Additionally, it should also be noted that there might be differences in the exact MT amplitude when stimulating with an interburst frequency of 10 Hz (which was used to determine the MT used in the experiment) as compared to an interburst frequency of 40 Hz (which was used for final delivery of Burst-DRGS), given the lower duty cycle of 10 Hz stimulation. As it is near impossible to objectively assess MTs using higher frequencies, including 40 Hz, preclinical studies tend to use lower frequencies for assessment of MT (both in DRGS and [Burst-]SCS studies). The latter is needed to obtain a clear and easy to observe MT, even though final delivery of stimulation happens at higher frequencies (23,30,47). Third, in the present study we chose to only include female Sprague–Dawley rats. Female rats reach their mature body weight and nerve conduction status faster as compared to their male counterpart, or either sex of other strains (24). As such, one should be cautious when extrapolating these results to the male sex (48). Fourth, there are fundamental differences in the clinical and preclinical manifestation of PDPN. In patients, diabetes is a chronic disease where complications often only arise after many years. STZ injection in rats relies on destruction of beta cells in the pancreas (24). The injection leads therefore to a very swift development of diabetes (within one week), and subsequent development of mechanical allodynia (within four weeks) (20,27,29,49). Although the injection of streptozotocin is the most common used diabetes model in rats, one should be cautious when extrapolating these results to a human situation. Lastly, the results presented here are limited to reflex-based outcome measures (Von Frey) in experimental PDPN. Future studies using spontaneous or operant behavioral tests and/or different animal models should be conducted to verify these results, as it is very well possible that Burst stimulation only targets specific components of pain, not observable by the Von Frey test (50).

Altogether, our findings indicate that there is a nonlinear relationship between Burst-DRGS amplitude and behavioral outcome,

as Burst-DRGS at 50% MT and 66% MT resulted in optimal pain relief and highest responder rates, with an estimated optimal pain relief at 52% MT. A further increase in Burst-DRGS amplitude up to 80% MT did not result in better pain relief, even though Burst-DRGS at amplitudes of 33% MT–80% MT significantly attenuated mechanical hypersensitivity in PDPN animals. Also, a significant wash-out of Burst-DRGS was observed 15 min following Burst-DRGS cessation at these amplitudes. Further optimization and analysis of DRGS driven by insights into the underlying mechanisms related to the various stimulation paradigms is warranted.

## Authorship Statements

Glenn Franken performed the experiments, analyzed the data, and wrote the manuscript. Elbert A.J. Joosten and Glenn Franken conceived and designed the experiment. Jacques Debets performed the DRGS implantations. All authors have approved the final version of the manuscript.

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