Burst-like conditioning electrical stimulation is more efficacious than continuous stimulation for inducing secondary hyperalgesia in humans.

AUTHORS AND AFFILIATIONS
Gousset S¹, Mouraux A¹, van den Broeke E.N.¹*.

1: Institute of Neuroscience (IONS), Division Cognition and Systems (COSY), Université catholique de Louvain (UCL), Brussels, Belgium.

*CORRESPONDING AUTHOR:
E.N. van den Broeke, PhD
Université catholique de Louvain (UCL), Institute of Neuroscience (IONS), Division Cognition and Systems (COSY), Brussels, Belgium.

Downloaded from www.physiology.org/journal/jn at Southern Cross Univ (203.002.032.209) on December 14, 2019.
NEW AND NOTEWORTHY

Burst-like electrical conditioning stimulation of cutaneous nociceptors is more efficacious than continuous stimulation for inducing heterosynaptic facilitation of mechanical nociceptive input in humans.

KEYWORDS

Electrical stimulation, burst-like; continuous; nociception, secondary hyperalgesia
The aim of the present study was to compare the efficacy of burst-like conditioning electrical stimulation versus continuous stimulation of cutaneous nociceptors for inducing increased pinprick sensitivity in the surrounding unstimulated skin (a phenomenon referred to as secondary hyperalgesia). In a first experiment (N=30) we compared the increase in mechanical pinprick sensitivity induced by 50 Hz burst-like stimulation (N=15) versus 5 Hz continuous stimulation (N=15), while maintaining constant the total number of stimuli and the total duration of stimulation. We found a significantly greater increase in mechanical pinprick sensitivity in the surrounding unstimulated skin after 50 Hz burst-like stimulation compared to 5 Hz continuous stimulation ($p=.013$, Cohen’s $d=.970$). Importantly, to control for the different frequency of stimulation we compared in a second experiment (N=40) 5 Hz continuous stimulation (N=20) versus 5 Hz burst-like stimulation (N=20), this time while keeping the total number of stimuli as well as the frequency of stimulation identical. Again we found a significantly greater increase in pinprick sensitivity after 5 Hz burst-like stimulation compared to 5 Hz continuous stimulation ($p=.009$, Cohen’s $d=.868$). To conclude, our data indicate that burst-like conditioning electrical stimulation is more efficacious than continuous stimulation for inducing secondary hyperalgesia.
INTRODUCTION

Long-term potentiation (LTP) refers to a long-lasting activity-dependent increase in synaptic strength and was first demonstrated by Bliss and Lømo (1973) following brief trains of stimulation of the perforant path to dentate granule cells in the hippocampus of anaesthetized rabbits. Interestingly, results seem to indicate that burst-like stimulation is more effective for inducing LTP than continuous stimulation (see Fig. 3 in Larson and Munkácsy, 2015).

Activity-dependent LTP can also be induced within spinal nociceptive pathways (Ikeda et al. 2003; 2006). For example, Ikeda et al. (2003) showed that brief trains of high frequency stimuli (HFS; 100 Hz for 1 s three times at 10-s intervals), further referred to as burst-like stimulation, applied to the rat sciatic nerve triggers homosynaptic LTP at the synapse between peripheral C fibers and spinal cord lamina I neurons projecting to the parabrachial area in the brainstem. Also low frequency continuous stimulation (2 Hz for 2 min) triggers homosynaptic LTP but at the synapse between peripheral C fibers and spinal cord lamina I neurons projecting to the periaqueductal grey (Ikeda et al. 2006). Besides homosynaptic LTP, HFS also triggers heterosynaptic LTP at remote C-fibers, which is induced through the activation of spinal glial cells releasing TNF-α and D-serine (Kronschläger et al., 2016). A recent study in rodents suggests that microglia mediated LTP could be one mechanism responsible for the development of chronic pain (Zhou et al., 2019).

In humans, HFS (100 Hz for 1 s five times at 10-s intervals) delivered to the skin to intensively activate skin nociceptors increases the perception elicited by single weak electrical stimuli delivered through the same electrode at which HFS was delivered. Moreover, it increases the perception elicited by mechanical pinprick stimuli delivered to the surrounding
unstimulated skin (Klein et al., 2004; Henrich et al., 2015). It has been suggested that the increase in perception elicited by the weak electrical stimuli after HFS is a perceptual correlate of homosynaptic LTP (also referred to as “homotopic pain LTP”), while the increase in mechanical pinprick stimuli is a perceptual correlate of heterosynaptic LTP (also referred to as “heterotopic pain LTP”) (Klein et al., 2004; Henrich et al., 2015). Regarding the pattern of conditioning stimulation, it is at present unclear if burst-like stimulation is more efficacious than continuous stimulation for inducing heterotopic pain LTP.

A previous study in humans compared the heterotopic pain LTP induced by 10 Hz continuous stimulation and 100 Hz burst-like stimulation (HFS), while keeping the total number of stimuli and total duration of stimulation the same for both protocols (Xia et al., 2016). Both conditioning stimuli induced a significant increase in mechanical pinprick sensitivity of the surrounding skin. However, although the average increase in pinprick ratings was greater after burst-like stimulation (49%) as compared to continuous stimulation (27%), these differences were not statistically significant. In contrast to these results, De Col and Maihöfner (2008) found that 20 Hz continuous stimulation resulted in a decreased sensitivity to mechanical pain, i.e. increased mechanical pain thresholds in the area surrounding the stimulated skin, suggesting that continuous stimulation could induce hypoalgesia rather than hyperalgesia. Finally, both Biurrun Manresa et al. (2010) and Vo and Drummond (2014) did not observe any significant changes in pinprick sensitivity after 1 Hz continuous conditioning stimulation.

The aim of the present study was to test if continuous nociceptive conditioning stimulation is more effective than burst-like stimulation in inducing “heterotopic pain LTP”. To test this we compared the change in mechanical pinprick sensitivity induced by 50 Hz burst-like
stimulation with the change in mechanical pinprick sensitivity induced by 5 Hz continuous stimulation (Experiment 1). Both protocols have the same total number of stimuli and the same total duration of stimulation. Furthermore, to control for a possible effect of frequency of stimulation, we also compared the change in mechanical pinprick sensitivity induced by 5 Hz continuous stimulation with the change in mechanical pinprick sensitivity induced by 5 Hz burst-like stimulation (Experiment 2). In this case, both the frequency and the total number of stimuli remained the same but the total duration was greater for burst-like stimulation.

METHODS AND MATERIALS

Participants

Thirty healthy volunteers took part in Experiment 1. Participants were randomly assigned to either 5Hz continuous conditioning stimulation (N=15, 5 men and 10 women; aged 19 – 27 years; 22.4 ± 2.4 years [mean ± SD]) or to 50 Hz burst-like conditioning stimulation (N=15, 5 men and 10 women; aged 21 – 36 years; 23.8 ± 4.0 years). In Experiment 2, forty healthy volunteers were included (5 Hz burst-like stimulation: N=20, 7 men and 13 women; aged 18 – 40 years; 23.0 ± 4.9 years; 5 Hz continuous stimulation: N=20, 7 men and 13 women; aged 19 – 27 years; 22.6 ± 2.3 years). Part of the data was re-used from the 5 Hz continuous stimulation condition of experiment 1 (N=15) and the 5 Hz burst-like stimulation condition of our previous collected dataset (N=15, van den Broeke et al., 2019). Comparison of these two groups of 15 participants did not reach statistical significance. However, the effect size calculated using the means and SDs of the increase in pinprick sensitivity (compared to baseline and control site) was moderate (Cohen’s d = .50), which could indicate that we did not have sufficient power to detect a difference. Therefore, to reduce the risk of making a
type II error, we increased the sample from 15 to 20 by collecting 5 new participants per

The experiment was conducted according to the declaration of Helsinki (except
preregistration of the trial). Approval for the experiments was obtained from the local
Ethical Committee (comité d'éthique hospitalo-facultaire des Cliniques universitaires Saint-
Luc-UCLouvain) of UCLouvain (B403201316436). All participants signed an informed consent
form and received financial compensation for their participation.

Experimental design

In all experiments, electrical conditioning stimulation was applied to the dominant or non-
dominant volar forearm, counterbalanced across participants (10 cm distal to the cubital
fossa, Fig. 1). Handedness was assessed using the Flinders Handedness Survey (Nicolls et al.,
2011). Before ('pre') and twenty minutes ('post') after the end of the conditioning
stimulation pinprick sensitivity of the skin was assessed by applying mechanical pinprick
stimuli (128 mN) to the skin surrounding the site where the conditioning stimulation was
delivered ('pinprick test area') and to the corresponding skin area of the contralateral arm
serving as control.

Conditioning stimulation

In all experiments, the conditioning stimulation consisted of biphasic charge-compensated
electrical pulses that were delivered to the ventral forearm using a constant-current
electrical stimulator (Digitimer DS5, Welvyn Garden City, UK) and a specifically designed
electrode built at the Centre for Sensory-Motor Interaction (Aalborg University, Denmark).

The biphasic pulses consisted of a 2-ms square-wave pulse followed, after a 0.1 ms delay, by a 4-ms compensation pulse of opposite polarity having half the intensity of the first pulse.

The electrode consists of 16 blunt stainless-steel pins (diameter: 0.2 mm) protruding 1 mm from the base (Fig. 1). The pins are placed in a 10 mm diameter circle and serve as cathode. A stainless-steel anode electrode is concentrically located around the steel pins (inner diameter: 22 mm; outer diameter: 40 mm).

The intensity of conditioning stimulation was individually adjusted to 20x the detection threshold to a single non-charge-compensated monophasic pulse (pulse width: 2 ms). The electrical detection threshold was determined after the pre measurement of pinprick sensitivity using a staircase procedure.

In all conditions the total number of electrical stimuli delivered during the conditioning stimulation was the same (i.e. 500). The 50 Hz burst-like stimulation consisted of 10 trains each including 50 pulses delivered at 50 Hz. The trains lasted 1 second and were separated by a 10 second inter-train interval (total duration: 100 seconds). The 5 Hz continuous stimulation consisted of 1 train of 5 Hz stimulation for 100 seconds. Finally, the 5 Hz burst-like stimulation consisted of 100 trains, each including 5 pulses, and lasting for 1 second. The trains were delivered in a 10 second inter-train interval (total duration: ±17 minutes).

The electrical pulses were triggered by a National Instruments digital-analogue interface (NI, National Instruments, Austin, Texas, USA), controlled by custom Matlab code (Matlab 2014B, Mathworks, USA).
Quantifying changes in the perceived intensity of mechanical pinprick stimuli

To assess changes in pinprick sensitivity, we followed the same method as described in our previous study (van den Broeke et al., 2019) and which is summarized here. A calibrated pinprick stimulator exerting a normal force of 128 mN using a 0.25 mm probe (MRC Systems, Heidelberg, Germany) was applied perpendicular to the skin. Before applying the conditioning stimulation and 20 minutes after having applied the conditioning stimulation a total of three pinprick stimuli were applied inside the pinprick test area of the conditioned arm and the contralateral control arm. The target of each pinprick stimulus was displaced after each stimulus. Participants were asked to report the intensity of perception elicited by the pinprick stimulation on a numerical rating scale (NRS) ranging from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from non-painful to painful domains of sensation.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). In Experiment 1, the change in perceived pinprick intensity induced by the 5 Hz continuous stimulation and 50 Hz burst-like stimulation was compared using a mixed two-way repeated measures ANOVA with ‘arm’ (HFS vs. control) and ‘time’ (post vs. pre) as within-subject factors and ‘condition’ (5 Hz continuous vs. 50 Hz burst-like) as between-subject factor. Post-hoc, an independent t-test was used to test for differences in the increase in pinprick
sensitivity (compared to baseline and control site) between the 5Hz continuous stimulation and the 50 Hz burst-like stimulation. In Experiment 2, the change in perceived pinprick intensity induced by the 5 Hz continuous stimulation and 5 Hz burst-like stimulation was compared using a mixed two-way repeated measures ANOVA with ‘arm’ (HFS vs. control) and ‘time’ (post vs. pre) as within-subject factors and ‘condition’ (5 Hz continuous vs. 5 Hz burst-like) as between-subject factor. Post-hoc, an independent t-test was used to test for differences in the increase in pinprick sensitivity (compared to baseline and control site) between the 5 Hz continuous stimulation and the 5 Hz burst-like stimulation. In all tests, the level of significance was set at p<.05.

RESULTS

Electrical detection thresholds

Experiment 1

The electrical detection thresholds to a single monophasic non-charge-compensated pulse were 0.29 ± 0.08 mA (mean ± SD) for the 5 Hz continuous stimulation, 0.26 ± 0.07 mA for the 50 Hz burst-like stimulation. An independent t-test revealed no statistical significant difference in electrical detection thresholds.

Experiment 2

The electrical detection thresholds to a single monophasic non-charge-compensated pulse were 0.26 ± 0.09 mA for the 5 Hz burst-like stimulation and 0.30 ± 0.08 mA for the 5 Hz
continuous stimulation. An independent t-test revealed no statistical significant difference in electrical detection thresholds.

Changes in mechanical pinprick sensitivity

Experiment 1

5 Hz continuous stimulation vs. 50 Hz burst-like stimulation

The means and standard deviations of the intensity of perception elicited by pinprick stimuli delivered before and after conditioning stimulation at both arms (control vs. conditioned) in both conditions (5 Hz continuous stimulation vs. 50 Hz burst-like stimulation) are shown in Figure 2. The mixed repeated-measures ANOVA revealed a significant time x arm x condition interaction (F(1,28)=7.062, p=.013, \(\eta^2=.201\)). Separate repeated-measures ANOVAs for the 5 Hz continuous stimulation and the 50 Hz burst-like stimulation revealed a significant time x arm interaction for both protocols (5 Hz continuous: F(1,14)=13.883, p=.002, \(\eta^2=.498\) and 50 Hz burst-like stimulation: F(1,14)=32.859, p<.001, \(\eta^2=.701\)). The increase in perceived intensity was significantly greater for the 50 Hz burst-like stimulation compared to the 5 Hz continuous stimulation (t (28)=2.658, p=.013, Cohen's \(d=.970\); Fig. 2).

Experiment 2

5 Hz continuous stimulation vs. 5 Hz burst-like stimulation
The means and standard deviations of the intensity of perception elicited by pinprick stimuli delivered before and after conditioning stimulation at both arms (control vs. conditioned) for both the 5 Hz burst-like stimulation and the 5 Hz continuous stimulation are shown in Figure 3. The mixed repeated-measures ANOVA revealed a significant time x arm x condition interaction ($F(1,38)=7.543, p=.009, \eta^2=.166$). Separate repeated-measures ANOVAs for the 5 Hz continuous stimulation and the 5 Hz burst-like stimulation revealed a significant time x arm interaction for both protocols (5 Hz burst-like stimulation: $F(1,19)=37.421, p<.001, \eta^2=.663$ and 5 Hz continuous stimulation: $F(1,19)=20.519, p<.001, \eta^2=.519$). The increase in perceived intensity was significantly greater for the 5 Hz burst-like stimulation compared to the 5 Hz continuous stimulation ($t(38)=2.746, p=.009, \text{Cohen's } d=.868; \text{Fig. 3}$).

**DISCUSSION**

The aim of the present study was to compare the efficacy of continuous versus burst-like electrical stimulation of cutaneous nociceptors for the induction of heterotopic pain LTP. Our data shows that, when controlled for frequency of stimulation, burst-like conditioning stimulation induces a significantly greater increase in pinprick sensitivity than continuous stimulation.

In Experiment 1 we showed that the increase in mechanical pinprick sensitivity induced after 50 Hz burst-like stimulation was significantly greater compared to the increase in mechanical pain sensitivity induced after 5 Hz continuous stimulation. In this experiment, the total duration of the conditioning stimulation and the total number of applied stimuli were identical, but the stimuli were delivered at different frequencies. Hence, differences between the two conditions could have been related to differences in stimulation frequency.
(van den Broeke et al. 2019) rather than the use of burst-like versus continuous patterns of stimulation. For this reason, we conducted a second experiment in which we compared the increase in mechanical pinprick sensitivity induced by 5 Hz continuous stimulation and 5 Hz burst-like stimulation. In this experiment, frequency of stimulation and total number of stimuli were identical across conditions. Again, we found that 5 Hz burst-like stimulation induced a greater increase in pinprick sensitivity as compared to 5 Hz continuous stimulation.

When studying LTP in the hippocampus, Larson and Munkácsy (2015) found that burst-like stimulation induced a relatively larger homosynaptic LTP than continuous tetanic stimulation. If heterotopic pain LTP is indeed a manifestation of heterosynaptic LTP, our results suggest that burst-like stimulation is more efficacious than continuous stimulation in inducing spinal heterosynaptic LTP. Studies conducted in rodents have shown that high frequency burst-like stimulation of the sciatic nerve can activate microglia (Kronschräger et al., 2016) and activated microglia can release brain-derived neurotrophic factor (BDNF, Zhou et al., 2019). The release of BDNF is thought to contribute to central sensitization (Retamal et al., 2018; Zhou et al., 2019), and may decrease the activity of the potassium-chloride co-transporter (KCC2), which would result in an increase in intracellular chloride concentration leading to a loss of inhibition and as consequence increased excitation (Coull et al., 2003; Dedek et al., 2019). Interestingly, studies in rats have shown that unlike burst-like stimulation, continuous stimulation does not lead to the release of BDNF (Lever et al., 2001).

Xia et al. (2016) also compared changes in pinprick sensitivity induced by continuous stimulation and burst-like stimulation in humans. Specifically, they compared 10 Hz continuous stimulation with 100 Hz burst-like stimulation, while keeping the total number of
stimuli and total duration of stimulation the same. They observed a significant increase in pinprick sensitivity after both stimulation protocols, however, and in contrast to our results, no statistical significant difference was observed, although the increases were not the same (10 Hz: 27%; 100 Hz: 49%). Xia et al. used an intensity of stimulation corresponding to 10 times the detection threshold while in the present study we used an intensity of 20 times the detection threshold. Moreover, in the present study we compared the two conditions (5 Hz vs. 50 Hz) with respect to baseline and contra-lateral control arm, while in the study of Xia et al. they compared three conditions (10 Hz continuous, 100 Hz burst-like, and 200 Hz burst-like) with respect to baseline and a control condition in which the electrode was attached to the skin but no stimulation was delivered.

We also show that continuous stimulation induces an increase in mechanical pinprick sensitivity. Klein et al. (2004) also observed hyperalgesia to pinprick stimulation in surrounding unstimulated skin after 1 Hz continuous conditioning stimulation, but only when the intensity of stimulation was 20 x the detection threshold. The total duration of their conditioning stimulation was around 16 minutes and the stimulation was applied to the ventral forearm. Also Torta et al., (2019) showed an increase in pinprick sensitivity of the skin after 2 minutes of 2 Hz continuous stimulation at the forearm.

In contrast, De Col and Maihöfner (2008) showed that 20 Hz continuous stimulation applied to the ventral forearm induced hypoalgesia rather than hyperalgesia of the skin surrounding the site at which the conditioning stimulation was delivered. However, there are differences between the present study and their study. In the study of De Col and Maihöfner, the continuous stimulation lasted for 35 min, while our continuous conditioning stimulation lasted 100 seconds only. Moreover, the electrode the authors used to deliver the
conditioning stimulation is different from ours. Whereas their electrode had only two pins, our electrode has 16 pins. It is likely that our stimulation activated a larger number of afferents. Furthermore, the frequency of stimulation is different. Whereas it was 20 Hz in their study, it was 5 Hz in the present study. Finally, the intensity of stimulation was different. In the study of De Col and Maihofner, the intensity was continuously adjusted during the conditioning stimulation to a pain intensity of 5 on a numeric rating scale ranging from 0 (no pain) to 10 (worst imaginable pain), while in our study the intensity of stimulation was set at 20 x the detection threshold.

Finally, Biurrun Manresa et al. (2010) and Vo and Drummond (2013) also used 1 Hz continuous conditioning stimulation but did not observe any significant changes in pinprick perception in the unstimulated surrounding skin. In both studies their conditioning stimulation was delivered at 10 times detection threshold. Moreover, in the study of Biurrun Manresa et al. they applied the conditioning stimulation to the dorsum of the foot instead of the forearm.

To conclude, the present study provides evidence that burst-like conditioning stimulation is more efficacious in inducing increased pinprick sensitivity in the surrounding unstimulated skin than continuous stimulation. These results show that the pattern of peripheral nociceptive input (i.e. not only the total amount of input) is an important determinant of how much central sensitization will be induced. Our results may have important implications for neurostimulation in the context of pain therapy.

REFERENCES


**GRANTS**

EvdB is supported by the Fonds de Recherche Clinique (FRC) provided by UCLouvain, Belgium. AM is supported by the ERC “Starting Grant” (PROBING PAIN 336130).

**DISCLOSURE**

The authors declare no conflict of interest.

**FIGURE LEGENDS**

**Fig. 1. Experimental set-up. A.** Conditioning stimulation is applied to the dominant or non-dominant volar forearm. Pinprick stimulation (128 mN) was applied to the skin surrounding the area onto which conditioning stimulation was applied (“pinprick test area”) as well as to the same skin area on the contralateral control arm. **B.** Characteristics of the conditioning electrode. **C.** Time-line of the experiment. The perceived intensity elicited by the pinprick stimulation was assessed at two different time-points: before conditioning stimulation (“Pre”) and twenty minutes after applying conditioning stimulation (“Post”).

**Fig. 2. Experiment 1. A.** Intensity of perception elicited by the mechanical pinprick stimulation (128 mN) before and twenty minutes after applying 5 Hz continuous...
conditioning electrical stimulation (left) or 50 Hz burst-like stimulation (right). Shown are the
group-level average and standard deviation of the numerical rating scale (NRS) scores. B.
Group-level average and standard deviation increase in NRS compared to baseline and
control site. The $P$-value shows the result of the independent t-test on the individual
changes in perception.

**Fig. 3. Experiment 2.** A. Intensity of perception elicited by the mechanical pinprick
stimulation (128 mN) before and twenty minutes after applying 5 Hz continuous
conditioning electrical stimulation (left) or 5 Hz burst-like stimulation (right). Shown are the
group-level average and standard deviation of the numerical rating scale (NRS) scores. B.
Group-level average and standard deviation increase in NRS compared to baseline and
control site. The $P$-value shows the result of the independent t-test on the individual
changes in perception.