Conditioning pain modulation reduces pain only during the first stimulation of the temporal summation of pain paradigm in healthy participants

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Published in:
European Journal of Pain

DOI (link to publication from Publisher):
10.1002/ejp.1408

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

CONDITIONING PAIN MODULATION REDUCES PAIN ONLY DURING THE FIRST STMIMULATION OF TEMPORAL SUMMATION OF PAIN PARADIGM IN HEALTHY PARTICIPANTS

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Running head: Does conditioning pain modulate temporal summation?

Original paper for: *Eur J Pain*

Funding sources: Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). Further, Shionogi Science Program and Daiichi Sankyo TaNeDS Europe supported this study.

Conflicts of interest: Nocitech is partly owned by Aalborg University.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ejp.1408

Significance: Current knowledge on the interaction effect of pro and anti-nociceptive paradigms are the lacking. The current study indicates that conditioning pain modulation does not modulate summation effect of temporal summation of pain, when evaluated by computerized pressure algometry. This finding was independent of the mild, moderate and severe painful conditioning intensities.

ABSTRACT

Background: Pro-nociceptive and anti-nociceptive mechanisms are commonly assessed in clinical and experimental pain studies, while their potential interaction is not well understood.

Objectives: Investigate the effect of conditioning pain modulation (CPM) on temporal summation of pain (TSP).

Methods: Twenty healthy participants underwent cuff algometry assessment on the lower legs to establish the pressure pain tolerance threshold (PTT). For the TSP assessment, ten stimuli at the level of the PTT were delivered by computerised cuff inflations (1-s stimulation, 1-s break) while participants rated pain intensity on a 10-cm electronic visual analogue scale (VAS). This TSP paradigm was repeated with a simultaneous conditioning stimulus induced by a cuff on the contralateral leg, inflated to a constant pressure corresponding to 30% (mild), 70% (moderate) or 100% (severe) of the PTT. These were assessed in a randomised order, with a fifteen-minute break between tests and a final TSP test without conditioning was reassessed in the end (post-recording).

Results: An interaction between stimuli (1-10) and repetition ($P<0.05$) was found for VAS scores. VAS scores for the first stimulus were decreased during 30%, 70%, and 100% conditioning intensities, compared to baseline ($P<0.05$). There was a significant increase in TSP during conditioning ($P<0.05$). There were no significant differences between baseline and post-recordings for any stimuli ($P>0.05$).

Conclusions: The current study indicates that mild to severe stimuli administered by cuff algometry does not modulate summation effect of temporal summation of pain, which could indicate that pain facilitatory mechanisms are more potent compared with pain inhibitory mechanisms.

Key-words – experimental pain, pain assessment, psychophysical evaluation, pro-nociceptive

INTRODUCTION

Affected pro-nociceptive and anti-nociceptive mechanisms are thought to be implicated in the poor prognosis of pain, such as pain chronicity, spreading and persistence across different types of chronic pain complaints. Quantitative sensory testing (QST) provides opportunity to assess measures of both pro-nociceptive and anti-nociceptive responses, and is widely used to evaluate pain sensitisation, both in patients with chronic pain conditions (Arendt-Nielsen, 2015, Baad-Hansen et al., 2015, Arendt-Nielsen et al., 2010, Graven-Nielsen and Arendt-Nielsen, 2010) as well as in experimental pain studies(Arendt-Nielsen et al., 2011, Graven-Nielsen and Arendt-Nielsen, 2002).

The diffuse noxious inhibitory control (DNIC) paradigm(Le Bars et al., 1992), known as the 'pain inhibits pain' phenomena (Le Bars et al., 1979), assessed in animals reflects the descending control from supra-spinal levels from the brainstem, which selectively inhibit wide dynamic range neurons, although is also mediated through other inhibitory mechanisms, including spinal components (Le Bars et al., 1979, Nahman-Averbuch et al., 2014). In humans, psychophysical pain testing assesses the DNIC-effect through conditioning pain modulation (CPM)(Yarnitsky et al., 2015). In the presence of this painful conditioning stimulus, a reduction in pain perception, or increase in pain threshold induced compared to with no conditioning reflects a net inhibitory CPM effect, but both a reduction or increase in pain perception were possible. CPM has been shown to be intensity dependent, where a more intense conditioning stimuli yields a larger CPM effect (Razavi et al., 2014, Oono et al., 2011, Graven-Nielsen et al., 2016).

Temporal summation of pain (TSP) is defined as the increase in pain to repeated noxious stimulation, and is thought to be the psychophysiological correlate of wind-up in the central nervous system (Arendt-Nielsen and Graven-Nielsen, 2011). In animals this is measured as the increase in response from dorsal horn wide dynamic range neurons to repeated noxious stimulation of the same intensity (Herrero et al., 2000) and is mediated by the co-release of glutamate/aspartate and substance P, and their activation of N-methyl-d-aspartate (NMDA) and neurokinin-1 receptors, which leads to prolonged depolarisations (Price et al., 1994, Woolf and Salter, 2000). In humans upregulated central integrative pain

mechanisms may result in facilitated temporal summation of pain, as is seen in chronic pain patients (Arendt-Nielsen et al., 2010, Petersen et al., 2017, Goubert et al., 2017).

Given that both TSP and CPM act on wide-dynamic range neurons, the ability of CPM to modulate TSP is plausible. Recent evidence has demonstrated inconsistent and conflicting results regarding this interaction (Horn-Hofmann et al., 2018, Lautenbacher et al., 2008, Serrao et al., 2004, Haack et al., 2012). Therefore, further research is needed to understand this and the factors which may modulate the interaction. Research to date has primarily focused on heat stimuli, leaving a gap in other modalities. In addition, other factors influencing the paradigm such as the influence of differing conditioning intensities have not been investigated.

Therefore, current study aims to investigate whether TSP can be modulated by CPM using automated pressure algometry in healthy controls with different conditioning intensity paradigms. It was hypothesised that moderate and intense conditioning would modulate repeated painful stimuli and decrease the degree of summation observed during TSP.

METHODS

Participants

Twenty healthy male volunteers age 18-45 years were recruited. Exclusion criteria included any previous history of musculoskeletal pain, neurologic or chronic pain conditions, mental illnesses (e.g. depression) and current use of medication (e.g. analgesics and anti-inflammatories) or recent history of acute pain affecting the lower limb. Participants were given both written and verbal information about the study and all participants provided written informed consent prior to participation. The study was approved by the local ethics committee (N-20150055) and was conducted in accordance with the Declaration of Helsinki.

Protocol

Participants underwent a full cuff algometry assessment protocol on the lower legs, including cuff pain sensitivity, TSP (repeated cuff stimuli on the non-dominant leg), CPM (cuff pain sensitivity assessment on one leg in parallel with conditioning on the contralateral leg), and TSP in parallel with mild, moderate, and sever conditioning intensities. The non-dominant leg was assigned as the test leg, and the dominant was the contralateral leg. First

bilateral pain sensitivity (cuff pressure pain detection and pain tolerance thresholds; PDTs and PTTs) were assessed, followed by baseline assessment of TSP and CPM (separately). After a fifteen-minute break PDTs and PTTs were re-evaluated immediately prior to TSP was assessed on the leg during conditioning on the contralateral leg (mild, moderate and intense conditioning; 30%, 70% and 100% of the PTT, respectively). The different conditioning stimuli intensities were performed in a randomized order (randomly generated by random.org) with a fifteen-minute break between each assessment. After the three TSP plus conditioning trials, a final post-conditioning TSP recording was assessed after 15 minutes break. Participants were blinded to the conditioning intensity and the study hypothesis.

Cuff algometry

Pressure detection and tolerance threshold were assessed using a computer controlled cuff algometry (Graven-Nielsen et al., 2016, Graven-Nielsen et al., 2015) (Nocitech, Denmark). An inflatable cuff tourniquet (VBN, Germany) was used to apply the pressure stimulus. Two cuffs were first applied below the heads of the gastrocnemius muscle. At the beginning of the test, the cuffs were inflated at a rate of 1 kPa/s (to a maximum safety threshold of 100 kPa). While the cuff inflated, subjects rated the first onset of pressure pain, and continued to rate the pain throughout the test using an electronic 10 cm visual analogue scale (VAS; “0 cm” representing “no pain” and “10 cm” representing “maximal pain”). Subjects were instructed to push a hand-held switch when the pressure was not tolerable (defined as PTT). If PTT was not reached before 100 kPa, the PTT was defined as 100 kPa for the further analysis. The cuff PDT was defined as when the VAS reached 1 cm (Imai et al., 2016)**.** This procedure was completed bilaterally in a randomised order at baseline, and subsequently re-evaluated before each conditioning test.

Conditioned pain modulation

CPM was assessed by changes in pressure detection threshold on the non-dominant leg, in the presence of a painful conditioning stimulus applied to the contralateral leg. The conditioning stimulus was induced by inflation of a second tourniquet around the lower leg contralateral to the test leg and to maintain 70% of the PTT (moderate conditioning) during the test. The cuff on the test leg began to inflate simultaneously at a rate of 1kPa/s, and was used to reassess the PDT in the presence of the painful conditioning stimulus. Both cuffs

were released once the subject terminated the test using the hand-held switch (or the system reached the maximum 100 s or the 100 kPa limit). The CPM-effect was quantified by examining the change in PDT from baseline, to during the conditioning stimulus (i.e. a positive CPM-effect indicating CPM), which has been shown as a reliability assessment(Graven-Nielsen et al., 2016).

Temporal summation of pain

The computer controlled cuff algometer (NociTech, Denmark) was used to assess TSP. Ten short-lasting stimuli (1 s each) at the level of PTT on the non-dominant leg were given with a 1s break between stimuli. The PTT intensity used for TSP was assessed immediately before the TSP protocol. Participants were instructed to continuously rate the pain intensity of each of these sequential 10 stimuli using the electronic VAS. There was no indication given to participants regarding the stimulus intensity or expected pain. They were instructed that the cuff would inflate ten times in a row and that should rate each subsequent stimuli, and that if they felt it was more painful than the previous, they should move the slider up, if it was less painful, they should move the slider down accordingly, and if they felt no change in pain, then to leave the slider where it was. Participants were also instructed not to move the slider or to return to zero in the breaks between stimuli to ensure a recording was not missed during the rapid sequential stimuli.

The VAS score from each of the ten cuff stimuli were extracted. The average VAS score was calculated based on the first to the fourth VAS score (VAS-I) and for the final three normalised VAS scores (VAS-II) as these have demonstrated excellent reliability (Graven-Nielsen et al., 2015). The TSP-effect was defined as the difference between VAS-I and VAS-II (i.e. VAS-II minus VAS-I), which has been use in similar studies previously (Petersen et al., 2016). This procedure was completed at baseline, and repeated again at the end of the test session.

Conditioned temporal summation of pain

To assess TSP with conditioning, the TSP protocol was performed, in the presence of the conditioning stimuli. Subjects were instructed to rate the pain from the TSP paradigm only and not the pain from the conditioning stimuli. This was repeated three times, at 30%, 70%

and 100% of the preceding PTT, in a randomised order with a fifteen-minute break between assessments.

Statistics

All data is reported as mean (± standard deviation, SD or 95% confidence interval, 95%CI) in figures and tables. Statistical analyses were completed in SPSS Statistics (v24.0; IBM, Armonk, NY). Data was assessed for normality using Q-Q plots, and parametric and non-parametric statistics were used as appropriate. CPM efficiency was assessed by comparing PDTs with and without the conditioning stimulus using a repeated measures analysis of variance (RM-ANOVA) with the within-subjects factor *condition* (baseline *versus* during conditioning). To examine the stability of PDTs and PTTs across the session, two-way RM-ANOVAs were used, with the within subjects' factors of *limb* (dominant versus non-dominant) and *time* (time1, time2, time 3, and time4). To investigate the impact of conditioning stimuli on TSP, were analysed using a two-way RM-ANOVA, with factors *stimuli* (1-10) and *conditioning* (baseline [no conditioning], 30%, 70%, 100%, and post-recordings [no conditioning]) on the VAS scores. To examine the effect of conditioning on the TSP-effect (VAS-II minus VAS-I), a RM-ANOVA with the factor *conditioning* (as above) was used. Greenhouse-Geisser corrections were used in case of sphericity violations. Post-hoc analysis for multiple comparisons with Fisher's least significant differences were used. The significance level was set to $P<0.05$.

RESULTS

Pain sensitivity assessed by cuff algometry

There was no significant interaction effect on PDT for *limb*time* ($F_3 = 0.037$, $P > 0.05$) or main effect for *limb* ($F_1 = 0.003$, $P>0.05$). There was significant main effect on PDT for time ($F_3 = 6.418$, $P < 0.01$). Post hoc testing revealed that the second and third PDT assessments were increased relative to baseline (Table 1; $P < 0.05$).

For PTT, seven participation reached the 100kPa safety limit. There was no significant main effects for *limb* ($F_1 = 0.126$, $P > 0.05$), *time* (Table 1; $F_3 = 2.68$, $P > 0.05$), or *limb*time* interaction ($F_3 = 0.586$, $P > 0.05$).

Conditioning pain modulation

Baseline CPM demonstrated there was an increase in PDTs, compared to without conditioning (F_1 = 4.73, P < 0.05; mean difference = 12.7 kPa, 95%CI: 0.475 to 25.0 kPa) indicating an efficient CPM-response at baseline.

Pain intensity during repeated stimulations with and without conditioning

There was a significant main effect for stimulation ($_{F1.8}$, = 79.6, P < 0.001). Post hoc testing revealed an increase in VAS scores across stimuli. All pairwise stimulations were significantly different from stimulation 1 (P<0.005; mean difference between first and last stimulus = 3.4 cm, 95%CI 2.7 -4.1, P < 0.001).

Two-way ANOVA of the VAS scores demonstrated a significant *stimulus*conditioning* interaction ($F_{6.2}$ = 2.8, P < 0.05). Post-hoc testing revealed that VAS scores during the first stimulus was significantly decreased during 30%, 70%, and 100% conditioning intensities, compared to both baseline and post-recording TSP assessments (P < 0.05, Fig. 1). There were no significant differences for VAS scores after stimuli 2 – 10 during any conditioning intensity, compared to either baseline or post-recordings (Fig. 2, P > 0.05).

Temporal pain summation with and without conditioning

There was a significant effect of the different levels of condition for the TSP-effect ($F_{3.5}$ = 4.0, P < 0.05). Post-hoc testing revealed that the TSP-effect was increased at 30% and 70% conditioning compared to baseline (Table 2).

DISCUSSION

CPM and TSP are two commonly used paradigms in experimental pain testing, both for basic studies and identifying clinical subgroups of patients. Despite this, the interaction of CPM and TSP has not been comprehensively established. The results of this study indicate that a painful conditioning stimulus attenuates pain intensity at the beginning of a temporal summation paradigm, rather than continuing to have an effect on pain throughout the TSP paradigm. Participants had an increase in pain intensity across the ten stimuli, indicating the TSP effect superseded the effect of the conditioning stimulus. The intensity of the conditioning stimuli (mild, moderate and severe conditioning) did not seem to influence the final pain ratings of the sequential stimulus used to assess TSP.

Conditioned temporal summation of pain

In line with previous studies(Graven-Nielsen et al., 2015), the TSP paradigm elicited temporal summation, with the tenth stimulus being significantly higher than the first, and there a progressively increase in VAS scores across consecutive stimuli.

The fact that CPM inhibited only the first stimulus of the TSP paradigm indicates that while the conditioning stimuli induced an initial inhibitory effect, which did not offset the progressively increasing VAS scores following sequential stimuli. In contrast, the TSP-effect was actually facilitated during mild and moderate conditioning when the first stimulus resulted in reduced VAS scores due to conditioning.

Despite this is the first study to examine if CPM can inhibit TSP using computerised cuff pressure algometry, previous studies have tried to elucidate this using different modalities (primarily heat) and demonstrated conflicting findings. Lautenbacher et al. (Lautenbacher et al., 2008) found a lack of interaction between CPM and TSP in healthy subjects. They demonstrated that a painful heat conditioning paradigm reduced overall pain rating in both single pressure pulse and five pulses at 0.5 Hz. Interestingly, CPM worked on both single or repeated stimuli to the same extent (Lautenbacher et al., 2008) in contrast to the current study. Using hot water as conditioning reduced pain ratings during sequential heat stimuli to a greater extent than a single heat pulse (Staud et al., 2003). Using cold pressor test as conditioning, Serrao et al.(Serrao et al., 2004) demonstrated that CPM attenuate TSP induced by electrical stimulus in healthy participants. The greater influence of CPM on heat induced TSP compared to pressure induced TSP(Horn-Hofmann et al., 2018) may explain the lack of effect beyond the first stimulus in the current study.

One of the primary reasons for the potentially surprising result that CPM did not modulate TSP (as has been shown) may be due to the difference in modalities used. A recent study found the modulation of TSP by CPM, depended on whether TSP induced by heat or pressure stimuli(Horn-Hofmann et al., 2018). CPM was more successful in inhibiting TSP induced by heat pulses than pressure pulses(Horn-Hofmann et al., 2018).

Another consideration is the frequency of stimulation. The current study employed a stimulation frequency of 0.5Hz. Staud (Staud et al., 2003) on the other hand employed a 0.7s duration with a total of 2seconds from onset to onset. These paradigms are both quicker compared to Lautenbacher (Lautenbacher et al., 2008) and Horn Hoffmann (Horn-Hofmann et al., 2018) used one stimulation every two seconds. This may be important as

research shows that pain intensity increases progressively more with 1s inter stimulus durations compared to slower (Nie et al., 2005), and may have implications on the differing results between paradigms.

Another possible explanation which would fit the pattern of results would be a strong attention focus on the TSP stimuli. Previous designs have only asked participants to rate average pain *after* completion of the TSP paradigm, which is contrary to the current study where participants rated each successive stimuli. The observation of an inhibitory CPM effect on the first stimulus might be due to initial attentional capture by the conditioning stimulus shortly after its onset which disappeared later on with decreasing novelty of the conditional stimulus. Indeed, attentional focus has been shown to influence CPM and this should be considered and compared in future paradigms(Hermans et al., 2016).

Findings in clinical populations

There are some data available examining the interaction between conditioning and TSP in patient populations. In healthy controls and those with chronic headache, Cathart et al. (Cathcart et al., 2010) found that healthy controls but not patients inhibited TSP during painful cuff compression compared to those with chronic headache. While healthy controls may be able to inhibit temporal summation of pain, results may not be directly comparable (as Cathart etc al. also used a stress task). Haack et al.(Haack et al., 2012) found that in healthy controls, a CPM and distraction condition both inhibited pain during ten sequential heat pulses, but those with primary insomnia did not experience such an inhibitory effect. Similarly Staud et al. (Staud et al., 2003) found an effect of CPM on TSP in healthy males, which was impaired in female fibromyalgia sufferers. Interestingly, the females in their study did not attenuate TSP during painful conditioning.

Stability of recordings

The current findings indicate that PDT changed at time point 2 and 3 compared to baseline (time point 1). It is unclear if this is an adaptation, or another possibility this may have been a carry over from the CPM paradigm. However this is not likely, as there has been no carryover with cuff algometry(Graven-Nielsen et al., 2016), and a 15-minute wash-out period was ensured between assessments. In any case, previous studies have demonstrated

that the CPM effects generally is significantly reduced within 15 min(Kennedy et al., 2016). There were also no significant differences in PTT across the session which supports this. Both the conditioning stimulus and TSP intensity were relative to the preceding PTT, so the intensity would therefore be similar if the baseline PTT was used instead. The order in which the conditioning (mild, moderate and severe) were applied was randomised throughout the session, mitigating any potential systematic effect of testing order on the results of the different conditioning paradigms. There were no differences in the degree of TSP-effects (increase across the ten stimuli) between the final post-recording and baseline, indicating a stable and robust TSP across the session, which was not influenced by time or the repeated assessments. This is in line with previous studies showing that cuff algometry is capable and reliable method of inducing temporal summation of pain in healthy individuals (Graven-Nielsen et al., 2015).

Strengths and limitations

This is the first study to use computerised cuff-algometry to examine the effect of conditioning on temporal summation of pain using pressure stimuli and multiple conditioning intensities. Participants were blinded to the main aim of the experiment, and to the intensity of conditioning and temporal summation stimuli. All participants were tested by the same female tester, which may decrease the generalisability. Further, the tester was not blinded to the conditioning intensities or study hypothesis. However, the cuff algometry approach is user-independent limiting potential non-blinding effects. The sequence of testing was randomised, to minimise any effect of order of tests, with a fifteen-minute break between tests. The lack of difference between baseline TSP and post-recording TSP, indicates no carryover from the testing procedures. Another potential limitation is the inclusion of an all-male sample, as previous studies have demonstrated differences between the sexes(Popescu et al., 2010).

Conclusion

Mild to severe conditioning stimuli administered by cuff algometry attenuated pain intensity at the beginning of the sequential stimulus paradigm used for TSP assessment, an effect which was not sustained to the end. This indicates that TSP may surmount the CPM effects.

Further research is needed to investigate if similar effects are present in conditions of facilitated TSP in clinical conditions.

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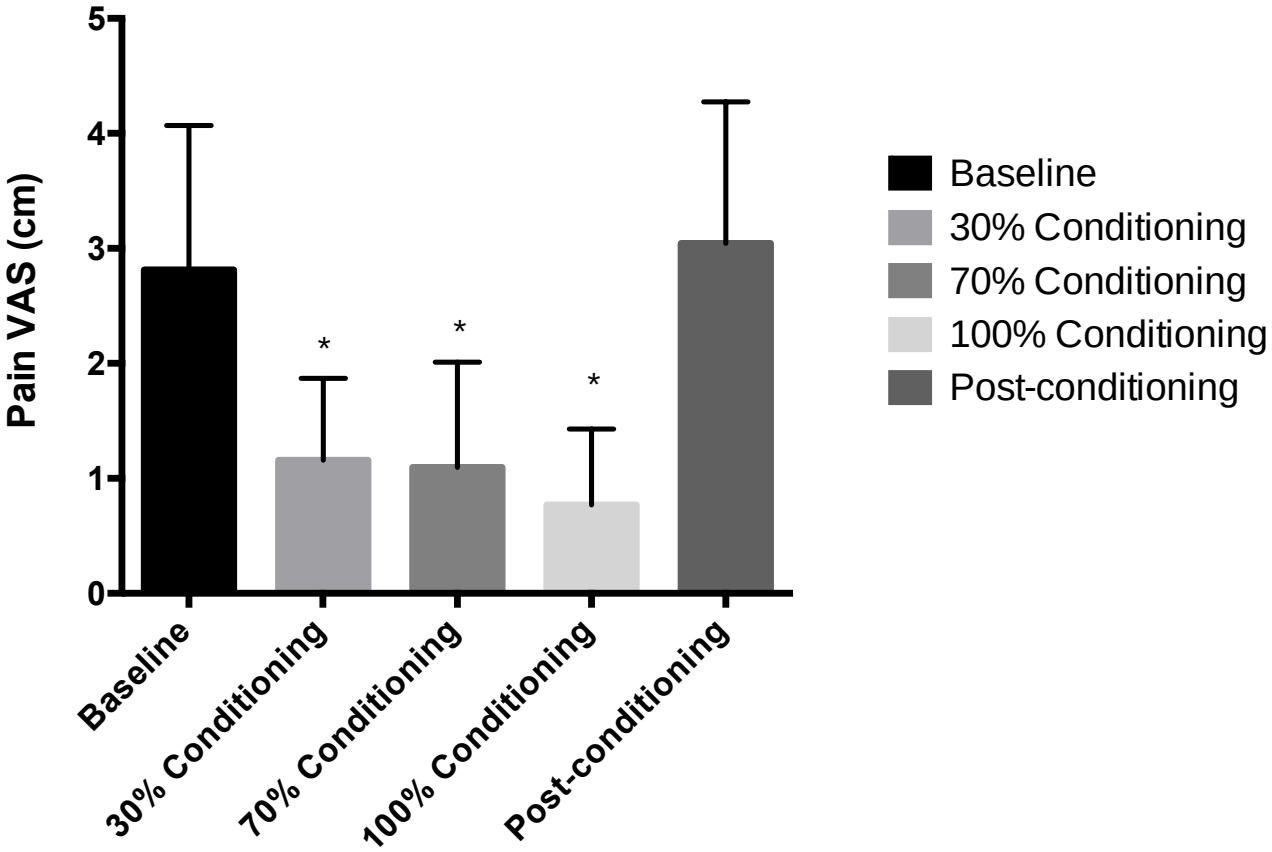

Figure 1. Mean (+ 95%CI) visual analogue scale (VAS) scores of the pain intensity for the first of ten stimuli used for assessment of temporal pain summation, at baseline, during different conditioning intensities and post-conditioning. Significantly reduced compared with baseline and post recordings (*, $P < 0.05$).

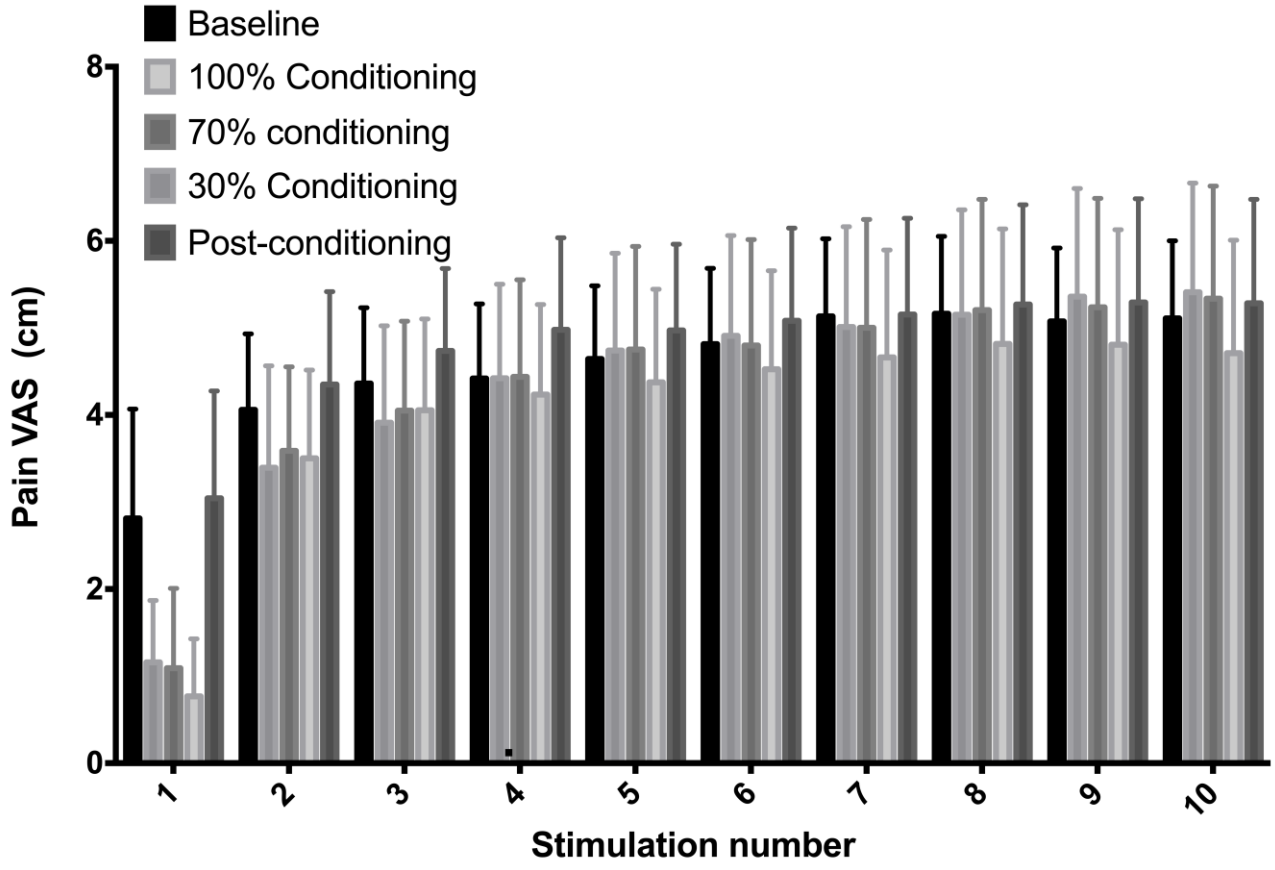

Figure 2. Mean (±95%CI) visual analogue scale (VAS) scores of the pain intensity for each of the ten stimuli at baseline, during conditioning, and at the post-conditioning assessment.

Table 1

Assessment	Pressure Detection Threshold	Pressure Tolerance Threshold
Time-1	31.5 (24.8 to 38.1)	79.4 (69.4 to 89.4)
Time-2	39.6 (48.4 to 30.8)*	84.4 (74.7 to 93.9)
Time-3	42.0 (51.2 to 33.0)*	84.9 (75.8 to 94.1)
Time-4	38.4 (47.5 to 29.2)	81.9 (72.2 to 91.6)

Table 1. Mean (95% CI) pressure detection threshold and pressure tolerance thresholds across four time points (before assessment of temporal summation of pain). Significantly different from Time-1 (*, $P < 0.05$).

Table 2

Repetition	TSP–effect (cm)
Baseline	0.9 (0.3 to 1.5)
30% Conditioning	2.1 (1.4 to 2.8)*
70% Conditioning	2.1 (1.4 to 2.8)*
100% Conditioning	1.6 (0.7 to 2.4)
Post-conditioning recording	1.3 (0.7 to 1.8)

Table 2. Mean (95%CI) TSP-effect (VAS-II minus VAS-I) at baseline, during conditioning and post-conditioning recordings. Significantly different from baseline (*, $P < 0.05$).