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Measurements of Temporal Summation of Heat Pain: a Pilot Investigation in Healthy Humans

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Temporal summation of pain (TSP) is a promising tool for measuring the pain modulation processes in healthy subjects and patients with chronic pain. We tried to find optimal stimulation parameters in order to elicit a robust reproducible TSP phenomenon. Twenty healthy volunteers (15 women and 5 men) completed four sessions/conditions of pulsating heat pain stimulation, applied to the left forearm with a frequency of 0.33–0.4 sec^{-1} using a contact heat-evoked potential stimulator. The stimulation temperature (step +0.5°C or +1.0°C up to the pain threshold, pain tolerance), pulse duration (500, 800, or 1000 msec), and number of stimuli (60 or 90) were varied. The participants rated the pain intensity at the first and every 10th heat pulse, using a numeric rating scale (NRS) 0–100. The TSP was calculated as the difference between the lowest rating and the rating of the last stimulus and was compared between conditions. The optimal condition (19 out of 20 participants responded with TSP) showed temperature at pain tolerance, pulse duration of 800 msec, and 90 stimuli. In addition, this condition showed weaker side effects (painful discomfort) than those with less (60) but longer (1000 msec) stimuli presented 1.0 degree above the pain threshold. The protocol with a relatively high stimulus repetition and a moderate pulse duration seems to be the optimal protocol to reproduce TSP. Heat stimulation with longer pulse durations and higher stimulation temperatures was less feasible.

Keywords: experimental heat pain, temporal summation, heat pain modulation, healthy subjects

INTRODUCTION

Repetitive noxious stimulation evoking pain in humans results in an increased perceived intensity of the painful stimuli [1]. This phenomenon is known as temporal summation of pain (TSP); it has been widely studied in both healthy subject and patients with chronic pain [2–8]. In animal models, direct repetitive stimulation of afferent unmyelinated C fibers led to facilitation of neuronal responses (a "wind-up" phenomenon) in the spinal cord dorsal horn [9]. The increase of neuronal discharges in the dorsal horn of anesthetized rats required a stimulation frequency of at least 0.3 sec^{-1}, and these discharges were strongly facilitated for the first five stimuli [10]. Such an experimentally induced form of central sensitization has been suggested to be a possible model for one of the mechanisms of chronic pain disorders [9, 11]. While the C fiber-mediated "wind-up" phenomenon was observed in animals and humans under normal conditions, the respective phenomenon for Aδ fiber-mediated signals was only shown in the states of hyperalgesia and mechanical allodynia [11].

Temporal summation of the perceived pain intensity is claimed to be a perceptual correlate of the neuronal "wind-up" phenomenon [3, 7], being mediated by the central neuronal mechanisms [12]. In humans, a phenomenon of temporal summation of "second" pain (TSSP) has been regarded as a perceptual correlate of C fiber activation [6]. The TSSP phenomenon is commonly used in experimental heat pain models to understand the underlying mechanisms of pain processing and central pain sensitivity in regard to chronic pain disorders [8, 14].

However, there is a considerable methodological variability in experimental paradigms commonly used to test temporal summation of heat pain, leading to a substantial variability of the TSSP-related responses [1–3, 6, 7, 14]. Common experimental TSSP protocols using at maximum 20 repetitions of heat stimuli with a fixed peak temperature revealed that the proportion of healthy subjects who responded

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with TSSP to repetitive noxious stimulation varied between 30 and 60% [15]. Therefore, an optimum protocol should combine previously established and newly determined stimulation parameters in order to elicit robust temporal summation of heat pain.

Our pilot study was aimed at identifying optimal experimental parameters, such as stimulation temperature, duration of stimuli, and number of repetitions that will reliably induce the TSP phenomenon in a vast majority of human subjects without differentiating into first and second pain experiences.

METHODS

Participants. The tests were performed in a laboratory room of the Department of Anesthesiology at the University Medicine Greifswald. Healthy volunteers with the physical status I or II (American Society of Anesthesiologists), without abnormal skin conditions (infection, scars, psoriasis, eczema, etc.) at the sites of the stimulation and at least two preceding days free of consumption of recreational drugs were included.

All participants were free to withdraw from participation in this study at any time and for any reason. At the beginning of the first study session, the participants were informed in detail about and familiarized with the study procedure.

Investigation Design. The study was performed in two steps (Fig. 1). Step 1 was performed using the stimulation parameters from previous reports, where the TSP phenomenon could be consistently reproduced [1–3, 7, 14]. After the interim analysis of results from TSP induction under conditions I – III, the optimal stimulation parameters were chosen for condition IV (step 2).

During step 1, the first sampling of 20 volunteers (15 women and 5 men) (Sample 1) aged 28 ± 8 years (mean ± s.d.) underwent stimulation under conditions I – III in a randomized crossover manner. The peak temperature of 60 heat pulses with +0.5°C increments was adjusted to the individual pain thresholds, and the duration of the pulse plateau was 500 msec (Condition I) and 800 msec (Condition II, Table 1). In Condition III, the stimulation temperature of the 60 heat pulses was adjusted to the individual pain threshold with increments of +1.0°C and a pulse plateau of 1000 msec (Table 1). Each condition was tested during one of three sessions within an interval of at least 48 h in between to prevent carryover effects. Interim analysis revealed a substantial TSP effect under Condition III, whereas three participants reported that the long stimuli elicited intolerable pain sensations.

We have limited the number of potential combinations of the peak temperature and pulse plateau duration to these three conditions only after our preliminary results and after careful analysis of previous methodological investigations [1–3, 7, 14].

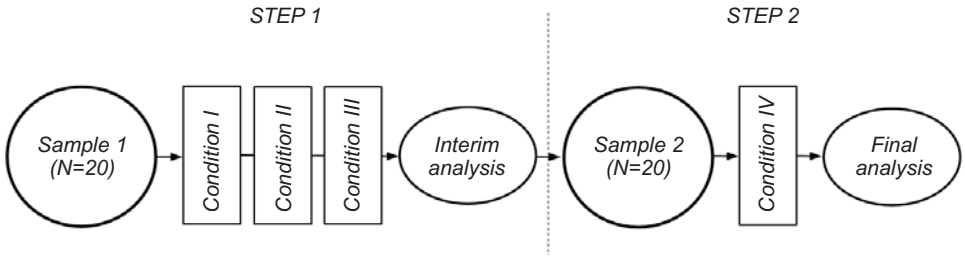

Fig. 1. Scheme of the investigation. Step 1 was performed in 20 participants (Sample 1), who underwent heat pain stimulation under three different stimulation conditions in a random crossover manner. After interim analysis, Condition IV was configured and tested in other 20 volunteers (Sample 2), who were matched according to gender with Sample 1.

T a b l e 1. Stimulation Parameters for Conditions I–IV.

Condition	*n*1 = 20			*n*2 = 20
	I	II	III	IV
Peak temperature, °C	PTh + 0.5	PTh + 0.5	PTh + 1.0	PTo
Duration of the pulse plateau, msec	500	800	1000	800
Number of repeated heat pain stimuli	60	60	60	90

Footnotes: PTh, pain threshold; PTo, pain tolerance. Sample 1 performed Conditions I to III in a randomized crossover manner; Sample 2 (gender-matched) underwent Condition IV.

The interim analysis of the results from TSP induction under conditions I – III led to the configuration of Condition IV (Fig. 1), where another sampling of 20 gender-matched volunteers (Sample 2) aged 24 ± 4 years was tested. The number of heat stimuli with a peak temperature of individual pain tolerance and 800-msec pulse plateau duration was increased to 90 (Table 1).

Heat Pain Stimulation Procedure. Heat stimulation was applied using a contact heat-evoked potential stimulator (Medoc Advanced Medical Systems, CHEPS, Israel). The CHEPS thermode, containing a Peltier element and an external heat foil with a circular area of 5.73 cm², was placed at the left ventral forearm 45 mm below the elbow crease. In order to provide comparable stimulation conditions among the participants throughout the study, the CHEPS thermode was fixed with a blood pressure cuff inflated to a pressure of 30 mm Hg. The thermode was calibrated before the experiments using a precision thermometer (Advanced Industrial Systems, YSI 4600, USA).

In each 35-min-long session, the individual pain threshold (or pain tolerance for Condition IV) was estimated before the performance of the heat stimulation procedure with one of the four stimulation conditions (Table 1). For determination of the pain threshold and tolerance temperatures, the contact surface of the thermode was automatically heated at a rate of 1°C/sec from a baseline temperature (38°C) until a participant determined her/his individual pain threshold (as soon as she/he felt pain for the first time) or the pain tolerance temperature (as soon as she/he felt intolerable pain) by pressing a button of the response unit that cooled down the contact surface to the baseline temperature at a rate of 1°C/sec [10]. Individual pain thresholds and tolerances were calculated as the mean peak temperature of 10 stimulations with a 3-sec interval in between.

The stimulation parameters for TSP induction protocol were chosen according to previous investigations [4, 10, 16, 17] and according to the results of TSP induction during step 1 (Fig. 1).

Repetitive heat pulses with a frequency of 0.4 sec^{-1} were applied to the left ventral forearm. For each pulse, the temperature was increased at a rate of 20°C/sec from the baseline temperature of 38°C to a determined peak temperature (Table 1), held for a defined plateau time, and then returned to the baseline temperature at a cooling rate of 40°C/sec. In order to attenuate the perception of the A fiber-mediated first pain and thus leave the feeling of the C fiber-mediated second pain for TSP [6], the method of adjusted heat pulses described by Staud et al. [10] was used at the beginning of each stimulation. Briefly, the pulse temperature was raised within 8 pulses from the baseline temperature by ≈ 1°C/pulse until the condition-dependent peak temperature was reached in order to mimic the rising skin temperature seen during repetitive contacts with a preheated probe in previous studies (Fig. 2) [4, 7, 10, 16].

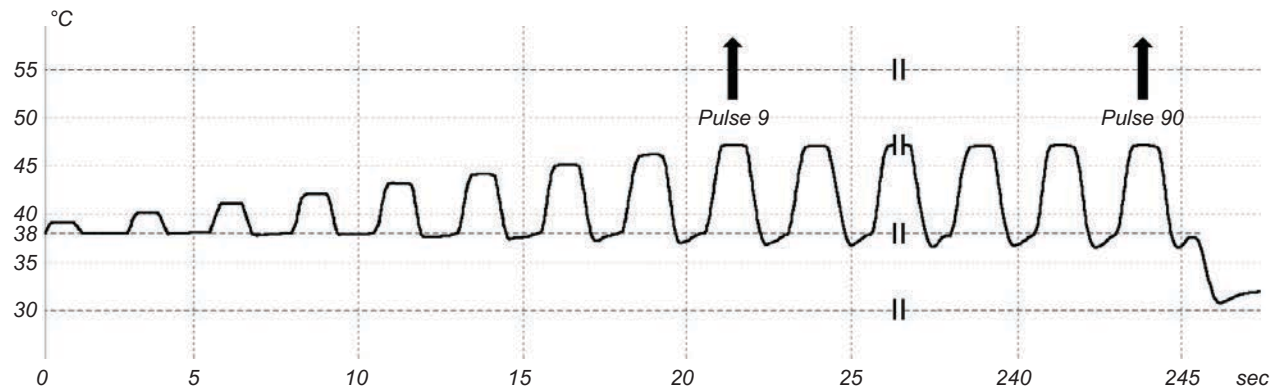

Fig. 2 Modified trace of heat pulses used to elicit temporal summation of pain (TSP) with a 0.33 sec^{-1} frequency. During the first eight pulses, the peak temperature increased from the baseline temperature (38°C) by ≈ 1°C/pulse until the condition-dependent peak temperature (Table 1) was reached. For Conditions I, II, and III, 60 repetitions (in total) of heat pulses were applied; Condition IV required 90 repetitions (the last pulses of Condition IV are demonstrated at the right side of the Figure). For each pulse, the temperature increased at a rate of 20°C/sec from the baseline temperature to a determined peak temperature held for a defined plateau time and then returned to the baseline temperature with a cooling rate of 40°C/sec. Participants reported the intensity of perceived pain initially at the 9th heat pulse (first pulse, which reached the target temperature) and every 10th heat pulse (black arrows) of the peak stimulation temperature, using a previously validated numeric rating scale NRS-100 (explanation in the text).

The measurement of local skin temperature was performed in randomly selected five volunteers before and immediately after the last stimulation pulse under Conditions III and IV, as well as 1, 3, 5, and 10 min after termination of heat stimulation. The temperature was measured using a noncontact infrared laser thermometer (Shenzhen Graigar Technology, CASON CA380, China) from a distance of 12 cm within a circular area (diameter 1 cm) in the middle of the stimulation site.

Outcome Measures and Data Analysis. Subjects verbally rated the intensity of perceived pain initially at the 9th heat pulse (the first heat pulse that reached the peak target temperature) and every 10th heat pulse on a numeric scale ranging from 0 (no pain at all) to 100 (intolerable pain) (Fig. 2). The rating scale, described in detail and used in previous studies [10,18], was translated with associated verbal descriptors into the German language and was displayed on a monitor in front of the subjects.

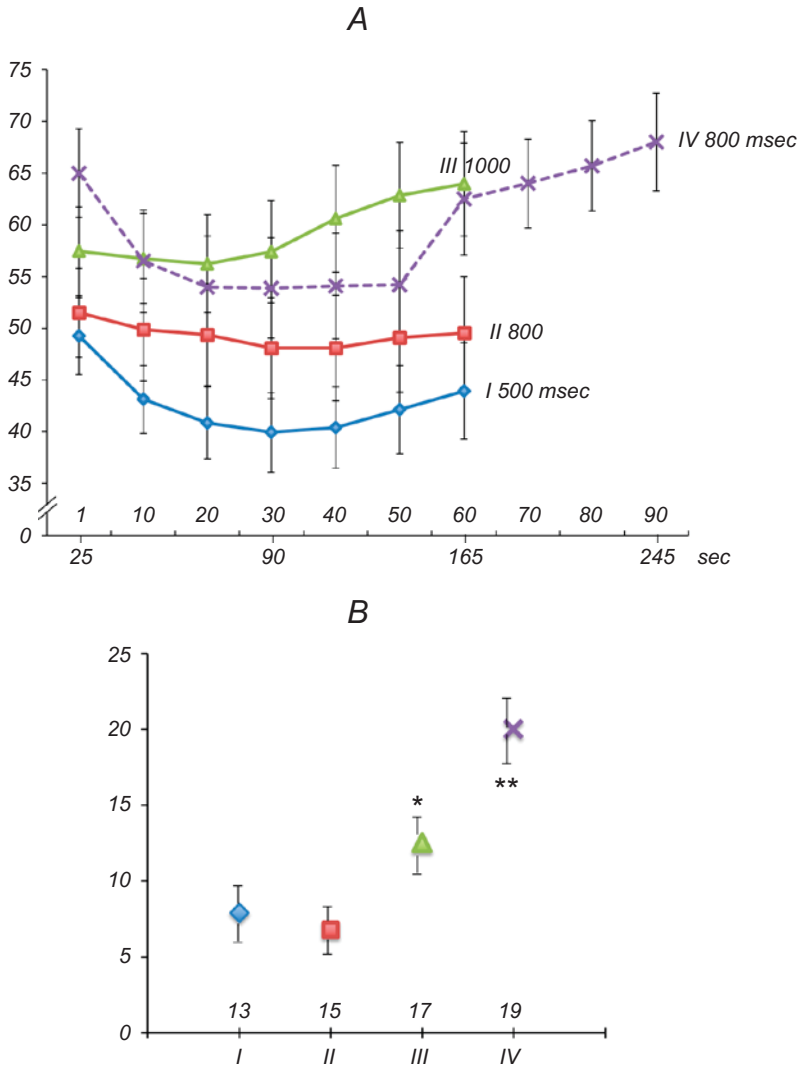

Fig. 3. Results of the tests. A) Pain intensity values under heat stimulation during each condition, presented as means ± s.e.m. Conditions I, II, and III had 60 repetitions of the heat stimuli; Condition IV required 90 repetitions. The duration of the pulse plateau for Conditions I–III is given as numbers in bold letters with respective colors. * $P < 0.05$ (Student's *t*-test for paired samples in comparison of the heat pain intensity at Condition I *vs.* Condition III and Condition II *vs.* Condition III); ** $P < 0.05$ (Student's *t*-test for unrelated samples for comparison of the heat pain intensity at Condition I *vs.* Condition IV and Condition II *vs*. Condition IV). B) Slopes of temporal summation of pain (TSP), calculated as the difference between the last and the lowest pain intensity rating for each participant and condition given as means ± s.e.m. The number of participants who reacted with TSP during each study condition is given on the abscissa. * $P < 0.05$ (Holm-Bonferroni corrected; Wilcoxon signed rank test) for comparison of TSP at Condition III *vs*. Condition I and Condition II; ** $P < 0.05$ (Holm–Bonferroni corrected; Mann–Whitney *U* test) for comparison of TSP at Condition IV *vs.* Condition I and Condition II.

The intensity of heat pain induced during Conditions I–III was analyzed using the Student's *t*-test for paired samples. The pain intensity induced during Conditions I–III was compared to Condition IV using the Student's *t*-test for unrelated samples.

Temporal summation of pain (TSP) was calculated as the difference (slope) between the last and the lowest pain intensity ratings for each participant and condition. The skewed temporal summation slopes were compared between the conditions using the Wilcoxon signed-rank test for the case of related samples (step 1) and with the Mann-Whitney *U* test when unrelated samples (step 2). Changes in normally distributed values of skin temperature were analyzed using the Student's *t*-test for paired samples; *P*-values were adjusted for multiple comparisons with the Holm-Bonferroni procedure, and corrected *P* values < 0.05 were considered as significant. Two-sided *P* values are reported below, and data are presented as means ± s.d., unless otherwise stated. Data analysis was performed with the Statistical Package for Social Sciences, Version 22 (IBM Corporation, SPSS, USA).

RESULTS

Pain Intensity during Stimulation under Conditions I–IV. In step 1 (Sample 1), the most intense pain response (of 59 ± 21 points, on average, on the NRS-100) was achieved with the peak individual pain threshold temperature (PTh) + 1°C and a pulse plateau duration of 1000 msec (Condition III, Fig. 3 A). The pain response under Condition III was higher than that under Condition II (PTh + + 0.5°C and a 800-msec plateau) with 49 ± 22 points (t_{19} = 2.2; *P* = 0.04 *vs.* Condition III). Condition I (PTh + 0.5°C and a pulse 500-msec plateau) yielded the lowest pain intensity (43±16, on average, t_{19} = = 4.3, *P* < 0.001 *vs.* Condition III). Condition IV induced a pain intensity comparable to that under Condition III (60 ± 19 vs. 59 ± 21, t_{38} = 0.1, *P* = 0.9) Table 2).

Slopes of Temporal Summation of Pain. The TSP slopes were induced in 19 out of 20 participants from Sample 2 (step 2 of the study), who underwent the procedure under Condition IV. Conditions I, II, and III induced TSP in 13, 15, and 17 participants, respectively (Fig. 3 B). Condition IV yielded the largest TSP slope of 20 ± 13.8 points on the NRS-100 (Fig. 3 B). This slope was higher in comparison with that under Condition I (–7.9 ± ± 9.6; *U* = 310; *P* = 0.006) and in comparison with the TSP slope under Condition II (6.8 ± 6.7; *U* = 319; *P* = 0.004; Fig. 3 B).

Condition III yielded the TSP slope with 12.5 ± ± 10.4 points, on average, with the NRS-100, which was higher in comparison with the slopes under Condition I (*Z* = 2.41; *P* = 0.03), and under Condition II (*Z* = 2.30; *P* = 0.02; Fig. 3 B). The TSP slopes comparing Conditions III and IV, as well as comparing Conditions I and II, did not differ from each other significantly. Spearman's ρ analysis revealed no significant correlations between the age and TSP in both samples. The numeric values of TSP slopes, including s.d. and interquartile range, are given in Table 3.

Skin Temperature under the Thermode. Skin temperature under the CHEPS thermode immediately after stimulation was higher at Condition III (mean difference 6.4°C; 95% CI 1.6–11.1°C; *P* = 0.016) and at Condition IV (mean difference 7.5°C; 95% CI 3.5–11.5°C; *P* = 0.004; Fig. 4) when compared to the baseline values. The observed skin temperatures were comparable to the baseline values 3 min after the last pulse of heat stimulation at Condition III and 5 min after the last heat pulse at Condition IV (Fig. 4).

T a b l e 2. Comparisons of the Heat Pain Intensity Induced under Conditions I–IV.

Conditions compared	*t*-value	P-value	mean difference	95% confidence interval
I *vs.* II	1.8	0.08	6.6	–0.8–14.0
I *vs.* III	4.3	<0.001	16.5	8.5–24.5
II *vs.* III	2.2	0.04	9.9	0.5–19.4
I *vs.* IV	3.0	0.005	17.0	5.6–28.5
II *vs.* IV	1.6	0.12	10.4	–2.7–23.7
III *vs.* IV	0.1	0.9	0.5	–12.3–13.4

Footnotes: Heat pain intensity at Conditions I to III was analyzed using the Student's t-test for paired samples; the pain intensity at Conditions I to IV was analyzed using Student's t-test for unrelated samples.

T a b l e 3. Results of Experimental Heat Pain Stimulation

Condition	Sample 1, *n* = 20			Sample 2, *n* = 20
	I	II	III	IV
Pain threshold/tolerance, °C	44.9 ± 1.8	44.9 ± 1.4	45.2 ± 1.8	*46.6 ± 1.5*
Number of subjects with TSP	13	15	17	19
TSP Slopes	7.9 (9.6)	6.8 (6.7)	12.5 (10.4)	20.0 (13.8)
IQR	5 (0–10)	5 (0.5-10)	10 (6-30)*	17.5 (5-19)**

Footnotes: TSP, temporal summation of pain; IQR, interquartile range. Data given as number of subjects, means ± s.d., and medians (IQR). Sample 1 performed Conditions I to III in a randomized crossover manner; Sample 2 (gender-matched) underwent Condition IV. The pain threshold was measured in volunteers from Sample 1, whereas pain tolerance was measured in volunteers from Sample 2; numbers of subjects that displayed temporal summation of pain slopes ≥ 0 are shown. The TSP slopes were calculated as differences between the last and lowest ratings.
* Mann–Whitney *U* test for Condition III compared to Condition I and II with Holm–Bonferroni adjusted *P* values < 0.05. ** Wilcoxon signed rank test for Condition III compared to Condition I and II with Holm–Bonferroni adjusted *P* values < 0.01.

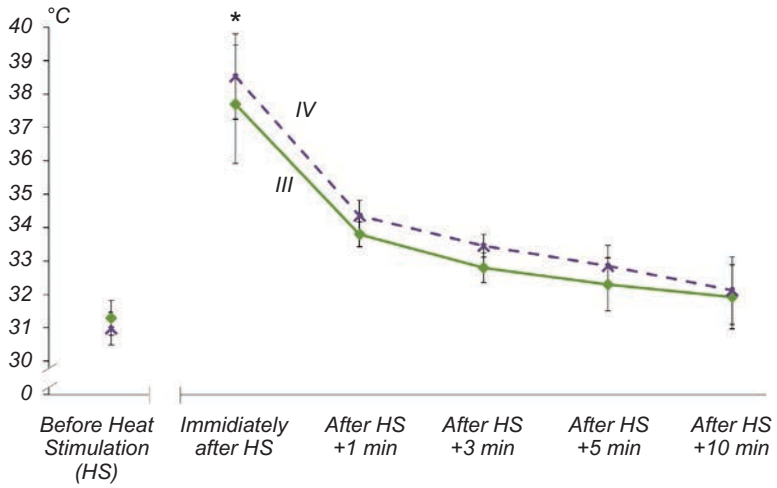

Fig. 4 Skin temperature under the CHEPS thermode of five randomly selected participants before, directly after, and 1, 3, 5, and 10 min after the last pulse of the heat stimulation procedure from Condition III (60 stimuli of pain threshold temperature +1°C and 1000-msec pulse plateau duration) and from Condition IV (90 stimuli of pain tolerance temperature and 800 msec pulse plateau duration). Data are presented as means ± s.d.; * $P < 0.05$ (Holm–Bonferroni corrected; Man–Whitney *U* test) for comparison *vs.* baseline (before heat stimulation).

DISCUSSION

In our pilot experimental study of healthy volunteers, we examined four conditions that included different combinations of heat stimulation parameters, namely stimulation temperature, pulse duration, and number of stimulus repetitions, to find a method that can consistently demonstrate temporal summation of the first and second pain phenomena. Condition IV with a pulse plateau of 800 msec and a greater number of repetitions (90 stimuli) seems to be optimal in comparison with the three other conditions. There were no significant differences between Conditions I and II, except for a difference in the plateau time of pulses. Three participants reported intolerable pain at Condition III, which included the greatest duration of the pulse plateau and a stimulation temperature of the pain threshold increased by 1.0°C. No subjects reported intolerable pain under any other condition. The shift of the pain intensity responses from a low to a higher intensity, Condition I < Condition II < Condition III (Fig. 3 A), was probably due to the longer durations of the pulse plateau throughout the conditions, respectively 500, 800, and 1000 msec.

In our investigation, pain experiences were not separated into first and second pain, as was reported in other studies [7,10,11,15,18]. Most of the wide dynamic range (WDR) neurons in the CNS receive inputs from Aδ and C fibers, whereas both types of nerve fibers are involved in the conduction of noxious stimuli elicited by a contact heat thermode [19]. Therefore, it seems plausible that the observed increase of perceived pain intensity in our study

may be, at least in part, based on neuronal wind-up processes in the central nervous system in both types of fibers. Thus, this heat pain stimulation might be an experimentally induced form of central sensitization, which mimics better clinical pain than stimulation methods based only on the measurement of temporal summation of second pain, respectively. Interestingly, in comparison with other studies, where pain summation was normally saturated after repetitions of 5 to 10 stimuli [4, 7, 10, 11, 15, 18], we found an initial decrease in the pain ratings after the first stimulus. This is in line with findings of habituation of first pain experiences after repetitive stimulation [7, 13, 20, 21] followed by a re-increase of the perceived pain intensities under our Condition IV, suggesting temporal summation of pain responses as a sign of central sensitization (Fig. 3 A). This response pattern could reflect an adaptive reaction to repetitive painful stimulation, whereas an initial strong "warning" signal (indicated by high pain intensity ratings) is followed by a pain-regulating process, during which the subjects adapt to stimulation, probably due to a decrease in the peripheral nociceptive responsiveness and/or central suppression of responses in primary nociceptive neurons [20]. With further ongoing stimulation, a central sensitization process occurs; it is manifested as intensification of the perceived pain, which at some point urges the subject to withdraw from/fight the painful stimuli to prevent injuries. Although the participants from Sample 1 were, on average, about 5 years older than the participants from Sample 2, no correlation was found between the age and TSP slopes in these samples. Thus, age was not a source of bias in our results; this agrees with previous investigations where age-related effects were found to be rather small [16, 22]. It is well known that healthy women demonstrate, as a rule, greater temporal summation of experimental pain in comparison with men [23, 24]. The crossover design in step 1 of our investigation (Fig. 1), where each participant served as her/his own control, prevented gender bias for comparison of Conditions I–III within Sample 1. In step 2, the potential bias in comparison of TSP magnitude between Sample 1 and Sample 2 was prevented by balancing the samples according to gender. Anyway, the same pattern of the robust TSP effect could be reproduced in a larger sample of 50 healthy volunteers (25 females) in a subsequent interventional investigation [25]; in this study, the effect of transcutaneous vagus nerve stimulation (TVNS) on the TSP phenomenon was studied. No gender difference in the TSP reaction was found in this investigation in the case of conditions without TVNS (no intervention) and placebo; under TVNS conditions, however, women, reported decreased TSP, if compared to conditions with no intervention [25].

Although the surface skin temperature under the thermode increased from 31°C by 6.4°C after 60 heat pulse stimuli and by 7.5°C after 90 such pulse stimuli; the absolute value of skin temperature was about 38°C (which was the baseline level of stimulation temperature within our heat pain stimulation paradigm). Nevertheless, these changes of skin temperature disappeared already during 3 min following the end of stimulation. Interestingly, while Lautenbacher et al. [4] did not find any changes in surface skin temperature after 5 min of tonic stimulation with a peak temperature of pain threshold + 1°C, Granot et al. [17] described increased skin temperature to almost 45°C during both tonic and phasic heat stimulation for 60 sec with peak temperatures of about 60% of individual pain threshold repetitions. Both groups, however, ruled out the influence of peripheral skin heating and suggested the central origin of the temporal summation of pain.

It should be recognized that our study has a number of limitations. It is not possible to conclude whether the results are based on differences in single parameters or due to emergent interaction of changes in multiple parameters, since the conditions differ from each other in several parameters each. Furthermore, Sample 2 under Condition IV may be somewhat different from subjects in Sample 1 that underwent stimulation under Conditions I, II, and III, since allocation to the groups (Sample 1 and Sample 2) was not randomized. Thus the potential bias due to a between-subject variability was not excluded in our investigation. Unfortunately, we did not compare both studied samples in regard to the anxiety level and pain catastrophizing. Both these characteristics may influence the size of temporal summation of pain [17] and should be specially measured in future investigations.

Another potentially limiting factor is that pain ratings were assessed verbally. Therefore, these ratings could be biased due to interaction between the participant and the investigator. The pain intensity was also only measured at every 10th pulse. To prevent a lack of information about changes in pain perception over time and to minimize interaction with the investigator, ratings

should be assessed continuously with a special computerized visual analog-scale device that can be synchronized with the CHEPS system. Moreover, in order to confirm the robust TSP effect under Condition IV, the test-retest variability in the same group of participants over time, as well as in clinical populations, should be tested in future studies.

Thus, we found that a protocol with 90 repeated stimuli, a pulse plateau of 800 msec, and a peak temperature of individual pain tolerance limit was suitable to reproduce most stably the TSP responses in a great majority (19 of 20) of healthy subjects. Heat stimulation with longer pulse durations and high stimulation temperatures (Condition III) was less feasible because it caused intolerable pain experiences in some participants. The reliability of our new temporal summation of pain-inducing protocol has to be further tested and refined in future studies, with larger gender-balanced samples. Nonetheless, this may be an obviously promising method to examine central sensitization and effects of hypoalgesic interventions in experimental investigations.

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Ethical approval: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national Research Committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The local Ethics Committee approved the study design (approval Nr. BB 093/16), and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

The authors, T. I. Usichenko, N. Möller, H. Janner, M. Lotze, and K. Hahnenkamp, declare the absence of any conflict in commercial or financial relations, relationships with organizations or persons that in any way could be related to the study, and also in interrelations of the co-authors.

REFERENCES

1. D. D. Price, “Characteristics of second pain and flexion reflexes indicative of prolonged central summation,” *Exp. Neurol*., **37**, 371–391 (1992)
2. L. Arendt-Nielsen, “Central sensitization in humans: assessment and pharmacology,” *Handb. Exp. Pharmacol.,* **227**, 79–102 (2015).
3. J. J. Barrel and D. D. Price, “The perception of first and second pain as a function of psychological set,” *Percept. Psychophys.,* **17**, 163–166 (1975).
4. S. Lautenbacher, S. Roscher, and F. F. Strian, “Tonic pain evoked by pulsating heat: temporal summation mechanisms and perceptual qualities,” *Somatosens. Mot. Res.,* **12**, 59–70 (1995).
5. W. Maixner, R. Fillingim, A. Sigurdsson, et al., “Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain: evidence for altered temporal summation of pain,” *Pain*, **76**, 71–81 (1998).
6. A. E. Olesen, T. Andresen, C. Staahl, and A. M. Drewes, “Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs,” *Pharmacol. Rev.,* **64**, 722–779 (2012).
7. D. D. Price, L. W. Hu, R. Dubner, and R. H. Gracely, “Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses,” *Pain*, **3**, 57–68 (1977).
8. C. Staahl and A. M. Drewes, “Experimental human pain models: a review of standardised methods for preclinical testing of analgesics,” *Basic Clin. Pharmacol. Toxicol.*, **95**, 97–111 (2004).
9. L. M. Mendell and P. D. Wall, “Responses of single dorsal cells to peripheral cutaneous unmyelinated fibers,” *Nature*, **206**, 97–99 (1965).
10. R. Staud, D. D. Price, and R. B. Fillingim, “Advanced contineous-contact heat pulse design for efficient temporal summation of second pain (windup),” *J. Pain,* **7,** 575–582 (2006).
11. R. Staud, M. E. Robinson, and D. D. Price, “Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients,” *J. Pain,* **8**, 893–901 (2007).
12. J. Schouenborg, “Functional and topographical properties of field potentials evoked in rat dorsal horn by cutaneous C-fiber stimulation,” *J. Physiol.,* **356**, 169–192 (1984).
13. J. F. Herrero, J. M. A. Laird, and L. A. Lopez-Garcia, “Wind-up of spinal cord neurons and pain sensation: much ado about something?,” *Prog. Neurobiol.,* **61**, 169–203 (2000).
14. R. Staud, “Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions,” *Expert. Rev. Neurother.,* **12**, 577–585 (2012).

15. R. J. Anderson, J. G. Craggs, L. E. Bialosky, et al., "Temporal summation of second pain: variability in responses to a fixed protocol," *Eur. J. Pain,* **17**, 67–74 (2013).
16. R. R. Edwards and R. B. Fillingim, "Effects of age on temporal summation and habituation of thermal pain: Clinical relevance in healthy older and younger adults," *J. Pain,* **2**, 307–317 (2001).
17. M. Granot, Y. Granovsky, E. Sprecher, et al., "Contact heat-evoked temporal summation: Tonic versus repetitive-phasic stimulation," *Pain,* **122**, 295–305 (2006).
18. C. J. Vierck, R. L. Cannon, G. Fry, et al., "Characteristics of temporal summation of second pain sensations elicited by brief contact of glabrous skin by a preheated thermode," *J. Neurophysiol.,* **78,** 992–1002 (1997).
19. R. Dubner and M. A. Ruda, "Activity-dependent neuronal plasticity following tissue injury and inflammation," *Trends Neurosci.,* **15**, 96–103 (1992).
20. W. Greffrath, U. Baumgärtner, and R. D. Treede, "Peripheral and central components of habituation of heat pain perception and evoked potentials in humans," *Pain,* **132,** 301–311 (2007).
21. D. D. Price and R. Dubner, "Mechanisms of first and second pain in the peripheral and central nervous systems," *J. Invest. Dermatol.,* **69,** 167–171 (1977).
22. S. Lautenbacher, M. Kunz, P. Strate, et al., "Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain," *Pain,* **115,** 410–418 (2005).
23. R. B. Fillingim, W. Maixner, S. Kincaid, and S. Silva, "Sex differences in temporal summation but not sensory-discriminative processing of thermal pain," *Pain,* **75,** 121–127 (1998).
24. E. Sarlani and J. D. Greenspan, "Gender differences in temporal summation of mechanically evoked pain," *Pain,* **97,** 163–169 (2002).
25. H. Janner, C. Klausenitz, N. Gürtler, et al., "Effects of electrical transcutaneous vagus nerve stimulation on the perceived intensity of repetitive painful heat stimuli: a blinded placebo- and sham-controlled randomized crossover investigation," *Anesth. Analg.,***126**, 2085–2092 (2018).