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Methodological Considerations for the Temporal Summation of Second Pain

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Abstract

Temporal summation of second pain (TSSP) is a psychophysical indication of a central pain encoding mechanism, potentially enhanced in pathological pain conditions. Low frequency repetitive stimulation of unmyelinated (C) nociceptors results in a progressive increase of pain intensity when thermal stimulation intensity remains constant. However, when using different methods of nociceptive delivery to the skin, both regularity and rate of pain enhancement with repetition varies between experiments. Specifically, repetitive ramping up and down from a neutral to a painful temperature has produced weak and inconsistent pain summation. In contrast, repetitive contact of the skin with a preheated probe has generated substantial pain summation. In the present study, TSSP by the intermittent contact (IC) with a preheated thermode and constant contact, ramp and hold (CC) methods were compared during 10 iterations of stimulation of glabrous skin of the hand or hairy forearm skin, with an onset to onset interval of 3.3 sec and stimulus interval of 0.8 sec. Significantly greater TSSP was observed for IC stimulation at both sites ($p < .001$). Differential activation of myelinated and unmyelinated nociceptors by ramping and tapping may account for different rates of temporal summation of heat pain.

Perspective—This article presents direct evidence suggesting the constant contact, ramp and hold stimulus may underestimate the level of Temporal Summation of Second Pain. This evidence

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suggests the re-evaluation of stimulation techniques utilized for Temporal Summation tests, especially within clinical models.

Keywords

Temporal Summation; Second Pain; Pain; Intermittent Contact; Constant Contact

1. Introduction

Temporal summation of second pain (TSSP) results from repetitive stimulation of peripheral C-fibers by short discrete heat pulses[27]. Specifically, long latency C-fiber mediated responses of nociceptive and wide dynamic range dorsal horn neurons, and the sensation of second pain, can undergo a progressive increase during a similar time course. The progressive increase in pain intensity represents a behavioral correlate of “wind-up” of spinal dorsal horn wide dynamic range neurons[3; 25; 27; 28]. TSSP using heat stimulation has become a standard psychophysical model to study pain processing, and this method is often used to inform of central sensitization[2; 6; 17; 45]. Early studies demonstrated that TSSP increases with temperature[41], is maximally produced using intervals of 3-sec or less[26], and summation becomes minimal by 6-sec[41].

Recently, the validity and robustness of TSSP have been questioned, due to troubling variability within subjects’ responses to ramping thermal stimulation[7]. For example, in a study reporting data from over 300 healthy subjects, Anderson combined data from five published studies and found that a high proportion of trials did not display a positive summation profile[1]. Other studies have also reported a substantial number of trials failing to show a consistent increase in pain ratings[10; 13; 31]. Various methods have attempted to increase the percentage of participants who experience temporal summation with ramp and hold stimulation; including adjustment of stimulus parameters, through individualized temperature[1; 9; 10; 16].

However, the influence of the mode of heat delivery is typically not considered. Two methods are commonly applied: 1) intermittent contact of the thermode (IC), alternatively known as “tapping” within previous literature, with the skin, which is preheated prior to gentle contact; and 2) continuous-contact of the thermode with the skin (CC), which heats and cools rapidly to create heat pulses. To date, studies on TSSP have been performed using either IC[41] or CC[1] methods, but no studies have compared these methodologies within a single sample. This remains an important comparison, as each methodology has demonstrated different TSSP responses.

Another parameter not considered is the tested body site, despite both the glabrous skin at the palm or hairy skin of the forearm being commonly probed. Studies using CC stimulation have compared other sites such as the forearm and knee in patient with knee osteoarthritis or healthy participants and have resulted in similar ratings, but differences were not directly tested[8; 32]. Raphael[31] did make statistical comparisons and failed to find TSSP differences when comparing the palm with a site directly over the masseter muscle in temporomandibular disorder patients. The tested body site is potentially important

physiologically, since both sensory organ (A – fibers vs C- fibers) distribution and activation varies[21; 40].

The aim of this study is to compare heat evoked TSSP during intermittent (IC) and constant contact (CC) stimulation of the glabrous and hairy skin. The overall magnitude and percentage of positive and negative TSSP profiles were evaluated in healthy pain-free adults.

2. Methods

2.1 Participants

Seventy-nine healthy adults (37 males/42 females) between the ages of 18–34 years (mean = 21.2, SD = 1.6) were recruited using flyers and word of mouth to participate in a study investigating the influence of concurrent mechanoreceptor activation with thermal stimulation. Subjects were given \$25 in Visa Debit cards per session for their participation. The target population were healthy younger adults free from any health conditions which could influence testing. Exclusion criteria included any chronic or systemic disease; chronic/frequent pain complaint or neurological disorders relating to changes in somatosensory and pain perception; any uncontrolled psychiatric conditions; or daily analgesic medication use. The sample was recruited as part of SPARTAN, a parent study examining age-related changes in pain inhibitory and facilitatory function. The University of Florida Institutional Review Board approved this protocol.

2.2 Apparatus and testing environment

All subjects were tested in the Pain Clinical Research Unit at the University of Florida's Pain Research and Intervention Center of Excellence (PRICE); in isolated, climate-controlled rooms under the supervision of experienced pain investigators. Temporal summation was tested utilizing two different testing methodologies: 1) IC and 2) CC.

The IC stimuli were delivered using a Thermo-Electric Stimulation System (TESS, Neuroanaytics, Gainesville FL). For this system, the 40×40mm thermode is housed in a free-standing stimulation module that is adjustable for height. At rest, the thermode is recessed within a thermally neutral plastic housing, out of contact with the skin; which rests on the plastic surface. The thermode is brought into reproducible light skin contact and withdrawn through a small cutout in the plastic housing by a solenoid-powered mechanism, with temperature controlled by a computer. Experimental pain ratings were measured using an electronic visual analog scale (eVAS) that consisted of a low-friction sliding potentiometer of 100 mm travel. The eVAS was mounted into the surface of a small inclined desk positioned to facilitate precise operation with minimal fatigue. Each stimulation cycle consisted a 0.8-second contact interval and a 2.5-sec inter-contact interval resulting in onset to onset of 3.3 sec.

CC stimuli were delivered using a computer-controlled Medoc Pathway Thermal Sensory Analyzer (Ramat Yishai, Israel) using the CHEPS (Contact Heat-Evoked Potential Stimulator) 30×30mm thermode. The thermode is hand held and placed at the site of testing using light pressure by the experimenter. Experimental pain ratings were recorded using the Pathway's electronic visual analog scale (COVAS) by moving a sliding pointer attached to a

low-friction sliding potentiometer of 100 mm travel across the housing. The COVAS was placed in front of each subject using a table positioned just above the subject's lap. Each stimulation cycle consisted trapezoid heat pulses using 0.4 sec rise time, 0.8 sec peak time, and 0.4 sec return to baseline of 38°C with an onset to onset of 3.3 sec.

Both eVAS and COVAS scoring methods allowed the subjects to fully concentrate on the task at hand and give continuous real-time ratings[29]. The left endpoint of the scale was identified as “no pain”, while the right endpoint was defined as “intolerable pain” with nine hash marks between these two anchors. All testing was conducted with the subject seated upright, the upper extremity supported.

2.3 Experimental Protocol

Individuals who were interested in the study were provided information about the procedures, informed about privacy regulations, and they reviewed and signed an Informed Consent Form. Eligibility for the study was determined after subjects completed a health history questionnaire, supplemented by interview and blood pressure measurement.

The study consisted of two pseudo-randomly ordered identical sessions on different days, using one method of stimulation during each session lasting 60–90 minutes. At the beginning of each session, subjects watched a Microsoft PowerPoint presentation (Microsoft Corporation, Redmond, WA, USA; version 2016) with imbedded video and audio overlay that described each testing methodology, the 0–100 pain rating scale, and the temporal nature of first and second pain. Next, subjects were given multiple familiarization trials on the glabrous skin of the palm and hairy skin on the volar forearm of the self-reported dominant hand consisting of 7 ascending heat stimuli from 45 to 51°C using a contact time/peak time of 0.8 sec using an onset to onset interval of 6.8 sec using the testing. Subjects practiced rating first or second pain on separate trial. The trials for rating first pain were provided during familiarization only, serving to reinforce differences in the temporal nature of first and second pain. For first pain, they were instructed to rate pain during the stimulation period. For second pain, subjects were instructed to rate pain after the stimulation period ended.

The investigators noted the temperature for elicitation of 25/100 second pain on the electronic VAS score for each site, and this was the initial temperature for temporal summation testing for that session and method of stimulation. During the test trials, if this temperature failed to produce second pain within the first three stimulations, the trial was stopped, and the test temperature was increased by 1 degree Celsius. The goal was to find a temperature that allowed participants to complete the trial without severe pain yet maximize the potential for the demonstration of summation, as higher temperatures generally result in greater summation[24; 41]. This process was followed during each session so that the testing temperature was optimized for each site and testing method. This adjustment occurred a maximum of 2 times to minimize acute sensitization before testing trials. Subjects were instructed to remove their arm/palm from the thermode should the pain became too intense. Subjects were not informed as to whether the testing temperature would rise/decline during testing.

The study consisted of a total of four temporal summation trials on the limb contralateral (Fig 1a) to the familiarization trials. Single trials were conducted on both the glabrous skin at the palm and the hairy skin at the volar forearm (Fig 1b), for both IC and CC stimulation (Fig 1c–d). The temporal summation trials consisted of 10 heat stimuli, starting with the predetermined temperature, a 0.8-second peak/contact interval (PI, CI) and a 2.5-second inter-pulse- or inter-contact interval (IPI, ISI) (see Figure 1e). Participants were unaware of the testing temperature profiles. Subjects were reminded to focus on the second pain sensation before each trial, and to leave the pointer of the electronic VAS in place for the next stimulation.

2.4 Data processing and analysis

Data were analyzed in MATLAB (Mathworks, MA, USA, Version 2014b). The Pathway processor records multiple lines of data per second for both thermode temperature and COVAS rating in real time. Upon data review, the onset of each pulse (stimulation event) was defined as the onset of thermode temperature ascension through a threshold of 38.3°C (+0.3°C change from baseline); while the end point of each pulse was defined as descending through the threshold of 38.3°C toward the baseline. The single rating for each pulse for data analysis was determined by adding a defined period of time to the end of stimulation. This time period was based on conduction velocities of unmyelinated afferents as well as the sampling rate for the testing protocol (second pain – 156 data points = 2.5 secs.; e.g., at the end of each ISI right before the next stimulation) (see Figure 1e)²⁷. A similar analysis protocol has been determined effective and used in previous publications[5]. The TESS makes a single recording of thermode temperature and eVAS rating per contact at a preprogrammed time point and was set to record at the same time interval as the Pathway. In the case that a subject terminated a trial before reaching the end of the 10 stimulations, the subject's final score was carried forward for the remaining stimuli.

The primary aim of this study was to test for differences between sensations evoked by IC and CC stimulation of glabrous skin (palm) and hairy skin (forearm). First, a repeated measures 2×2 ANOVA was performed on “temperature”, defined as the temperature used to elicit the temporal summation effect, using the procedures described above. The independent variables were “method” (IC and CC) and “site” (glabrous skin at the palm and hairy skin at the volar forearm). To control for temperature differences between IC and CC protocols, we employed linear mixed modeling with thermode testing temperature entered as a time-dependent covariate. Models included “method” (IC and CC) and “site” (glabrous skin at the palm and hairy skin at the volar forearm) and their interaction term. The first dependent variable was “summation”, defined as (1) the 10th pain rating minus the initial pain rating; (2) the 5th pain rating minus the initial pain rating; (3) the maximum pain rating minus the initial pain rating. An initial set of analyses included sex as a covariate; however, sex was not significant in any analysis and was subsequently dropped from the final reported models. The frequencies of positive and zero or negative slopes for each stimulation methodology by site were also calculated and method differences were tested with the McNemar Test. Significant differences were determined at $p = 0.05$. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0. (Armonk, NY: IBM Corp.)

3. Results

Table 1 presents the frequencies of positive and zero/negative slopes as well as means and standard deviations by method and site.

3.1 Methodology differences in temperature by site

Table 1 presents the temperatures for each stimulation methodology by site. The method x site interaction term was not significant ($p = .141$), indicating that the magnitude of differences between temperatures was not dependent upon site. On the temperature variable, there was a main effect of method ($F_{1,84} = 81.359$, $p < .001$) but not site. Inspection of the marginal means indicated that temperatures for CC stimulation were lower (subjects more sensitive) than for IC stimulation, at each site. Testing temperature was correlated with TSSP scores as follows: $P10 - P1$, $r = .19$, $p = .002$; $P5 - P1$, $r = 0.15$, $p = .007$; $PMax - P1$, $r = .24$, $p < .000$. Findings support the use of testing temperature as a covariate.

3.2 Methodology differences in summation by site

Figure 2 presents the average pain ratings for each trial across both sites and stimulation methodologies. The method x site interaction term was not significant ($p = .294$) indicating that summation was not dependent on site. When comparing stimulation methodologies and site tested for the 10th pain rating (P10) minus the initial pain rating (P1), there was a main effect of method ($F_{1,123} = 55.941$, $p < .001$) but not sites ($p = .796$). Inspection of the marginal means indicated that summation ($P10 - P1$) for CC stimulation was lower than for IC stimulation, at each site. Table 1 presents the frequencies of positive slopes for each stimulation methodology by site. McNemar Tests indicated that for $P10 - P1$ resulted in more positive profiles for the IC method than CC at the palm ($p < .001$) and forearm ($p < .001$).

Comparing the summation for the 5th pain rating (P5) minus P1 by site, the method x site interaction term was not significant ($p = .248$); indicating that summation was not dependent upon site. There was a main effect of method ($F_{1,146} = 28.835$, $p < .001$) and site ($F_{1,134} = 4.370$, $p = .038$). Inspection of the marginal means indicated that the summation ($P5 - P1$) for CC stimulation was lower (less sensitization) than for IC stimulation, at each site. In addition, when collapsed across method, summation was lower at the palm compared to the forearm (mean = 10.7 and 13.9 respectively). Table 1 presents the frequencies of positive slopes for each stimulation methodology by site. McNemar Tests indicated that for $P5 - P1$ resulted in a greater frequency of positive summation profiles for the IC method than CC at the palm ($p < .001$) and forearm ($p = .001$).

Lastly, when comparing summation for the maximum pain rating (PMax) minus P1 by site, the method x site interaction term was not significant ($p = .101$); indicating that summation was not dependent upon site. A main effect existed for method ($F_{1,123} = 58.125$, $p < .001$) but not sites ($p = .167$). Inspection of the marginal means indicated that summation ($PMax - P1$) for CC stimulation was lower (less sensitization) than for IC stimulation, at each site. McNemar Tests indicated that for $PMax - P1$ there was a no difference in the number of

positive summation profiles between stimulation methods at the forearm ($p < .001$) but not the palm ($p = .109$).

Looking across scoring methods (P10-1, P5-P1, PMax-P1) within each method of stimulation, TSSP scores were highly correlated at $p < .001$ (CC: Palm $r = .86$ to $.74$, at Forearm $r = .91$ to $.83$; IC: Palm $r = .96$ to $.77$, Forearm $r = .98$ to $.78$). However, across stimulation methods (CC, IC), TSSP scores were not associated (Palm, P10-P1, $r = .20$, P5-P1, $r = -.03$, PMax-P1, $r = .19$; Forearm, P10-P1, $r = .03$, P5-P1, $r = -.03$, PMax-P1, $r = .10$, all at $p > .05$) suggesting that TSSP tested by each method of stimulation are influenced by different factors.

4. Discussion

Previously, psychophysical studies have demonstrated variable increases in TSSP pain ratings[1];[9; 31]. This issue has led to attempts to maximize TSSP scores through modification of stimulation techniques (i.e. fixed temperature vs. individualized temperature protocols)[9; 16], as well as introduction of IC techniques. The current study sought to investigate this issue by directly comparing heat evoked TSSP during IC and CC stimulation of glabrous skin of the palm and hairy forearm skin in healthy pain-free adults. The results indicate that the use of IC stimulation presents greater levels of TSSP under all conditions and quantification methods. The ability to reveal valid TSSP scores within different pain populations versus healthy controls may potentially provide greater understanding of the importance of TSSP for development of clinical pain [1; 7; 38].

Commonly, evoking TSSP has been achieved through the use of a protocol employing 10-CC trapezoidal heat pulses. However, this method has produced results leading to assumptions such as saturation of pain intensity after four pulses[11]. Additionally, individual reports have demonstrated a reduction in pain responses after the first 5 heat pulses in a variety of populations[23]. Accordingly, total summation magnitude has been defined as Pulse Max – Pulse 1 or Pulse 5 – Pulse 1 for this study [1]. This approach fails to demonstrate the true level of second pain summation. Within the current study, IC stimulation by a preheated probe (alternatively known as intermittent tapping) produced greater TSSP than repetitive CC stimulation. Testing site did not differ between glabrous and hairy skin over short series of 5 stimuli (T1-T5) or 10 stimuli (T1-T10), comparing the initial and the maximum pain ratings in series of 10 stimuli (T1-TMax), and comparing the slopes of ratings across 10 stimuli.

Differences between stimulation of glabrous skin of the hand or hairy forearm skin were not found despite differences in various sensory receptor distributions and activation patterns across both glabrous skin hairy skin[14; 21; 40]. Initially it must be noted that this study optimized the testing temperature for each subject and stimulation site to maximize the TSSP effect. This action alone may “wash out” differences seen across testing sites. Although differences in sensory receptor distribution exists across sites, optimizing the temperature at each site may compensate for these distribution differences. This optimization technique is supported by previous work; noting that thermal stimulation designed to elicit similar psychophysical ratings required higher nominal thermal

temperatures for glabrous skin when compared with hairy skin[12]. However, employing the use of temperature optimization for testing may in effect eliminate the ability to detect site differences as theoretically expected. Importantly, the lack of a site difference in the current study is also consistent with our earlier study where site differences were found between the palm and forearm for the temperature for first pain threshold but not second pain threshold[5].

With previous attempts to assess TSSP using CC stimulation, pain ratings have not reliably increased with each repetition of the stimulus[13; 34; 36; 37; 39]. This difficulty was apparent in the present study, directly comparing the reliability of increases in ratings for each method. The reliability of increased second pain intensity with each successive thermal pulse was high for IC (96% and 95%) when compared to CC (70% and 67%) stimulation. Across sites, the percentage of positive progressions through 10 stimuli for IC was 95.5%, compared to 68.5% for CC pulses. As a result, the slopes of rating increases across sites and 10 stimuli averaged 30.5 for IC and 8.5 for CC. The plots of ratings across 10 stimuli (Figure 2) for CC are notable for a plateau beyond the third stimulus, compared to a nearly linear increase in pain for IC. These results fall in line with other investigations utilizing preheated, IC stimulations. For example, TSSP has shown a consistent rise across 10 stimuli through IC stimulation in both older and younger participants across genders, as well as in persons with fibromyalgia[22; 35; 36].

4.1 Mechanisms of action

When investigating TSSP, several features of CC stimulation are concerning. First, ramping up to a nociceptive temperature from a neutral temperature preferentially activates myelinated (A-delta) nociceptors[15; 24; 42; 46], whereas TSSP depends upon activation of C nociceptors[11; 17; 19; 20; 27]. Second, activation of A-delta nociceptors is inhibitory to C nociception at spinal[18; 33; 42] and cortical[43] levels of nociceptive processing. Therefore, ramping thermal stimulation elicits first pain with possible suppression of subsequent second pain in a temporal summation paradigm. In contrast, slow passive distribution of heat to deep layers of the skin following IC stimulation is an effective natural stimulus for unmyelinated, but not for myelinated nociceptors[41; 47]. Substantial summation of late sensations can progress from warmth to VAS ratings of 80 over a series of 20 stimuli[41], when first pain appears late or not at all in series of IC stimuli.

Preferential activation of C nociceptors by intermittent contact likely accounts for high rates of temporal summation with this technique. In contrast, plateaus during series of ramping stimulation[11] appear to reflect inhibition of second pain by input from myelinated nociceptors. Consistent with this possibility, blockade of input from A-delta nociceptors enhances TSSP with ramping stimulation[27]. In the present study, subjects were more sensitive to a ramped 0.8 sec stimulus than to the same duration of IC. Lower ramping temperatures were rated at an eVAS of 25. Accordingly, the first ramping stimulus in the repetitive series on glabrous and hairy skin was rated higher than the initial tapping stimulus in the temporal summation series. If this enhanced sensitivity were specific to C nociceptors, it would have increased the amount of TSSP throughout the series[24]. However, the amount of TSSP was less for CC than IC on glabrous and hairy skin, with the last CC stimulus

producing lower VAS pain ratings than the 10th IC stimuli at both sites. Thus, the TSSP of normal subjects using the standard method of CC stimulation, is both variable and troublesome; thereby raising concerns about the extent to which TSSP is altered among clinical populations. This assertion is supported by the lack of association between CC and IC scores in this study.

A common hypothesis for clinical studies of TSSP has been that central sensitization by NMDA activation has somehow become hyper-responsive[45]. Confirmation of this expectation, that central NMDA activation can pathologically increase pain more than normally, would be provided if the slope of TSSP from IC stimulation is associated with hyperalgesia. This result has yet to occur in research; and evidence linking abnormal temporal summation of thermal pain to clinical pain conditions is lacking. For example, pharmacological blockade or interference with NMDA activation does not differentially reduce TSSP for thermal stimulation of patients with chronic, widespread pain, relative to normal controls[28; 30; 37]. However, the slope of TSSP for repetitive pressure stimulation of a muscle is dramatically increased for individuals with fibromyalgia[34]. Deep muscular stimulation directly activates the C nociceptors that are sensitized by fibromyalgia (i.e. primary hyperalgesia)[44], compared to thermal stimulation, which activates spinal neurons receiving convergent input from the sensitized muscle nociceptors (i.e. secondary hyperalgesia). Thus, the hypothesis that central sensitization can account for clinical pain independent of peripheral sensitization of the C nociceptors that drive TSSP remains unproven. Increased sensitivity to a given thermal stimulus intensity increases ratings of the first of these stimuli in a repetitive series and increases the overall amount (but not the rate) of summation. For example, ratings of the first stimulus in a repetitive series of IC stimuli are elevated for subjects with fibromyalgia, but the slope of temporal summation matches that of control subjects[10]. A result such as this represents sensitization (increased pain sensitivity) but does not indicate that the NMDA-dependent process of TSSP is altered.

4.2 Limitations and Strengths

The current study directly compares evoked heat pain through the use of IC and CC stimulation. A potential study limitation results from dissimilar thermode size, consequently spatial summation may play a role in the findings. Nevertheless, we have previously reported similar TSSP using IC stimulation with a 24mm thermode compared with the current 40mm version[4]. The current study also fails to address issues such as day-to-day variations in TSSP, pain sensitivity and ongoing pain levels of normal subjects and individuals with different clinical conditions. However, strengths of this study comparison include a within-subjects design isolating the influence of each testing protocol on pain intensity. The use of young healthy subjects provides a clear normative picture of TSSP characteristics within upper-limb testing sites. Additionally, the results of this study parallel previous reports on TSSP, increasing validity for the use of IC stimuli to effectively evoke TSSP[41]. Additionally, previous concerns of mechanical sensory influence and changing skin temperatures inhibiting TSSP pain scores[9; 20] are satisfied within the presented results.

4.3 Conclusions

The current study compared heat evoked TSSP during ramp and hold constant contact and tapping, intermittent contact with the glabrous skin of the palm and hairy forearm skin. While previous concerns have been raised about intermittent, tapping protocols, the results of the current study clearly demonstrate a superior effectiveness of intermittent tapping protocols for evoking heat TSSP. Comparison of previous reports support this claim, and when put together, suggest constant contact ramping protocols underestimate TSSP. Attenuated TSSP may be a product of greater A-delta nociceptor activation; which, in turn increases central inhibition of C-nociceptors. This is consistent with the lack of significant association between CC and IC scores as supports that each are influenced by different factors. To evoke optimal TSSP, we suggest that future studies investigating TSSP, particularly among clinical populations, should consider implementing intermittent contact protocols.

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Highlights

- Direct comparison of temporal summation of second pain stimulation techniques.
- Constant contact ramping protocols may underestimate temporal summation.
- Models using temporal summation should consider intermittent tapping techniques.

Figure 1.

Graphical representation of the experimental setup including **A)** the limbs associated with familiarization and then experimental testing trials; **B)** location of the sites tested; **C)** stimulation profile associated with intermittent, tapping stimulation; **D)** stimulation profile associated with constant contact, ramping stimulation; and lastly **E)** the timing associated with each individual heat stimulus and of second pain ratings.

Figure 2.

Figure representing the average TSSP pain ratings across ten pulses for each site and methodology for all trials.

Table 1

Frequencies of positive and zero/negative slopes and mean (standard deviations) for thermode testing temperature by stimulation, method, and site

Site	Palm		Forearm	
Method	Constant Contact (CC)	Intermittent Contact (IC)	Constant Contact (CC)	Intermittent Contact (IC)
	% (n) or mean ($\pm$ SD)	% (n) or mean ($\pm$ SD)	% (n) or mean ($\pm$ SD)	% (n) or mean ($\pm$ SD)
Testing temperature ^a	47.7 (2.1)	49.0 (3.2)	47.1 (2.1)	49.4 (2.6)
1 st Testing temperature ^b adjustments	15% (n=13)	10% (n=9)	9% (n=8)	12% (n=10)
2 nd Testing temperature ^c adjustments	8% (n=7)	0% (n=0)	1% (n=2)	1% (n=2)
Frequency of positive profiles				
P10 - P1	69% (n=54)	95% (n=75)	67% (n=53)	95% (n=75)
P5 - P1	71% (n=56)	90% (n=71)	70% (n=55)	95% (n=75)
Pmax - P1	89% (n=70)	96% (n=76)	76% (n=60)	98% (n=77)
Slopes ^d				
P10 - P1	10.1 (21.8)	29.5 (19.0)	6.8 (27.6)	31.5 (21.0)
P5 - P1	6.0 (14.3)	15.4 (12.2)	7.4 (21.4)	20.4 (16.0)
Pmax - P1	17.1 (15.5)	30.7 (17.3)	17.9 (18.6)	33.1 (19.0)
Percentage increase				
T1 - T10	87%	433%	33%	266%
T1 - T5	76%	227%	36%	171%
T1 - TMax	147%	444%	88%	279%

^aTesting temperature was pre-established for each individual based on an eVAS rating of 25 out of 100.

^bPercentage and number (n) of subjects who needed a temperature adjustment.

^cPercentage and number (n) of subjects who needed a second temperature adjustment.

^dIncludes all positive, zero and negative trials.