ABSTRACT: Transient decrease in the excitability of a reflex circuit following its activation by appropriate stimuli is a well-recognized phenomenon, but it is unclear how this applies to thermoalgesic stimuli during quantitative sensory testing (QST). We examined the effects induced by a thermoalgesic (conditioning) stimulus on the response to a subsequent (test) stimulus of the same characteristics. All tests were done using a Peltier thermode with a surface area of 12.5 cm² using ramp rates of 2°C/s and variable interstimulus intervals (ISIs) ranging from 10 to 60 s. Perception was measured with an electronic visual analog scale. No changes were observed in latency of pain perception. However, latency of warm perception was significantly delayed and pain perception intensity was significantly reduced with respect to conditioning stimuli at ISIs below 60 s. Our results indicate a transient saturation of warm and heat pain perception systems after a thermoalgesic stimulus. We therefore recommend that time intervals of >1 min be used between two consecutive thermoalgesic stimuli when examining QST.


TRANSIENT DECREASE OF SENSORY PERCEPTION AFTER THERMOALGESIC STIMULI FOR QUANTITATIVE SENSORY TESTING

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Quantitative sensory testing (QST) of warm and heat pain sensations is often used in clinical practice for functional evaluation of small peripheral nerve fibers and their central nervous system projections.4,26 In the method of limits,7 the conventional assessment of warm and heat pain sensory perception is done by applying a Peltier thermode over relevant areas of the skin, and requesting the subject to press a switch to mark when the sensation is perceived. In clinical practice, assessment of sensory thresholds with the method of limits is done by repeating the same stimulus a few times for consistency of the results.4,29,31 Usually, sensory perception changes slightly from one stimulus to the next, even in cooperative subjects. These small variations are attributable at least in part to oscillations in attention and affect,15,19,28 but transient refractoriness of the system could also theoretically have some effect if the interval between stimuli is short enough.

Although it is relevant for a better physiological understanding of the effects of warm and heat pain stimuli, the temporal profile of refractoriness in the perception system has not been properly analyzed. We reasoned that, as in many other physiological systems, the application of a thermoalgesic stimulus should be followed by transient refractoriness of the sensation induced by a subsequent stimulus of similar characteristics, as is the case for other forms of sensory processing.3,6,22,25 The aim of our study was to define the time course of recovery of sensory perception after thermoalgesic stimuli, using variable interstimuli intervals (ISIs).
MATERIALS AND METHODS

The study was carried out in 22 healthy subjects, 11 women, aged 21–43 years. Subjects who agreed to participate in the experiment were invited to undergo the thermoalgesic stimulus so that they could be confident of tolerating the stimulus when doing the actual recording. The study was approved by the ethics committee of the Hospital Clinic of Barcelona and accorded with the Helsinki Declaration, and all subjects gave their written informed consent.

Thermoalgesic stimuli were delivered through a Peltier thermode with a surface area of 12.5 cm² (Thermotest; Somedic, Stockholm, Sweden) controlled by Stim-it software. Baseline temperature was always set at 31.5°C, and ramp rate was fixed at 2°C/s. We first determined the individual warm and heat pain thresholds, using the method of limits and standardized procedures.²,²³ The thermode was then attached with a Velcro strip to the ventral aspect of the subject’s left mid-forearm. Conditioning and test stimuli were delivered through the same thermode, using ISIs of 10, 20, 40, and 60 s from the end of conditioning to onset of test temperature stimuli. Peak temperature was set according to each individual’s pain threshold. We used subthreshold intensity (80%) in one session and suprathreshold intensity (120%) in another. The order of the two sessions was randomized and they were always separated by at least 1 day of rest. Three trials were applied for each ISI, at slightly different sites of thermode contact in the forearm, and separated by at least 5 minutes.

Psychophysical assessment was made using an electronic visual analog scale (VAS) made from a 10-cm linear analog potentiometer (RSA0N11S9002; Alps, Munich, Germany). This device was installed in a metallic box where it could be activated by a lever. We marked seven labels on the side of the lever: no temperature sensation; light warm; medium warm; high warm; light pain; medium pain, and high pain. Subjects were requested to pay attention to the thermal sensation and to avoid speaking during the experiment. Care was taken to ensure that the subjects were awake with eyes open during the whole session. They were instructed to move the lever with their right hand as soon as they felt any change in temperature, and to keep marking the changes in the intensity of their sensations until the stimulus was over. The lever could be moved without resistance along its course, and the use of intermediate positions was encouraged. Signals from the lever were recorded together with the temperature signal generated by the Thermotest during the entire trial. The signals were digitized at a sampling frequency of 200 Hz and fed into a computer equipped with software for off-line analysis (Acknowledge; Biopac Systems, Bionic Iberica, El Masnou, Spain).

**FIGURE 1.** Latency measurements of psychophysical events with respect to onset of temperature change (time 0): (a) warm onset; (b) pain onset; (c) MaxVAS-onset; (d) MaxVAS-dur; and (e) MaxVAS-level.

Data Reduction, Measurements, and Statistical Analysis. In all trials, we measured time variables with respect to onset of temperature change (time 0), as the moment when subjects marked the following psychophysical events (Fig. 1): first perception of light warm (warm onset); first perception beyond high warm (pain onset); highest VAS score (MaxVAS-onset); and onset of the descent after reaching maximum (MaxVAS-end). We calculated the duration of maximum perception (MaxVAS-dur) by subtracting MaxVAS-end from MaxVAS-onset. The corresponding thermode temperature was noted at each psychophysical event. We also assessed the relative intensity of the sensation by measuring the individual’s VAS score at MaxVAS-onset (MaxVAS-level), as a percentage of the maximum possible VAS lever displacement. We calculated the mean value of the responses to the conditioning and test stimuli grouped according to ISI and subject. For each subject we ended with five descriptors of VAS for the responses to each stimulus: four time variables (warm onset, pain onset, MaxVAS-onset, and MaxVAS-end) and one magnitude variable (MaxVAS-level). Normality of distribution of the data was assessed using the Kolmogorov–Smirnov test. Statisti-
tistical analyses were done with a paired Student’s t-test for group comparisons between data obtained in the same subjects for sub- and suprathreshold conditions. Repeated-measures one-factor analysis of variance (ANOVA) was used to determine the effects of ISI as an independent variable on VAS descriptors. Bonferroni’s post hoc test was used to explore the nature of significant effects found by ANOVA. The level of significance was set at $P = 0.05$.

**RESULTS**

Mean data are summarized in Table 1 for VAS descriptors measured in the responses to conditioning and test stimuli, for both sub- and suprathreshold stimulus conditions. Because there was no occurrence of pain sensation in the subthreshold stimulus, no values are given for variables related to pain in this condition.

Warm onset in response to conditioning stimuli was not different between sub- and suprathreshold stimulus conditions ($t$-test; $P = 0.6$). As expected, MaxVAS-level was higher in the suprathreshold stimulus condition (mean 98.52 ± 2.2% vs. 52.02 ± 1.8%; $t$-test; $P = 0.001$). A few subjects reported a ceiling effect in their marking of MaxVAS during suprathreshold stimuli. Responses to test stimuli were different from those to conditioning stimuli.

Statistical analysis showed that ISI accounted for significant differences in a number of VAS descriptors in both stimulus conditions. In the subthreshold stimulus condition, significant effects of ISI were seen in warm onset ($F[4, 105] = 13.8, P = 0.001$) and MaxVAS-level ($F[4, 105] = 27.5; P = 0.01$). In the suprathreshold stimulus condition, significant effects of ISI were seen in warm onset ($F[4, 105] = 20.8; P < 0.001$), MaxVAS-level ($F[4, 105] = 22.0; P = 0.02$), MaxVAS-end ($F[4, 105] = 10.1; P = 0.002$), and MaxVAS-dur ($F[4, 105] = 16.6; P < 0.001$). Figure 2 shows representative recordings of the paired suprathreshold stimulus condition in all ISIs.

Bonferroni’s post hoc analysis indicated that warm onset was delayed and MaxVAS-level was lower at ISIs of 10, 20, and 40 s for both sub- and suprathreshold stimuli ($P < 0.05$ for all comparisons). In the suprathreshold stimulus condition, MaxVAS-end and MaxVAS-dur were delayed at ISIs 10, 20, and 40 s ($P < 0.02$ for all comparisons).

No significant effects of ISI were found on pain onset ($F[4, 105] = 0.08; P = 0.9$) and MaxVAS-onset ($F[4, 105] = 0.17; P = 0.2$) in the suprathreshold stimulus condition, nor on MaxVAS-onset ($F[4, 105] = 0.35; P = 0.1$), MaxVAS-end ($F[4, 105] = 0.09; P = 0.09$), and MaxVAS-dur (ANOVA; $F[4, 105] = 0.80; P = 0.1$) in the subthreshold condition.

**DISCUSSION**

Our results indicate that a single stimulus with a Peltier thermode induces a consistent delay in the
perception of warm sensation to a subsequent stimulus applied at intervals of <60 s. Therefore, an immediate implication of these results is that it would be appropriate in clinical practice to allow for a resting interval of >1 minute between two successive stimuli in QST measurements. A longer ISI would require a longer time for QST assessment in clinical practice. This may lead to decreased levels of attention, but would avoid residual effects of the preceding stimulus on subjective perception of thermal sensation. We have been unable to find in the reviewed literature, or even in instruction manuals from the most commonly used QST devices, any specific recommendation for the minimum ISI required to avoid the influence of effects carried out by the preceding stimulus. Some investigators utilized ISIs between 20 and 30 s to assess heat pain thresholds in thenar eminence and averaged the results. According to our results, such stimulus repetition would not have caused a significant effect on heat pain thresholds, but warm thresholds would have been increased significantly.

We did not specifically evaluate how possible subgroup differences in thermal thresholds related to age or gender could affect recovery of thermal perception. It is known that thermal thresholds remain relatively stable in adult humans up to the age of 60 years.12 Thermal thresholds in our subjects, aged 21–43 years, showed little dispersion and, therefore, we did not carry out a subgroup analysis of age differences. We also did not perform a subgroup analysis of gender differences. Whether the thresholds for men and women are different is debatable and, according to Riley et al., a minimum sample size of 41 per group would be needed to guarantee adequate statistical power.

The transient decrease in the efficacy of input transmission after a thermoalgesic stimulus may be just one aspect of the habituation phenomenon that takes place if a subject is repeatedly presented with a stimulus. This is probably related to the reduced levels of selective attention and arousal that occur after a series of thermal stimuli. It is also possible that the conditioning stimulus causes refractoriness in the receptors mediating warm sensation or subsequent changes in perception, such as those related to context updating and context closure. The delay in perception of warm sensation occurred in both sub- and suprathreshold stimulus conditions, indicating that it is independent of whether heat pain receptors are stimulated. This could be an intrinsic feature of the warm, but not pain perception system, because, at the intervals tested in our study, the delay was only significant for perception of warm sensation. In fact, studies of refractoriness of Aδ-nociceptor afferents, involving application of two consecutive pinprick laser stimuli, have shown full recovery of the cortical evoked potentials at intervals beyond 2 seconds.

Interestingly, whereas pain onset did not change significantly in our study, the pain intensity, revealed by MaxVAS-level, was reduced in the responses to test stimuli in comparison to those to the conditioning stimuli. This dissociation suggests that there is a different physiological mechanism accounting for heat pain perception threshold and intensity of sensation. Heat pain perception threshold depends on Aδ-mediated nociception, whereas heat pain intensity may be also influenced by C-fiber-mediated nociception. Therefore, the differences in warm perception threshold and heat pain sensation intensity between conditioning and test stimuli found in our study could be due to refractoriness in circuits mainly dependent on C-fiber receptors.

Our finding of a decrease in the perception of pain intensity in test stimuli is opposite to the pain amplification reported in studies of temporal summation. Such an effect would have required a stimulus frequency of >0.3 Hz. However, the fact that MaxVAS-dur was longer in test than conditioning stimuli may indicate a longer period of aftersensation. Both the aftersensation phenomenon and temporal summation may reflect enhancement of neuronal discharges in wide-dynamic-range neurons and are found to be altered in neuropathic pain states.

We conclude that after a thermoalgesic stimulus there is a transient delay of warm perception and a
transient decrease of pain intensity perception. This is probably due to a refractoriness in the pathway of the C-fibers carrying inputs from both warm and heat pain receptors. This effect declines progressively and is practically overcome at about 60 s after the end of the stimulus. Our results suggest that ISIs of >1 min should be used between consecutive thermoalgesic stimuli during QST assessment of small-fiber function. Further studies are needed to assess transient changes in perception using different thermode sizes and body regions.

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