The Influences of Inter-Trial Interval on the Thermal and Thermal Pain Thresholds in Quantitative Sensory Testing

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Abstract-

Purpose: Thermal threshold and thermal pain threshold determinations are part of quantitative sensory tests. Usually the average of many repeated trials is defined as the threshold. The inter-trial interval (ITI) may have an effect on the later trials. The main purpose of this study was to investigate the influence of ITI on the test results.

Methods: Forty-two healthy subjects were recruited. The limit method was adopted. Each subject received 4 sessions of testing, with one ITI equal to 10, 20, 40 and 60 seconds, respectively. In each session, all four modalities (warm and cold thresholds and cold and hot pain thresholds) were performed. Thermal thresholds were the averages of four trials and thermal pain thresholds were the averages of three trials. The ITI order and the modality order were pseudo-randomized. Analyses of variance were utilized to test the influence of ITI, the modality order and the trial order.

Results: The results implied that the test results by the limit method are independent of ITI in the range of 10 to 60 seconds, but the results may not be the true thresholds.

Conclusion: The results showed that while ITI and the modality order did not have significant effects on the test results, the trial order did have effects on the results of all modalities.

Key Words: quantitative sensory test, inter-trial interval, thermal pain, thermal threshold, pain threshold

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INTRODUCTION

Quantitative sensory testing (QST) is a noninvasive technique to evaluate thermal thresholds and thermal pain thresholds. QST, a psychophysical examination equivalent to audiometry in hearing evaluation, requires alertness and the cooperation of the subjects. Over the past few decades QST has been widely used in detecting sensory deficits resulted from small nerve fiber injury, especially in patients with normal routine nerve conduction studies [1]. QST has been proven to be a useful tool in examining and monitoring diabetic polyneuropathy [2,3], and correlates well with clinical symptoms. Recently, the role of QST is heightened in surveys about neuropathic pain for its ability to capture and quantify stimulus-evoked negative and positive sensory phenom-

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ena [4,5]. Some newly-developed standard protocols and normative data are available to facilitate the clinical applications of QST [6].

The limit method is the most commonly used 'reaction time-inclusive' paradigm in computerized QST processing [7] and is practiced by applying a decreasing or increasing ramp stimuli starting from the baseline temperature, and asking the individual to report when the warm or cold sensation is first detected. In using the limit method, the clinicians usually noted that the thermal threshold changed slightly from one stimulus to the next, even in cooperative subjects. These small variations were thought to be mainly related to the fluctuation of attention and concentration [1].

Thresholds are usually defined as the average of a series of trials. Topographic difference of body parts, the rate of temperature change, the size of thermode and the interval between consecutive trials are known to influence the results of thermal thresholds [8]. However, there is no concrete data about the impact of inter-trial intervals (ITI) in QST. A study [9] indicated that the latency of warm perception was delayed and pain perception intensity was reduced after a conditioning stimulus at ITI below 60 seconds. Based on these results the authors suggested that the ITI for QST should be greater than 60 seconds. However, the experiment's procedures were different from the ones commonly implemented in clinical practice.

The main goal of this study was to investigate the effects of ITI on QST results under procedural conditions that are commonly implemented in clinical practice.

METHODS

Forty-two healthy subjects, aged 23-48 years and equal in number of males and females, were recruited for this study. Inclusion criteria included clear consciousness with good cooperative attitude. Exclusion criteria included a medical history of neurological diseases such as stroke, myelopathy and peripheral neuropathy or systemic diseases such as diabetes and renal and hepatic diseases. No subject had the prior experience of similar QST in the past. A brief history taking and complete neurological examination was performed by one of the staff physicians (HW Huang and CCK Lin) on each subject to ensure the absence of past neurological events and current neurological deficits. The study protocol was approved by the NCKUH ethics committee on human subject study. Before an experiment, the purpose, the potential hazards and the procedure of the experiment were fully explained to the subject. A written informed consent was provided by each participant. The body weight and body height were first measured, and QST of four limbs was performed.

In our routine QST, the test is performed in an isolated room with room temperature control. All four modalities, warm threshold (WT), cold threshold (CT), hot pain (HP) and cold pain (CP), were evaluated sequentially in a session. The subject sat comfortably in an armchair in front of a personal computer. All four modalities were evaluated with a sensory and pain threshold evaluation system (Pathway, Medoc Advanced Medical Systems, Ramat Yishai, Israel) following the established standard protocols. The test thermode (3×3 cm) was secured on the target skin surface with a band with minimal tension. The test sites were the dorsal surfaces of the hand and foot. We adopted the limits method (modified Marstock method) as the test algorithm, in which the machine delivered changing temperature stimuli at a constant rate (1˚C/sec) starting from the baseline value of 32˚C. The temperature was increased to detect WT and decreased to detect CT. When the stimulus was perceived, the subject immediately pushed the left mouse button with the right hand, and the machine stopped delivering stimuli. If the button was not pushed and the temperature reached the predefined limits (0 or 50˚C), the machine terminated the trial and returned the temperature to the baseline automatically. The average of 4 successive trials was defined as the thermal threshold [10-12]. HP and CP were obtained similarly, except that the subject pushed the left mouse button to stop the temperature change when pain was first perceived, the ramping rate was 1.5 ˚C/sec, and 3 trials were averaged to obtain the thermal pain thresholds.

In this study, because the main goal was to investi-

Table 1. The effects of gender, age and limb on the thermal and thermal pain thresholds

	UE [#1]	LE	P_{Gender} [#2]	$P_{U/L}$	P_{Age}
HP [#1]	44.1±1.7	44.4±1.8	<0.001	0.36	0.04
	44.0±2.1	43.9±3.1			
WT	36.3±1.7	37.5±1.7	0.38	<0.001	<0.001
	36.3±1.7	37.5±1.7			
CT	28.1±1.6	27.4±1.8	0.05	0.66	<0.001
	27.9±1.8	27.2±2.0			
CP	8.8±5.5	9.1±5.4	0.39	0.12	<0.001
	10.6±5.3	10.8±5.7			

[#1]HP: heat pain, WT: warm threshold, CT: cold threshold, CP: cold pain and UE and LE: upper and lower limbs, respectively. Upper and lower rows of each item are for men and women, respectively.
[#2]PGender: p value for the factor gender, $P_{U/L}$: p value for the factor of upper versus lower limbs and P_{Age}: p value for age.

gate the effects of ITI, one of 10, 20, 40 and 60 seconds was chosen as the ITI and kept constant in a session. The interval between consecutive modalities was identical to ITI. A subject had to receive 4 sessions, each with a different ITI. Consecutive sessions were separated by 5 minutes. While the modality orders in the four sessions were identical for a subject, the modality order and ITI order were pseudo-randomized among subjects. One-way ANOVA with ITI as the independent variable was performed first. In this analysis, the test results (dependent variables) were the mean of the repeated trials in a session. The linear correlation between ITI and the test results were evaluated through the slopes in the linear regressions. In the next step, two-way repeated measures ANOVA with ITI and the trial order as the independent variables were performed. In this analysis, the raw test results were used. The purpose of this step was to investigate the effects of trial order. In the third step, two-way ANOVA with ITI and the modality order as the independent variables were performed. The purpose of this step was to assess the effects of modality order. In the fourth step, three-way repeated measures ANOVA with ITI, modality order and trial order as the independent variables was performed. In all steps, probabilities of $P < 0.05$ were considered to be statistically significant. All statistical analyses were performed by using the software Statview (SAS Institute Inc., Cary, NC, USA).

RESULTS

In total, 42 healthy subjects were recruited (M/F: 21/21), with age between 20 and 50 year-old (35±7.4). The effects of gender, age and (upper and lower) limb on the thermal and thermal pain thresholds, by analysis of covariance, are shown in Table 1. Hot pain threshold is influenced by gender, while warm threshold is different between upper and lower limbs. In consistence with our previous report [13], age has effects on all four modalities. In this study, the effects of age and gender were minimized by randomization of subjects.

One-way ANOVA with ITI as the single independent variable showed that ITI was not a significant factor for all thermal and thermal pain thresholds (Table 2). Respective linear regressions with each threshold as the dependent variable and ITI as the independent variable were performed and the results showed that no tendency (slope) was significant for any threshold. The results of

Table 2. Group means and p values of ITI (unit: second)

	10	20	40	60	p
HP_U [#1]	43.8±1.8	43.9±1.9	44.2±2.0	44.1±1.9	0.74
WT_U	36.1±1.6	36.3±1.9	36.4±1.7	35.8±1.7	0.51
CT_U	28.2±1.5	27.7±1.9	28.1±1.7	27.6±1.7	0.19
CP_U	10.4±5.7	9.6±5.1	10.0±5.6	9.1±5.4	0.78
HP_L	44.2±2.0	44.1±1.8	44.1±4.1	44.2±1.7	0.99
WT_L	37.6±1.8	37.6±1.9	37.6±1.7	37.1±1.7	0.61
CT_L	27.3±1.8	27.2±2.0	27.3±2.0	27.9±1.8	0.44
CP_L	9.6±5.3	9.8±5.9	10.1±5.5	10.9±5.8	0.83

[#1]Suffix U and L for upper and lower limb, respectively.

Table 3. Results (*p* values) of one-way repeated measures ANOVA

	ITI	Trial order
HP_U	0.68	<0.001
WT_U	0.84	<0.001
CT_U	0.14	<0.001
CP_U	0.50	<0.001
HP_L	0.99	<0.001
WT_L	0.99	<0.001
CT_L	0.96	<0.001
CP_L	0.96	<0.001

repeated measures two-way ANOVA with ITI and the trial order as the independent variables are shown in Table 3. While ITI was again not a significant factor for all thermal and thermal pain thresholds, the trial order significantly affected all the thresholds.

Two-way ANOVA with ITI and session order as the independent variables showed that either ITI or session order was not a significant factor for all thermal and thermal pain thresholds. The results of three-way repeated measures ANOVA with ITI, modality order and trial order as the independent variables are summarized in Table 4. For all the thermal and thermal pain thresholds, either the ITI or the modality order had significant effects while the trial order within a session was a significant factor. There was no interaction in independent variables. Plots with the trial order as the single independent variable were shown in Figure 1. For both the upper

Table 4. Results (p values) of three-way repeated measures ANOVA with ITI, modality order and trial order as the independent variables

	ITI	Modality order	Trial order
HP_U	0.93	0.51	<0.001
WT_U	0.96	0.54	0.006
CT_U	0.52	0.08	<0.001
CP_U	0.71	0.81	<0.001
HP_L	0.98	0.29	<0.001
WT_L	1.00	0.54	<0.001
CT_L	0.76	0.85	<0.001
CP_L	0.81	0.47	<0.001

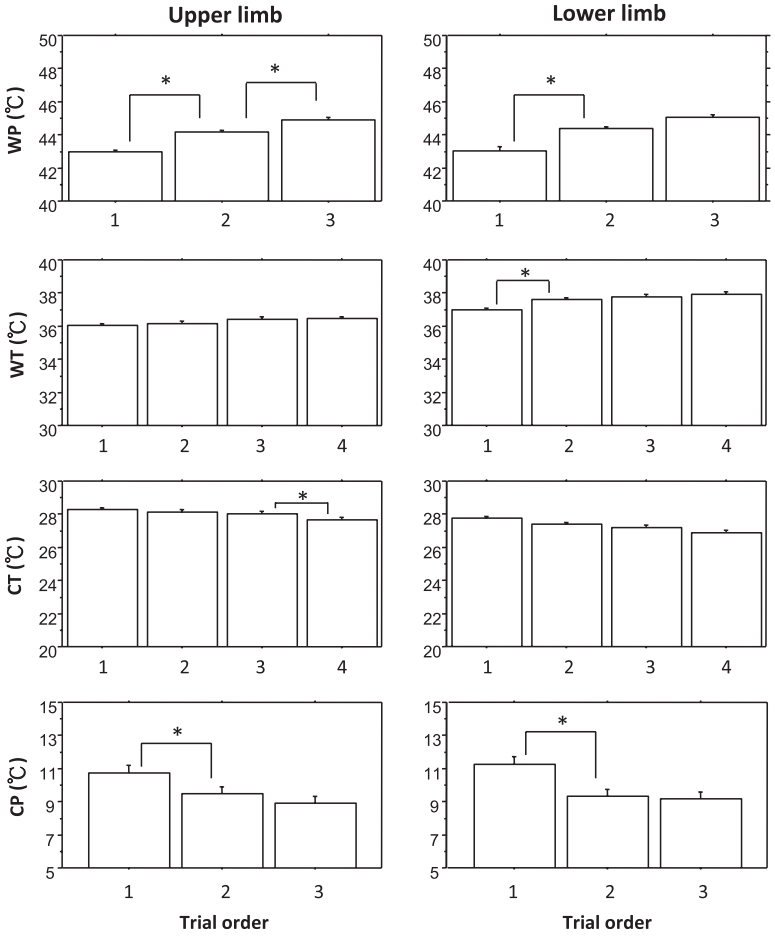

Figure 1. The effects of trial order on the thermal and thermal pain thresholds of upper and lower limbs. The trends were toward larger deviation from the baseline temperature for all the modalities. Asterisks mark a significant difference ($p < 0.05$) by post hoc tests.

and lower extremities, in the later trials, cold threshold and cold pain threshold decreased, while warm threshold and hot pain threshold increased. In summary, the statistical analyses indicated that, when the limit method was adopted, the test results changed with the trial order but not with the modality order or ITI.

DISCUSSION

QST is gradually becoming a clinical routine for diseases of both the central and peripheral nervous systems. However, there are only scarce documented guidelines or objective data to guide the selection of ITI for QST. Many QST studies used ITI in the range of 5 to 10 seconds for thermal thresholds and 10 to 30 seconds for thermal pain thresholds [14-16] and some did not explicitly specify values for ITI [17]. Schestatsky et al. [9] recommended that an ITI of at least 60 seconds should be used between two consecutive thermoalgesic stimuli when performing QST. However, their study protocol was different from the clinical routine in performing QST using the limit method. In addition, the study only investigated the warm threshold and the hot pain threshold. We deliberately designed this study to match the whole procedure of QST in the clinical routine, except the control variables the ITI and the modality order.

The statistical analyses in this study showed that, when the limit method was used, the test results changed with the trial, even when the ITI was 60 seconds. The changes in all thresholds followed a trend toward larger absolute values, i.e., more away from the baseline, with the later trials. This trend might be due to physical factors, such as habituation of perception, or psychological factors, such as reduction of concentration. The post hoc tests (Figure 1) revealed that most of the significant differences (5/7) were between the first and second trials. This trend was compatible with a previous study that investigated habituation in QST [18].

The modality order was also adopted in this study as a control variable to investigate the influence of cross-modality effects. The documented studies about the modality order are again scarce. A study [14] using the limit method reported that the modality order did not influence the test results, except that the thermal thresholds (WT and CT) were higher when performed after the thermal pain threshold determination. In contrary, the modality order in our results did not have effects on the test results. The interval between the modalities was set to be identical to ITI, which changed in sessions from 10 to 60 seconds and was longer than the ones commonly adopted in other studies. We think this might be the reason that the modality order did not have influence on the test results.

Our results about ITI that were needed to obtain trial-interaction free QST results were inconsistent with those of a prior study [9]. There are many possible explanations for this discrepancy. First are the differences in testing procedures. While only two stimuli were given in a session and 2 sessions were repeated in the previous study, 4 stimuli for thermal thresholds and 3 stimuli for thermal pain thresholds were given in a session and 4 sessions were repeated in this study. Second, while the latency of perception was the response variable in the previous study, the threshold was directly the investigated variable in this study.

Because the limit method has been most popularly adopted in clinical routine, our results implied that the thresholds obtained by clinical practice may not be the true thresholds in a strict sense and, in order to obtain QST results devoid of inter-trial interactions, ITI has to be longer than 60 seconds. Currently, the time needed to perform a complete routine QST, including thermal and thermal pain thresholds, using an ITI of 5-10 seconds is approximately 30 minutes. If an ITI longer than 60 seconds is required, the time for completing a QST will be longer than 1 hour, leading to an increased cost and discomfort for the patient. The main practical utilization of QST is in differentiating the pathological state from the normal baseline. Therefore, relative values may be sufficient for this purpose. From this view point, our results that the test results by the limit method were independent of ITI in the range of 10 to 60 seconds instead provided evidence of the robustness of the limit method. However, for some scientific research or other purposes, if absolute determination of thresholds is needed, a longer ITI should be adopted.

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