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Original experimental

The inter- and intra-individual variance in descending pain modulation evoked by different conditioning stimuli in healthy men

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ABSTRACT

Background and purpose: Conditioned pain modulation (CPM) is a phenomenon in which pain is inhibited by heterotopic noxious stimulation. It is not known how the experimental condition affects the magnitude of the CPM response and the inter- and intra-individual variations. It is important to get the information of the test–retest reliability and inter-individual variations of CPM to apply CPM as a diagnostic tool or for screening analgesic compounds. This study evaluated (1) the magnitude of CPM, (2) the inter-individual coefficient of variation (inter-CV) and (3) the intra-individual coefficient of variation (intra-CV) to (A) different stimulus modalities to evoke CPM and (B) different assessment sites.

Methods: Twelve healthy men (age 19–38 years) participated in this study. Cold pressor pain (CPP) (immersing the hand into cold water), tourniquet pain (cuff around the upper arm) and mechanical pressure pain (craniofacial region) were used in randomized order as conditioning stimuli (CS). The test stimulus (TS) was pressure pain applied to the right masseter muscle, left forearm and leg (bilateral tibialis anterior: TA). The responses were pressure pain thresholds (PPT), pressure pain tolerance (PPTol) thresholds and the pain intensity which was assessed on a visual analogue scale (VAS, 0–10 cm) following 1.4 and 1.6× PPT applied to TA. The TS was applied before, during and 10 min after the CS. The intra-individual CV was estimated between different days.

Results: CPP induced the most powerful CPM on PPT (66.3 ± 10.0% increase), VAS ratings (41.5 ± 5.3% reduction) and PPTol (32.6 ± 4.6% increase), especially at TA, and resulted in the smallest inter-CV (41.4–60.1%). Independently of the CS, the inter-CV in general showed that the recordings from the orofacial region and the forearm had smaller values than from the leg. The smallest intra-CV value was obtained in pain ratings with CPP (27.0%).

Conclusions: This study suggests that (1) the CPP evokes the largest CPM, (2) the leg as the assessment site results in the largest CPM responses and (3) the CPP causes the smallest inter- and intra-CV.

Implication: The present investigation implicates that the CPP is the most efficient conditioning stimulus to induce CPM when assessed by pressure pain thresholds.

1. Introduction

Diffuse noxious inhibitory control (DNIC) is a phenomenon in which the activities of convergent neurons (wide dynamic range neuron; WDR neuron) in the spinal dorsal horn and trigeminal nucleus are selectively and powerfully inhibited by the application of a heterotopic noxious stimulation. Such inhibitory phenomena were initially described in animals [1–3] and subsequently in humans [4–8]. It has recently been suggested that the DNIC-like effects in humans should be termed "conditioned pain modulation (CPM)" [9].

It has been reported that there are various methodologies for inducing and evaluating CPM [10]. To estimate CPM effects in healthy subjects, various experimental pain modalities, such as thermal (cold [11,12] and heat [13,14]), electrical [15], chemical [6,7,16] and ischemic [17], have been applied to various body regions. The effects of CPM are known to differ depending on the magnitude and nature of the conditioning stimulus (CS) and the modality of the test stimulus (TS) [10,18,19]. There are indications that the magnitude of the CPM effect is related to the intensity of the CS [3,18–22]. It has been reported that the approximated median magnitude of the CPM effect is 29% [10]. However, in these reports the data are derived from different TS and CS applied to different assessment sites.

Recent research [23] suggests that the evaluation of CPM may identify patients at risk of developing chronic pain. For further

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application of CPM as a diagnostic tool or for screening of analgesic compounds, the test–retest reliability and inter-individual variations of CPM should be determined.

The aim of the present study was to evaluate (1) the magnitude of conditioned pain modulation (CPM), (2) the inter-individual coefficient of variation (inter-CV) and (3) the intra-individual coefficient of variation (intra-CV) to (A) different stimulus modalities to evoke CPM and (B) different assessment sites.

2. Methods

2.1. Subjects

Twelve healthy men (mean ± SEM age: 25.6 ± 1.5 years, age range: 19–38 years) participated in the current study. Besides, one subject withdrew from the experiment because he could not tolerate the cold pressor pain (CPP) (the data from this person were excluded). None of the subjects had current or previous injuries or psychiatric conditions, chronic pain or major medical conditions that could interfere with the normal somatosensory function. Informed consent was obtained from each subject. The study followed the Helsinki Declaration and was approved by the local ethics committee (VN20090047).

2.2. Experimental protocol

The subjects were sitting in a reclined position in a bed. During assessment the leg was positioned below the computer-controlled algometer probe and stabilized in a vacuum-packed kapok-filled pillow. CPP (2–4 °C) to the right hand, ischemic muscle pain (pressure of 36 kPa) to the right upper arm or tonic mechanical pressure to the craniofacial region were applied as CS after 5 min break following TS recording (baseline). Each CS was applied for 10 min, and the three different CS were applied in randomized order on different days separated by at least 2 days. The subjects were asked to rate the pain intensity of the CS continuously on a 0–10 cm electronic visual analogue scale (VAS). In order to test the intra-individual variance, the experiments were repeated on two different days (session I and II) separated by at least 2 days. As TS, pressure pain thresholds (PPT) and pressure pain tolerance (PPTol) thresholds were measured at the right masseter muscles (MAR) and left flexor carpi radialis muscle (forearm) using a hand-held pressure algometer (Somedic, Sweden). PPT and PPTol recorded from the right and left tibialis anterior (TA) were assessed by a computer-controlled pressure algometer (Aalborg University, Denmark) [24]. The mechanical pressures with pain intensities of 1.4 and 1.6× PPT applied to the right and left TA were rated on an electronic VAS (0–10 cm). All parameters were recorded before the application of the CS, during the application of the CS and 10 min after the termination of the CS. PPTs by the hand-held algometer were determined in triplicate, and PPTs by the computer-controlled algometer were determined in duplicate. PPTol was recorded only once each time to avoid excessive stimulation and sensitization.

2.3. Conditioning stimulus (CS)

2.3.1. Cold pressor pain (CPP)

The right hand up to the wrist was immersed into stirred cold water (2–4 °C, measured in the water) for 10 min. All subjects had no previous experience of CPP. Almost all subjects felt severe pain for CPP and could not keep the hand into cold water on the first try. After a brief pause, they were asked to try again until they could tolerate the immersion. The subjects rated the CPP intensity on an electronic VAS (0–10). The TS was applied after the pain intensity on the VAS exceeded 6 and became stable (to show no change in the VAS values for 30 s). When the measurements of all parameters had been finished, subjects withdrew the hand from the cold water.

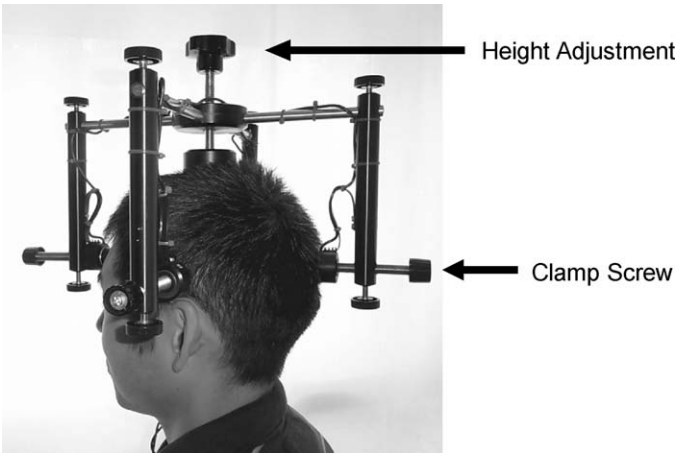

Fig. 1. The compressive device of experimental craniofacial pain. The device was set on the vertex. It was height-adjustable by a downwardly directed screw. Compression of the craniofacial region was achieved by tightening four, horizontally opposed clamp screws with a force transducer.

2.3.2. Tourniquet pain

Ischemic muscle pain was induced by inflation of a 13 cm wide tourniquet applied around the right upper arm (VBM Medizintechnik GmbH, Germany) [25]. The lower rim of the tourniquet was 3 cm proximal to the cubital fossa. The cuff control unit (Aalborg University, Denmark) was programmed to maintain the pressure at 36 kPa (above the systolic pressure) throughout the inflation period of 10 min. After the target pressure was reached, the subject was asked to repeat hand grips 10 times or more until 6 was reached on the VAS. The subjects rated the contraction-evoked pain on an electronic VAS. When 6 was reached on the VAS, all measurements were assessed. The tourniquet was released once all measurements had been finished.

2.3.3. Mechanical pressure on head

Craniofacial conditioning pain was provoked with a specially designed compressive device (head band) (Fig. 1) [26]. Briefly, the model is based on a mechanical compressive device which is positioned over the vertex and can be fastened over the skull by four probes (10 mm radius) each separated by 90° mounted on two clamps. A strain gauge force transducer is attached on the four probes, and pressure can be adjusted over time using the VAS feedback from the subject to maintain the pain intensity at a given level (target level, VAS5).

2.4. Pain ratings

The subjects rated the pain intensity of the three CS on a 0–10 electronic VAS (0 = no pain, 10 = worst pain imaginable), and the ratings were sampled and stored on a computer every 5 s. The pain ratings were recorded from the start of the CS until they returned to zero. The VAS peak pain values were used for further analysis.

2.5. Test stimulus (TS)

2.5.1. Pressure pain thresholds (PPT) and pressure pain tolerance (PPTol) thresholds

The pressure pain thresholds (PPT) and pressure pain tolerance (PPTol) thresholds were recorded from the right masseter muscles (MAR) and left flexor carpi radialis muscle (forearm) by a hand-held pressure algometer. The PPT was defined as the amount of pressure

(kPa) which the subject first perceived to be painful. The algometer probe (1 cm^2 area) was applied with a constant application rate of 30 kPa/s [8]. The subject pushed a button to stop the pressure stimulation when the threshold was reached. The PPT was repeated three times with about 1 min in between, and the average value was used for further analysis. The PPTol was defined as the highest pressure (kPa) the subject could tolerate.

A computer-controlled pressure algometer applied the mechanical stimuli perpendicular to the skin surface of the right and left TA [24]. A round aluminium footplate with a padded contact surface of 1 cm^2 was fixed to the tip of the piston. The pressure stimulation was feedback controlled via a built-in force transducer. The tissue of the TA was compressed against the interosseous membrane of the leg. A mechanical pressure stimulus with an ascending pressure gradient of 0.3 kg/s (30 kPa/s) was applied continuously until the subject felt pain and pressed a button. The PPT was defined as the mean of two trials separated by 10–15 s and used for further analysis. The PPTol was defined as the most painful pressure (kPa) the subject could tolerate.

The PPT and PPTol were recorded before the application of the CS, during the application of the CS, and 10 min after the termination of the CS. Threshold determinations at the right and left TA by a computer-controlled pressure algometer were performed in randomized order. Then thresholds were recorded by a hand-held pressure algometer at the MAR and subsequently left forearm. PPT measurement was followed by PPTol measurement. The measurements always followed the same sequence except the difference of order at the right or left TA (PPT at right or left TA, PPTol at right or left TA, PPT at right or left TA, PPTol at right or left TA, PPT at MAR, PPT at forearm, PPTol at MAR, PPTol at forearm).

2.5.2. VAS ratings of pressure pain on tibialis anterior (TA)

After the recording of PPT and PPTol at TA, each subject scored the pain sensations during the sequential stimulation for 5 s at the intensity of 1.4 and 1.6 times of the averaged PPT for each TA. The scoring was performed continuously during the stimulation on a 10 cm electronic VAS (0 = no pain, 10 = worst pain imaginable). The VAS intensities were sampled at 200 Hz by a computer. The VAS scores over time were split into five epochs (20% of the total duration) from which the maximum score was extracted. The maximum of the extracted value was defined as VAS1.4 and VAS1.6 and was used for further analysis.

2.6. Statistical analysis

The VAS peak pain values were analyzed by two-way ANOVA to test the effect of CS (cold, tourniquet, head band) and the effect of session (session I and II) as the repeated factor. Absolute PPT and PPTol values at baseline were analyzed by three-way ANOVA to test the effects of CS (cold, tourniquet, head band), assessment site (masseter, forearm, right TA, left TA) and session (session I and II) as repeated factors. Absolute VAS1.4 and VAS1.6 ratings at baseline were analyzed by three-way ANOVA to test the effects of CS (cold, tourniquet, head band), assessment site (right TA, left TA) and session (session I and II) as repeated factors. Then the PPT, PPTol, VAS1.4 and VAS1.6 ratings were normalized to the baseline values. The relative changes in PPT and PPTol were analyzed by three-way ANOVA to test the effects of CS (cold, tourniquet, head band), assessment site (masseter, forearm, TA) and time (baseline, during the CS and 10 min after the CS) as repeated factors. The relative changes in VAS1.4 and VAS1.6 ratings were analyzed by two-way ANOVA to test the effects of CS (cold, tourniquet, head band) and time (baseline, during the CS and 10 min after the CS) as repeated factors. The ANOVAs were followed by the post hoc Student Newman–Keuls (SNK) tests to compensate for multiple comparisons.

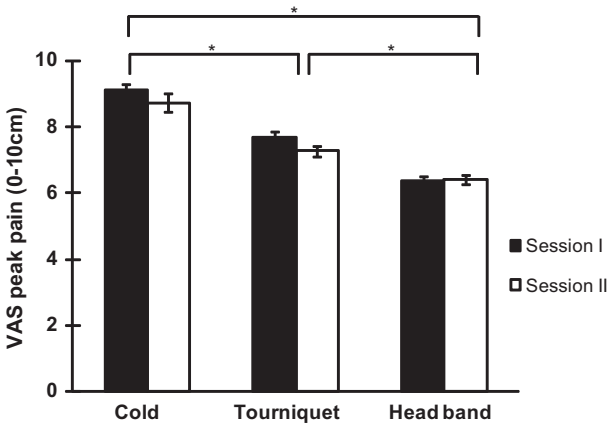

Fig. 2. The visual analogue scale (VAS) peak pain values (mean ± SEM) from three modalities of conditioning stimuli in each session (session I and II). *$P < 0.001$ vs other modalities of conditioning stimuli. There were significant differences among the three conditioning stimuli (cold pain > tourniquet > head band).

The subjects who did not have any inhibitory CPM (i.e., relative decreases in PPT and PPTol values and relative increases in VAS1.4 and VAS1.6 = increases in sensitivity to test stimulus) during the CS were defined as "non-responders". Only data from CPM-responders were included in the analysis. The CPM effect during the CS was also analyzed by Wilcoxon signed-rank test.

Inter-individual coefficient of variation (inter-CV) was calculated as standard deviation/mean × 100 (%). Intra-individual coefficient of variation (intra-CV) was calculated as $CV = \left(\sqrt{\left[\left(\sum d^2\right)/2n\right]}\right)/x \times 100(\%)$, in which d is the difference between two results obtained from one subject, n is the number of subjects, and x is the mean of the results obtained from all the subjects [27]. The number of non-responders was analyzed with the Mann–Whitney U-test to get the information which is more appropriate for TS; PPT or PPTol, and VAS1.4 or VAS1.6.

The correlations between the VAS peak pain values and relative PPT changes during the CS obtained from all responders in all sessions (correlation coefficient; R) were calculated by means of a least-squares regression analysis.

All data are presented as mean values and standard errors of mean (SEM). The level of significance was set at $P < 0.05$.

3. Results

3.1. Pain ratings of the conditioning stimuli

The two-way ANOVA showed that the VAS peak pain values were significantly dependent on the CS ($F = 90.018$, $P < 0.001$) but not on the session ($F = 2.664$, $P = 0.104$). The post hoc tests showed that there were significant differences among the three CPM techniques; significantly higher ($P < 0.001$) in the cold stimulus compared to the tourniquet and head band, and significantly higher ($P < 0.001$) in the tourniquet compared to the head band (cold stimulation: 8.9 ± 0.2, tourniquet: 7.5 ± 0.1, head band: 6.4 ± 0.1) (cm, mean ± SEM) (Fig. 2).

3.2. Test stimulus

3.2.1. Baseline values (Table 1)

The ANOVA indicated an effect of assessment sites on PPT and PPTol values (Table 1). These differences among assessment sites necessitated a normalization of the PPT and PPTol values to directly compare the effects of CPM. As there were no significant differences

Table 1
Baseline values of test stimulus for PPT, PPTol, VAS1.4 and VAS1.6.

Assessment site	PPT (kPa)	PPTol (kPa)	VAS1.4 (mm)	VAS1.6 (mm)
Masseter	201.0 ± 6.1	380.7 ± 20.9	–	–
Forearm	555.4 ± 22.5**	1044.4 ± 46.3**	–	–
Right TA	310.3 ± 18.5*	921.9 ± 42.5*	2.6 ± 0.3	3.0 ± 0.3
Left TA	292.5 ± 16.5*	906.3 ± 45.2*	2.9 ± 0.3	3.3 ± 0.3

* $P < 0.05$ compared to the masseter.
** $P < 0.05$ compared to the masseter, right TA and left TA.

between the right and left TA, the values from both TA were added up and used for further analysis.

Concerning VAS1.4 and VAS1.6, the ANOVA indicated no main effect of CS, session or assessment site. As there was no significant difference between the right and left TA, the values from both TA were added up and used for further analysis. Also the VAS1.4 and VAS1.6 ratings were normalized to directly compare the effects of CPM and used for further analysis.

3.2.2. CPM effect in normalized values

As there were no significant differences between the sessions in baseline values of PPT, PPTol, VAS1.4 and VAS1.6, the data from both sessions were pooled and used for analysis.

Table 2 shows the rank of the CPM effect during the CS on PPT, PPTol, VAS1.4 and VAS1.6. All tests showed significantly positive changes (CPM effects) compared with baseline (Wilcoxon signed-rank test; $P < 0.05$). The cold stimulus induced the most powerful CPM effects on PPT (up to 66.3 ± 10.0% increase), VAS1.4 ratings (up to 41.5 ± 5.3% reduction), VAS1.6 ratings (up to 37.2 ± 4.4% reduction) and PPTol (up to 32.6 ± 4.6% increase). In all the measurements, the largest CPM effect was induced by the cold stimulus followed by the tourniquet and head band. Concerning the PPT, the CPM effect from TA was bigger than the effect from the masseter and forearm. For PPT, VAS1.4 and VAS1.6, the condition with cold hand pain as CS and leg as the assessment site induced the largest CPM effects.

The ANOVAs on the normalized PPT values indicated a main effect of time ($F = 56.009$, $P < 0.001$) with assessment site and time ($F = 11.170$, $P < 0.001$) and CS and time interaction ($F = 5.156$, $P < 0.001$). The post hoc tests revealed that the normalized PPT values were significantly increased during the CS (31.8 ± 2.8%, $P < 0.001$) and 10 min after the CS (12.6 ± 2.1%, $P < 0.001$) compared with baseline values. The increment of normalized PPT values during the CS was significantly higher with the cold stimulus (43.6 ± 6.2%) than with the tourniquet (31.0 ± 3.7%, $P < 0.05$) and head band (20.5 ± 3.4%, $P < 0.001$). The post hoc tests also showed that the increment of normalized PPT values during the CS was significantly higher at TA (46.6 ± 4.7%) compared

Table 2
Rank of the CPM effect on PPT, PPTol, VAS1.4 and VAS1.6.

Rank	Conditioning stimulus	Assessment site	CPM effect (%)	Number and proportion (%) of non-responders
PPT				
1	Cold	TA	66.3 ± 10.0*	0 (0)
2	Tourniquet	TA	43.4 ± 5.8*	2 (8.3)
3	Head band	TA	29.1 ± 5.7*	1 (4.2)
4	Cold	Masseter	23.3 ± 4.3*	1 (8.3)
5	Tourniquet	Masseter	20.7 ± 3.4*	1 (8.3)
6	Cold	Forearm	16.7 ± 2.8*	0 (0)
7	Tourniquet	Forearm	15.1 ± 2.6*	2 (16.6)
8	Head band	Forearm	13.8 ± 4.7*	1 (8.3)
9	Head band	Masseter	10.1 ± 2.7*	0 (0)
*$P < 0.01$ compared with baseline				
PPTol				
1	Cold	Masseter	32.6 ± 4.6*	1 (8.3)
2	Tourniquet	Forearm	24.7 ± 4.9*	5 (41.6)
3	Cold	TA	24.6 ± 2.3*	0 (0)
4	Head band	Masseter	24.4 ± 4.9*	1 (8.3)
5	Tourniquet	Masseter	20.5 ± 3.7*	2 (16.6)
6	Tourniquet	TA	20.2 ± 2.7*	5 (20.8)
7	Cold	Forearm	19.8 ± 2.4*	0 (0)
8	Head band	TA	18.7 ± 4.8*	8 (33.3)
9	Head band	Forearm	15.0 ± 3.4*	3 (25.0)
*$P < 0.05$ compared with baseline				
VAS1.4				
1	Cold	TA	41.5 ± 5.3*	2 (8.3)
2	Tourniquet	TA	26.7 ± 5.1*	4 (16.6)
3	Head band	TA	24.0 ± 3.8*	7 (29.1)
*$P < 0.001$ compared with baseline				
VAS1.6				
1	Cold	TA	37.2 ± 4.4*	1 (4.2)
2	Tourniquet	TA	33.2 ± 5.4*	3 (12.5)
3	Head band	TA	21.3 ± 3.2*	4 (16.6)
*$P < 0.001$ compared with baseline				

The subjects who did not have any inhibitory CPM (i.e., relative decreases in PPT and PPTol values and relative increases in VAS1.4 and VAS1.6 = increases in sensitivity to test stimulus) in any of the sessions were defined as non-responders. Before the exclusion of non-responders, $N = 12$ for the recording from masseter and forearm and $N = 24$ for the recording from tibialis anterior (TA).

Table 3
Rank of the inter-individual coefficient of variation (inter-CV) for CPM effect.

Rank	Conditioning stimulus	Assessment site	Inter-CV (%)	Number and proportion (%) of non-responders
PPT				
1	Tourniquet	Forearm	54.0	2(16.6)
2	Tourniquet	Masseter	54.3	1(8.3)
3	Cold	Forearm	57.4	0(0)
4	Cold	Masseter	61.2	1(8.3)
5	Tourniquet	TA	62.7	2(8.3)
6	Cold	TA	74.1	0(0)
7	Head band	Masseter	80.5	0(0)
8	Head band	TA	100.6	1(4.2)
9	Head band	Forearm	103.2	1(8.3)
PPTol				
1	Cold	Forearm	41.4	0(0)
2	Cold	TA	45.1	0(0)
3	Cold	Masseter	46.9	1(8.3)
4	Tourniquet	Forearm	52.1	5(41.6)
5	Tourniquet	Masseter	57.5	2(16.6)
6	Tourniquet	TA	58.9	5(20.8)
7	Head band	Masseter	66.0	1(8.3)
8	Head band	Forearm	68.7	3(25.0)
9	Head band	TA	101.7	8(33.3)
VAS1.4				
1	Cold	TA	60.1	2(8.3)
2	Head band	TA	64.8	7(29.1)
3	Tourniquet	TA	86.2	4(16.6)
VAS1.6				
1	Cold	TA	56.8	1(4.2)
2	Head band	TA	67.7	4(16.6)
3	Tourniquet	TA	74.3	3(12.5)

The subjects who did not have any inhibitory CPM (i.e., relative decreases in PPT and PPTol values and relative increases in VAS1.4 and VAS1.6 = increases in sensitivity to test stimulus) in any of the sessions were defined as non-responders. Before the exclusion of non-responders, $N = 12$ for the recording from masseter and forearm and $N = 24$ for the recording from tibialis anterior (TA).

to the masseter ($17.8 \pm 2.2\%$, $P < 0.001$) and forearm ($15.3 \pm 2.0\%$, $P < 0.001$).

The ANOVAs on the normalized PPTol values indicated a main effect of time ($F = 105.172$, $P < 0.001$). The post hoc tests revealed that the normalized PPTol values were significantly decreased during the CS ($22.3 \pm 1.2\%$, $P < 0.001$) and 10 min after the CS ($6.9 \pm 1.5\%$, $P < 0.05$) compared with baseline values. The ANOVAs on the normalized PPTol values indicated no effect of CS or assessment site during the CS.

Though ANOVAs on normalized VAS1.4 ratings showed no main effect of time ($F = 2.438$, $P = 0.092$), the ANOVAs on normalized VAS1.6 ratings indicated a main effect of time ($F = 58.305$, $P < 0.001$). The post hoc tests revealed that the normalized VAS1.6 ratings were significantly increased during the CS ($30.9 \pm 2.7\%$, $P < 0.001$) compared with baseline values. No time effect was observed at 10 min after the CS either for VAS1.4 or VAS1.6.

The ANOVAs on the normalized VAS1.4 ratings indicated an effect of CS ($F = 3.757$, $P = 0.029$) during the CS. The post hoc tests revealed that the reduction of the normalized VAS1.4 ratings with cold stimulus ($41.5 \pm 5.3\%$) was significantly higher than the ratings with head band ($24.0 \pm 3.8\%$, $P < 0.05$).

The ANOVAs on the normalized VAS1.6 ratings also indicated an effect of CS ($F = 3.386$, $P = 0.040$) during the CS. The post hoc tests revealed that the reduction of the normalized VAS1.6 ratings with cold stimulus ($37.2 \pm 4.4\%$) was significantly higher than the ratings with head band ($21.3 \pm 3.2\%$, $P < 0.05$).

As all tests showed significantly positive CPM effects during the CS, those data were used for further analysis.

3.2.3. The inter-individual CV

Table 3 shows the rank of the inter-individual coefficient of variation (inter-CV) for the CPM effect. Independently of the CS, the inter-CV from PPT and PPTol in general showed that the recording from the orofacial region and the forearm had smaller values than from the leg. In general, the cold conditioning stimulus caused smaller inter-individual variation (41.4–60.1%) than the tourniquet and head band.

3.2.4. The intra-individual CV

Table 4 shows the rank of the intra-individual coefficient of variation (intra-CV) for the CPM effect. The smallest intra-CV value was obtained in pain ratings with cold stimulus (27.0%). Concerning the threshold determinations, the smallest variability was obtained from the masseter or the forearm regardless of the CS (35.2–40.1%). As for the CS, there was a general pattern that the cold stimulus led to smaller intra-CV (27.0–42.4%).

3.2.5. CPM non-responders

The subjects who did not have any inhibitory CPM (i.e., relative decreases in PPT and PPTol values and relative increases in VAS1.4 and VAS1.6 = increases in sensitivity to TS) were defined as non-responders. In the result for the CPM effect (Table 2) and inter-CV (Table 3) the subjects who did not have any inhibitory CPM in any of the sessions were defined as non-responders. In the result for the intra-CV (Table 4) the subjects were defined as non-responders if they did not have any inhibitory CPM even in one of the two sessions. Before the exclusion of non-responders, $N = 12$ for the recording from masseter and forearm and $N = 24$ for the recording from both TA ($N = 12$ for right TA and left TA, respectively). The number and proportion of non-responders are shown in Tables 2–4.

Though the number of non-responders was less in PPT recording than PPTol recording, as well as less in VAS1.6 compared with VAS1.4, there were no significant differences between PPT and PPTol ($P = 0.133$) and VAS1.4 and VAS1.6 ($P = 0.376$) for Tables 2 and 3, and PPT and PPTol ($P = 0.062$) and VAS1.4 and VAS1.6 ($P = 0.127$) for Table 4 (Mann–Whitney U-test).

Table 4
Rank of the intra-individual coefficient of variation (intra-CV) for CPM effect.

Rank	Conditioning stimulus	Assessment site	Intra-CV (%)	Number and proportion (%) of non-responders
PPT				
1	Cold	Forearm	40.1	5 (41.6)
2	Cold	TA	40.9	4 (16.6)
3	Tourniquet	Masseter	54.5	5 (41.6)
4	Cold	Masseter	65.9	3 (25.0)
5	Head band	TA	64.3	11 (45.8)
6	Tourniquet	Forearm	66.6	2 (16.6)
7	Head band	Forearm	72.9	5 (41.6)
8	Tourniquet	TA	84.1	12 (50.0)
9	Head band	Masseter	84.1	7 (58.3)
PPTol				
1	Head band	Masseter	35.2	8 (66.6)
2	Tourniquet	Forearm	38.6	10 (83.3)
3	Cold	Masseter	42.4	4 (33.3)
4	Cold	TA	59.0	7 (29.2)
5	Tourniquet	TA	66.7	16 (66.7)
6	Cold	Forearm	70.9	8 (66.7)
7	Head band	Forearm	72.2	7 (58.3)
8	Tourniquet	Masseter	81.5	9 (75.0)
9	Head band	TA	99.6	17 (70.8)
VAS1.4				
1	Cold	TA	27.0	7 (29.2)
2	Tourniquet	TA	42.4	19 (79.2)
3	Head band	TA	70.3	18 (75.0)
VAS1.6				
1	Cold	TA	36.9	5 (20.8)
2	Tourniquet	TA	55.5	13 (54.2)
3	Head band	TA	66.3	16 (66.7)

The subjects who did not have any inhibitory CPM (i.e., relative decreases in PPT and PPTol values and relative increases in VAS1.4 and VAS1.6 = increases in sensitivity to test stimulus) even in one of the two sessions were defined as non-responders. Before the exclusion of non-responders, $N = 12$ for the recording from masseter and forearm and $N = 24$ for the recording from tibialis anterior (TA).

3.2.6. Correlation between pain ratings of the CS and CPM effects

The correlations between the VAS peak pain values versus relative PPT changes during the CS obtained from all responders in all conditions were calculated. A significantly positive correlation was detected between the VAS peak pain values and the relative PPT changes ($R = 0.176$, $P = 0.009$). There was also a significantly positive correlation in the data recorded from TA ($R = 0.261$, $P = 0.005$).

4. Discussion

The present study shows that the CPM induced by CPP elicits the strongest responses, and the leg as the assessment site results in the largest responses. Moreover, the CPP causes the smallest inter- and intra-CV in general.

4.1. Methodological considerations

Various methodologies have been utilized to evoke and characterize CPM [10]. Pressure pain threshold (PPT) by hand-held algometer tends to be the most sensitive for the test measurement to detect CPM responses [8,28,29]. Moreover, PPT values are stable within the same individual [30] and have a good inter-examiner reliability [31]. Hence it was used in the present study.

The computer-controlled pressure algometry [24,32] allows estimation of the stimulus-response function which is more difficult to obtain by hand-held algometer. The automated system was used for assessing responses from TA, whereas for the other locations it was more adequate to use the hand-held algometer. Therefore, the CPM effects and the inter- and intra-individual CVs were not compared directly between the two algometers.

There were no significant differences in the number of non-responders between PPT and PPTol recording and VAS1.4 and VAS1.6. These results imply that both of these measurements are useful as TS to evaluate CPM.

Gender differences [28] and age differences [33,34] can affect the CPM. When muscle pain is used as CS, more efficient CPM is induced in men [28]. Research on the gender differences of the CPM effect has yielded inconsistent findings [10]. Moreover, it has been reported that the CPM effects of women differ with menstrual phase, suggesting hormonal influence on the CPM [22,35]. In addition, the menstrual phase may also influence the pain response which is lower pain threshold during the luteal phase compared with the follicular phase by CPP [36]. An age-associated decrement of CPM has been reported [33,34]. As only young men have been tested in the study, the issue of gender and age differences was not addressed. However, in the present study we deliberately excluded older subjects and women as these factors would influence the CPM effects. Overall, we consider that we could evaluate the CPM effects more accurately without the influence of age and hormonal factors.

The sample number of this study was 12 for the masseter and forearm and 24 for TA. In view of the fundamental aspects of CPM, we only included the data from CPM-responders in the analysis. However, it is also important to state the proportion of CPM non-responders. Therefore the number and proportion of CPM non-responders are shown in Tables 2–4. We cannot deny the possibility of overestimation of the CPM effects and the low reliability because of the low sample. Therefore, additional investigations would be required before the results can be substantiated.

4.2. Modality and assessment site effects of CPM

The CPP [8], tourniquet pain [25,37] and skull pressure pain [26] were applied in the present study as CS. To our knowledge this is the first study to evaluate the CPM effects comparing three different CS modalities together with several assessment sites. In the research concerning CPM and the risk of developing chronic pain, heat pain was applied as both CS and TS [23]. These differences in pain modalities should be noted.

The effects of CPM are known to differ depending on the magnitude and nature of the CS and the stimulated nerve fibers [10,18,19]. The median magnitude of the CPM effect was 29% [10]. In the present study, the CPP induced the most powerful CPM effects (43.6%) in all the measurements.

It seems that the effect of CPM is more intense with stronger intensity of the CS [3,18–22]. In this study, the target level of CS was different among the three modalities of the CS (6 for CPP and tourniquet and 5 for head band) because the pain intensity of mechanical pressure on the head continues to increase gradually for the duration of the compression [26], and actually the VAS peak pain value of head band was 6.4. However the VAS peak pain value of CPP was significantly higher than for the tourniquet and head band. Moreover, there was a significantly positive correlation between the intensity of the CS and the CPM effect. This difference in the intensity of the CS might affect the magnitude of CPM.

Immersion in cold water produces arterial pressor responses [38]. Duschek et al. [39] demonstrate an inverse relationship between blood pressure levels and pain intensity triggered by CPP. From that point of view, hot water seems to be a more appropriate model for tonic pain compared to CPP [40]. On the other hand, CPP, ischemic pain and mechanical pressure have excellent reliability and validity as well as thermal pain [41]. Thus, we consider that the modalities of CS employed in this study were all adequate, and the bigger increases in the threshold determinations and reductions in the VAS ratings induced by CPP are mainly due to the CPM effects.

The central pain-modulating system can be activated not only by noxious stimuli, but also by psychological factors, such as attention, distraction and the environmental demands (emotional arousal) [42–45]. Both distraction and stress can reduce pain [42] and contribute to pain-evoked hypoalgesia, as painful stimuli are not only painful but also distracting and stressful [43]. Also factors such as task difficulty and emotional arousal are implicated in moderating the interruptive function of pain [44]. The intense cold stimulus might shift the subject's attentional level and have some influence on the CPM effect.

There were significant differences in the CPM effects among the assessment sites; a significantly higher CPM effect at TA than the masseter or forearm. The differences in the CPM effects among the assessment sites might partly be related to the method of evaluation (hand-held or computer-controlled algometer) but also the size of the muscle could influence the response.

4.3. The inter- and intra-individual CV

The inter-individual variation for CPM is important for the experimental and clinical pain research. However, there is no such information available for the reference. One study [46] reported the test–retest reliability of CPM with intraclass correlations (ICCs) and coefficient of repeatability (CR) using hand-held algometry (mechanical TS to the middle finger and trapezius) and occlusion cuff (CS). The results demonstrated that the CPM effect showed no significant differences across test–retest occasions. However, the cuff resulted in large inter-individual variation between test and retest measurements (CR was 1.69 for finger CPM). This is consistent with our result that in general the cuff has larger inter-individual variation than CPP.

In the present study, CPP was found to evoke smaller inter-individual variation compared to the tourniquet pain and mechanical craniofacial pressure. Concerning the factors which might affect the inter-individual CV for CPM, the individual difference of the sensitivity to painful stimuli could be considered besides gender and age differences. Even though we cannot exclude the possibility of variation due to a sensitization of peripheral nociceptors, it would be neglected because any experimental methods might have the differences derived from the general experimental intervention [47].

Regarding the intra-individual variation for CPM, the smallest threshold variability was obtained from the masseter or the forearm regardless of the CS (35.2–40.1%). And for pain ratings, the smallest variability was obtained with CPP (27.0%). There was a general pattern that the CPP leads to smaller intra-CV.

A previous study [21] mentioned that the CPM effect is relatively free of individual variability. A significantly positive correlation of the CPM effect was noted between the two induced temperatures of 46.5 °C and 12 °C, suggesting that individuals who show greater CPM with heat also show greater CPM with cold. The finding supports that the extent of CPM is related to the individual characteristics of the pain modulation response rather than to the modality of CS.

The possible influential factor on the intra-individual variation for CPM is habituation [48]. Though there might be an influence of that between the first and second session, it would be similar for all measurements. Another possible parameter is the influence of examiner [31]. Although the possible bias introduced by different examiners should be taken into account, it does not influence as this experiment was performed by the same examiner (woman). Moreover, the duration of the TS was kept constantly within the study cohort as the same examiner performed the whole experiment.

Another parameter to be investigated for inter- and intra-individual variation is the factor from PPT testing. Previous studies reported that the test–retest reliability of the PPT technique can be guaranteed [46,49]. It has been shown that the CVs for PPT ranged from 20.1% (right parietal) to 47.8% (right temporal) [49] and are relatively large for all measures when compared with other CV findings (e.g.; 18% after 15 min, 14% after 45 min and 29% after 5 weeks [31,50]), but are of similar magnitude to the findings of PPT intra-individual variation [30]. Brennum et al. [30] report that the inter-individual CV for PPT measured by a hand-held algometer was 28% for women and 33% for men, and the intra-individual CV based on repeated PPT measurements with a 1 week interval was 14%. Pud et al. [10] report that the approximated median magnitude of the CPM effect measured by the suprathreshold test-pain reduction was 29% (ranging from 10% to 55%); the increase in the test-pain thresholds was 25% (ranging from 3% to 100%); and the change in the neurophysiological measures was 28.5% (ranging from 10% to 60%). These figures imply that due to the high variation within the 15 studies which measured the changes by test-pain thresholds, continued use of this method in future studies may be questionable.

5. Conclusions

The present investigation has demonstrated that the cold pressor pain is the most efficient conditioning stimulus to induce CPM when assessed by pressure pain thresholds.

Declaration of financial relationship

There are no relationships to be declared.

Conflict of interest

It is confirmed that there are no financial or other arrangements that might lead to a conflict of interest for this paper.

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