# Conditioned pain modulation is not associated with thermal pain illusion

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Original Experimental

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**Abstract**

**Objectives:** Paradoxical sensations, known as thermal pain illusions, can be evoked by painful cold-heat pulse stimulation. They may provide diagnostic value; however, the possible interaction between conditioned pain modulation and thermal pain illusions has not been explored. The present study examined: (1) whether conditioned pain modulation could be induced by alternating tonic painful cold-heat pulse stimulation; and (2) whether the presence of thermal pain illusions during the conditioning stimulus influences the degree of conditioned pain modulation.

**Methods:** This study was approved by the Ethics Committee of Meikai University (A1507). Conditioned pain modulation was provoked using alternating painful cold-heat pulses delivered at 20-s intervals applied to the forearm. Thermal pain illusions were qualitatively evaluated, and conditioned pain modulation was assessed quantitatively using the pressure pain threshold as a test stimulus. Differences in the conditioned pain modulation effect between the participants who experienced thermal pain illusions and those who did not were analysed using Student's *t*-test.

**Results:** A significant positive conditioned pain modulation effect (51.0 ± 4.7%, overall effect) was detected. There was no significant difference in conditioned pain modulation between the participants who experienced thermal pain illusions and those who did not (44.3 ± 6.0% and 55.5 ± 6.8%, respectively; p = 0.255).

**Conclusions:** Conditioned pain modulation induced by alternating painful conditioning cold-heat pulse stimulation was identical during the conditioning stimulation in volunteers with and without thermal pain illusions. Conditioning cold-heat pulse stimulation is useful to evaluate conditioned pain modulation. Moreover, conditioned pain modulation is not influenced by the presence of thermal pain illusions, indicating partially different underlying supraspinal, neuronal networks.

**Keywords:** conditioned pain modulation; healthy volunteers; painful cold-heat pulse stimulation; thermal pain illusion.

## Introduction

Conditioned pain modulation (CPM) is a phenomenon in which a tonic painful conditioning stimulus affects a painful test stimulus [1]. CPM was originally termed 'diffuse noxious inhibitory controls (DNIC)' in animals to describe a specific inhibitory mechanism mediated by the lower brainstem [2, 3]. CPM is considered a centrally processed measure of

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the net effect of the descending inhibitory and facilitatory pathways [4] from supraspinal structures, such as the subnucleus reticularis dorsalis (SRD) in the medulla [3, 5].

Experimental evidence suggests that the magnitude of the CPM effect depends on the intensity of the painful conditioning stimuli [6]. In terms of the modality of the conditioning stimulus, cold-water immersion seems to trigger the strongest CPM effect compared to ischaemic and pressure pain [7]. However, stimulation of cold-water immersion is bothersome in a clinical setting, and alternative methods are warranted. Furthermore, no gold standard for CPM testing has been established, which makes comparison between studies complicated [8]. Recently, we developed a Peltier-based quantitative thermal stimulator device as a useful, simple, bedside tool for use in clinical settings [9]. In addition, the thermal stimulator could deliver simultaneous or alternating tonic painful cold-heat pulse stimulation [9]. The Peltier device has advantages of no expectation, prediction, or attention to a visible input [10] and no habituation [11] as observed with cold-water hand immersion. Alternating Peltier-delivered tonic painful cold-heat pulse stimulation can evoke a thermal pain illusion (TPI) in approximately 35% of volunteers [9]. TPI is a paradoxical phenomenon; for example, a heat sensation is experienced when a cold stimulus is applied (paradoxical heat sensation), and the cold and heat sensations are not observed when tonic painful cold-heat pulse stimulation is applied. TPI is similar to the thermal grill illusion (TGI) [12], where pain is caused by innoxious cold and heat stimulation. Brain imaging studies have identified the cortical structures that are activated during TGI, including the anterior cingulate cortex (ACC), and the thalamic and insular region [13–15]. The experimental paradigms, and the types of stimuli employed in TGI and TPI are different [9]; (1) TGI is caused by a grill, whereas the TPI is caused by pulse stimulation, and (2) TGI is induced by innocuous stimuli, whereas the TPI is induced by painful cold and heat stimuli. TPI and TGI are two separate illusory phenomena caused by cold and heat stimulation, however, they may include central and peripheral gating mechanisms [9, 12–20].

As the underlying mechanisms of CPM and TGI are known to include supraspinal structures, such as the SRD, ACC, and the thalamic and insular regions, it is possible that TPI caused by conditioning alternating painful cold-heat pulse stimulation potentially influences the effect of the conditioning stimulus, resulting in an influence on CPM measurements. Therefore, analyses of the influence of TPI during conditioning cold-heat pulse stimulation on CPM measurements are warranted.

Therefore, the present study aimed to investigate: (1) whether CPM could be induced by alternating tonic painful cold-heat pulse stimulation and (2) if the presence of TPI during the conditioning stimulus could influence the degree of CPM.

# Methods

## Participants

This study was conducted in accordance with the Declaration of Helsinki at the Division of Dental Anesthesiology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry and was approved by the Ethics Committee of Meikai University (A1507). Written informed consent was obtained from all participants before inclusion in the study. The study was registered as a University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) Clinical Trial (Unique ID: UMIN000037547).

The inclusion criteria for the study were: (1) age >20 years old, (2) healthy and pain-free, and (3) able to provide informed consent. The exclusion criteria were: (1) the presence of a serious medical condition, such as any acute or chronic pain, or neurological, psychiatric, or neuromuscular diseases; (2) current use of any pain medication within 24 h prior to the start of the investigation; and (3) inability to provide informed consent.

## Quantitative thermal stimulator device

A customised quantitative thermal stimulator device (VTH-3500; VICS, Tokyo, Japan) was designed to deliver tonic painful pulse stimulation [9]. The quantitative thermal stimulator device comprised a ceramic contact plate (30 × 30 mm) that was cooled or heated with a Peltier element. The temperature was measured continuously using a thermometer placed on the surface of the Peltier element. The baseline temperature was 32 °C (neutral temperature). Temperatures ranging from 10 to 40 °C were obtained with a ramp time of 5 °C/s; temperatures ranging from 0 to 10 °C and 40–45 °C were obtained with a ramp time of 0.5 °C/s.

## Assessment of temperatures for cold and heat stimuli ('setting for cold and heat stimuli')

The subjective assessment of temperatures for the cold and heat stimuli was determined on the inside of the non-dominant forearm (5 cm from the fossa) using a quantitative thermal stimulator device. Stimuli were delivered in a randomised order. The probe temperature was set at a neutral temperature (32.0 °C), and subsequently manually increased or decreased via a computer interface [9]. For the continuous evaluation of subjective assessments of pain intensity induced by hot and cold temperatures, a custom-made electronic visual analogue scale (VAS) (0–100 mm) was applied, employing a sliding resistor and the sampled data. The left endpoint (0) indicated 'no pain', and the right endpoint (100) indicated the 'worst pain imaginable.' Participants who did not experience a pain intensity of 70/100 mm on VAS before reaching the cut-off temperature of 0 or 45 °C were excluded from the study. The temperatures that induced cold and heat pain at

an intensity of approximately 70/100 mm on VAS were applied in the subsequent experiment ('CPM period').

Qualitative sensations were assessed via open questioning after evaluating the temperatures for the cold and heat stimuli.

## CPM evaluation ('CPM period')

Painful cold-heat pulse stimulation comprised a sequence of repeated, alternating cold and heat stimuli delivered at 20-s intervals (0.025 Hz) [9]. The stimuli were applied to the inside of the non-dominant forearm (5 cm from the fossa) as a conditioning stimulus for 5 min: 2-min stimulation without the test stimulus and 3-min stimulation with the test stimulus (Figure 1) [9]. The cold and heat temperatures for painful conditioning alternating cold-heat pulse stimulation were determined at baseline.

Pressure pain thresholds (PPTs) were applied as a test stimulus [6, 7, 21–23] to the inside of the dominant forearm to assess CPM potency. PPTs were assessed using a custom-made electronic pressure algometer (AIKOH Engineering, Osaka, Japan) with a probe area of 1 $cm^2$. PPT was defined as the amount of pressure (N) perceived as painful by the participant. Pressure was applied at a steadily increasing rate of 3 N/s (30 kPa/s) [6, 7, 21–23].

The participant pressed the stop button on reaching the threshold. PPT measurements were repeated thrice with 1-min intervals. The mean value of the three recordings was used for subsequent analysis. PPT was recorded before (baseline), during, 10 min after, and 20 min after the conditioning stimulus.

The CPM effect was defined as follows: [(PPT during the conditioning stimulus, 10 min after the conditioning stimulus, or 20 min after the conditioning stimulus)/(PPT at baseline) – 1] × 100 (%).

## TPI evaluation

TPI is defined as: (1) heat sensation during cold stimulus (paradoxical heat sensation), (2) cold sensation during heat stimulus (paradoxical cold sensation), (3) no cold sensation in the cold stimulus phase, and (4) no heat sensation during the heat stimulus phase [9]. The TPI phenomena are evaluated by applying tonic painful cold-heat pulse stimulation [9]. In the current study, TPI was qualitatively evaluated during the 5-min conditioning tonic painful cold-heat pulse stimulation. Subjective sensations were assessed via open questioning at every 20-s interval in the CPM period (total duration of 5 min) (Figure 1).

## Experimental protocol

All experiments were performed at a constant room temperature (25 °C) (Figure 1). During the setting of cold and heat stimuli, temperature assessments for the cold and heat pain stimuli were

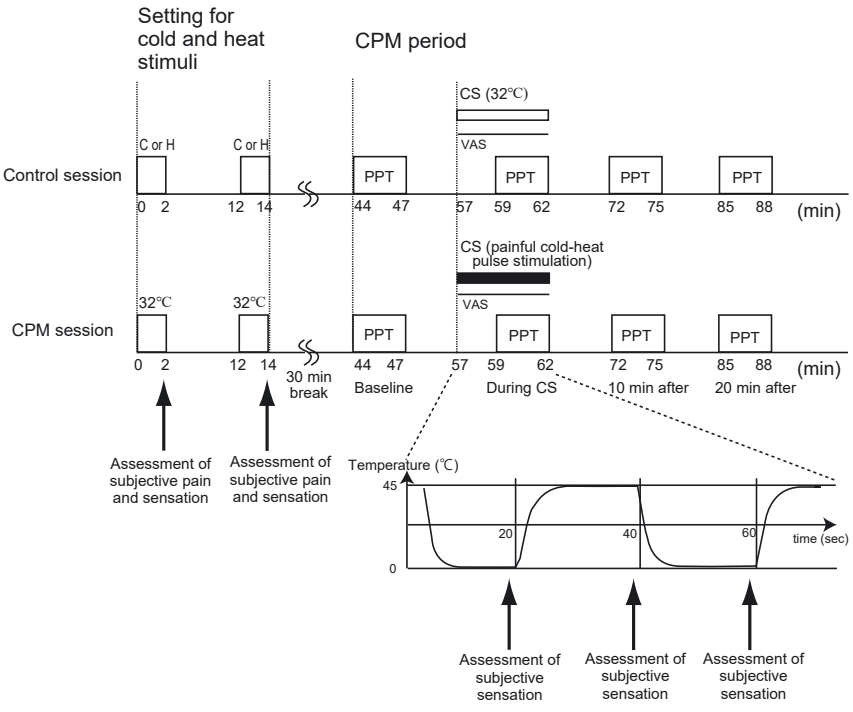


**Figure 1:** Timeline of the study. In the CPM session, assessment of temperatures for the painful cold and heat stimuli is conducted in a random order using a quantitative thermal stimulator device with 10 min between the cold and heat stimuli setting. The CPM evaluation (CPM period) is initiated 30 min after the cold and heat stimuli. The pressure pain threshold (PPT) is recorded before (baseline), during, 10 min after, and 20 min after the conditioning stimulus. Subjective sensations are assessed via open questioning after evaluating the temperatures for the cold and heat stimuli during the cold and heat stimuli setting, and for every 20-s interval in the CPM period (total duration of 5 min). The pain intensity resulting from painful thermal pulse stimulation is rated continuously using the electronic visual analogue scale (VAS). In the control session, a neutral temperature (32 °C) is applied, and the same protocol is performed. The order of the two sessions (CPM session and control session) is randomly assigned, with a 1-week interval. C or H, assessment of temperature for cold and heat stimuli; CPM, conditioned pain modulation; VAS, visual analogue scale; PPT, pressure pain threshold, CS, conditioning stimulus.

conducted in a randomised order at 10-min intervals using a quantitative thermal stimulator device. Initiation of the CPM sequence (CPM period) occurred 30 min after setting for cold and heat stimuli.

CPM and control sessions were performed in the CPM period. Painful cold-heat pulse stimuli were applied as conditioning stimulus in the CPM session, whereas the same protocol was performed in the control session but with a neutral temperature (32 °C) as the stimulus.

Subjective sensations for cold-heat pulse stimuli were evaluated using an open-ended interview question after setting the temperatures for the cold and heat stimuli as well as for every 20-s interval during conditioning cold-heat pulse stimulation in the CPM period. The phrasing of the open-ended interview question was as follows: 'Please describe the sensation evoked by the thermal stimuli'. The participants were oriented to the subjective sensations for cold-heat pulse stimuli during the interview before starting the evaluation. The pain intensity resulting from painful thermal pulse stimulation was rated continuously using the custom-designed electronic VAS (0–100 mm). Participants were instructed to move the VAS indicator to describe the pain evoked by painful thermal pulse stimulation. The temperature of the quantitative thermal stimulator device was continuously recorded.

PPT was recorded before (baseline), during, 10 min after, and 20 min after the conditioning stimulus. The application of the conditioning stimulus began 2 min before the test stimulus until the end of the measurement period (for 5 min).

All participants underwent two experimental sessions (CPM session and control session) 1 week apart, with the order decided randomly.

#### Data analysis

Two-way repeated measures analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare differences in CPM effects at each point (baseline, during the conditioning stimulus, 10 min after the conditioning stimulus, or 20 min after the conditioning stimulus) with two sessions for inter-session comparisons. One-way ANOVA and Tukey's multiple comparison tests were used to compare the differences in CPM effects at each point (baseline, during the conditioning stimulus, 10 min after the conditioning stimulus, or 20 min after the conditioning stimulus). Differences in the CPM effect between the two groups (with/without TPI) were analysed using Student's *t*-test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using EZR (version 1.54, Jichi Medical University, Tochigi, Japan) [24].

The temperature and VAS values are presented as mean ± standard deviation. PPT and CPM effects are presented as the mean ± standard error of the mean.

# Results

## Participants

A total of 38 participants (20 men, 18 women, aged 24–57 years) participated in the study. Eight participants who did not report VAS values over 70/100 mm for the cold and heat stimuli were excluded from the study; therefore, 30 participants (15 men and 15 women, aged 25–45 years) were included in the final analysis.

## Setting for cold and heat stimuli (before CPM period)

#### Temperature for the cold and heat stimuli

In the CPM session, the temperatures for the cold and heat pain stimuli were 2.9 ± 1.8 °C and 42.9 ± 1.4 °C, respectively. In the control session, the neutral temperatures of the two measurements were 31.7 ± 0.2 °C and 31.7 ± 0.2 °C, respectively.

#### VAS values for the cold and heat stimuli

In the CPM session, the VAS values for the cold and heat stimuli were 76.7 ± 6.0 mm and 79.7 ± 8.6 mm, respectively. In the control session, the VAS values for the neutral temperatures of the two measurements were 0.3 ± 1.6 mm and 0.6 ± 2.2 mm, respectively.

#### Assessment of subjective perceptions

In the CPM session, one participant reported a heat sensation during cold pain skin surface temperature measurement (Table 1). The other participants did not experience a paradoxical phenomenon. In the control session, no participant experienced a paradoxical phenomenon.

## CPM period

#### Temperature for the cold and heat stimuli

In the CPM session, the peak temperatures for the cold and heat stimuli were 3.3 ± 2.1 °C and 43.0 ± 1.3 °C, respectively. In the control session, the neutral temperature for the neutral stimuli was 31.8 ± 0.2 °C.

#### VAS values for the cold and heat stimuli

In the CPM session, the VAS values for the cold and heat stimuli were 47.5 ± 16.9 mm and 74.1 ± 21.0 mm, respectively. In the control session, the VAS value at the neutral temperature was 0.9 ± 4.0 mm.

**Table 1:** Conditioned pain modulation effects and subjective sensation for painful cold-heat pulse stimulation (thermal pain illusion).

| Participants number | Gender (M/F) | CPM effects during CS (%; mean ± SE) | CPM effects at 10 min after CS (%; mean ± SE) | TPI | Heat sensation at cold stimulus phase during cold-heat pulse stimulation (paradoxical heat sensation) | Loss of cold and/or heat sensation during painful cold-heat pulse stimulation | Heat sensation during assessment of subjective sensation | Cold sensation during assessment of subjective sensation |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 109.4 | 52.3 | – | – | – | – | – |
| 2 | M | 101.8 | 60.3 | – | – | – | – | – |
| 3 | M | 95.5 | 23.2 | – | – | – | – | – |
| 4 | F | 80.4 | 29.8 | – | – | – | – | – |
| 5 | M | 76.4 | 34.1 | – | – | – | – | – |
| 6 | M | 70.2 | 32.3 | + | + | + | – | – |
| 7 | F | 68.1 | 21.8 | – | – | – | – | – |
| 8 | F | 63.6 | −15.6 | + | – | + | – | – |
| 9 | F | 63.3 | 27.8 | + | – | + | – | – |
| 10 | F | 61.3 | 40.3 | + | + | – | – | – |
| 11 | M | 59.8 | 27.8 | – | – | – | – | – |
| 12 | M | 55.9 | 22.7 | – | – | – | – | – |
| 13 | M | 55.2 | 22.4 | – | – | – | – | – |
| 14 | M | 48.2 | 11.2 | – | – | – | – | – |
| 15 | F | 47.9 | 41.5 | + | + | + | – | – |
| 16 | F | 46.8 | 10.9 | + | + | – | – | – |
| 17 | M | 46.2 | 23.1 | – | – | – | – | – |
| 18 | F | 44.2 | 27.2 | + | + | – | + | – |
| 19 | M | 42.2 | 0 | – | – | – | – | – |
| 20 | F | 38 | −7.1 | + | – | + | – | – |
| 21 | M | 37.6 | −2.5 | + | + | – | – | – |
| 22 | F | 36.3 | 37.3 | – | – | – | – | – |
| 23 | M | 35.3 | 13.7 | – | – | – | – | – |
| 24 | M | 35.3 | −20 | + | + | – | – | – |
| 25 | M | 35 | 14.9 | – | – | – | – | – |
| 26 | F | 30.4 | 26 | – | – | – | – | – |
| 27 | M | 30.3 | −6.1 | + | – | + | – | – |
| 28 | F | 14.9 | 5 | – | – | – | – | – |
| 29 | F | 7.5 | 1.5 | – | – | – | – | – |
| 30 | F | −7.2 | −9.6 | + | + | – | – | – |

CPM, conditioned pain modulation; M, male; F, female; TPI, thermal pain illusion; CS, conditioning stimulus; SE, standard error of the mean.

### Assessment of TPI during CPM (Table 1)

The following qualitative TPI sensations were reported: (1) heat sensation when the cold stimulus was applied (paradoxical heat sensation) (eight of 30 participants) and (2) no cold and/or no heat sensation when the cold-heat stimulation was applied (six of 30 participants). The remaining 18 participants did not report TPI (Table 1). No participant reported a cold sensation when the heat stimulus was applied (paradoxical cold sensation). The subjective sensations changed during assessment of TPI. If the participant reported TPI sensations at least once, they were counted as a participant who exhibited TPI.

## CPM effects (Table 1, Figure 2)

In the CPM session, the PPT at baseline, during the conditioning stimulus, 10 min after the conditioning stimulus, and 20 min after the conditioning stimulus were 14.2 ± 0.4 N, 21.1 ± 0.6 N, 16.4 ± 0.4 N, and 14.2 ± 0.4 N, respectively. The CPM effects during the conditioning stimulus, at 10 min after the conditioning stimulus, and 20 min after the conditioning stimulus were 51.0 ± 4.7%, 18.2 ± 3.6%, and 1.0 ± 2.3%, respectively.

In the control session (natural temperature), the PPT at baseline, during the conditioning stimulus, 10 min after the conditioning stimulus, and 20 min after the conditioning

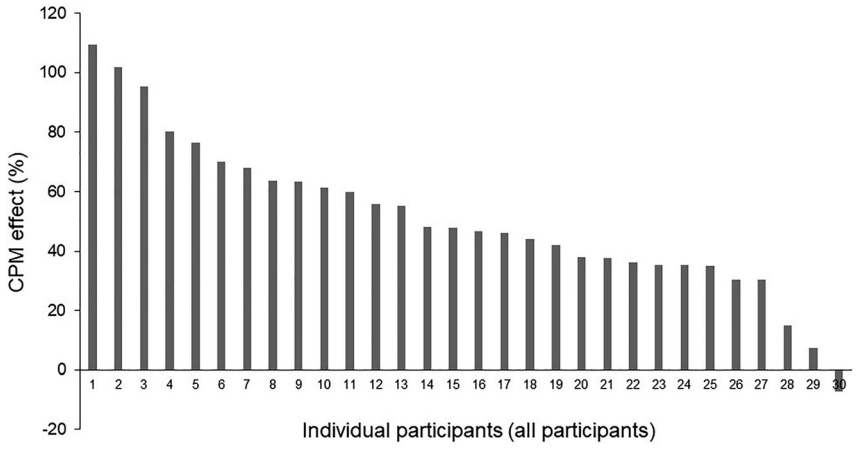


**Figure 2:** Frequency plot of individual CPM effects during the conditioning stimulus in 30 participants. Positive scores indicate a CPM effect, as defined by an increased PPT during the conditioning stimulus. The number of individual participants is consistent with "participants number" in Table 1. CPM, conditioned pain modulation; PPT, pressure pain threshold.

stimulus was 13.5 ± 0.4 N, 14.0 ± 0.4 N, 13.6 ± 0.4 N, and 13.4 ± 0.4 N, respectively. The CPM effects during the conditioning stimulus, at 10 min after the conditioning stimulus, and 20 min after the conditioning stimulus were 3.8 ± 1.1%, 0.2 ± 1.1%, and −0.7 ± 0.7%, respectively.

Performing two-way repeated measures ANOVAs on the CPM effect indicated a significant session ($F = 56.444$, $df = 1$, $p < 0.001$) and time ($F = 82.750$, $df = 3$, $p < 0.001$) effect with a significant session and time interaction ($F = 59.495$, $df = 3$, $p < 0.001$). As expected, the CPM effects in the CPM session were significantly larger than that in the control session ($p < 0.001$). Post-hoc tests showed that the CPM effects significantly increased during the conditioning stimulus ($p < 0.001$) and 10 min after the conditioning stimulus ($p < 0.001$) but not at 20 min after the conditioning stimulus ($p > 0.05$).

In the CPM session, a significant positive CPM effect was detected during the conditioning stimulus (51.0 ± 4.7%, $p < 0.000001$), compared to baseline. A significant positive CPM effect was detected at 10 min after the conditioning stimulus (18.2 ± 3.6%, $p = 0.0006$), compared to baseline. At 20 min after the conditioning stimulus, no significant difference was detected (1.0 ± 2.3%, $p = 0.9963$), compared to baseline. A frequency plot of individual CPM effects during the conditioning stimulus is shown in Figure 2.

In the control session (natural temperature), the statistical analysis showed a significant CPM effect during the conditioning stimulus (3.8 ± 1.1%, $p = 0.0095$), compared to baseline. No significant CPM effects were detected at 10 min after the conditioning stimulus (0.2 ± 1.1%, $p = 0.9969$) and 20 min after the conditioning stimulus (−0.7 ± 0.7%, $p = 0.9406$), compared to baseline.

### Relationship between CPM effects and TPI (Figure 3)

There were no significant differences in the CPM effects between participants who experienced TPI and those who did not (44.3 ± 6.0% and 55.5 ± 6.8%, respectively; $p = 0.255$). Frequency plots of the individual CPM effects during the conditioning stimulus in participants who experienced TPI and those who did not are shown in Figure 3.

## Discussion

The present study found that CPM can be induced by painful alternating cold-heat conditioning stimulation. CPM was not affected by the experience of TPI during the conditioning stimulation.

### Conditioning painful cold-heat pulse stimulation and CPM

Conditioning heat or cold stimuli have been applied for the assessment of CPM [7, 25, 26]. Nilsen et al. reported a CPM effect of 39.5% in response to heat pain stimulation as a test stimulus with a cold pressor test as the conditioning stimulus [27]. Furthermore, two studies reported CPM effects, which were evaluated using somatosensory-evoked potentials induced by electric pulp stimulation as the test stimulus and a $CO_2$ laser stimulation as the conditioning stimulus of 31.3–51.6% [28, 29]. One study that used PPT

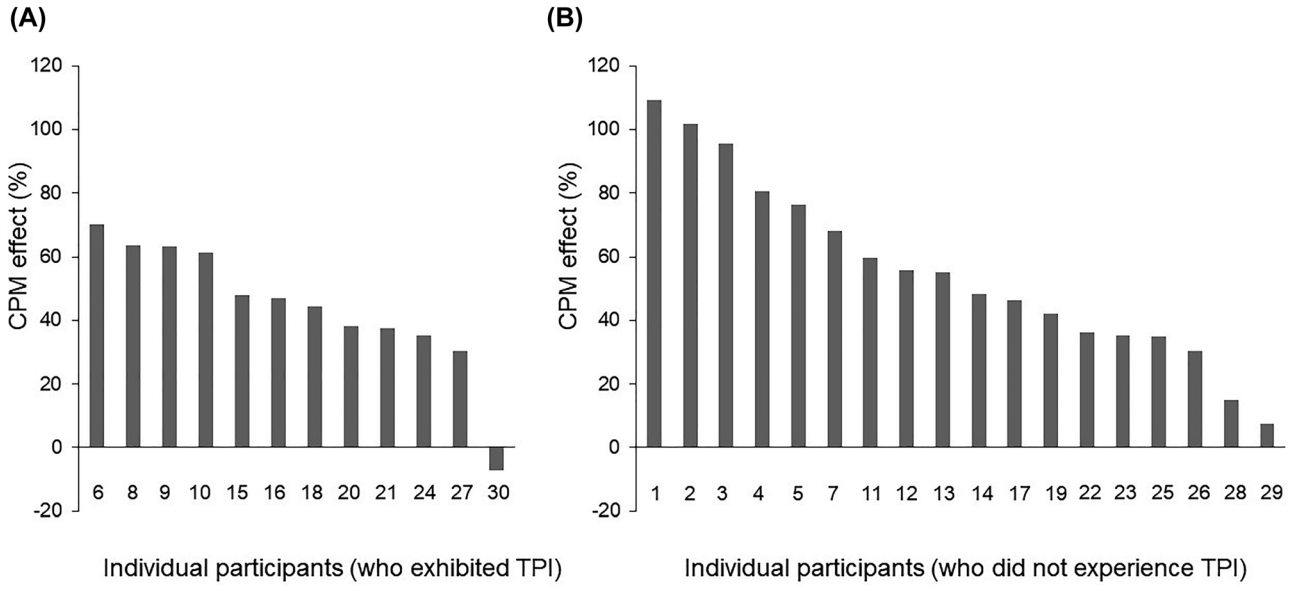


**Figure 3:** Frequency plots of individual CPM effects during the conditioning stimulus in participants who exhibited TPI (A) and participants who did not experience TPI (B). Positive scores (CPM responder) indicate a CPM effect, as defined by an increased PPT during the conditioning stimulus. The number of individual participants is consistent with "Participants number" in Table 1. CPM, conditioned pain modulation; PPT, pressure pain threshold.

stimuli as the test stimulus reported that cold pressor pain triggered CPM effects of 65.3–66.3% [7].

To date, there have been no reports on the induction of the CPM effect using conditioning alternating painful cold-heat pulse stimulation. The current study showed a significant positive CPM effect (average 51.0%) during the cold-heat stimuli in healthy volunteers. The CPM effect has been found to be dependent on the intensity of the conditioning stimulus [6]; therefore, it is sensitive to the modality of the conditioning stimulus. The current study was performed with a conditioning pain intensity of 70/100 mm on the VAS. One literature review reported an approximate median magnitude of the CPM effect of 25% (range: 3–100%) [25]. The average CPM effect (51% in healthy volunteers) obtained via conditioning alternating cold-heat pulse stimulation in the current study was consistent with this literature review [25]. The VAS values for the cold stimuli before CPM period was 76.7 ± 6.0 mm, VAS values for the cold stimuli in the CPM period was 47.5 ± 16.9 mm. Cold-heat pulse stimulation, consisting of a sequence of repeated, alternating cold and heat stimuli delivered at 20-s intervals, may lead to smaller VAS values for the cold stimuli in the CPM period compared to before CPM period, which was evaluated with only cold stimuli and not with cold-heat pulse stimulation. Concerning the VAS values for the heat stimuli in CPM period (74.1 ± 21.0 mm), the heat stimulus, which was applied after cold stimulus, might affect the smaller VAS values compared with the VAS values for heat stimuli in the before CPM period (79.7 ± 8.6 mm).

The current study showed a small but significant CPM effect during the conditioning stimulus in the control session. CPM is defined as 'the phenomenon through which the conditioning stimulus affects the test stimulus' [1]. Non-painful conditioning stimulus will cause a CPM effect [1]. Testani et al. reported that non-painful electrical stimulation reduced the amplitude of the vertex N2/P2 laser-evoked potentials component and the laser pain rating [30]. The result of our study implies that non-painful thermal conditioning stimuli will cause a CPM effect although this triggered CPM effect is very small.

In this study, a CPM after-effect was obtained 10 min after the conditioning stimulus but not 20 min after the conditioning stimulus. Willer et al. demonstrated a CPM after-effect lasting from 6 to 9 min after the end of a conditioning moderately noxious temperature (46 °C) stimulus to the hand in humans [31]. In contrast, selective Aδ-fibre stimulation with a $CO_2$ laser produces a CPM effect without an after-effect at 7.5 min after the conditioning stimulation [28].

### Individual responses

The frequency plots of individual CPM effects during the conditioning stimulation showed an overall distribution of responses with no clear differences in terms of CPM effect between participants who did not experience TPI and participants who did. It is important to know the distribution, as different distributions can provide the same average number; therefore, this has been suggested as an important step in the interpretation of CPM [32].

### Association between TPI and CPM

The following qualitative TPI sensations were reported: (1) heat sensation when the cold stimulus was applied (paradoxical heat sensation) and (2) no cold and/or no heat sensation when the cold-heat stimulation was applied; however, TPI did not affect CPM. These findings could be indicative of different neural networks, and both peripheral and central mechanisms could be involved in TPI and CPM. CPM involves descending pathways and central serotonergic [29] and adrenergic mechanisms [33, 34]; nevertheless, it is not known whether TPI could be modulated by similar mechanisms.

ACC as well as the thalamic and insular regions are involved in TGI [13–15]. In addition, N-methyl-D-aspartate neurotransmission is believed to be involved in TGI [35]. These TGI mechanisms are different from the underlying mechanisms of CPM.

Based on these previous studies and the current data, it seems that CPM and TPI work independently via different supraspinal neuronal networks, although there may be some pathways that have not yet been identified.

## Conclusions

Examining CPM with painful conditioning alternating cold-heat pulse stimulation is an option to provoke CPM, even though the altered cold-heat pulse stimulation may induce TPI.

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**Competing interests:** The authors declare no competing interests.

**Informed consent:** Informed consent has been obtained from all individuals included in this study.

**Ethical approval:** The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board.

**Ethical committee number:** The study was approved by the Ethics Committee of Meikai University (A1507).

**Trial registry number:** The study was registered as a University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) Clinical Trial (Unique ID: UMIN000037547).

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