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Title: Effect of types and anatomical arrangement of painful stimuli on conditioned pain modulation

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Abstract

Reduced pain perception during painful stimulation to another body region (conditioned pain modulation [CPM]) is considered important for pain modulation and development of pain disorders. The various methods used to study CPM limit comparison of findings. We investigated the influence of key methodological variations on CPM, and the properties of CPM when the back is used for the test (TS) or conditioning (CS) stimulus. Two different TS (pressure pain threshold [PPT] and pain response to suprathreshold heat [Pain-45]) were assessed before and during application of a noxious or non-noxious (sham) CS. Eight blocks of trials varied the anatomical location (back and forearms) and arrangement (body side) of the stimuli. PPT (as the TS) increased during application of noxious, but not non-noxious CS when stimuli were applied to opposite body sides or heterotopic sites on one body side. Inconsistent with pain-induced CPM, Pain-45 decreased during both noxious and non-noxious CS. These findings indicate; (i) PPT can be more confidently interpreted with respect to CPM evoked by a painful stimulus than Pain-45, (ii) the back and forearm are equally effective as sites for stimuli; and (iii) stimuli arrangement does not influence CPM, except for identical anatomical regions on the same body side.

Perspective

This study indicates PPT as the TS provides a more valid measure of pain-induced CPM than pain response to a suprathreshold heat stimulus. Induction and magnitude of CPM is independent of stimuli arrangement, as long as ipsilateral homotopic sites are avoided. Findings clarify methods to study CPM.

Research Highlights

- CPM is more confidently interpreted using pain threshold than intensity as test stimuli
- Back and forearms are equally effective as sites for the test and conditioning stimuli
- Test and conditioning stimuli for CPM should not be applied to a single body region

Key words
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CPM; pain modulation; pain threshold; suprathreshold pain.

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1. Introduction

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The perceived intensity of pain is reduced in the presence of a painful stimulus in another area of the body [24]. This phenomenon is referred to as conditioned pain modulation (CPM) [47]. The mechanisms that underlie CPM are only beginning to be understood and are likely to involve multiple inhibitory and facilitatory pathways related to pain processing. Exploration of these mechanisms is important because a range of pain disorders and syndromes have been linked to abnormal CPM [2, 16, 31, 43, 48] and this has been proposed to reflect altered pain inhibitory function [44]. Although CPM is extensively studied, the use of a large range of methods to explore this phenomenon has created methodological uncertainty and made it difficult to compare and/or interpret findings.

CPM is quantified as a reduction in pain intensity to a standard stimulus or increased threshold for a stimulus to become painful (test stimulus – TS) during or after the application of a second noxious stimulus (conditioning stimulus – CS) to another body region [32, 47]. The TS used to study CPM has involved several pain modalities (thermal, mechanical, electrical, chemical) and test types (threshold stimulus to evoke pain [e.g. pressure pain threshold – PPT] vs. pain intensity reported on a visual analogue scale [VAS] in response to a standardized suprathreshold stimulus [e.g. pain reported in response to a stimulus sufficient to evoke a pain of 45 out of 100 on a VAS [Pain-45]] [4]). CPM has also been induced using different combinations of body regions (opposite/same body side; homotopic/heterotopic anatomical sites). It is unclear whether all procedures provide comparable results. Moreover, it is difficult to draw conclusions regarding the influence of many of these procedural variants on CPM as studies have differed with respect to multiple parameters, which makes direct comparison difficult. Recent data shows that CPM responses vary greatly in the same subjects when different TS are used, and that greater magnitude of CPM is detected using PPT than other TS, including suprathreshold measures [26]. However, these findings are limited to a single stimuli arrangement (left and right arm) and CPM can vary with respect to the spatial configuration of the TS and CS [7, 28, 40]. Here we investigated the effect of

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variation of sites used for the TS and CS; and differences between two test types used for the TS – with the aim of identifying whether CPM could be elicited with equal confidence with TS based on both pain threshold and intensity measures in response to a painful CS. As our primary interest was optimization of assessment of CPM in people with back pain, we explored combinations of body regions including the forearm and back. Specific objectives were to compare CPM when TS and the CS were applied to: (i) heterotopic regions on the same body side; (ii) a homotopic region on the same body side; (iii) homotopic regions on opposite body sides; and (iv) heterotopic regions on opposite body sides – reverse arrangement of stimuli in this condition allowed us to examine whether CPM was influenced by use of the back as a site for the TS or CS. We also studied the effect of a painful and non-painful (sham) CS on the TS. *A priori* we proposed that we could only be confident of the validity of the measure if the TS was modified by a painful, but not non-painful CS. In addition, we compared two typically used TS (PPT and pain reported from a standardized painful heat stimulus [Pain-45]) that differed by sensory mode and test type for each stimuli arrangement.

2. Materials & methods

2.1 Participants

Thirty-one participants (14 males and 17 females) aged 25±6 (mean±SD) years volunteered for the study. Participants were included if they had no arm or back pain in the last three months, no history of chronic pain, no known medical conditions, no medication use on a regular basis (except oral contraceptives) and no pain relieving medications in the last seven days. They were also required to communicate in English and understand the study purpose and instructions. Participants were recruited by local advertisement around the University campus. Ethical clearance for the study was obtained from the University Medical Research Ethics Committee and participants provided written informed consent.

2.3 Conditioning stimulus (CS)

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The CS involved contact heat pain produced by a computerized Peltier based contact stimulation device (PATHWAY Pain and Sensory Evaluation System, Medoc Ltd., Israel) with a 30 x 30 mm probe. Unlike limb immersion techniques (e.g. hand immersion in painfully hot or cold water), this form of stimulation enabled application of a CS to the back. CS intensity was determined at the start of the session for each test site using a heat pain threshold (HPT) paradigm. Beginning at a temperature of 32 °C, eight ascending heat stimuli were applied with a rate of temperature change of 0.7 °C/s, and an inter-stimulus interval of 10 s. Participants signalled the onset of pain by pressing a button, and the temperature immediately returned to baseline. HPT was identified as the mean of the final five trials [31]. The CS was set at 1°C above the HPT. Some participants were unable to tolerate this CS during CPM trials, and the CS was reduced at 0.5 °C increments until reported pain scores were below "80" out of 100 on a numerical rating scale (NRS) anchored with "no pain" at 0 and "worst pain imaginable" at 100. If the CS intensity was less than "45" on the NRS, the temperature was increased until pain was reported above "45" on the NRS. This "revised" temperature was then used for all remaining CPM trials that involved the same CS test site unless further modifications were required (i.e. increase or decrease temperature). This procedure ensured the CS was safe and sufficiently intense to induce CPM over a short application time.

During CPM testing the CS was applied at an initial temperature of 32 °C before rising at a rate of 0.7 °C/s to the predetermined intensity. Temperature was returned to baseline at 7°C/s immediately following the completion of both TS measurements (~90 s). During exposure to the CS, participants reported the pain intensity caused by the CS on the NRS three times: at 0 s, 30 s, and just prior to cessation of the CS (i.e. after the last TS recording). In the event that a participant's pain response to the CS could not be maintained at or above 35/100 (NRS) for 30 s the trial was excluded on the basis that a CS of at least moderate intensity (~35/100 NRS) is considered necessary to induce CPM [27].

2.4 Test stimulus – PPT

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A pressure algometer (Somedic A/B, Stockholm, Sweden) with a 1 cm disc-shaped probe head was used to assess the PPT. Pressure was delivered perpendicular to the skin and increased at a rate of 30 kPa/s to the pressure at which the participant reported the stimulus changed from one of pressure to one of pain. Three trials were performed at each site, separated by less than 10 s, and a mean score in kPa was calculated.

2.5 Test stimulus – Pain-45 test (Pain-45)

Heat at a suprathreshold pain intensity was applied using contact heat generated by a separate device (Thermal Sensory Analyzer 2001 system with a 30 x 30 mm Peltier contact probe [Medoc Ltd., Ramat Yishai, Israel]). The temperature required for the participant to report a pain of "45" out of 100 on the NRS was identified [12]. Participants were exposed to a series of heat stimuli of ~10-s duration. The first stimulus was set at 1 °C below the HPT, followed by stimuli of increasing intensity (1 °C increments) separated by a 60-s inter-stimulus interval to minimize skin sensitization. After each stimulus, participants verbally rated their pain on the NRS. When a stimulus induced pain of at least 45/100, the test was discontinued and the temperature was selected as the TS.

The protocol used for application of the TS involved an increase in temperature at a rate of 4°C/s up to the "Pain-45" temperature and maintained for ~10 s before returning to a baseline temperature of 28°C to facilitate the reduction of skin temperature and avoid changes in skin sensitivity. Three trials were performed at each site, separated by less than 10 s, with the pain intensity rated after each TS and a mean score out of 100 was calculated.

2.6 Procedure

Four body regions were selected for testing: (1) right forearm, (2) left forearm, (3) right side of the lower back, and (4) left side of the lower back (Fig 1). Anatomical landmarks used for the assessment of PPT were the proximal region of the muscle belly of extensor carpi radialis longus for the forearm, and 2 cm lateral to the spinous process of the L3 vertebrae for the lower back. The locations used for the Pain-45 test were at the proximal volar aspect of the forearms, and 2 cm

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lateral to the spinous process of L1 in the lower back. The CS was delivered 10 cm from each TS site. This is the minimal distance between two concurrent stimuli that has been shown to induce CPM [34].

Trials were conducted in eight test blocks (Table 1) performed in random order in a single session with the TS and CS applied to:

(1) Homotopic regions on opposite sides of the body (back or forearms)

(2) Homotopic regions on the same side of the body (back or forearms)

(3) Heterotopic regions on opposite sides of the body (back and forearm)

(4) Heterotopic regions on the same side of the body (back and forearm)

The CPM paradigm commenced 15 min after determination of the Pain-45 and CS temperatures. Each block of trials involved three repetitions of the two TS (i.e. PPT and Pain-45 test), in random order (allocated *a priori*), after which the CS commenced, and the two TS were reapplied 30 s after onset of the CS, again in random order. The CS was maintained until all TS measurements had been completed (~90 s). The sham procedure to test the validity of the CPM measures was undertaken in an additional block with the CS intensity below the HPT (thermode set at 32 °C) applied to the right forearm and the TS applied to the left forearm. The sham block was randomised amongst the other blocks in the experiment. A 15-min rest was enforced between blocks to eliminate any unresolved CPM effects (Fig 2) [44].

2.7 Data analysis

Data were analysed in two ways. First, the TS values before and during the CS were compared to determine whether the TS changed. Second, absolute change scores (difference between TS scores obtained before and during the CS [48]) were calculated to compare the magnitude of CPM between conditions. A decrease in pain evoked in the Pain-45 test or an increase in the PPT during exposure to the CS is consistent with CPM, and were both expressed as positive values. We controlled for potential gender differences in pain thresholds and perception (e.g. females have lower PPTs and are less tolerant to thermal and pressure pain for a specific stimulus intensity [35])

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by virtue of our repeated measures design and individualisation of the stimulus intensities used for the TS and CS, hence data for males and females could be pooled for analysis. Although there may be minor differences in the magnitude of CPM between genders we did not power the study to investigate this as our primary aim was to compare the relative efficacy of different stimulus combinations to evoke CPM.

2.8 Statistical analysis

Statistical analyses were conducted using STATISTICA (version 10, StatSoft, Inc, OK, USA) with significance set at $\alpha = 0.05$. An initial analysis was conducted to test whether a CPM response could be induced using our protocol (Table 1, "sham" condition). We considered our data would be consistent with a CPM response if PPT increased and/or Pain-45 decreased during the noxious CS, but not the non-noxious (sham) CS. This was evaluated with a separate repeated measures analysis of variance (ANOVA) for each TS. Using data from the left forearm (TS) and right forearm (CS) arrangement, we compared TS measures before and during the CS (CONDITIONING) and between trials with a painful and non-painful (sham) CS (CONDITION TYPE).

To compare the effect of CPM according to anatomical location, repeated measures ANOVAs were undertaken on TS scores (PPT and Pain-45 test ratings), with the factors CONDITIONING (two levels – before vs. during the CS) and ARRANGEMENT (eight levels – eight different stimuli arrangements [see Fig 1 and Table 1]). If there was a significant CONDITIONING x ARRANGEMENT interaction, Duncan's multiple range test was used for post-hoc analysis. This test was chosen over more conservative post-hoc tests (which are less protective against false negatives) to confirm, with greater certainty, whether the sham CS had an effect on the TS and thus verify the legitimacy of a CPM effect according to our definition.

To address specific questions posed in this study, change scores were compared in three separate repeated measures ANOVAs (Table 2). As the validity of the Pain-45 measure was not confirmed (there was no difference between trials with a painful and non-painful CS [see "Results"]) this analysis was only undertaken on data using PPT as the TS. These analyses

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investigate whether CPM differed between stimulus configurations based on: (1) side of the body (SIDE: two levels – same vs. opposite body sides arrangement) and anatomical site (ANATOMY: two levels – forearm vs. back) used for the TS and CS, (2) matched or unmatched regions on opposite sides of the body (REGION: two levels – homotopic vs. heterotopic anatomical sites) and TS location (TS-LOCATION: two levels – forearm vs. back), and (3) matched or unmatched regions on the same side of the body (REGION: two levels – homotopic vs. heterotopic anatomical sites) and TS location (TS-LOCATION: two levels – forearm vs. back).

3. Results

All 31 participants completed the eight-block experimental CPM paradigm. Despite efforts to maintain the CS intensity above 45/100 (NRS), pain ratings fell below 35/100 (NRS) in 12 TS trials (out of a total of 217 noxious CS trials) across eight different participants. These data were removed given the intensity of pain was considered not sufficient to induce CPM [10].

3.1 Effects of CS on PPT

Fig 3 presents PPT values before and during the CS. Analysis of the validity of PPT to detect CPM shows that a change in baseline PPT during application of the CS (Main effect: CONDITIONING – $F[1, 28] = 0.1$, $p = 0.815$) depended on whether a noxious or non-noxious sham CS was used (Interaction: CONDITIONING x CONDITION TYPE – $F[1, 28] = 8.1$, $p = 0.008$). That is, although the PPT for the left forearm was greater during application of the noxious CS to the right forearm than prior to the CS (post hoc $p < 0.001$), there was no difference in PPT with the identical stimuli arrangement but with a non-noxious sham CS (post hoc $p = 0.390$). This suggests the increase in PPT in the presence of a painful CS is consistent with pain-induced CPM.

Change in PPT in the presence of the CS depended on the location of stimuli (Main effect: ARRANGEMENT – $F[7, 161] = 69.8$, $p < 0.001$; Interaction: CONDITIONING x ARRANGEMENT – $F[7, 161] = 2.6$, $p = 0.015$). Post hoc tests showed that PPT was higher than baseline during a noxious CS when the TS and CS were applied to heterotopic anatomical sites on the same side of the body (TS –

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left forearm, CS – left back, p = 0.006) or opposite sides of the body, regardless of anatomical site (TS – right back, CS – left back, p = 0.014; TS – right back, CS – left forearm, p < 0.001; TS – left forearm, CS – right forearm, p = 0.003), with exception of the left forearm (TS) and right back (CS) combination. No CPM effect was found when the TS and CS were applied to homotopic anatomical sites on the same side of the body (TS – left back, CS – left back, p = 0.857; TS – left forearm, CS – left forearm, p = 0.918).

3.2 Effects of CS on the heat Pain-45 test

Unlike PPT, pain reported during the Pain-45 test (left forearm) reduced when the right forearm was exposed to either the noxious CS (Main effect: CONDITIONING – F [1, 28] = 19.0, p < 0.001; post hoc p = 0.008) or non-noxious CS (p < 0.001), and the reduction in pain scores were of similar magnitude (Main effect: CONDITION TYPE – F [1, 28] = 0.4, p = 0.532). Pain reported during the Pain-45 test was reduced during the noxious CS with all stimuli arrangements (Main effect: CONDITIONING – F [1, 28] = 38.9, p < 0.001; post hoc: all p < 0.026) (Fig 4). The magnitude of the reduction in pain (change scores) did not differ between arrangements (Main effect: ARRANGEMENT – F [7, 154] = 0.4, p = 0.914). As our data questioned the validity of the Pain-45 test to study a CPM effect we undertook no further analysis.

3.3 Effect of side of the body and anatomical site (back vs. forearm) on CPM

The magnitude of CPM, measured using PPT as the TS, was greater when the TS and CS were applied to homotopic sites on opposite sides of the body than the same side of the body (Main effect: SIDE – F [1, 25] = 7.1, p = 0.013) regardless of whether the back or forearms were used (Fig 5). No difference in the magnitude of CPM was found between the back and forearms regardless of body side arrangement (Main effect: ANATOMY – F [1, 25] < 0.1, p = 0.991).

3.4 Effect of homotopic and heterotopic anatomical sites on opposite sides of the body on CPM

No difference in the magnitude of CPM was found between stimuli combinations that involved homotopic sites on opposite sides of the body (e.g. TS – back, CS – back) and heterotopic sites on opposite sides of the body (e.g. TS – back, CS – forearm) (REGION – F [1, 26] = 0.4, p =

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0.509), irrespective of whether the TS was applied to the back or forearm (TS-LOCATION – F [1, 26] = 0.8, p = 0.366) (Fig 6). Post hoc analyses show that the amplitude of CPM was not affected by reversal of the TS and CS (p = 0.192).

3.5 Effect of homotopic and heterotopic anatomical sites on the same side of the body on CPM

Fig 7 displays the comparison between homotopic and heterotopic stimuli combinations on the same side of the body on CPM. Although CPM effects were induced when stimuli were positioned on heterotopic regions (TS – left forearm, CS – left back), this was not apparent for stimuli applied to a homotopic region (TS – left forearm, CS – left forearm) on the same side of the body (Main effect: REGION – F [1, 26] = 7.8, p = 0.01; post hoc p = 0.010).

4. Discussion

This study has three main findings. First, we show that changes in PPT (as the TS) provide a valid measure of CPM, but this could not be confirmed for when the TS is the pain intensity to a suprathreshold heat stimulus (Pain-45 test). Although heat Pain-45 scores reduced during the painful CS, inconsistent with pain-induced CPM they also reduced during the non-painful (sham) CS. Second, CPM was best evoked when the TS and CS were applied on opposite body sides (for the same [homotopic] and different [heterotopic] anatomical sites), and heterotopic, but not homotopic, anatomical sites on the same body side. Third, CPM magnitude was similar whether the TS or CS were applied to back or forearm sites. These data have implications for future investigations of CPM.

4.1 Effect of TS type on CPM

These data provide evidence of validity of PPT as the TS for measurement of CPM but not suprathreshold pain measures. Although a recent study that compared different TS support this finding [26], our conclusion differs from that of Pud et al. [32] who, after review of the literature, argued that suprathreshold measures were preferable to threshold measures. Pud et al.'s [32] argument was based on greater variability of threshold measures (pain threshold increased by between 3% to

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100%) than suprathreshold measures (pain intensity decreased by between 10 to 55%). We propose that variation in percentage TS change is not an optimal criteria to judge relative merits of TS types as the scoring methods of the TS measures differ in terms of the variance structure. Suprathreshold pain measures use a scale bounded by 0 to 10 or 100, whereas, threshold measures are recorded with variable boundaries guided by the tolerance of the participant and could yield higher percentage change scores. Thus, justification of preference for one method based on magnitude of variation is not ideal and we argue that evaluation of the validity of detection of CPM provides a better means to compare methods. Consistent with our observations, Pud et al. [32] noted that supratheshold pain measures, but not threshold measures, had failed to detect CPM in healthy controls in at least one study [39]. Other work supports our view that threshold-based TS provides a more valid test of CPM [22, 42] (e.g. CPM measured using both TS methods more consistently showed age-related decline in CPM when assessed using a threshold measure [18]).

The two TS used in the current study differed in modality, intensity and nature of the response (threshold vs. intensity). Others concur that CPM varies with different TS [26]. This beckons the question whether differences in psychophysical properties of each method contributed to disparity in their validity as a CPM measure. This has been implied in earlier studies. CPM increases when attention is directed to the TS and CS [7, 39] and distraction away from the stimuli has a small but significant effect on CPM [20]. Distraction might differ between stimuli. As suprathreshold pain intensity measures are strongly correlated with psychological factors such as fear of pain and anxiety [36], and can be substantially modulated by simple cues (e.g. visual cues [25]) it is reasonable to speculate that a suprathreshold painful stimulus could be more threatening and have a greater effect on attention. Large changes in Pain-45 test values in the current study, even with the non-noxious CS, support this notion.

Differences in CPM might also be partially explained by variability of the TS. Although assessment of pain threshold and pain reported for a standard stimulus involve similar somatosensory pathways (e.g. spinothalamic) [13], the process involved in interpretation of each is

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distinct; one involves decision regarding a change from non-noxious to noxious, whereas the other requires interpretation relative to an abstract scale, and variability is inherent in both. Numerous studies have reported variability in PPT in people with [23, 38] and without [5, 6, 29] clinical pain conditions. Perception of pain to a standard painful stimulus also has inherent variability; pain intensity varies between repeat assessments [3, 5, 8, 15] and with demographic and psychological variables [36, 37]. In summary, both measures have some inherent variation, however in our study, only the threshold measure (PPT) yielded a change consistent with pain-induced CPM, which we argue is a better comparison to determine preference for TS measure.

4.2 Effect of anatomical test site on CPM

With the exception of homotopic sites on the same side of the body, the combination of anatomical regions selected for the TS and CS had no effect on CPM. This suggests an application site in the lower back region for the TS and/or CS is equally effective as a forearm region for induction of CPM. Although various body regions have been explored (e.g. legs, arms, neck and head – see Pud et al. [32]), direct comparison between different sites is limited to a few studies, and the results are inconsistent. Although some report no difference between arm and leg sites [40] others reported greater CPM for leg sites [28]. The present study is the first to validate the back, against a commonly used region (forearm), as a site for TS or CS application.

Confirmation that stimulation of the back can generate CPM in healthy controls provides an opportunity to explore whether patients with and without back pain yield different results. This is important for two reasons; first, it is not known whether back pain involves altered CPM, as has been shown in fibromyalgia [17] and some people with thorax pain post-thoracotomy [48]; and second, sensory disturbances have been reported in back pain (e.g. hyperalgesia [11] and allodynia [9]) that implicate disturbed nociceptive processes. The only study of CPM in back pain tested whether patient's clinical pain (interpreted as a CS) was associated with higher pain threshold for a TS in another body region [30]. Although that study found no difference in pain thresholds when compared to pain-free controls, it is unclear how this relates to CPM because the TS was not measured in

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patients prior to having back pain, and this comparison is critical for interpretation of CPM. Further, this would be contrary to the common observation of reduced pain thresholds, consistent with sensitization [14]. Studies that investigate TS before and during a CS are required.

4.3 Effect of the arrangement of the TS and CS on CPM

Consistent with Pud et al. [32], CPM magnitude was similar when the TS and CS were applied to opposite body sides regardless of whether homotopic or heterotopic anatomical sites were used. CPM magnitude was also similar when heterotopic, but not homotopic, anatomical sites were used on the same body side. Larger CPM magnitudes have however been reported for heterotopic sites (arm and leg) on opposite body sides than the same side [40]. The effects may differ whether a limb or the trunk is used. Although we showed no CPM when stimuli were placed 10 cm apart on the forearm or back, one study induced CPM with stimuli separated by 10 cm on the legs [34], and another showed increasing CPM magnitude as the distance between stimuli increased from 30 cm, regardless of the body side or region [7]. Taken together with evidence that spatial summation of thermal noxious stimuli (an effect opposite to CPM) occurs with a separation of 5 cm or less [7], separation of the TS and CS by 10 cm is on the borderline of that required to induce CPM. Interestingly, recent work from our group using transcutaneous electrical stimulation delivered to the forearm shows that spatial summation can occur for distances up to 20 cm (Reid et al., 2013 unpublished data).

4.4 Effect of CS on CPM

There is disagreement whether the CS must be painful to induce CPM. Our data show CPM using PPT was elicited when the CS was painful but not in the non-painful sham condition. Although Lautenbacher et al. [19, 21] demonstrated a strong but non-painful CS (hand immersion in 42 °C water) reduced the pain provoked by thermal stimulus, Granot et al. [12] did not; pain only reduced (i.e. CPM) when the CS was painfully hot (46.5 °C) or painfully cold (12 °C), and not after non-painful stimuli (15 °C, 18 °C and 44 °C). Further, some studies have shown increased magnitude of CPM with CS intensity (temperature) within an individual [41, 45], whereas others report no difference in

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CPM magnitude between moderate and intense CS intensities [27] and that the pain induced by the CS is unrelated to CPM magnitude, once the CS becomes painful [12, 33]. Although our data showed reduced Pain-45 test ratings during a non-painful CS, we contend that TS involving reports of pain to a suprathreshold stimulus provides a less sensitive measure of CPM in response to a painful CS.

4.5 Study limitations

It is important to note that a sham CS was only studied for the left and right forearm arrangement. A priori we decided that a valid measure of CPM could only be considered to be valid if the TS reduced during painful, but not sham CS. Although it could be argued that evaluation of the validity of the interpretation of presence of CPM would be more thorough if we studied a sham in all configurations, we considered it necessary to limit the number of conditions to avoid any adverse effects of repeated exposure to tests. We chose the left (TS) and right (CS) forearm combination for the sham condition because it is consistent with the paradigm studied in most of the existing literature. Finally, we accept that comparison of two psychophysically different TS (test type - threshold vs. intensity; and sensory mode - thermal vs. pressure) preclude any direct conclusions regarding whether the “test type” or “sensory mode” was responsible for the reduced confidence in detection of CPM using the heat pain-45 method than PPT as the TS.

4.6 Conclusion

Differences in the experimental methods employed to evoke CPM in separate studies complicate interpretation of experimental findings. The present findings suggest that PPT can be more confidently interpreted with respect to pain-induce CPM than pain reported for a suprathreshold heat stimulus as the TS for the configurations and set up tested here. Further, the back and forearm are equally effective as sites for application of the TS and CS. Our data also suggest arrangement of the TS and CS does not influence CPM, as long as identical anatomical regions on the same side of the body are avoided. These findings further clarify the methods by which CPM is effectively activated.

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Disclosures

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Figure legends

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Figure 1. Locations used for the test and conditioning stimuli.

Figure 2. Schematic representation of the experimental protocol for the induction and assessment of conditioned pain modulation over 8 testing blocks (only 4 blocks are shown).

Figure 3. PPT scores (mean + SD) before (baseline) and during the CS for all eight stimuli arrangements. “TS”, test stimulus (PPT); “CS”, conditioning stimulus; “R Back”, right side of back; “L Back”, left side of back; “R Arm”, right forearm; “L Arm”, left forearm; *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 4. Pain ratings (NRS [mean + SD]) from the Pain-45 test before (baseline) and during the CS for all eight stimuli arrangements. “TS”, test stimulus (Pain-45 test); “CS”, conditioning stimulus; “R Back”, right side of back; “L Back”, left side of back; “R Arm”, right forearm; “L Arm”, left forearm; *, P<0.05; **, P<0.01; ***, P<0.001.

Figure 5. Conditioned pain modulation (Mean + SD) assessed with stimuli configured using two back conditions and two forearm conditions: Conditions from left–to–right: (1) PPT and CS applied to the left back, (2) PPT applied to the left back and CS applied to the right back, (3) PPT and CS applied to the left forearm, and (4) PPT applied to the left forearm and CS applied to the right forearm. “TS”, test stimulus (PPT); “CS”, conditioning stimulus; “CPM”, conditioned pain modulation.

Figure 6. Conditioned pain modulation (Mean + SD) assessed using homotopic and heterotopic anatomical sites on opposite body sides. Measures of PPT (TS) are either recorded from the back or forearm. Conditions from left-to-right: (1) TS – right side of the back, CS – left side of the back, (2) TS – right side of the back, CS – left forearm, (3) TS – left forearm, CS – right forearm, (4) TS – left

forearm, CS – right side of the back. "TS", test stimulus; "CS", conditioning stimulus; "CPM", conditioned pain modulation.

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Figure 7. Conditioned pain modulation (Mean + SD) assessed using homotopic and heterotopic anatomical sites on the same body side. Conditions from left-to-right: (1) TS – left forearm, CS – left forearm, (2) TS – left forearm, CS – left side of the back. "CPM", conditioned pain modulation; **, P=0.01.

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Table 1. Locations of the test and conditioning stimuli for each experimental block.

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	Arrangement	
Condition	Test Stimuli	Conditioning Stimulus
Homotopic regions on opposite body sides	L Forearm	R Forearm
	L Back	R Back
Homotopic regions on the same side	L Forearm	L Forearm
	L Back	L Back
Heterotopic regions on opposite body sides	L Forearm	R Back
	R Back	L Forearm
Heterotopic regions on the same side	L Forearm	L Back
Sham	L Forearm	R Forearm (skin temp)

"L", left; "R", right; "temp", temperature.

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Table 2. Repeated measures ANOVA models used to compare change scores (CPM) with different stimuli configurations.

	TS	CS	TS	CS
Anatomy vs. side				
	ANATOMY			
SIDE	Arm		Back	
Same	L Forearm	L Forearm	L Back	L Back
Opposite	L Forearm	R Forearm	R Back	L Back
Region (opposite body sides) vs. TS location				
	REGION			
TS-LOCATION	Homotopic		Heterotopic	
Arm	L Forearm	R Forearm	L Forearm	R Back
Back	R Back	L Back	R Back	L Forearm
Region (same body side) vs. TS location				
	REGION			
TS-LOCATION	Homotopic		Heterotopic	
Arm	L Forearm	L Forearm	L Forearm	L Back

"TS", test stimuli; "CS", conditioning stimulus; "L", left; "R", right.

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Figure 1.

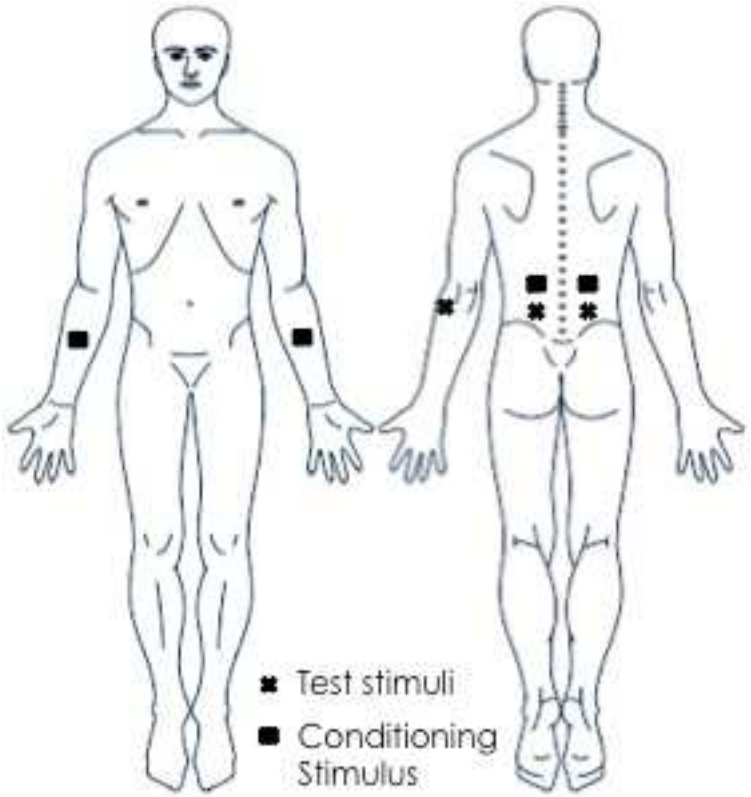

Figure 2.

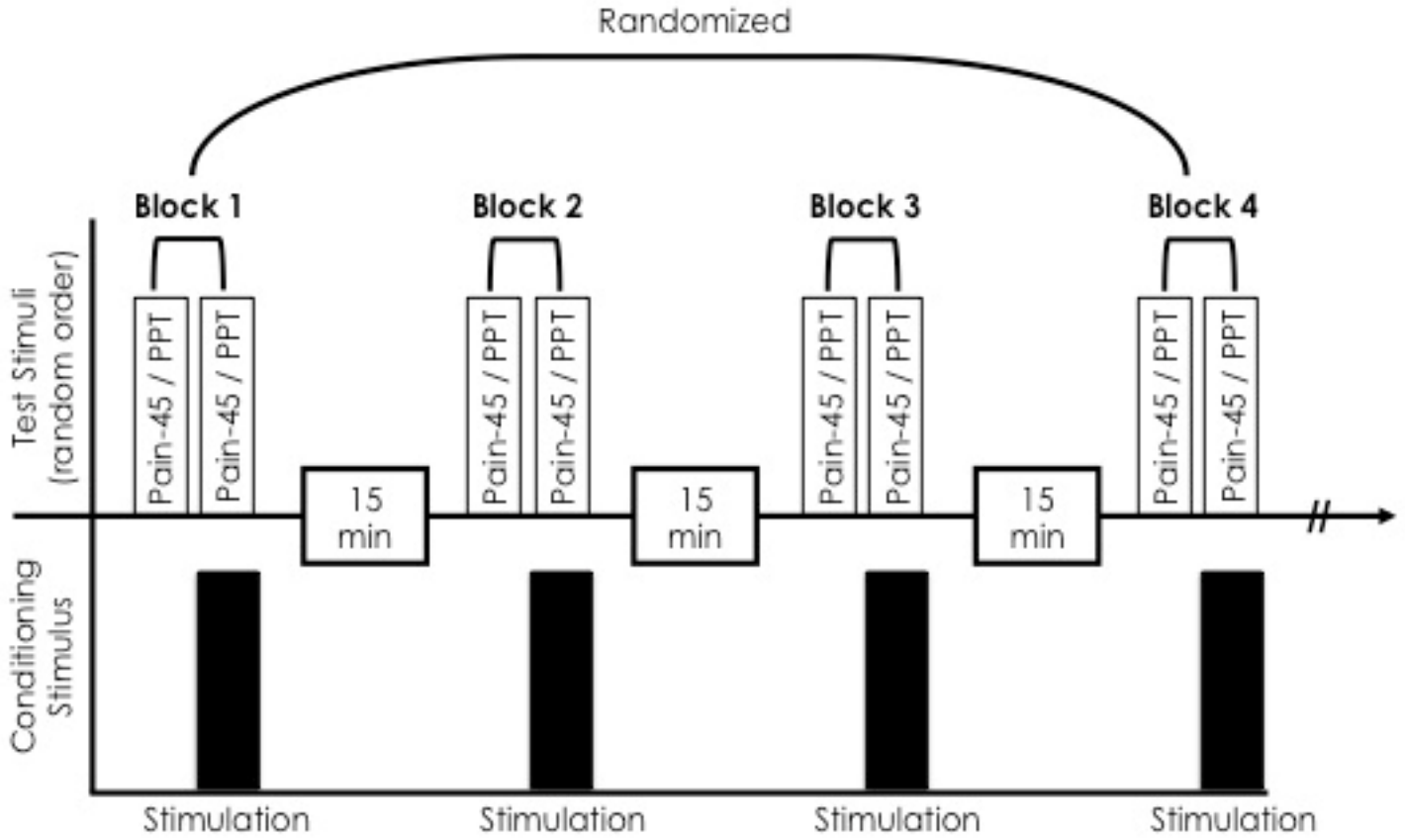
Randomized
Block 1
Block 2
Block 3
Block 4
Test Stimuli
(random order)
Pain-45 / PPT
Pain-45 / PPT
15
min
Conditioning
Stimulus
Stimulation
Stimulation
Stimulation
Stimulation

Figure 3.

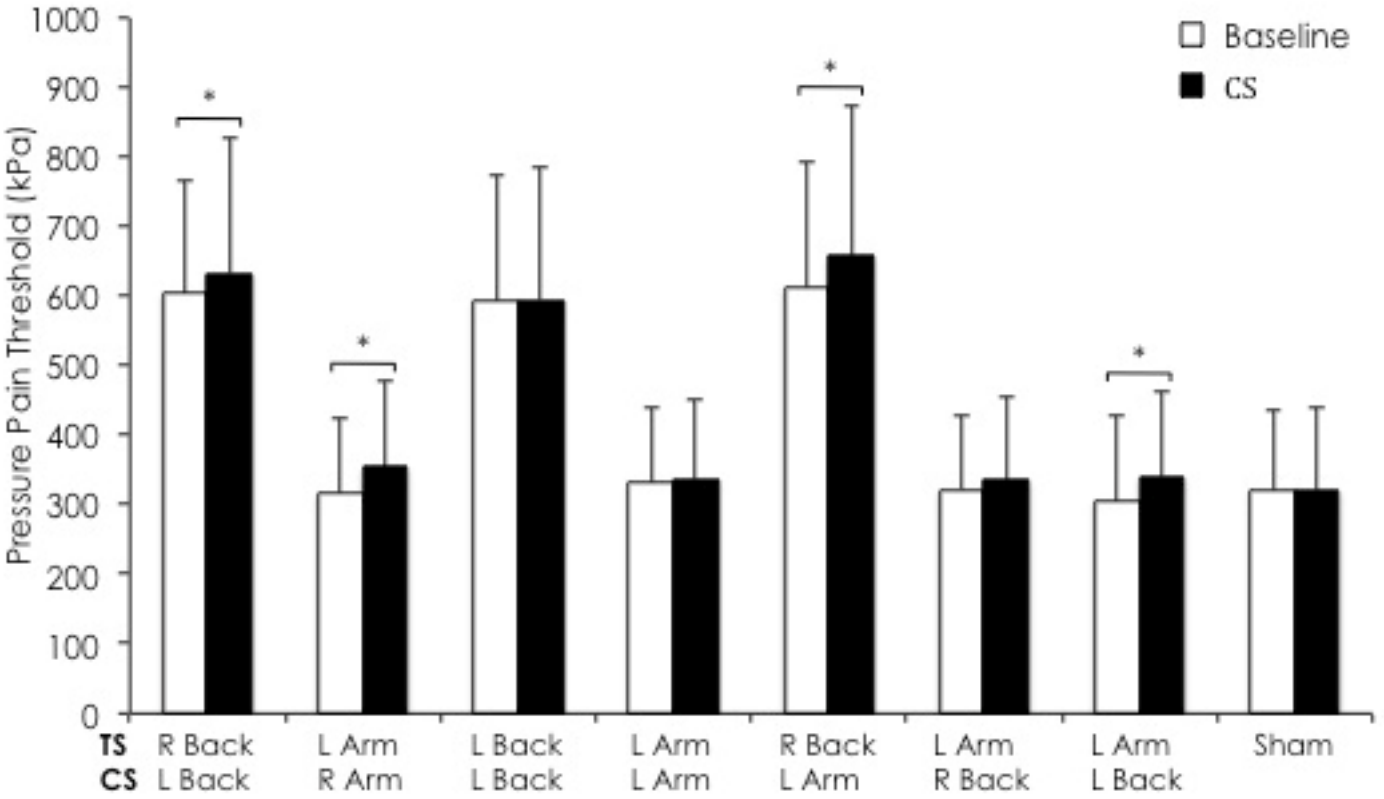
Pressure Pain Threshold (kPa)
1000
900
800
700
600
500
400
300
200
100
0
Baseline
CS
*
*
*
*
TS R Back L Arm L Back L Arm R Back L Arm L Arm Sham
CS L Back R Arm L Back L Arm L Arm R Back L Back

Figure 4.

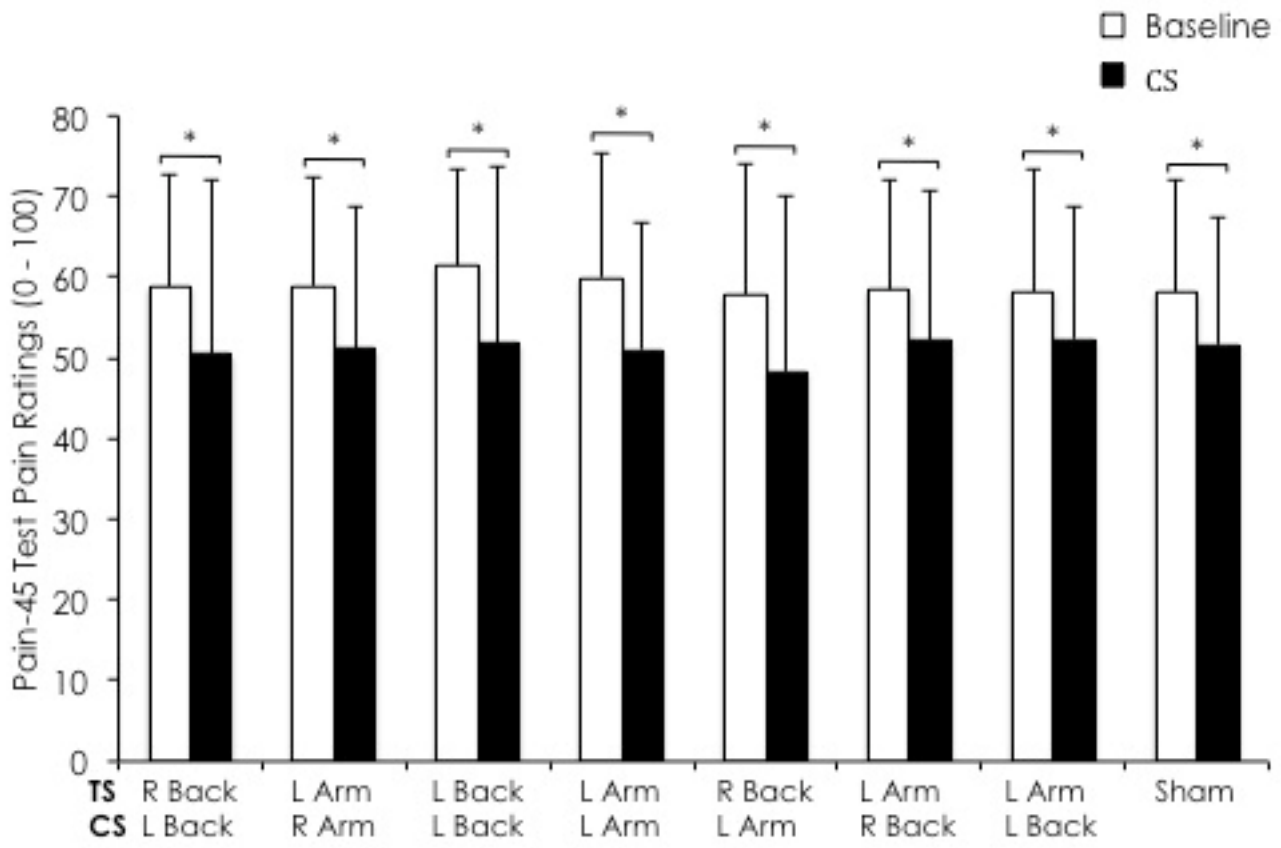
Baseline
CS
Pain-45 Test Pain Ratings (0 - 100)
80
70
60
50
40
30
20
10
0
*
TS R Back L Arm L Back L Arm R Back L Arm L Arm Sham
CS L Back R Arm L Back L Arm L Arm R Back L Back

Figure 5.

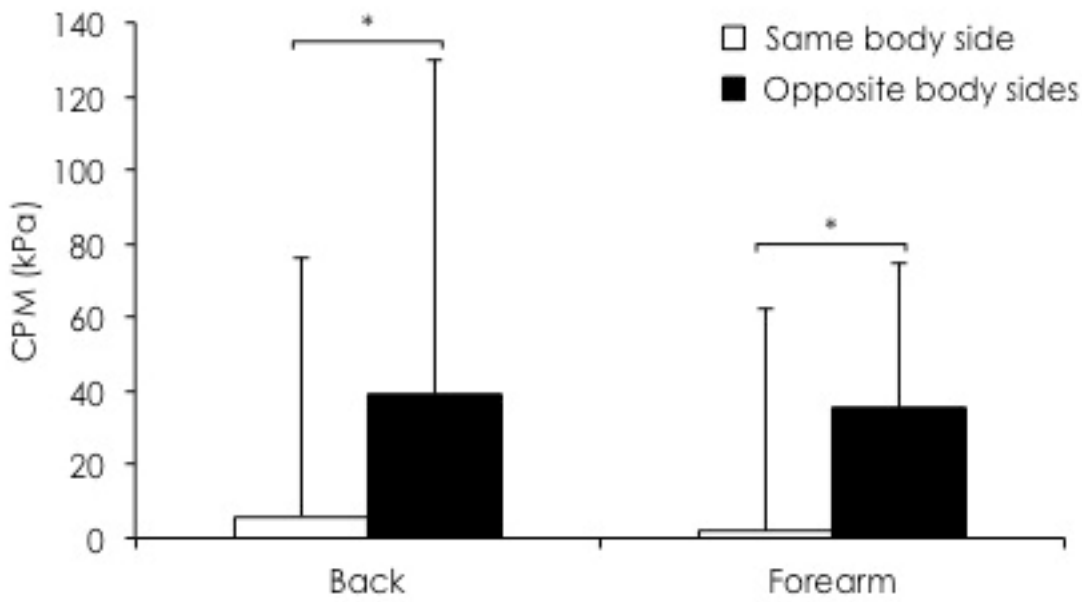

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Figure 6.

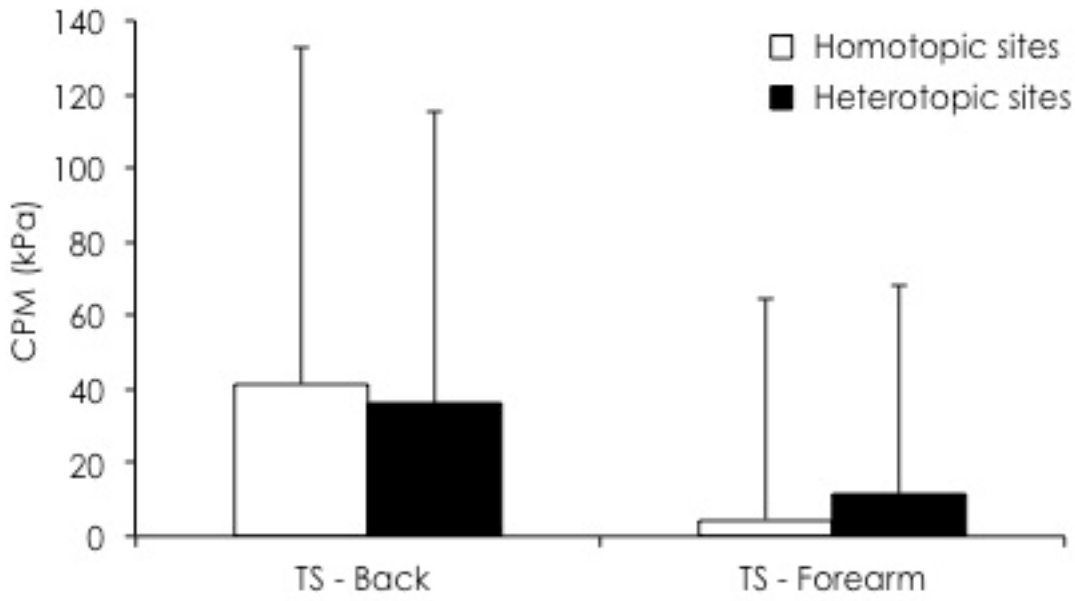

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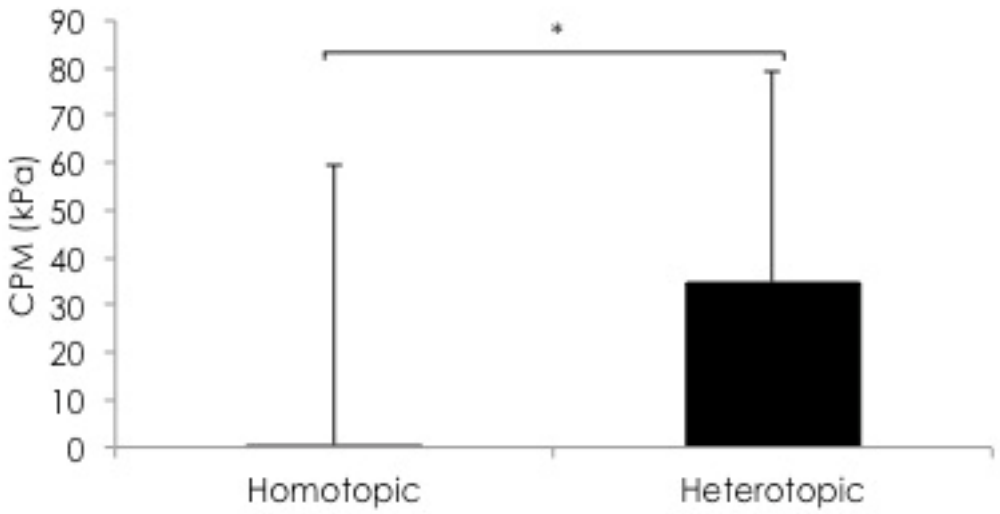
Figure 7.



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