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Exploration of conditioned pain modulation effect on long-term potentiation-like pain amplification in humans

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Abstract

Background: This study aimed to explore conditioned pain modulation (CPM) effect on long-term potentiation (LTP)-like pain amplification induced by cutaneous 10-Hz conditioning electrical stimulation (CES).
Methods: Conditioned pain modulation was induced by cold pressor conditioning stimulus (CPCS) (4 °C) which was applied immediately before CES in the active session. In the control session, water with a temperature of 32 °C was used. Twenty subjects participated in two sessions in a randomized crossover design with at least 1-week interval. Perceptual intensity ratings to single electrical stimulation (SES) at the conditioned skin site and to pinprick and light-stroking stimuli in the immediate vicinity of the CES electrodes were measured . Superficial blood flow (SBF), skin temperature (ST) and heat pain threshold (HPT) were measured covering both homotopic and heterotopic skin. The pain intensities during CES process were measured and short-form McGill Pain Questionnaire (SF-MPQ) was used for assessing CES pain experience.
Results: Cold pressor conditioning stimulus reduced pain perception increments to weak pinprick and light-stroking stimuli after 10-Hz CES compared with the control session. Moreover, CPCS resulted in lower pain intensity ratings during CES process but without affecting the SF-MPQ scores between two sessions. The SBF and ST increased after CES and then gradually declined but without differences between CPCS and control sessions. CPM did not affect HPT and pain intensity increments to SES.
Conclusions: The CPCS inhibited heterotopic perception amplification to weak mechanical stimuli after CES. The results indicate that endogenous descending inhibitory systems might play a role against development of non-nociceptive perception amplificatory states (e.g. allodynia).
Significance: Conditioned pain modulation (CPM) may play a role in inhibiting the pain amplificatory process at the central nervous system and prompting central desensitization. CPM has a special inhibition effect for the development of perception amplification to non-painful mechanical stimuli.

1. Introduction

Characterization of endogenous pain modulation is an important aspect in understanding the mechanisms underlying chronic pain. Spinal long-term potentiation (LTP) is long-lasting enhancement of excitatory synaptic transmission at the synaptic connections in the spinal cord dorsal horn following conditioning noxious stimulation (Willis, 1993; Liu and Sandkühler, 1997; Ikeda et al., 2003). LTP-like phenomena have been considered to be a mechanism underlying the neurogenic pain amplification such as persistent post-operative pain and chronic pain conditions initiated by a painful event, for example, peripheral inflammation or neuropathy (Sandkühler, 2000; Ji et al., 2003; Ruscheweyh et al., 2011; Sandkühler and Gruber-Schoffnegger, 2012; Price and Inyang, 2015). Moreover, the central sensitization concept describes increased excitability and synaptic efficacy in central nociceptive pathways and may play a major role in several chronic pain conditions (Woolf, 2011). Sustained low frequency discharging of C-fibre nociceptors during neuropathic or inflammatory pain conditions has been considered to contribute to the elevated responsiveness and activity of dorsal horn neurons (Puig and Sorkin, 1996; Han et al., 2000; Xiao and Bennett, 2007; Drdla and Sandkühler, 2008). This is manifested in patients as increased response to noxious stimuli (hyperalgesia) and pain resulting from normally innoxious tactile stimuli (allodynia) (Latremoliere and Woolf, 2009). As a model of injury-induced hyperalgesia, heterotopic LTP-like pain amplification can be induced by continuous 10-Hz conditioning electrical stimulation (CES) or bursts of 100-Hz CES in healthy humans (Klein et al., 2004; Xia et al., 2016a,b). The afferent activity in the 10-Hz LTP model may resemble the low frequency discharging of C-fibre nociceptors following an injury. Hence, this model may involve a similar mechanism as in the development of chronic pain (Handwerker et al., 1987; Ji et al., 2003; Drdla and Sandkühler, 2008; Hathway et al., 2009).

In contrast to the pain amplification caused by the conditioning noxious stimulation, a distant conditioning painful stimulus can inhibit the nociceptive response evoked by a test stimulus. This is named 'diffuse noxious inhibitory control' (DNIC) (Le Bars et al., 1979b). Later, the term 'conditioned pain modulation' (CPM) has been introduced involving a broader description of inhibitory pain modulatory phenomena in humans. The CPM effect refers to the phenomenon that a remote tonic painful stimulus (conditioning stimulus) decreases the perceived pain intensity caused by a test stimulus (Yarnitsky et al., 2010). As an important manifestation of an endogenous inhibitory system, the CPM has been shown to inhibit nociceptive spinal neuronal activity leading to decreasing hyperalgesia and nociceptive responses in animals (Bouhassira et al., 1992) and pain perception in humans (Meeus et al., 2008; Villanueva, 2009; Roussel et al., 2013). In human studies, the cold pressor test is most often used as the conditioning stimulus to induce the CPM because of better reliability compared with other methods such as pressure pain or tourniquet pain (Oono et al., 2011; Lewis et al., 2012). The mechanisms underlying CPM is thought to involve descending inhibitory serotonergic and noradrenergic systems leading to inhibition of wide dynamic range (WDR) neurons in the spinal dorsal horn (Le Bars et al., 1979b; Bouhassira et al., 1992; Le Bars, 2002; Piché et al., 2009; Nir et al., 2011; Sprenger et al., 2011).

The endogenous pain inhibition mechanisms are still not fully known and an effective chronic pain treatment strategy remains a challenge. In this study, the CPM is hypothesized to have an inhibitory effect on the induction of LTP-like pain amplification by 10-Hz CES in healthy humans. This will help to provide new theoretic methods to understand the endogenous perceptual modulation on pain amplification.

2. Methods

2.1 Subjects

The experiments were performed on 20 subjects (6 females and 14 males; 20–37 years; mean age 27 years) after obtaining approval from the local ethical committee (N-20120046). All subjects participated in a training session and two experimental sessions. The subjects were seated in a reclining chair with the right arm placed comfortably on the table. The room temperature was 23~26 °C. Exclusion criteria were prior or current skin disease, neurological disease, any history of chronic pain as well as drug abuse or suffering from ongoing pain. All subjects gave their written informed consent prior to their inclusion in the study. The study was performed according to the Declaration of Helsinki.

2.2 Conditioning electrical stimulation (CES)

Cutaneous electrical stimulation from a constant current stimulator (DS5; Digitimer Ltd; Welwyn Garden

City, UK) was applied to the right forearm, 7 cm distal to the cubital fossa. The stimulations were applied using an epicutaneous pin electrode (EPE) consisting of a circular array (diameter: 10 mm; area: 79 mm^2) of 15 cathodal electrodes, each with a diameter of 0.2 mm, protruding 1 mm from the base. A large circular stainless steel plate served as the anode with an inner diameter of 20 mm and an outer diameter of 40 mm and was placed concentrically around the cathodes (Fig. 1A) (Biurrun Manresa et al., 2010). This electrode has been verified to induce pain/stinging at lower stimulation intensity compared with conventional cutaneous patch electrodes because the diameter of the cathodes is smaller; thus, achieving a high current density in the epidermal layers where the nociceptive Aδ- and C-fibres terminate (Hansen et al., 2007; Mørch et al., 2011). The individual electrical detection threshold (DTh) was determined using the method of limits: three series of electrical pulses with increasing and decreasing intensity at a step size of 3% present stimulation intensity. The final DTh was determined by the geometric mean value of the three assessments. 10-Hz CES (pulse duration: 1 ms) was used for induction of LTP-like pain amplification (Xia et al., 2016a,b). This CES process lasted 50 s and consisted of 500 rectangular 1 ms pulses. The intensity of the CES was 10× DTh which evoked a clearly painful sensation.

2.3 Experimental protocol

Three sessions were arranged for each subject. The first session (training) aimed to familiarize the subjects with the different stimulus modalities and gaining experience in rating the test stimuli using a visual analog scale (VAS). The data obtained during the training session were not analysed. Cold pressor conditioning stimulus (CPCS) and control experimental sessions were randomly assigned on two experimental days conducted at least 1 week apart for each subject in a crossover design. CPCS (left foot in an ice-filled water bath holding 4 °C) was used to activate the CPM. The cold and control water immersions of the foot were performed in a bucket filled with water to the ankle level for 2 min. A metallic net was placed in the water bucket to prevent direct contact between the foot and the ice. The CPCS induced a strong painful sensation (as the conditioning stimulus) and the control water was 32 °C which induced a warm comfortable sensation. All subjects were encouraged to put the foot back into the cold water as soon as possible if they withdrew it because of intolerable pain. 10-Hz CES was started immediately after the conditioning stimulus. A series of test stimuli was applied on the right forearm three times before and six times after the CES with intervals of 10 min (Fig. 1B). The test stimuli were pinprick and light-stroking stimulation surrounding the conditioned sites and homotopic single electrical stimulation (SES) at the conditioned sites using the same concentric electrode. The heat pain threshold (HPT) was measured at a skin site covering both the conditioned and the surrounding skin area. Neurogenic inflammatory responses were assessed using blood flow imagery and thermography. A VAS was used to assess the perception intensity. It was anchored at 0 (no sensation) and 100 (the most intense pain imaginable) with 30 indicating the pain

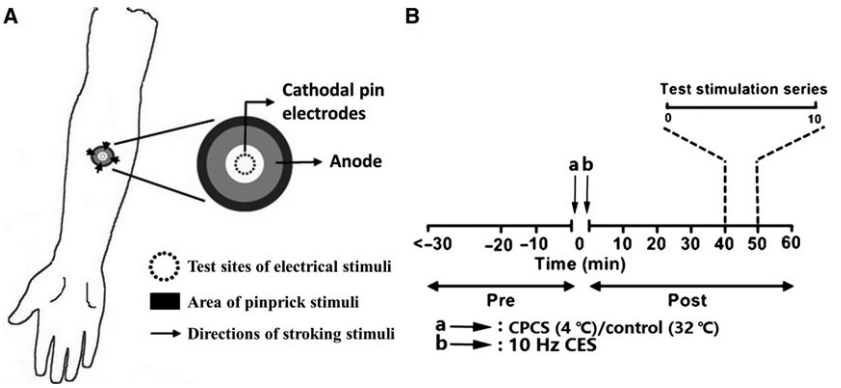

Figure 1 Experimental setup. (A) Continuous 10-Hz CES for inducing pain LTP was applied on the volar forearm via an EPE. The pain ratings to SES were measured at the conditioned site by the same EPE. Pinprick and light-stroking stimuli were applied in the surrounding skin area. (B) A series of assessments including neurogenic inflammation imaging (SBF and ST), heterotopic perception intensities to pinprick and light-stroking stimuli, homotopic pain to single electrical stimulation and HPT measurements were repeated with 10-min intervals three times before (pre-CES) and six times after CES (post-CES) in two sessions. In each session, the 10-Hz CES (b) was applied immediately after removing the conditioning stimulus (a), that is, CPCS or control water bath.

threshold. All experiments were performed by the same researcher to rule out the inter-rater variation.

2.4 Perception of CES process

The subjects were asked to continuously rate the magnitude of the pain intensity during the CES process by means of a handheld VAS device which was sampled by a computer. Afterwards, they were asked to describe the quality of the CES using the short-form McGill Pain Questionnaire (SF-MPQ). The SF-MPQ consists of sensory and affective dimensions of pain, evaluative overall intensity of total pain experience and present pain intensity (PPI) index of the standard MPQ. The PPI is the average pain intensity stated by the subjects after completing the rating of the conditioning process. All rating scores were added up to get a total quantitative value (Melzack, 1987).

2.5 Neurogenic inflammation imaging

To observe the possible excitation of peptidergic nerve fibres and assess the temporal changes of the superficial blood flow (SBF) during the entire observation period, a Full-Field Laser Perfusion Imager (FLPI) was used to assess the SBF index (MoorFLPI; Moor Instruments Ltd, Axminister, UK). Changes in the skin temperature (ST) were measured using infrared thermography (Thermovision A40; FLIR; Danderyd, Sweden). The SBF and ST were measured in a round area with a diameter of 15 mm concentrically to the circular pin electrodes which did not cover the area of pinprick stimuli.

2.6 Light-stroking stimuli

A cotton swab was used to deliver light-stroking stimuli (~100 mN) for assessing the perception sensitivity (dysesthesia) around the conditioned site. The stroking was performed in four directions moving from the outer region towards the centre of the conditioning pin electrodes and was stopped at 1 cm to the border of the circular pin electrodes (Fig. 1A). Each stroke was conducted at a speed of 1~2 cm/s with a distance of 1 cm. The subjects gave a perception rating to the light stroking using the VAS as mentioned above. An average of the four VAS ratings in the four directions was used as the perception intensity for the light-stroking stimulation.

2.7 Pinprick stimuli

Mechanical pinprick-evoked perception was assessed by three custom-made weighted pinprick stimulators (12.8, 30, 50.1 g, SMI, Aalborg University, rounded tip, 0.2 mm in diameter) applied on three different locations adjacent to the conditioned site (i.e. at 1.5~2 cm distance to the border of the cathodal electrodes) (Fig. 1A). The subjects rated the perceived intensity using the VAS scale.

2.8 Heat pain threshold

The heat pain threshold was measured using a thermode placed concentrically to the pin electrodes (Pathway; 30 × 30 mm ATS; Medoc Ltd.; Ramat Yishai, Israel). The area of the thermode covered the conditioned sites and the surrounding unconditioned skin. The baseline temperature was 32 °C and the temperature was increased at a rate of 1 °C/s until the subject indicated the perception of heat pain on a response button. Subsequently, the temperature returned to baseline at a rate of 8 °C/s. An average of three tests was used as the heat pain threshold.

2.9 Single electrical stimulation (SES)

A single rectangular constant current electrical stimulation (intensity: 10 × DTh) was applied as a homotopic electrical test stimulus using the same EPE placed at the conditioned sites (Fig. 1A). The subject rated the perceived intensity using the VAS scale. An average of three tests with 10 s intervals was used as the final homotopic pain rating to SES at the conditioned sites.

2.10 Data evaluation and statistics

The assessments of the outcome measures at nine time points (−30, −20, −10, 10, 20, 30, 40, 50, 60 min relative to the CES) were included in the statistical analysis. The perception intensity ratings to SES, pinprick and light-stroking stimuli and HPT were normalized by expressing the measurements as percentage of the average value of the preconditioning tests. The blood flow index was logarithmically transformed to obtain the lognormal distribution. The skin temperature used raw data which presented a normal distribution. The highest pain rating for each 10 s-interval was chosen to compare the perceived pain intensity during the 10-Hz CES process (i.e. five VAS ratings throughout the 50 s-conditioning period). A two-way repeated measures analysis of variance (Two-way RM-ANOVA; SPSS v. 21.0) (conditioning stimulus and time effects were within-subjects factors) was used for SBF, ST, pain ratings during the CES process, HPT, pain ratings to light-stroking and pinprick stimuli and SES to determine the temporal changes and differences between CPCS

and control session. Greenhouse–Geisser method was used for correction of non-sphericity, and Bonferroni–Holm adjustment was used for post hoc multiple comparisons if a main effect of CPCS or time was found. Paired t-test was used to determine the differences between the SF-MPQ scores of CES between the two sessions. All data are presented as mean values ± standard error of the mean (SEM). p-values < 0.05 were considered statistically significant.

3. Results

3.1 Baseline characteristics

The average electrical stimulation intensity of the single pulse used for 10-Hz CES and SES was 2.63 ± 1.8 mA (10 × DTh, mean ± SD, $n = 40$). This intensity was perceived as painful (35.2 ± 15; mean ± SD) by most of the subjects (18 out of 20 subjects) in the ratings to SES during the preconditioning period across both experimental sessions (pre-CES, $n = 40$). The average pain rating for 50.1 g pinprick stimulator was 25 ± 13 (mean ± SD; $n = 40$) at baseline and more than half of subjects (15 out of 20 subjects) perceived it as painful. The average pain rating for 30 g pinprick stimulator was 19 ± 11 (mean ± SD; $n = 40$) at baseline and nine subjects perceived it as painful. The average pain rating for 12.8 g pinprick stimulator was 12 ± 9 (mean ± SD; $n = 40$) at baseline and five subjects perceived it as painful. No visible skin injuries occurred following the electrical stimulation in any of the two sessions. For all outcome measures, no significant differences were found at baseline between the two sessions suggesting similar conditions before CES.

3.2 Perception of CES

The perception during the CES process in the CPCS and control sessions was found to decline (time effect, $F = 17.82$, $p < 0.01$), that is, the perception intensity rating in the first (0–10 s) and second (10–20 s) 10 s stimulation intervals was higher than the third (20–30 s), fourth (30–40 s) and fifth (40–50 s) rating ($p < 0.05$); the perception intensity rating in the third (20–30 s) 10 s stimulation interval was higher than the fourth (30–40 s) and fifth (40–50 s) rating ($p < 0.01$); the perception intensity rating in the fourth (30–40 s) 10 s stimulation interval was higher than the fifth (40–50 s) rating ($p < 0.05$). The pain perception evoked by the 10-Hz CES was lower in the CPCS session compared with the control session (CPCS effect, $F = 9.43$, $p < 0.01$) (Fig. 2A). However, the SF-MPQ scores and PPI were not found to be significantly different between the two sessions ($F = 0.011$, $p = 0.92$; $F = 0.892$, $p = 0.357$) (Fig. 2B). No interaction effect was found between the conditioning stimulus and time factors.

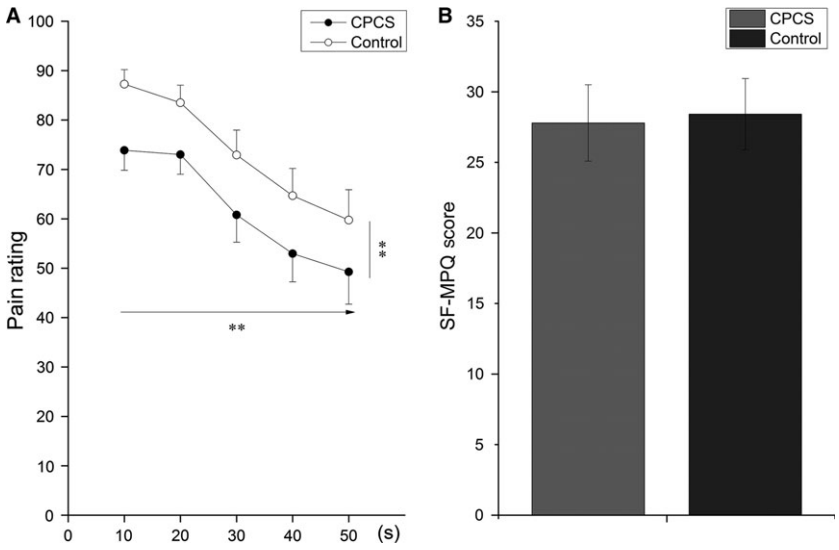

Figure 2 CPM effect on pain experience during the 10-Hz CES process. (A) Temporal changes of pain intensity during the conditioning process. 10-Hz CES elicited pain perception intensity decreased along the 500 impulses stimulation in both sessions. CPCS reduced the pain perception intensity compared with the control session. (B) Depiction of total SF-MPQ scores for CES. The SF-MPQ scores were not significantly different between the two sessions. Mean values ± SEM. $^{**}p < 0.01$.

3.3 Neurogenic inflammation

No difference was found between the CPCS session and the control session for the SBF changes ($F = 2.1$, $p = 0.164$). The SBF was found to significantly increase after CES; then gradually declined (time effect, $F = 141.058$, $p < 0.01$), that is, the SBF at 10-min post-CES was higher than at 30, 40, 50 and 60 min ($p < 0.0.05$); the SBF at 20 min post-CES was higher than at 40, 50 and 60 min ($p < 0.0.05$); the SBF at 30 and 40 min post-CES was higher than at 50 and 60 min ($p < 0.0.05$) (Fig. 3A). SBF had an average increase after the CES by 9.5% and 10.4% in the CPCS and control session, respectively. The increased SBF lasted for at least one hour after the CES (Fig. 3A). No interaction effect was found between conditioning stimulus and time factors.

No difference was observed between the CPCS session and the control session for the ST (CPCS effect, $F = 0.456$, $p = 0.508$). The ST was found to increase after the CES in both the CPCS and control sessions and then lasted till the end of the observation period (time effect, $F = 16.34$, $p < 0.01$), that is, ST at 30 min pre-CES was lower than in all the later time points; ST at 20 min pre-CES was lower than at 20, 30 and 40 min post-CES ($p < 0.05$) (Fig. 3B). ST had an average increase after the CES by 2% and 1.4% in the CPCS and control session, respectively (Fig. 3B). No interaction effect was found between conditioning stimulus and time factors.

3.4 Light-stroking perception intensity adjacent to the conditioned sites

The perception intensity increments to light-stroking stimuli around the conditioned sites in the CPCS session were found to be lower than in the control session showing a significant CPCS effect ($F = 5.341$, $p < 0.05$). The stroking perception intensity increased after the CES and lasted until the end of the observation period in both sessions (time effect, $F = 10.836$, $p < 0.01$), that is, the perception intensity increment at 30 min pre-CES was lower than at 30, 40, 50 and 60 min post-CES ($p < 0.05$); the perception intensity increment at 20 and 10 min pre-CES was lower than at 50 and 60 min post-CES ($p < 0.05$) (Fig. 4A). No interaction effect was found for conditioning stimulus and time factors.

3.5 Pinprick perception intensity adjacent to the conditioned sites

An interaction effect was found between conditioning stimulus temperature and time factors for 12.8 g pinprick stimulus ($F = 2.658$, $p < 0.05$). In the CPCS session, the pinprick perception increments at 40 min, and 50 min post-CES were found to be lower than in the control session (CPCS effect, $p < 0.05$) (Fig. 4B). No time effect for perception intensity was found for 12.8 g pinprick testing after the CES with Bonferroni–Holm adjustment.

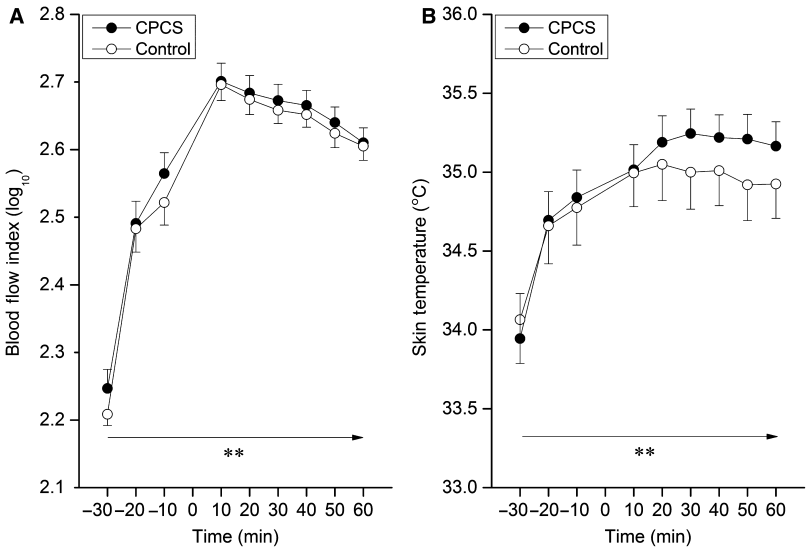

Figure 3 CPM effect on peripheral neurogenic inflammation. (A) Changes in SBF. SBF was found to be significantly increased after 10-Hz CES; then gradually declined. No difference was found between the CPCS session and the control session. (B) Changes in ST. ST was found to be significantly increased after 10-Hz CES in both sessions; then gradually declined. No difference was observed between the CPCS session and the control session. Mean values ± SEM. $^{**}p < 0.01$.

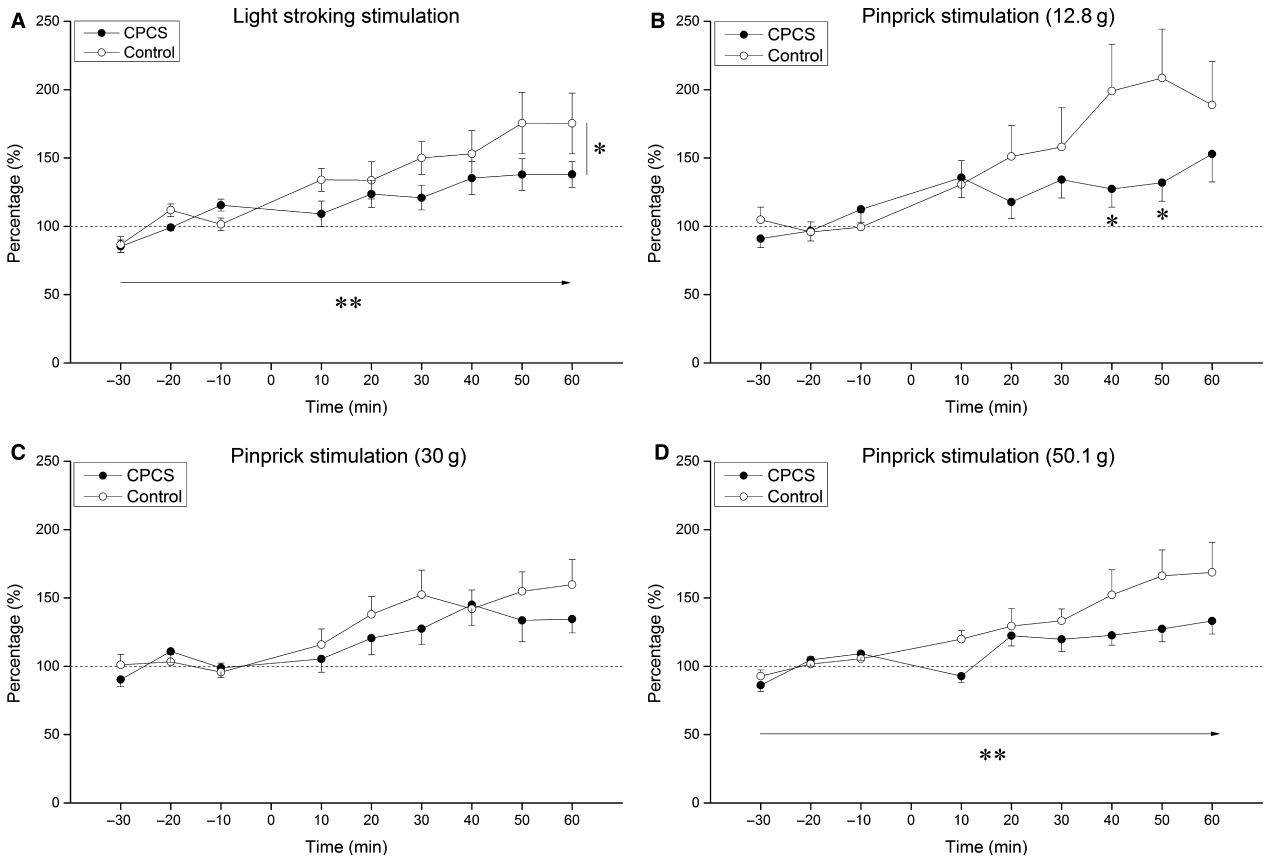

Figure 4 CPM effect on heterotopic pain LTP to mechanical stimuli (normalized data). (A) Light-stroking stimuli. The light-stroking perception intensity increased after 10-Hz CES which lasted until the end of the observation period in both sessions. The perception intensity to light-stroking stimuli increments around the conditioned site decreased in the CPCS session compared with the control session. (B,C,D) Pinprick stimuli. In 12.8 g pinprick testing, the perception intensity increments were lower at 40 and 50 min post-CES in the CPCS session compared with the control session. The pinprick perception intensity increased after CES only for 50.1 g pinprick testing. Mean values ± SEM. $^{**}p < 0.01$,$^{*}p < 0.05$.

A time effect was found for both 30 and 50 g pinprick stimulators ($F = 7.237$, $p < 0.01$; $F = 12.889$, $p < 0.01$). 50 g pinprick testing showed a significantly increased perception intensity, that is, pain ratings at 30 min pre-CES and 10 min post-CES were lower than at 20, 30, 40, 50 and 60 min post-CES; pain ratings at 20 and 10 min pre-CES were lower than at 50 min; pain rating at 20 min post-CES was lower than at 50 min post-CES (Fig. 4D). However, for 30 g pinprick testing, no significant difference in the perception intensity increments was found between any time points with multiple comparisons after Bonferroni–Holm adjustment (Fig. 4C). The CPCS effect showed no statistical significance for 30 and 50 g pinprick stimulators, and no interaction effects were found for conditioning stimulus and time factors (Fig. 4C,D).

3.6 SES perception intensity at the conditioned sites

No differences were found for the pain intensity increments by SES between the CPCS session and the control session ($F = 0.696$, $p = 0.415$) (Fig. 5A). The pain intensities to SES exhibited declining tendencies after CES in both sessions. However, no temporal changes were found for the perception increments of SES after Bonferroni–Holm adjustment in both sessions, although a time effect was found ($F = 3.65$, $p = 0.021$) (Fig. 5A). No interaction effect was found between conditioning stimulus temperature and time factors.

3.7 Heat pain threshold (HPT)

No differences were found for the HPT between CPCS and control sessions ($F = 0.16$, $p = 0.694$). In

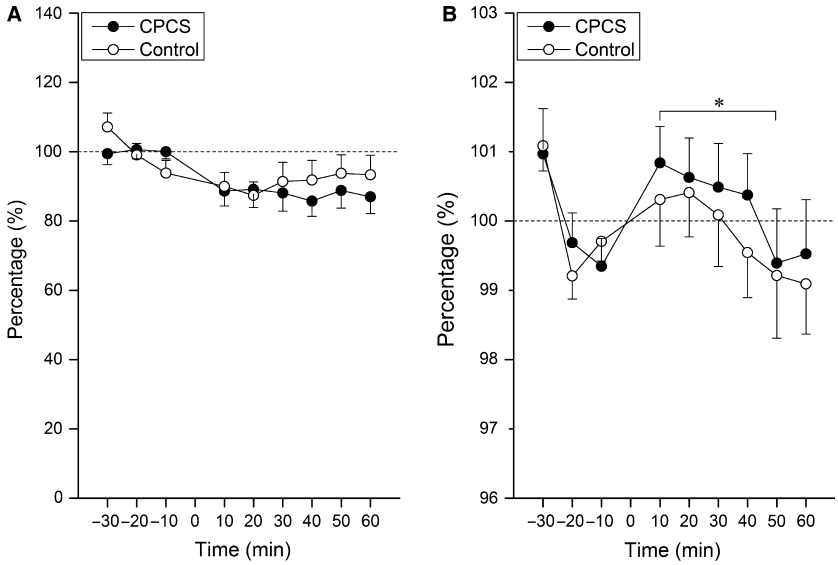

Figure 5 CPM effect on homotopic pain LTP induced by 10-Hz CES (normalized data). (A) Pain intensity evoked by SES at the conditioned site. The pain intensity increments by SES were not reduced by CPCS. No temporal changes were observed for the perception intensity of SES. (B) HPT. No differences were found for the HPT increments between CPCS and control sessions. The HPT increment at 10 min post-CES was higher than 50 min post-CES in both sessions. Mean values ± SEM. *$p < 0.05$.

both sessions the HPT showed temporal changes during the observation period (time effect, $F = 3.057$, $p < 0.05$), that is, the HPT at 10 min post-CES was higher than at 50 min post-CES ($p < 0.05$) (Fig. 5B). No interaction effect was found between conditioning stimulus temperature and time factors.

4. Discussion

This study is the first to investigate the effect of CPM on CES-induced neurogenic inflammation and pain amplification. The CPM was induced by a cold pressor conditioning stimulus applied on a remote body location (left foot) relative to the CES-stimulated sites (right forearm). The pain ratings during the CES process decreased with the immediately pre-applied CPCS. The CES induced heterotopic mechanical pain LTP but not homotopic pain LTP in the control session. The development of heterotopic perception intensity amplification including non-painful pinprick perception amplification and light-stroking dysesthesia could be inhibited in the CPCS session; however, the homotopic pain sensation to SES and HPT were not affected.

4.1 CPM effect on 10-Hz CES process

The perception intensity during the CES process in the CPCS session was lower than in the control session indicating that the CPM effect occurred, that is, the pain sensation of the test stimulus (i.e. CES) was inhibited by another extra-segmentally applied conditioning stimulus (i.e. CPCS). Moreover, this CPM inhibition took effect rapidly by decreasing the CES perception intensity when immediately applying the CPCS. The conditioning electrical stimulation paradigm used in this study consisted of a train of 10-Hz stimulation pulses which was considered to be more rational due to its close similarity to physiological firing rates of nociceptors (Xia et al., 2016a). In both the CPCS and the control session, the CES showed high pain intensity during the first 20 s, then gradually declined. This gradual reduction in the pain sensation during the 50 s conditioning process is probably due to habituation or triggered descending inhibition when the stimulus is applied repeatedly (Rankin et al., 2009; van den Broeke et al., 2012); in the CPCS session, the descending inhibition was enhanced reflecting the CPM effect.

CPCS was applied immediately before CES in order to show a 'cleaner' pain modulation eliminating the bias of distraction compared with CES during the cold pressor conditioning stimulation. Hence, the distraction and CPM on pain inhibition could employ separate physiological mechanisms (Moont et al., 2010). The duration of the inhibitory CPM effect is largely unknown but has been reported to last 10 min after termination of the conditioning

tonic pain (Reinert et al., 2000; Lewis et al., 2012). Therefore, the application of the CES in this study was within the time course of the CPM effect activated by CPCS. The pain ratings during the CES process were depressed by CPCS indicating that the pain transmission involving peptidergic C-fibre nociceptive pathways activated by the EPE was inhibited (Hansen et al., 2007). Furthermore, it may be speculated that the endogenous inhibitory effect could indeed depress a part of the spinal interneurons (mainly deep dorsal horn WDR neurons (Le Bars et al., 1979b)) which are also involved in the induction of LTP-like plasticity of nociceptive transmission in the spinal cord (Willis, 1993; Svendsen et al., 1997, 1999). The SF-MPQ scores in the two sessions were not different indicating that the overall pain experience for the CES process was not affected by the CPCS. However, the depressed pain ratings during the CES process support the hypothesis that CPM probably mainly depresses pain intensity without affecting pain qualities.

4.2 Neurogenic inflammation

Nociceptive electrical stimulation can activate peptidergic nerve endings (mainly C-fibres) causing the release of neuropeptides, for example, substance P, and calcitonin gene-related peptide (Sauerstein et al., 2000). These substances induce neurogenic inflammation including vasodilatation, plasma extravasation, attraction of macrophages or degranulation of mast cells (Lynn, 1996; Schaible et al., 2005; Schaible, 2007). In this study, SBF and ST were found to increase after CES. SBF increased immediately after CES while ST increased 10 min later indicating that SBF had a faster onset than ST. Significant increase of SBF and ST were found in the pre-CES period in both sessions which most likely were due to the process of determining DTh. This process used a series of electrical pulses with different stimulation intensities below pain threshold which may have activated the peptidergic part of Aδ-fibres and a small proportion of C-fibres (McCarthy and Lawson, 1989; Mouraux et al., 2010). However, the neurogenic inflammation responses were not affected by the CPCS. Therefore, the CPM effect inhibiting the pain transmission in the central nervous system could not affect the release of neurogenic mediators at peripheral nociceptive nerve endings. This indicates that the CPM inhibitory effect on pain LTP reflects a central mechanism with minimal impact on peripheral inflammatory processes.

4.3 CPM effect on heterotopic pain LTP

In this study, heterotopic pinprick hyperalgesia was induced 30 min after the CES for the 50.1 g pinprick testing in accordance with a recent reliability study (Xia et al., 2016b). However, no significant decreased pain amplification was observed on painful pinprick stimulation. This indicated that CPM inhibition on the central sensitization process might be insufficient to significantly prevent pain amplification following robust painful stimuli. In contrast, for the 12.8 g pinprick testing, a CPM effect was observed. CPCS also caused lower light-stroking perception increment compared with the control session. These observations indicate that the CPM could prevent the heterotopic perception amplification process, in particular for the light-weight pinprick hyperalgesia and light-stroking dysesthesia. Moreover, the decrease in non-painful mechanical perception amplification indicated that the CPM inhibitory effect might have promoted the processes of spinal desensitization. In this study, 12.8 g pinprick stimulus is on the edge between non-painful light-stroking stimuli and painful pinprick stimuli; so, it may be dynamic for 12.8 g stimulus to present the decreased perception intensity amplification. In our previous study (Xia et al., 2016a), a gradual increase in pinprick pain amplification was present until reaching the plateau 30 min after CES. Therefore, the significant decrease in perception amplification could be present when the amplification reached the plateau (i.e. with the maximum difference between CPCS and control sessions). This is the likely explanation why the CPM inhibition on CES facilitatory process resulted in decreased perception amplification 40 min after CES on the 12.8 g pinprick stimulus testing. However, from the tendencies of sensory changes after CES in three pinprick stimulators testing, lower perception intensities always seemed to be present in CPCS session compared with the control session.

Repetitive electrical stimulation of primary nociceptive C-fibres, most likely a part of the CES, could induce facilitation of non-nociceptive Aβ-fibre and nociceptive Aδ-fibre pathways resulting in heterotopic pain LTP (i.e. dynamic mechanical allodynia and secondary mechanical hyperalgesia) (Klein et al., 2004; Hansen et al., 2007; van den Broeke and Mouraux, 2014). In a previous study, TRPV1-positive C-fibres (major contribution) and TRPV1-positive A-fibres (minor contribution) were found to be the main inducers of heterotopic pain LTP; whereas, TRPV1-negative A-fibres were found to be

the main mechanism mediating secondary pinprick hyperalgesia (Henrich et al., 2015). Furthermore, the long-term increase in excitability of WDR neurons mediates mechanical and thermal hyperalgesia after injury of hairy skin which might contribute to pain chronification (Willis, 1993; Rygh et al., 1999; Kawamata et al., 2005). The supraspinal descending inhibitory neuronal pathways involved in the CPM could act post-synaptically on WDR convergent projection neurons receiving nociceptive C- and A-fibre stimuli (Le Bars et al., 1979b); and these WDR neurons are mainly located at lamina V of the spinal dorsal horn (Sorkin and Carlton, 1997). It has been shown that continuous 10-Hz CES can induce LTP at spinothalamic neurons (Kim et al., 2015) and most of these neurons are convergent cells (Le Bars et al., 1979a; Giesler et al., 1981). Moreover, DNIC has been reported to modulate the activity of the spinothalamic convergent neurons (Dickenson and Le Bars, 1983). The present findings could support that the CPM might inhibit WDR neurons involved in the facilitation of spinothalamic nociception transmission pathways. Therefore, the decreased heterotopic pain amplification is speculated to be a result of decreased sensitization of spinal cord neurons due to the CPM effect. However, the CPM did not present a complete inhibition as increased mechanical perception intensity was still maintained in the CPCS session.

Alternatively, other mechanisms could mediate the heterotopic pain facilitation such as (1) the diffusible neuropeptides, such as substance P or calcitonin gene-related peptide released from C-fibre central terminals causing expansion and facilitation of nearby A-δ and A-β neuropathways (Liu et al., 1994); (2) simultaneous activation of glutamatergic excitatory interneurons which may lead to sensitization of nociception projection neurons in the spinal cord (Santos et al., 2007); (3) serotonergic descending facilitation derived from the rostral ventromedial medulla of the brain stem causing the release of serotonins which could act on central terminals of Aδ-fibres to enhance the release of glutamate and neuropeptides (Pertovaara, 1998; Zeitz et al., 2002). However, the exact role of CPM in any of these alternative mechanisms is unknown.

4.4 CPM effect on homotopic pain intensity

Homotopic pain LTP to single electrical stimulation is most likely a far more complex phenomenon. Compared with the control session, the CPCS did not affect the pain perception intensity to SES or changed the HPT in the conditioned area. This seems to indicate that the CPM had no effect on the homotopic pain perception. Furthermore, the pain perception to the SES at the conditioned site was not found to increase after 10-Hz CES. In fact, van den Broeke et al.'s (2012) tested 100-Hz CES and observed a decreased pain intensity of SES in both conditioned and unconditioned skin sites despite the coexistence of enhanced event-related cortical potentials. Similarly, a declining perception intensity was also observed in another study with a minor change in the homotopic pain sensitivity (Matre et al., 2013). The HPT after CES was not found to decrease compared with the preconditioning assessments. This is in agreement with our previous reliability study showing that HPT even increased after 10-Hz CES (Xia et al., 2016b) which is also supported by the observations by Lang and colleagues (Lang et al., 2007). Together these observations indicate the absence of homotopic pain LTP by CES. However, 10-Hz CES has previously been shown to induce LTP in field potentials in nociception transmission neurons in the spinal dorsal horn in animals (Terman et al., 2001; Kim et al., 2015). The absence of homotopic pain LTP may be due to several reasons (1) the counter effects of LTP and long-term depression which could be activated by CES of C-fibre and A-δ fibre pathways, respectively (Liu et al., 1998; Pfau et al., 2011); (2) habituation or fatigue to repetitive electrical stimulations in the same area, that is, fatigue of C-fibre nociceptors to stepped stimuli (Slugg et al., 2000; Rankin et al., 2009); (3) hypoesthesia that has been observed following continuous 20-Hz CES at C-fibre intensity (De Col and Maihöfner, 2008); or (4) a methodological explanation related to movement of the electrode between tests which may mask the pain amplification to SES. However, movement of the pin electrodes could not be avoided in this study design because of the neurogenic inflammation measurements.

TRPV1-positive C-fibre nociceptors mainly distributed in the superficial layer of the dorsal horn have been reported to be the main contributors to induction of homotopic pain LTP (Valtschanoff et al., 2001; Yang et al., 2014; Kim et al., 2015). Moreover, superficial nociceptive specific neurons expressing neurokinin 1 receptors have been found to be crucial for generation of LTP-like changes in WDR neurons located in the deep spinal dorsal horn (Rygh et al., 2006); in addition, both the two groups of neurons are believed to be able to support the development of spinal LTP (Svendsen et al., 1999; Bester et al., 2000; Ikeda et al., 2003). Conditioning

peripheral electrical stimulation at C-fibre strength could induce an increased synaptic strength (i.e. LTP) in monosynaptic connections to superficial lamina neurons (Ikeda et al., 2006). In humans, homotopic pain LTP was thought to resemble this increased monosynaptic excitability (Klein et al., 2004). In this study, the absence of homotopic pain LTP renders it impossible to speculate whether the CPM could prevent homotopic pain amplification or not. However, CPM inhibition has been shown not to affect nociception-specific superficial spinal dorsal horn neurons (Le Bars et al., 1979a). These neurons play a central role in spinal LTP (Yang et al., 2014). This supports the assumption that the CPM might not depress homotopic pain LTP because of the failure to prevent homosynaptic LTP-like nociceptive facilitation in nociceptive C-fibre pathways.

4.5 Limitations

Several potential limitations of this study should be considered. First, a control non-CES session was not arranged in this study as homotopic pain amplification might have been covered by habituation to SES. However, homotopic pain amplification after CES was absent when compared with a control non-CES session (Xia et al., 2016a) and when compared with pre-CES values (Xia et al., 2016b), while heterotopic pain amplification was present in both studies. Second, repositioning of the EPE most likely will involve activation of different nerve fibres despite markers being made on the forearm aiming to place the electrode at the same location every time. Third, another test stimulus outside the skin area presumed to be affected by CES could have been added in order to document the duration of the CPM effect. With this study design it is unknown whether the conditioning stimulus inhibits generation of LTP only, or also the subsequent test stimuli.

5. Conclusions

This study found that CPM depressed heterotopic mechanical LTP-like perception facilitation of non-painful mechanical pinprick and light-stroking stimulation, whereas it did not affect the heterotopic pain amplification by painful pinprick stimulation. Furthermore, CPM did not modulate homotopic electrical stimulation and heat pain perception or peripheral neurogenic inflammation. On the whole, this study has provided a better understanding of the potential role of the endogenous pain inhibitory mechanism on the model of LTP-like pain amplification.

Author contributions

The study was designed by O.K. Andersen, C.D. Mørch and W. Xia. The measurements were performed by W. Xia. The analysis was performed by O.K. Andersen, C.D. Mørch, D. Matre and W. Xia. The manuscript was written by W. Xia, D. Matre, C.D. Mørch and O.K. Andersen. All authors discussed the results and commented on the manuscript.

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