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

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Published in:
European Journal of Pain

DOI (link to publication from Publisher):
10.1002/ejp.968

Publication date:
2017

Document Version
Accepted author manuscript, peer reviewed version

Abstract

Background: The current 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: CPM was induced by cold pressor conditioning stimulus (CPCS) (4℃) which was applied immediately before CES in the active session. In the control session 32℃ water was used. 20 subjects participated two sessions in a randomized crossover design with at least one 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: CPCS 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).

Key words: conditioned pain modulation; central sensitization; hyperalgesia; conditioning electrical stimulation; cold pressor conditioning stimulus

Exploration of Conditioned Pain Modulation Effect on Long-term Potentiation-like Pain Amplification in Humans

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Category: Original Article

Conflicts of interest
None declared

Funding sources
Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121); the China Scholarship Council.

What does this study add?

1. CPM may play a role in inhibiting the pain amplificatory process at the central nervous system and prompting central desensitization.
2. 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 & Sandkühler, 1997; Ikeda *et al.*, 2003). LTP-like phenomena have been considered to be a mechanism underlying the neurogenic pain amplification such as persistent postoperative pain and chronic pain conditions initiated by a painful event, e.g. peripheral inflammation or neuropathy (Sandkühler, 2000; Ji *et al.*, 2003; Ruscheweyh *et al.*, 2011; Sandkühler & Gruber-Schoffnegger, 2012; Price & 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-fiber nociceptors during neuropathic or inflammatory pain conditions has been considered to contribute to the elevated responsiveness and activity of dorsal horn neurons (Puig & Sorkin, 1996; Han *et al.*, 2000; Xiao & Bennett, 2007; Drdla & Sandkühler, 2008). This is manifested in patients as increased response to noxious stimuli (hyperalgesia) and pain resulting from normally innoxious tactile stimuli (allodynia) (Latremoliere & 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, 2016b). The afferent activity in the 10 Hz LTP model may resemble the low frequency discharging of C-fiber 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 & 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 the present 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 to 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℃. 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 7cm 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 fifteen 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. 1*A*) (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-fibers terminate (Hansen *et al.*, 2007a; 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, 2016b). 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 analyzed. Cold pressor conditioning stimulus (CPCS) and control experimental sessions were randomly assigned on two experimental days conducted at least one week apart for each subject in a crossover design. CPCS (left foot in an ice-filled water bath holding 4℃) 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 two minutes. 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℃ 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. 1*B*). 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 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 fibers 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 center of the conditioning pin electrodes and was stopped at 1 cm to the border of the circular pin electrodes (Fig. 1*A*). 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 (12.8g, 30g, 50.1g, 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. 1*A*). The subjects rated the perceived intensity using the VAS scale.

2.8 Heat Pain Threshold

The heat pain threshold (HPT) 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 un-conditioned skin. The baseline temperature was 32℃ and the temperature was increased at a rate of 1℃/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℃/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. 1*A*). 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 (preCES, 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, $F=17.82$, $p<0.01$), i.e. 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. 2*A*). 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. 2*B*). No interaction effect was found between the conditioning stimulus and time factors.

3.2 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, $F=141.058$, $p<0.01$), i.e. the SBF at 10 min postCES was higher than at 30 min, 40 min, 50 min and 60 min ($p<0.0.05$); the SBF at 20 min postCES was higher than at 40 min, 50 min and 60 min ($p<0.0.05$); the SBF at 30 min and 40 min postCES was higher than at 50 min and 60 min ($p<0.0.05$) (Fig. 3*A*). 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. 3*A*). 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 to the end of the observation period (time effect, $F=16.34$, $p<0.01$), i.e. ST at 30 min preCES was lower than in all the later time points; ST at 20 min preCES was lower than at 20 min, 30 min and 40 min postCES ($p<0.05$) (Fig. 3*B*). ST had an average increase after the CES by 2% and 1.4% in the CPCS and control session, respectively (Fig. 3*B*). No interaction effect was found between conditioning stimulus and time factors.

3.3 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, $F=10.836$, $p<0.01$), i.e. the perception intensity increment at 30 min preCES was lower than at 30 min, 40 min, 50 min and 60 min postCES ($p<0.05$); the perception intensity increment at 20 min and 10 min preCES was lower than at 50 min and 60 min postCES ($p<0.05$) (Fig. 4*A*). No interaction effect was found for conditioning stimulus and time factors.

3.4 Pinprick Perception Intensity Adjacent to the Conditioned Sites

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

A time effect was found for both 30g and 50g pinprick stimulators ($F=7.237$, $p<0.01$; $F=12.889$, $p<0.01$). 50g pinprick testing showed a significantly increased perception intensity, i.e. pain ratings at 30 min preCES and 10 min postCES were lower than at 20 min, 30 min, 40 min, 50 min and 60 min postCES; pain ratings at 20 min and 10

min preCES were lower than at 50 min; pain rating at 20 min postCES was lower than at 50 min postCES (Fig. 4*D*). However, for 30g pinprick testing, no significant difference in the perception intensity increments was found between any time points with multiple comparisons after Bonferroni-Holm adjustment (Fig. 4*C*). The CPCS effect showed no statistical significance for 30g and 50g pinprick stimulators, and no interaction effects were found for conditioning stimulus and time factors (Fig. 4*C,D*).

3.5 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. 5*A*). 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, even though a time effect was found ($F=3.65$, $p=0.021$) (Fig. 5*A*). No interaction effect was found between conditioning stimulus temperature and time factors.

3.6 Heat Pain Threshold (HPT)

No differences were found for the HPT between CPCS and control sessions ($F=0.16$, $p=0.694$). In both sessions the HPT showed temporal changes during the observation period (time effect, $F=3.057$, $p<0.05$), i.e. the HPT at 10 min postCES was higher than at 50 min postCES ($p<0.05$) (Fig. 5*B*). No interaction effect was found between conditioning stimulus temperature and time factors.

4. Discussion

The present 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; whereas 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, i.e. 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 the present 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-fiber nociceptive pathways activated by the EPE was inhibited (Hansen *et al.*, 2007a). 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-fibers) causing the release of neuropeptides, e.g. 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 the present 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. As a series of increasing and decreasing electrical pulses with low stimulation intensities below pain threshold was repeated which may have activated the peptidergic part of Aδ-fibers and a small proportion of C-fibers (McCarthy & 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 the present 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 of non-painful mechanical perception amplification indicated that the CPM inhibitory effect might have promoted the processes of spinal desensitization. In the present 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 gradually increase of pinprick pain amplification was present until reaching the plateau 30 min after CES. Therefore, the significant decrease of 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 facilitory 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 to the control session.

Repetitive electrical stimulation of primary nociceptive C-fibers, most likely a part of the CES, could induce facilitation of non-nociceptive Aβ-fiber and nociceptive Aδ-fiber pathways resulting in heterotopic pain LTP (i.e. dynamic mechanical allodynia and secondary mechanical hyperalgesia) (Klein *et al.*, 2004; Hansen *et al.*, 2007b; van den Broeke & Mouraux, 2014). In a previous study, TRPV1-positive C-fibers (major contribution) and TRPV1-positive A-fibers (minor contribution) were found to be the main inducers of heterotopic pain LTP; whereas, TRPV1-negative A-fibers were found to be the main mechanism mediating secondary pinprick hyperalgesia (Henrich *et al.*, 2015). Furthermore, the long-term increase of 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-synapticaly on WDR convergent projection neurons receiving nociceptive C- and A-fiber stimuli (Le Bars *et al.*, 1979b); and these WDR neurons are mainly located at lamina V of the spinal dorsal

horn (Sorkin & 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 & 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-fiber 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 deriving from the rostral ventromedial medulla of the brain stem causing the release of serotonins which could act on central terminals of Aδ-fibers 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's study (2012) tested 100 Hz CES and observed a decreased pain intensity of SES in both conditioned and unconditioned skin sites despite with 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-fiber and A-δ fiber pathways, respectively (Liu *et al.*, 1998; Pfau *et al.*, 2011); 2) habituation or fatigue to repetitive electrical stimulations in the same area, i.e., fatigue of C-fiber 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-fiber intensity (De Col & 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 the present study design because of the neurogenic inflammation measurements.

TRPV1-positive C-fiber 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 of 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-fiber 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 the present 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-fiber pathways.

4.5 Limitations

Several potential limitations of this study should be considered. First, a control non-CES session was not arranged in the present 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 fibers despite that markers were 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 the current study design it is unknown whether the conditioning stimulus inhibits generation of LTP only, or also the subsequent test stimuli.

5. Conclusions

The present 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. All in all, 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.

6. 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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8. Legends

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 (preCES) and six times after CES (postCES) in two sessions. In each session, the 10 Hz CES (b) was applied immediately after removing the conditioning stimulus (a), i.e. CPCS or control water bath.

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. ** $p<0.01$.

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 12.8g pinprick testing, the perception intensity increments were lower at 40 min and 50 min postCES in the CPCS session compared with the control session. The pinprick perception intensity increased after CES only for 50.1g pinprick testing. Mean values±SEM. ** $p<0.01$,* $p<0.05$.

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 postCES was higher than 50 min postCES in both sessions. Mean values±SEM. * $p<0.05$.

Figure 1

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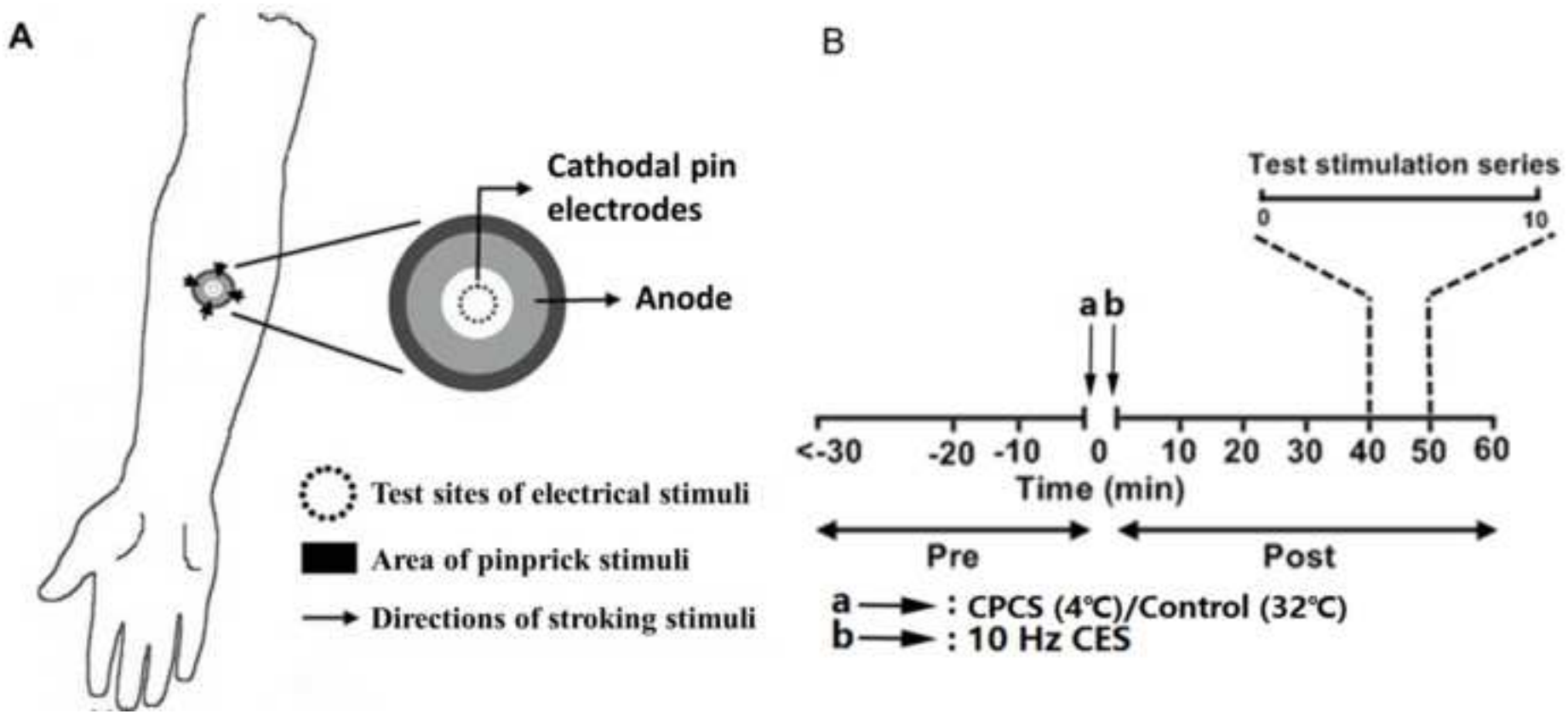

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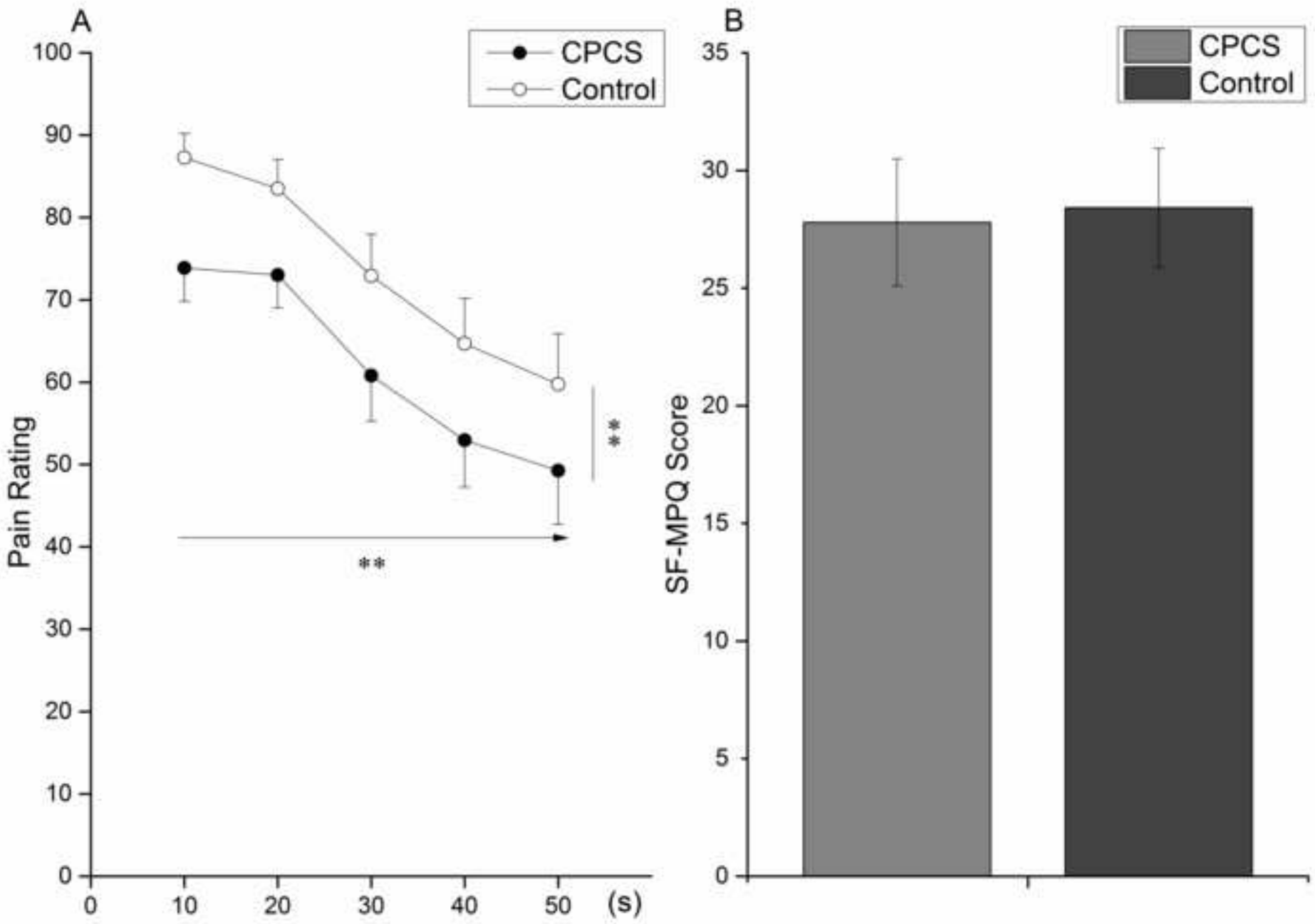

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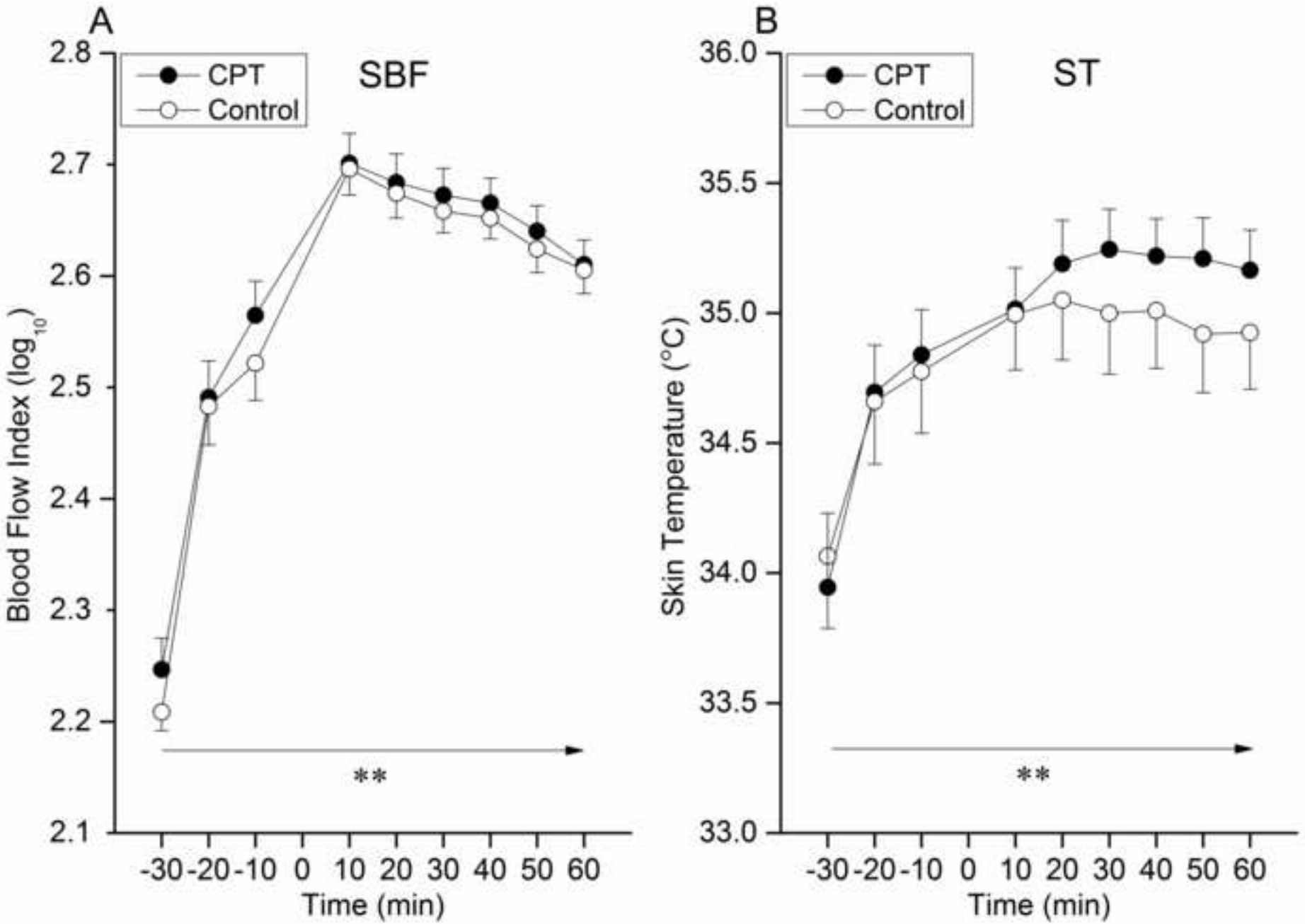

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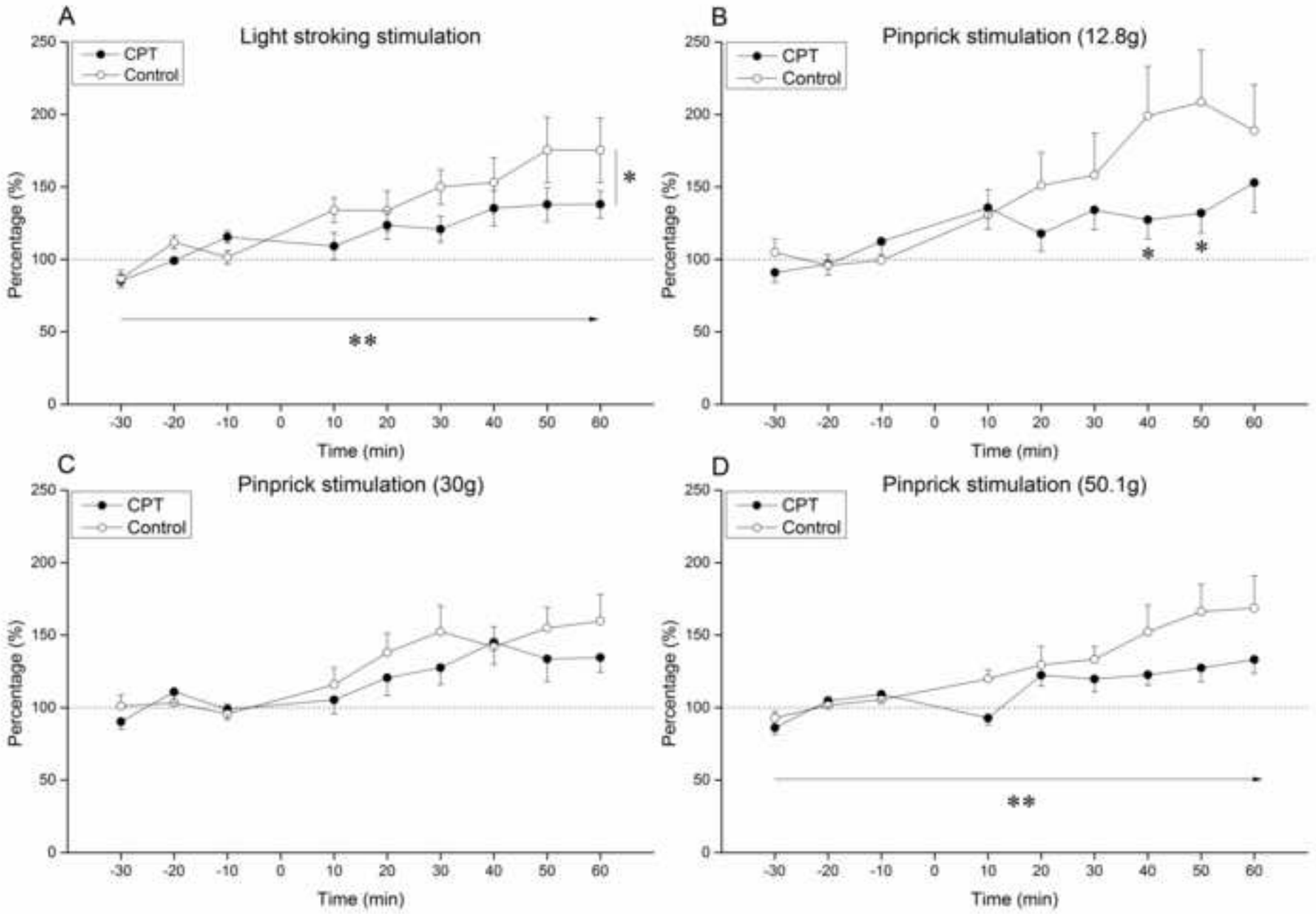

Figure 5

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