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Comparison of five conditioned pain modulation paradigms and influencing personal factors in healthy adults

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Significance: Hot water immersion, cold pressor test, and single and double ischemic occlusion result in comparable CPM-effects at the mm. trapezius and quadriceps. Anti-nociceptive effects of the cold pack are mainly a result of attention towards the cold pack. Chronic stress, attentional focus towards the conditioning stimulus and perceived pain of the conditioning stimulus influenced the anti-nociceptive effects at the m. trapezius. Gender and level of physical activity influenced the anti-nociceptive effects with the other methods at the m. quadriceps.

Abstract (249)

Background: Conditioned pain modulation (CPM) methods are experimental procedures to assess presumed descending nociceptive modulatory pathways. Various CPM-methods are currently used, making the comparison of results difficult. The aim of this study was to compare five conditioning stimuli and to evaluate the influencing effects of personal factors on CPM-efficacy.

Methods: 101 healthy pain-free adults (50 males, 51 females) participated in this cross-sectional study with repeated measures design. The CPM-method consisted of hot water immersion (46°C, HWI), cold pressor test (12°C, CPT), cold pack application, and single and double ischemic occlusion as conditioning stimuli in randomized order. Pressure pain threshold was used as test stimulus at the mm. trapezius and quadriceps for all CPM-protocols.

Results: All CPM-protocols resulted in effective CPM, although cold pack application revealed smaller CPM-magnitudes compared to all other methods at both muscles, except single ischemic occlusion at the m. quadriceps. A smaller CPM-effect at the m. trapezius was shown when CPM was provoked by single ischemic occlusion compared to the CPT.

Chronic stress, gender, attentional focus, age, physical activity and perceived pain are all influencing factors, in various conditioning stimuli at the mm. trapezius and quadriceps.

Conclusions: CPT and HWI seem to be the most appropriate conditioned pain modulation paradigms for research settings, while single and double ischemic occlusion seem to be more useful for clinical settings. Influencing factors to be considered are gender, age, stress, physical activity, perceived pain and attentional focus to the conditioning stimulus, but depend on the test site and exerted method.

Keywords: conditioned pain modulation (CPM) – endogenous pain modulation – diffuse noxious inhibitory controls (DNIC) – influencing factors

Introduction

Diffuse noxious inhibitory control (DNIC) is a descending nociceptive modulatory pathway and entails the ability of a noxious stimulus on one body site to inhibit another stimulus (noxious or innocuous) located in another body site (Le Bars et al., 1979). DNIC ensures that inputs from the periphery could inhibit sensations of pain from other areas (Le Bars et al., 1979). In humans, a DNIC-like effect can be considered by the reduction in pain intensity of a first stimulus (test stimulus) during or after the application of a second stimulus (conditioning stimulus) (Pud et al., 2009). A DNIC-like effect can be assessed with a paradigm called conditioned pain modulation (CPM) (Nir and Yarnitsky 2015). Assessment of CPM in clinical practice can document the (in)efficacy of the endogenous anti-nociceptive system.

At present, different experimental paradigms are used to assess CPM (Leone and Truini 2019). Both cold and hot water immersion as conditioning stimulus have been shown to result in effective CPM and seem to be useful in research settings (Granot et al., 2008; Lautenbacher et al., 2008; Tousignant-Laflamme and Marchand 2009; Nir et al., 2011; Oono et al., 2011; Lewis et al., 2012b; Kennedy et al., 2016; Nuwailati et al., 2019; Traxler et al., 2019). Other frequently used paradigms to effectively induce CPM and better applicable in clinical practice are among others cold pack application (Ladouceur et al., 2012; Cormier et al., 2013; Marouf et al., 2014), and various types of cuff occlusion (France and Suchowiecki 1999; Campbell et al., 2008; Cathcart et al., 2009; Oono et al., 2011; Lewis et al., 2012b; Daenen et al., 2013; Meeus et al., 2013; Graven-Nielsen et al., 2017). Nonetheless, no golden standard for CPM assessment is currently available (Yarnitsky et al., 2015; Kennedy et al., 2016) and comparison of the effects of these paradigms is difficult due to differences in exerted protocols. Studies using multiple CPM paradigms mainly determined the reliability (Imai et al., 2016; Vaegter et al., 2018). Furthermore, we are aware of only few studies that compared up to three conditioning stimuli (Granot et al., 2008; Oono et al., 2011).

Another interesting aspect is the influence that several personal factors might have on CPM. Based on a systematic review (Hermans et al., 2016) evidence was found for age as an influencing factor with various test and conditioning stimuli. Whereas conflicting evidence was found for catastrophizing (Hermans et al., 2016; Nahman-Averbuch et al., 2016), gender, distraction, attention to the conditioning stimulus (Hermans et al., 2016), and preliminary evidence for physical activity and expectations to influence CPM-efficacy (Cormier et al., 2013; Hermans et al., 2016). In contrast to acute stress, we are not aware of studies investigating the effect of chronic stress on CPM-efficacy.

The first objective of the current study was to compare five different commonly used conditioning stimuli (hot water immersion, cold pressor test, cold pack application, and single and double ischemic occlusion) in healthy people, and their effects upon the same test stimulus. If these five conditioning stimuli are equally effective for the assessment of DNIC, the least expensive and time-consuming method can be used in clinical practice. Based on previous studies (Granot et al., 2008; Oono et al., 2011) the hypothesis was that differences that will occur are small and not clinical relevant.

The second objective of this study was to examine the effect of personal factors (expectations regarding the conditioning stimuli, catastrophizing, chronic stress, and physical activity levels) on CPM-efficacy. We expected an influence of expectations, physical activity and stress on CPM. Participants expecting less pain will probably have a higher pain threshold (Cormier et al., 2013). Furthermore, higher levels of physical activity suggest more efficient CPM (Naugle and Riley 2014) and stress could lead to distraction from the pain or a physiologic arousal reaction and result in higher anti-nociceptive effects (Butler and Finn 2009; Fechir et al., 2009; Joels and Baram 2009; Olango and Finn 2014).

Methods

This cross-sectional study comprises of a comparison of five different CPM methods, applied to healthy participants in randomized order. The study took place at the research unit of the department of Rehabilitation Sciences and Physiotherapy at Ghent University (Belgium). The ethical committee of the University Hospital Ghent/Ghent University approved the study (B670201318187).

Participants

Healthy pain-free male and female participants, between the age of 18 and 65 years, were recruited for this study. Only participants who had no current pain or history of chronic pain complaints were eligible for study participation. Pregnant females or females who gave birth less than one year ago were not eligible for study participation. The participants were recruited via personal acquaintances of researchers, or employees of the department 'Rehabilitation Sciences and Physiotherapy' of Ghent University. All participants provided a written informed consent before the start of the experiments.

Procedure

The participants were assessed in a laboratory of the Ghent University Hospital. The assessment lasted two hours. First, participants were asked to complete a set of eight questionnaires. These included a general questionnaire, a questionnaire assessing expectations of pain relating to the test paradigm, the Long-term Difficulties Inventory (LDI), the List of Threatening Events (LTE), the short version of the International Physical Activity Questionnaire (IPAQ-sf), the Pain Catastrophizing Scale (PCS), the Pain Vigilance and Awareness Questionnaire (PVAQ), and the short version of the Fear of Pain Questionnaire (FPQ-9).

Subsequently, pressure pain thresholds (PPTs) were determined. These thresholds were the test stimuli in all CPM protocols. Then, five different experimental CPM protocols were delivered in random order determined by a computerized randomization protocol (research randomizer) (Urbaniak and Plous). Between subsequent test paradigms, a rest period of 10 minutes was provided (Granot et al., 2008). A summary of the procedure is shown in Figure 1.

[Insert figure 1 about here]

Materials

Questionnaires

General questionnaire

A general questionnaire was used to register age, intake of medication, alcohol, nicotine or caffeine, playing sports, experienced sport-related symptoms on the day of the experiments, and hormonal contraceptives.

Long-term Difficulties Inventory (LDI)

This 12-item questionnaire evaluates chronic stress related to household, occupation, social contacts, free time, finances, health, study and religion (Hendriks 1990). Situations were scored on a 3-point-scale (0 = “not stressful”, 1 = “slightly stressful”, 2 = “very stressful”) and the total score (range 0 – 24) was used as outcome measure, where higher scores indicate more problems. Reliability scores are sufficient (Rosmalen et al., 2012). The questionnaire is in Appendix 1.

List of Threatening Events (LTE)

Highly stressful situations in the past 12 months were assessed with a 12-item binary questionnaire, with total outcome score ranging between 0 (no stress) and 12 (highly stressful) (Brugha and Cragg 1990). The questioned life events are related to long-term consequences. This questionnaire showed sufficient reliability (Rosmalen et al., 2012). The questionnaire is in Appendix 2.

International Physical Activity Questionnaire – Short form (IPAQ-sf)

This questionnaire assesses heavy, moderate and light physical activities performed over the past 7 days (Booth 2000). The three categorical output scores (1) ‘inactive’, (2) ‘minimal active’, and (3) ‘health enhancing physical activity’ were used in the analysis of this study. Participants were assigned to category 3 if (a) they performed vigorous-intensity on at least 3 days in the last week and accumulated 1500 Metabolic Equivalent of Task (MET) or (b) performed a combination of activity-intensities in the last 7 days with a MET accumulation of 3000. Category 2 comprised: (a) 3 or more days of vigorous activity of at least 20 minutes per day or (b) 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes or (c) 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-minutes/week (Ainsworth et al., 2000). If participants did not meet the criteria for category 2 or 3, they were classified in category 1. This short form questionnaire has a moderate validity and test-retest reliability (Craig et al., 2003).

Pain Catastrophizing Scale (PCS)

This 13-item questionnaire, assesses worrying about pain and catastrophizing (Sullivan et al., 1995). Likert scales for each item range from 0 = “not”, to 4 = “always”, resulting in a total score range of 0-52. Higher scores are associated with higher levels of catastrophizing. This questionnaire has a sufficient validity and reliability (Osman et al., 1997).

Pain Vigilance and Awareness Questionnaire (PVAQ)

Attention to pain, awareness to pain, vigilance to pain and pain observation were evaluated using this 16-item questionnaire with good reliability (McCracken 1997; Roelofs et al., 2002). Items were scored on a 0-5 Likert scale, with 0 = “never” and 5 = “continuously”. The higher the total score between 0 and 80 is the more attention was focused on pain.

Fear of Pain Questionnaire-9 (FPQ-9)

In this short version of the Fear of Pain Questionnaire, nine painful situations are described and had to be scored on a 0-5 Likert scale, with 0 not afraid at all and 5 extremely afraid (Parr et al., 2012). The higher the total score, the more fearful. This short version is a sound alternative for the longer version for assessing fear and anxiety associated with pain (McNeil et al., 2018).

Expectations of pain

The expectations of the pain of the conditioning stimuli that were applied later in the procedure were assessed at the beginning of the experiment, before pain application. Answers were given on a 1-5 Likert scale, with the highest score representing the most pain expectations.

Attentional focus

After each conditioning stimulus, the attentional focus to the stimulus was scored on a 1-5 Likert scale with the highest score representing the most focus to the stimulus.

Conditioned Pain Modulation (CPM) test paradigms

The test stimulus was the same throughout the five test paradigms. The test stimulus was applied to the dominant side at the mm. trapezius and quadriceps, whereas the different types of conditioning stimuli were applied to the non-dominant side.

Test stimulus: Mechanical Pressure Pain Threshold (PPT)

The participant took place in a seat with armrests. The test stimulus was applied at two different body sites. PPTs were determined at the muscle belly of the m. trapezius, more specifically, the middle of the distance between C7-spine and the acromion (Cathcart et al., 2009; Meeus et al., 2013). The second site was the center of the m. rectus femoris, namely the middle of the distance between de spina iliaca anterior inferior and the upper edge of the patella (Daenen et al., 2014; Vaegter et al., 2014). These sites were chosen because they are often used in research, can both be tested in a sitting position and both upper and lower extremity were tested to distinct segmentally and to determine generalized pain modulation (Yarnitsky et al., 2015).

The assessor (LH) elicited mechanical pressure pain on the mm. trapezius and quadriceps using an analogue algometer with a rubber tip of 1 cm² (Wagner Force Dial FDK 40). To determine the PPT the assessor applied a gradually increasing pressure at a speed of 1kg/sec until the participant experienced the stimulus as annoying and uncomfortable. This was repeated a second time after 30 seconds. The average of two measurements was considered as the PPT for the respective test site. It was found that the average of two trials was sufficient to determine the PPT (Ohrbach and Gale 1989). The terms annoying and uncomfortable were chosen, because this are synonyms for unpleasant and to avoid possible anxiety effects of the word “pain”. The assessor was trained by a senior researcher with more than 10 year experience in PPT testing. Furthermore the assessor already had experience with PPT testing in a previous study. It was found that intra-rater reliability and the rate of force application are reliable after training (Kinser et al., 2009; Waller et al., 2015). During PPT assessment participants were blinded to the screen of the algometer and consequently they were unaware of the applied pressure. The PPT as test stimulus was chosen, because it is easy and quite fast to apply and thereby easy applicable in clinical practice. Compared to thermal, electrical or chemical test stimuli, PPT can be determined much faster. Moreover, PPT is frequently used (Pud et al., 2009) and excellent intra-session reliability for CPM assessment with PPTs has been reported (Lewis et al., 2012a).

Conditioning stimuli

Generally, the conditioning stimulus was applied for 2 minutes. Thirty seconds after application of the conditioning stimulus, the participant was requested to rate the intensity of the conditioning stimulus using a Visual Analogue Scale (VAS) for perceived pain of the conditioning stimulus, ranging from 0 = ‘‘no pain’’ to 10 = ‘‘the worst pain imaginable’’. Directly after the VAS-score for the conditioning stimulus, the first test stimulus (via PPT) was measured at the m. trapezius. After 60 seconds, the second test stimulus on the m. trapezius was assessed. Again 30 seconds later (at 1’30” after application of the conditioning stimulus), the first test stimulus on the m. quadriceps was applied (via PPT) and the last test stimulus at the m. quadriceps again 30 seconds later (at 2’).

CPM-effect was calculated as PPT baseline – PPT during conditioning stimulation. The percentage difference between test stimulus assessed during conditioning stimulation and the baseline PPT was calculated (PPT baseline – PPT during CPM / PPT baseline) and called the relative CPM effect. Negative scores indicate effective CPM and positive scores indicate no CPM-effect.

Hot water immersion (HWI)

The non-dominant hand up to the wrist (Arendt-Nielsen et al., 2008) was inserted for 10 seconds in a bucket water of 37°C for habituation. This was followed by open-hand immersion for 2 minutes in a circulated water bath (Techne TO-10D tempette) of 46° C. This temperature is in accordance with several previous studies (Granot et al., 2008; Lautenbacher et al., 2008; Nir et al., 2011).

Cold pressor test (CPT)

A similar habituation protocol as for the HWI was applied, followed by open-hand immersion in a circulating cold water basin (Huber Variostat) of 12 °C for 2 minutes. The water temperature was 12 °C and is similar as in several previous studies (Tousignant-Laflamme and Marchand 2009; Lewis et al., 2012b).

Cold pack application (CPA)

A cold pack was wrapped in a thin towel and applied to the forearm during two minutes (Ladouceur et al., 2012). Pressure on the cold pack was given by the assessor to ensure contact between the cold pack and the skin.

Single ischemic occlusion (SIO)

The participants were asked to perform a maximal voluntary contraction with a handheld dynamometer (Sanmons Preston (kg)) to obtain the 1-Repetion Maximum (1-RM) value for handgrip strength of the non-dominant hand with their elbow in 90 degrees flexion and lower arm horizontally supported. Next, they performed contractions for 30 seconds at 50% of the 1-RM value. Subsequently, the arm was positioned upwards for 15 seconds and the inflatable cuff (Welchyallyn or S'o'hngen, Boso, Germany (mm Hg)) was inflated to 240 mm Hg at the non-dominant upper arm. Finally, the participant put the arm back horizontally supported, while the cuff remained tightened for 2 minutes (France and Suchowiecki 1999; Lewis et al., 2012a).

Double ischemic occlusion (DIO)

This method is the bilateral version of the single, unilateral ischemic procedure described above. Hence, the procedure from above was applied to both arms simultaneously.

Statistical analysis

The data was analyzed using the IBM Statistical Package for Social Sciences for Windows version 23.0 (IBM CORP. Armonk, N.Y., USA). Based on the central limit theorem (Kwak and Kim 2017), parametric statistics were performed. CPM-effects for mm. trapezius and quadriceps were evaluated separately, which is in accordance with the literature (Rezaii and Ernberg 2010; Meeus et al., 2013).

Mean, standard deviation and range for the total group are presented for continuous variables, for ordinal variables the number and percentage are presented. The difference between the different conditioning stimuli for expected pain and attentional focus to the conditioning stimuli were analyzed with Friedman's test.

First, the effect of the five different conditioning stimuli on the PPT was analyzed with linear mixed models with fixed factor type of conditioning stimulus (no condition stimulation, HWI, CPT, CPA, SIO, and DIO) and covariate age. As older aging has plausible reducing effects on CPM magnitude (Hermans et al., 2016; Khan et al., 2018).

Second, linear mixed model analysis was applied to evaluate the differences between conditioning stimuli (fixed factor) on the outcome measure relative CPM-effect (in %). Inherent to the protocol, order of randomization was also included as fixed factor in the model. Post-hoc Bonferroni corrections for multiple analyses were used for comparisons of CPM-effects using the five different protocols. Since there was a significant difference in perceived pain between the conditioning stimuli, the analysis was repeated with the perceived pain as a covariate. In addition, the number and percentage of responders to each conditioning stimulus was determined. Responders are those who have a meaningful relative CPM effect of 5,3% (Locke et al., 2014). The differences in responders to the five different conditioning stimuli for each muscle were analyzed with Friedman's test, while the difference in responders for different locations for each of the five conditioning stimuli were analyzed with Wilcoxon signed ranks test.

Finally, the contribution of influencing factors on relative CPM-effects (dependent variable) at the mm. trapezius and quadriceps were analyzed via forward stepwise multiple linear regression. The following factors were used as regressors: age, gender, total score LDI, total score LTE, IPAQ-sf total score (with 2 dummy variables), total score PCS, total score PVAQ, total score FPQ-9, expectation of pain of the conditioning stimulus (with 4 dummy variables), attentional focus to the conditioning stimulus (with 4 dummy variables), and perceived pain of the conditioning stimulus. The criteria for inclusion in the model were set at 0,05 and for exclusion at 0,10. Significance level for all analyses was set at α <0,05.

Results

One-hundred and one healthy participants (50 males and 51 females, mean age 23,78 ± 6,68) participated in this study. All available data from these participants were used in the analyses. Group characteristics are presented in Table 1. Results regarding expected pain of the conditioning stimuli and attentional focus to the conditioning stimuli are presented in Table 2. Friedman's test showed a significant difference in pain expectations (Chi^2=21,816; df=3; p<0,001) and attentional focus to the conditioning stimuli (Chi^2=113,381; df=4; p<0,001).

[Insert Tables 1 and 2 about here]

2.1 Efficacy of conditioning stimulation

Figure 2 shows the PPT values at baseline and during the five different conditioning stimuli for both mm. trapezius and quadriceps. Linear mixed models revealed significant increases in PPT during all types of conditioning stimuli compared to baseline PPT, indicating statistically effective CPM during each conditioning stimulation (p<0.001).

[Insert Figure 2 about here]

2.2 Comparison of conditioning stimulation

Table 3 presents the comparison of the perceived pain intensity (on a VAS) and relative CPM effect for the five different conditioning stimuli at the mm. trapezius and quadriceps. The perceived pain intensity ranges from 1,15 to 4,49 (on a VAS 0-10), and the relative improvement in PPT ranges from 22% to 44% at the m. trapezius and from 17 to 32% at the m. quadriceps.

Linear mixed models revealed a significant difference in relative CPM-effect between the applied conditioning stimuli, when PPTs where measured at the m. trapezius (F 21,290; p<0,001). Subsequent analyses displayed significant inferior CPM-effects for CPA compared to all other conditioning stimuli. Also a lesser CPM-magnitude was shown when CPM was evoked by SIO and DIO compared to the CPT. Accordingly, the linear mixed models showed a significant influencing effect from type of conditioning stimulus on relative CPM-effect at the m. quadriceps (F=8,081; p<0,001). Post hoc comparisons revealed inferior CPM-effects during CPA compared to HWI, CPT and DIO. When perceived pain was added as a covariate, the CPA revealed inferior CPM-efficacy at the mm. trapezius and quadriceps compared to all other paradigms except SIO at the m. quadriceps. In addition, the CPT at the m. trapezius revealed a higher CPM-efficacy compared to SIO only.

The numbers of responders to each conditioning stimulus are presented in Table 4. Friedman test showed a significant difference in the applied conditioning stimuli for both the mm. trapezius (Chi^2 = 32,141; df = 4; p < 0,001) and quadriceps (Chi^2 = 11,341; df = 4; p = 0,023) in the number of responders. The highest number of responders was found with HWI as conditioning stimulus and the lowest with CPA as conditioning stimulus. When comparing the paradigms for different sites for the number of responders a significant effect of site was found for HWI (Z = -2,858; p = 0,004), CPT (Z = -3,402; p = 0,001) and SIO (Z = -2,121; p = 0,034), with a higher number of responders at the m. trapezius.

[Insert Table 3 and 4 about here]

The influence of personal factors on CPM magnitude

Regression models for the five conditioning stimuli and their influencing factors are shown in Table 5. Multiple linear regression was used to determine influencing factors on various conditioning stimuli at the mm. trapezius and quadriceps. This indicated a significant effect of **chronic stress** (LTE and LDI) with HWI, CPT and DIO as conditioning stimuli at the m. trapezius, with being more stressed resulting in a larger anti-nociceptive effect at the m. trapezius. **Gender** influenced the relative CPM-effect with all conditioning stimuli at the m. quadriceps, with being female achieving larger anti-nociceptive effects. **Attentional focus** to the conditioning stimulus had a significant influence on CPT at the m. trapezius and with CPA at both muscles. Herewith larger anti-nociceptive effects were achieved with more focus on the conditioning stimulus at the m. trapezius and larger effects were achieved at the m. quadriceps with less focus to the conditioning stimulus. **Physical activity** influenced the anti-nociceptive effect of CPM with CPT and DIO at the quadriceps, when participants were more physical active they achieved a larger anti-nociceptive effect. **Age** was found as an influencing factor with CPA as conditioning stimulus and PPT at the m. trapezius, with obtaining larger anti-nociceptive effects with higher age. Finally, **perceived pain intensity** of the conditioning stimulus influenced DIO as conditioning stimulus and PPT at the m. trapezius, with higher perceived pain resulting in a pro-nociceptive response. These factors explained 5,0% to 20,8% of the CPM-effect.

[Insert Table 5 about here]

Discussion

The first aim of this study was to compare CPM-effects of five different commonly used types of conditioning stimuli (i.e. HWI, CPT, CPA, SIO, and DIO). Our findings show, as hypothesized, that CPM activates the anti-nociceptive system in healthy participants with each of the five conditioning stimuli. However, with respect to the magnitude of the CPM-effect, the CPA revealed less pronounced CPM-effects at the mm. trapezius and quadriceps compared to the other conditioning stimuli (except with SIO at the m. quadriceps). Additionally, a smaller CPM-effect at the m. trapezius was observed when CPM was evoked by SIO compared to the CPT.

As a second aim, this study revealed the following personal factors as significant contributors to the CPM-effect assessed at the mm. trapezius and quadriceps: chronic stress, age, gender, physical activity, attentional focus towards the conditioning stimulus, and perceived pain intensity of the conditioning stimulus.

Efficacy and comparison of conditioning stimulation procedures

Although CPA revealed significant CPM-effects in the present and previous studies (Ladouceur et al., 2012; Cormier et al., 2013; Marouf et al., 2014) methodological differences are possibly responsible for the magnitudes of CPM. From a previous study it is known that attention to an innocuous conditioning stimulus resulted in anti-nociceptive effects (Ladouceur et al., 2012). In our study, multiple linear regression indicated that the variance in the CPM-effect of CPA can be explained by a certain attention towards the conditioning stimulus. This might explain the anti-nociceptive effects and not necessarily the conditioning stimulus itself.

SIO displayed smaller anti-nociceptive effects at the m. trapezius but not at the m. quadriceps compared to the CPT. The difference at the m. trapezius is in accordance with the findings of Oono et al. (2011) who reported the CPT to be superior to tourniquet pain as conditioning stimulus. The conflicting result within our study might be explained by an additive effect of attention to the CPT during the performance at the m. trapezius, as indicated by the linear regression. Although it is suggested that CPM is minimally related to attentional processes (van Wijk and Veldhuijzen 2010), several studies found an additive effect of attention to pain modulation (Moont et al., 2010; Ladouceur et al., 2012; Hermann et al., 2019).

Attention can be modulated by changed levels of cortisol (Schulz et al., 2013) and can alter inputs by selecting crucial information (Quevedo and Coghill 2007). By division of attention, the pain intensity can be lower but this does not automatically change the level of anti-nociception (Quevedo and Coghill 2007). The changed levels of cortisol (Schulz et al., 2013) (and/or blood pressure (Chalaye et al., 2013)) might be a consequence of the activation of the autonomic system by thermoregulatory stimuli (Fechir et al., 2009). In contrast, no change in blood pressure was found with ischemic occlusion (Campbell et al., 2008). Moreover, it seems that DNIC is more effective on C-fiber mediated pain (Kakigi 1994) and these fibers are activated with ischemic pain (Crews et al., 1994) or a higher level of thermal stimuli (Basbaum et al., 2009). Therefore, conditioning stimuli causing cardiovascular reactions or with a certain threshold from peripheral nociceptors result in anti-nociceptive effects (van Wijk and Veldhuijzen 2010; Chalaye et al., 2013).

The difference in responder rates might be a consequence of several factors. First, there is a difference in pain phenotype, which results in pro- or eu-nociception instead of anti-nociception (Yarnitsky et al., 2014). Second, different paradigms and test sites were used and there might be a modality specific response (inter-individual variation) (Yarnitsky et al., 2014; Vaegter et al., 2018). Finally, the perceived pain intensity might be insufficient (e.g. CPA) to reach the responder threshold (5,3%). Further research should investigate these possibilities.

Influencing factors of CPM

A striking finding is the positive influence of **chronic stress** on CPM magnitude. In this study the level of stress was determined with questionnaires (LDI and LTE), which displayed larger anti-nociceptive effects at the m. trapezius in case of higher stress levels with HWI, CPT, and DIO. This might be explained by the fact that non-pain-related stress may distract attention away from a concurrent painful stimulus (Vaegter et al., 2020). This factor should be confirmed in future studies by additional measures of cardiovascular and endocrine factors (Logan et al., 2001; Vassend and Knardahl 2004; Larra et al., 2015; Hermann et al., 2019). **Age** only had influence on CPM at the m. trapezius with the CPA as conditioning stimulus, however, the age range in the present study was relatively small, and the mean age indicated a rather young sample. The weakest CPM-effect was found in older females (Riley et al., 2020). In the current study there were only six participants (four women) above 40 years, which explains the limited influence of age. This is in line with the literature (Lariviere et al., 2007; Grashorn et al., 2013; Hackett et al., 2019; Leone and Truini 2019). **Gender** influenced only the CPM-efficacy at the m. quadriceps with all the conditioning stimuli. This is in line with the results of Skovbjerg et al. (2017), who found a difference in CPM-effects at the m. tibialis anterior with gender. The absence of gender as an influencing factor at the m. trapezius is in line with Khan et al. (2018), who found no influence of gender on CPM-efficacy at two locations in the upper body. A possible explanation for this difference is the segmentally divided locations, which react different. The positive effect of **physical activity** on CPM-efficacy at the m. quadriceps with the CPT and DIO is in accordance with the study of Umeda et al. (2016) that reported higher CPM magnitudes in participants with higher physical activity levels. **Attentional focus** towards the conditioning stimulus had an additive effect on CPM-magnitude with application of the CPT at the m. trapezius and CPA, which is in accordance with previous studies (Defrin et al., 2010; Ladouceur et al., 2012). It seems that attention driven analgesia uses partially overlapping mechanisms as those of pain-driven analgesia (Hoegh et al., 2019) and this might explain the CPM-effects for these paradigms. Although CPM-efficacy is usually independent of perceived pain intensity (Nahman-Averbuch et al., 2016), we found that **perceived pain intensity of the conditioning stimulus** influenced CPM-efficacy for DIO at the m. trapezius. This finding is in accordance with recent studies (Graven-Nielsen et al., 2017; Smith and Pedler 2018) that found that moderate to high pain intensity of cuff occlusion had a positive effect on CPM-magnitude.

Limitations and suggestions

Yarnitsky and colleagues (2015) recommended a mild to moderate conditioning stimulus intensity for activating the anti-nociceptive system (concrete VAS >2/10). The intensity of the CPA in our study can be interpreted as innocuous (mean VAS 1,15 ± 0,20), which is probably inadequate for evoking anti-nociception (Smith and Pedler 2018).

To make proper comparisons of CPM results, it is recommended to use either an additional test stimulus or an additional standard CPM protocol (Yarnitsky et al., 2015). In the current study five CPM protocols were used, which makes a proper comparison possible.

A 10-minute break is recommended between CPM-paradigms (Yarnitsky et al., 2015) and this time interval is sufficient to eliminate the effects of most conditioning stimuli (Lewis et al., 2012a). However, it cannot be ruled out that all effects of the conditioning stimuli were eliminated at the start of the next stimulus, but the influence is expected to be minimal because of the randomization of the paradigms.

The assessment sites of the test stimulus were always applied in the same order, which might have influenced the results. Therefore, the sensory adaptation to the conditioning stimuli and a possible change in temperature of the thermal stimuli throughout the application may interfere with the results of the m. quadriceps. It is advised for future research to randomize this order.

In the CPM-assessment with ischemic occlusion, a methodological contradiction can be noticed. The exercises aimed to induce more pain than by solely cuff occlusion, because required blood supply to recover is obstructed. However, endogenous pain inhibition might be activated in terms of exercise-induced hypo-algesia by performing exercises and might counteract the pain induction (Naugle et al., 2012; Foxen-Craft and Dahlquist 2017).

Our results apply for CPM assessment with PPTs as test stimulus and healthy, relative young subjects and cannot generalized to other paradigms or age-groups.

Clinical implications

The present study revealed large effects and high responder rates for HWI, CPT, SIO, and DIO as conditioning stimuli to experimentally induced CPM-effect upon a mechanical test stimulus. CPA is inferior, because the perceived pain intensity was probably not high enough to evoke CPM and the smaller effect might (partly) be explained by attentional focus towards the cold pack. SIO displayed only inferior results to the CPT when performed at the m. trapezius, but not at the m. quadriceps. Therefore, these four paradigms can be used for assessing CPM-efficacy. However, the expensive equipment and preparation time needed when using HWI or CPT make these types of conditioning stimuli less suitable for use in clinical practice and SIO or DIO are more appropriate. To evoke a clear CPM-effect an individualized conditioning stimulus intensity eliciting mild to moderate pain should be considered (Yarnitsky et al., 2015), implicating a VAS score of at least 3/10 (Boonstra et al., 2014). Next to these practical applications, attentional focus towards the conditioning stimulus and objective measures of chronic stress should be assessed in future studies investigating CPM. The positive influence of physical activity levels on CPM underlines the beneficial effects of physical activity on pain inhibitory pathways.

Conclusion

HWI, CPT, CPA, SIO, and DIO are all methods that induce true anti-nociceptive effects. However, the small positive effect of CPA seems to be a result of attention to the cold pack and insufficient perceived pain of the cold pack and is not recommended. Therefore, SIO and DIO can be used in clinical practice. Personal factors affecting CPM-efficacy in healthy adults depend on the submitted protocol and test site. Stress was found as a factor at the m. trapezius in three stimuli. Gender and physical activity were found as a factor at the m. quadriceps with all stimuli and with CPT and DIO at the m. trapezius.

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Appendix 1: The long-term Difficulties Inventory (LDI)

Below is a list of various aspects of life. We would like to know how you experience these aspects with respect to difficulty and stress in the past 12 months and the successive age categories. Fill the circle in on every row, which corresponds to how you felt: not stressful, slightly or very stressful.

1. Housing (e.g. house is too small, could not find a house, noise problems)
2. Work (e.g. too exacting, conflicts with boss, (threatening) resigned or sacked)
3. Relationship with friends or good acquaintances (e.g. arguments, not enough support)
4. Relationship with partner (e.g. jealousy, conflicts, doubts about relationship, arguments)
5. Relationship with your children (e.g. frequent conflicts, not showing enough respect)
6. Relationship with parents (e.g. regular conflicts, little or no acceptance)
7. Relationship with other family members (e.g. regular conflicts, little or no acceptance)
8. Free time (e.g. not enough, too much free time)
9. Finances (e.g. large debts, inadequate income)
10. Your health (e.g. regularly ill, chronically ill)
11. School/study (e.g. too difficult, not possible to combine with other tasks)
12. Faith, church or religion (e.g. doubt, conflict with clergyman/parson)

Appendix 2: The List of Threatening Events (LTE)

In the next questionnaire, 12 unpleasant events are listed. Please indicate if you have experienced these events in the past 12 months.

1. You yourself suffered a serious illness, injury or an assault
2. A serious illness, injury or assault happened to a close relative
3. Your parent, child or spouse died
4. A close family friend or another relative (aunt, cousin, grandparent) died
5. You had a separation due to marital difficulties
6. You broke off a steady relationship
7. You had a serious problem with a close friend, neighbor or relative
8. You became unemployed or you were seeking work unsuccessfully for more than 1 month
9. You were sacked from your job
10. You had a major financial crisis
11. You had problems with the police and a court appearance
12. Something you valued was lost or stolen

Figure legends

Figure 1**:** Study flow diagram.

Figure2: Pressure pain thresholds at baseline (no conditioning stimulus) and during the five different conditioning stimulation in kg/cm2 for both mm. trapezius and quadriceps.