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Conditioned pain modulation is affected by occlusion cuff conditioning stimulus intensity, but not duration

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Abstract

Background: Various conditioned pain modulation (CPM) methodologies have been used to investigate diffuse noxious inhibitory control pain mechanisms in healthy and clinical populations. Occlusion cuff parameters have been poorly studied. We aimed to investigate whether occlusion cuff intensity and/or duration influenced CPM magnitudes. We also investigated the role of physical activity levels on CPM magnitude.

Methods: Two studies were performed to investigate the role of intensity and duration of occlusion cuff conditioning stimulus on test stimulus (tibialis anterior pressure pain thresholds). In Study 1, conditioning stimulus intensity of 2/10 or 5/10 (duration <20 s) was evaluated using a paired-samples *t*-test. In Study 2, duration of 2/10 conditioning stimulus was 3 min. One-way repeated-measures ANOVA was used to investigate the effect of time (0, 1, 2 and 3 min) on CPM magnitude.

Results: In Study 1, 27 healthy volunteers (mean ± SD: 24.9 years (±4.5); eight female) demonstrated that an occlusion cuff applied to the upper arm eliciting 5/10 local pain resulted in a significant (mean ± SD: 17% ± 46%) increase in CPM magnitude, when compared to 2/10 intensity (−3% ± 38%, $p = 0.026$), whereas in Study 2, 25 healthy volunteers (22.5 years (±2.7); 13 female) demonstrated that 3 min of 2/10 CS intensity did not result in a significant change in CPM ($p = 0.21$). There was no significant relationship between physical activity levels and CPM in either study ($p > 0.22$).

Conclusions: This study demonstrated that an occlusion cuff of 5/10 conditioning stimulus intensity, when compared to 2/10, significantly increased CPM magnitude. Maintaining 2/10 conditioning stimulus for 3 min did not increase CPM magnitude.

Significance: Dysfunctional conditioned pain modulation (CPM) has been associated with poor health outcomes. Various factors can influence CPM outcomes. The role of occlusion cuff conditioning stimulus intensity and duration has not been previously investigated. Intensity (5/10), but not duration of lower intensity (2/10) conditioning stimulus, affects CPM magnitude.

1. Introduction

Conditioned pain modulation (CPM) is an experimental psychophysical paradigm used to test the phenomenon through which a conditioning stimulus (CS) influences perception of an applied test stimulus (Yarnitsky et al., 2010). This paradigm has been used in both healthy and clinical populations to investigate diffuse noxious inhibitory control (DNIC) pain mechanisms (Yarnitsky et al., 2014). Different methodologies are routinely used to investigate the various parameters of the CPM paradigm (Pud et al., 2009; Yarnitsky et al., 2010), including the modality (thermal, electrical or mechanical), timing (sequential or parallel), intensity, duration and location of the stimuli (Yarnitsky, 2015).

A large portion of research on CPM paradigms has focussed on application of a thermal CS modality (Willer et al., 1984; Lautenbacher et al., 2002; Granot et al., 2008; Nir et al., 2011; Razavi et al., 2014). An alternative procedure involves application of an occlusion cuff. Cuff occlusion is associated with ischaemic pain, which is generally associated with C-fibre transduction (Crews et al., 1994). It has also previously been demonstrated that C-fibre- more so than A-fibre-mediated pain results in greater DNIC effectiveness (Kakigi, 1994), indicating the possible utility of occlusion cuff CS compared to thermal CS.

Use of the occlusion cuff as a CS has previously been shown to be reliable for research purposes (Cathcart et al., 2009). However, research is lacking in regard to the influence of other occlusion cuff CS parameters, and in particular the effect of CS pain intensity or duration in relation to CPM outcomes. Three studies have demonstrated that the duration of a high-pain-intensity occlusion cuff CS (VAS: 5.6–7/10) influences the test stimulus outcome (Tuveson et al., 2006; Razavi et al., 2014; Graven-Nielsen et al., 2017). However, the comparative effect of variable occlusion cuff CS pain intensities on CPM outcomes does not appear to have been fully investigated, whilst the effect of durations (>1 min) of CS (using an occlusion cuff), at pain intensity (i.e. <5.6/10), has also yet to be fully investigated. Further research is warranted as both the pain intensity and duration of the CS have been demonstrated to influence the overall CPM effect for thermally applied CS (Lautenbacher et al., 2002; Pud et al., 2009; Nir et al., 2011; Razavi et al., 2014).

There is significant variability in the magnitude of CPM (Imai et al., 2016; Kennedy et al., 2016), which may relate to age, sex (Edwards et al., 2003; Ge et al., 2004; Magerl et al., 2010) and other variables. One variable that has recently demonstrated a relationship with CPM is physical activity levels. The association between physical activity levels and CPM is variable, with one study in active (≥30 min of moderate-to-vigorous physical activity/day) versus inactive post-menopausal women not demonstrating any relationship with CPM (Adrian et al., 2015), whilst another study demonstrated that individuals with higher physical activity levels demonstrated a greater magnitude of CPM; however, higher dose moderate-intensity physical activity did not add to the benefits obtained from vigorous-intensity physical activity (Umeda et al., 2016). Thus, as a result of the large unexplained spread in CPM effect between individuals, and the uncertain effect of physical activity levels on CPM, further investigation of the association of these variables is warranted.

Accordingly, two studies were performed to determine whether (1) the intensity and/or (2) the duration of pain evoked by the occlusion cuff (CS) influenced pain sensitivity in a group of healthy participants. We hypothesized that increased duration and pain intensity of the CS would result in increased CPM magnitude. We also investigated whether physical activity levels were associated with CPM magnitude. We hypothesized that lower physical outcome levels would be associated with reduced CPM magnitude.

2. Methods

2.1 Participants

Twenty-seven participants (mean (±SD): 24.9 years (±4.5)) for Study 1 and 25 participants (22.5 years (±2.7)) for Study 2 were recruited via advertisements placed over the local university campus, social media and word of mouth. Participants were self-reported healthy individuals aged between 18 and 65 years. Participants provided informed consent to participate in the study. Exclusion criteria included any type of current or chronic musculoskeletal disorder, a level of English that was not sufficient to allow completion of questionnaires, pregnancy and any type of neurological, inflammatory, cardiovascular or psychopathological disorder. Ethical clearance for this study was granted by Griffith University Human Research Ethics Committee (Ref.: 2016/198).

2.2 Outcome measures

2.2.1 Conditioned pain modulation

2.2.1.1 Test stimulus. The test stimulus was a pressure pain threshold (PPT) measured using a 1-cm^2-

tipped-diameter algometer (Somedic Production AB, Sollentuna, Sweden), applied at a rate of 40 kPa/s. This was measured over the tibialis anterior musculature opposite to the arm to which the occlusion cuff was applied, whilst the participant was lying in a relaxed supine position. The test stimulus (PPTs) was applied prior to and during application of the occlusion cuff. The PPT was determined by asking the participant to press and release a participant-controlled switch at the moment the sensation of pressure became painful.

2.2.1.2 Conditioning stimulus (CS). The CS was a single, 8.5-cm-wide chamber, occlusion cuff (Element, Shenzhen, China) applied to the nondominant arm, 2 cm superior to the cubital fossa. The cuff was inflated manually by the examiner via hand squeeze on the bulb. After each hand squeeze (approximately 10–15 mmHg increase in pressure), the participant was asked to provide a pain rating (/10). Inflation continued until the participant rated their arm pain as 2/10 or 5/10. As this study aimed to evaluate the effect of pain intensity on CPM magnitude, there was no intention to produce arm ischaemia with cuff inflation.

Conditioned pain modulation was calculated as follows: CPM (%) = $(PPT_{conditioning} - PPT_{baseline})/PPT_{baseline} \times 100$. The mean of triplicate baseline PPTs was used for analysis.

2.2.2 Physical activity levels

Physical activity levels were assessed using the International Physical Activity Questionnaire – Long Form (IPAQ; Craig et al., 2003). The IPAQ is made up of a set of four questionnaires that examine the physical activity levels of the individual in the last 7 days across a variety of domains including transport-related physical, work-related physical activity, domestic and gardening activities and leisure time physical activity. Each activity is weighted by the energy requirement required to undertake it, measured in METs (metabolic equivalents). The total physical activity score was used for analyses. The computation of this score equated to the sum of the duration (in min) and frequency (days) for all the types of activities in all domains. All cases in which the sum total was greater than 960 min (16 h) were excluded from further analysis. Maximum score possible is 21,420 METs/week.

2.3 Procedure

Participants completed demographics and the IPAQ prior to receiving testing instructions (e.g. blinding protocol of CS intensity) and undergoing the testing protocol (Fig. 1). The participants were placed in a relaxed, supine position. Initial baseline triplicate pressure pain thresholds (PPTs) were collected with a 20-s interstimulus interval. In Study 1, to avoid sensitization, a 1-min rest period was provided prior to the occlusion cuff pressure being applied. Participants were informed that the occlusion cuff pressure would increase until a perceived pain rating of either 2 or 5 of 10 was achieved (Fig. 1A). The intensity of this pressure was randomized. This was based on the numerical scale (NRS) of 0–10, with 0 being 'no pain at all' and 10 being the 'worst pain imaginable'. The test stimulus was repeated, whilst cuff inflation was maintained at the assigned pain intensity. The researcher applying the test stimulus was blinded to the pain level of CS being applied (i.e. was unable to see an assigned pain rating written on paper held by another research assistant). The cuff was immediately deflated following the completion of the test stimulus application. A 5-min rest period ensued. Triplicate PPTs were repeated. The occlusion cuff was re-inflated to the alternate pain intensity following a 1-min rest period. The test stimulus was repeated. Another 5-min rest period followed this testing, at which time a further three PPTs were collected.

In Study 2, triplicate PPTs were initially measured (20-s interstimulus interval). A 5-min rest period ensued. The occlusion cuff was then inflated until a pain of 2/10 was reported by the participant. The cuff was maintained at this pressure for 3-min duration. A PPT was measured immediately upon reaching the occlusion cuff pain intensity and then repeated at the 1-, 2- and 3-min marks. Participants were asked to report a numerical rating score (0–10) for the associated evoked CS pain for each time point. The cuff was deflated after the final PPT was recorded (Fig. 1B). Following a 5-min rest period, a further three PPTs were collected.

2.4 Statistical analysis

Initial data for both studies were analysed for normality through investigation of box and scatterplots. For both studies, possible sex differences were investigated using Mann–Whitney tests. To investigate any possible effects of sensitization or accommodation resulting from repeat application of the test stimuli in Study 1, one-way repeated-measures (RM) ANOVA was performed to test for the effect of time (three levels: prior to CS, following first CS and following second CS) on PPTs. Age and sex were entered as covariates to account for the possible

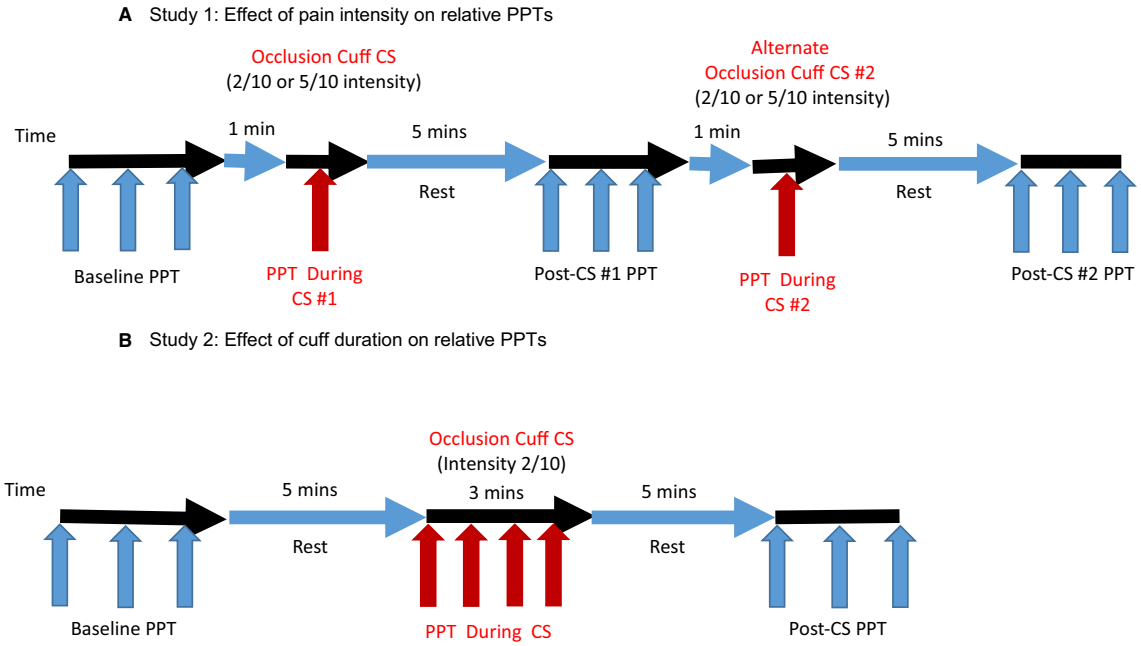

Figure 1 Flow of test protocol for Studies 1 and 2. Each vertical arrow indicates time points at which PPTs and pain scores were measured. There was a 20-s interstimulus interval between PPTs. The horizontal blue arrows indicate rest periods. PPTs, pressure pain thresholds; CPM, conditioned pain modulation; CS, conditioning stimulus.

effects of gender and age and the interactive effects of gender × age on PPT (Magerl et al., 2010).

For aim 1 investigating the influence of CS intensity on PPTs, it was first necessary to define CPM. CPM for each condition was calculated as a change in PPT from immediately prior to the application of the CS to the value taken during the CS. The change in PPT (CPM) was normalized to a percentage change relative to the PPT value taken immediately prior to the application of the CS to normalize CPM effects for baseline PPT values using the formula: CPM (%) = $(PPT_{conditioning} - PPT_{baseline})/PPT_{baseline} \times 100$. A positive value for CPM represents an increase in PPTs following application of the CS. Differences in the magnitude of CPM using a mild (2/10 VAS) and moderate (5/10 VAS) CS were assessed using a paired-samples *t*-test. To determine whether CS of 2/10 intensity significantly affected PPTs (and hence induced CPM), a paired-samples *t*-test was used.

For aim 2 investigating the influence of CS duration, one-way RM ANOVA was performed to test for the effect of time of cuff application in minutes (four levels: 0, 1, 2 and 3) on PPTs and CS pain. Age and sex were entered as covariates to account for the possible effects of gender and age and the interactive effects of gender × age on PPT (Magerl et al., 2010). Assumptions for repeated-measures (RM) ANOVA were tested, including Mauchly's test of sphericity. When Mauchly's test of sphericity was violated ($p < 0.05$), the Greenhouse–Geisser correction results are reported. To determine whether the first application of CS of 2/10 intensity significantly affected PPTs, a paired-samples *t*-test was used.

Bivariate analyses investigating CPM and physical activity levels were performed using Spearman's ρ. All analyses were performed using IBM SPSS Statistics 22 (IBM, Armonk, NY, USA). For all analyses, the significance level was set at $p \leq 0.05$.

3. Results

3.1 Study 1

3.1.1 Preliminary and descriptive analyses

Twenty-seven (eight female) volunteers participated in Study 1. Sixty-eight per cent of study volunteers demonstrated a 'high' level (>3000 MET min/week) of physical activity (median [interquartile range]: 4543 [3425, 6921]; Craig et al., 2003). There were no significant differences in physical activity levels between groups (Mann–Whitney *U*: $Z = -0.32$, $p = 0.75$). There were no significant differences in physical activity levels (Mann–Whitney *U*: $Z = -1.76$, $p = 0.085$) or baseline PPTs (Mann–Whitney *U*: $Z = -0.28$, $p = 0.81$) between sexes.

3.1.2 Effect of time

There was no significant main effect of time (prior to CS; following first CS; following second CS) on PPTs measured ($F_{1.30,27.3} = 0.017$, $p = 0.98$). There were no significant time × age ($F_{1.3,27.3} = 0.13$, $p = 0.88$) or time × gender interactions ($F_{1.3,27.3} = 0.72$, $p = 0.44$). Gender did not interact with age to affect PPTs ($F_{1.30,27.3} = 0.89$, $p = 0.42$). This suggests that individuals were not becoming sensitized or hypoalgesic over time as a result of repeated testing.

3.1.3 Primary statistical analysis

3.1.3.1 Effect of conditioning stimulus intensity. There was a significant difference in CPM magnitude measured for the CS of 5/10 intensity (mean (±SD): 17% (±46%)) when compared to 2/10 intensity (−3% (±38%)), $t_{25} = -2.36$, $p = 0.026$ (Table 1). This suggests that CPM magnitude is higher for increased (VAS: 5/10) CS intensity (Fig. 2). There was no significant difference between PPTs for CS of 2/10 intensity and those measured at baseline ($t_{25} = 1.12$, $p = 0.27$), indicating that this CS intensity did not induce CPM.

3.1.3.2 Relationship between physical activity and CPM. There was no significant relationship between magnitude of CPM response for occlusion cuff intensity 2/10 and IPAQ scores ($r = 0.089$, $p = 0.67$) or between magnitude of CPM response for occlusion cuff intensity 5/10 and IPAQ ($r = -0.074$, $p = 0.37$).

3.2 Study 2

3.2.1 Preliminary and descriptive analyses

Twenty-five (13 female) participants volunteered for Study 2. Sixty-four per cent of study volunteers were participating at a 'high' level of physical activity (median [IQR]: 4503 [1761, 10199]; Craig et al., 2003). The mean (±SD) systolic blood pressure applied to induce 2/10 arm pain in the participants was 172 (±39) mmHg. There was no significant sex difference between baseline physical activity (Mann–Whitney *U*: Z = −0.76, $p = 0.45$) or PPT measures (Mann–Whitney *U*: Z = −1.14, $p = 0.25$).

3.2.2 Secondary statistical analysis

3.2.2.1 Effect of conditioning stimulus duration on CPM. There was no significant main effect of (cuff inflation) time on CPM ($F_{3,63} = 0.36$, $p = 0.78$) (Fig. 3). Sex × age × gender ($F_{3,63} = 0.30$, $p = 0.83$), sex × time ($F_{3,63} = 0.30$, $p = 0.83$) or age × time ($F_{3,63} = 0.35$, $p = 0.79$) interactions did not significantly affect PPTs. There was no significant effect of initial cuff application on PPTs ($t_{24} = 0.66$, $p = 0.52$), indicating that CS of 2/10 intensity did not induce CPM.

3.2.2.2 Effect of Conditioning Stimulus Duration on CS Pain. There was a significant main effect of time on the intensity of arm pain ($F_{1.73,37.56} = 10.35$, $p < 0.001$). Post hoc analysis indicated that arm pain did not change between time of initial application (Numerical Pain Rating Scale: NRS mean (SE): 2.2 (0.13)) and 1 min (NRS: 2.64 (0.27), $p = 0.23$), but arm pain progressively and significantly increased by 2 min (NRS: 3.08 (0.27), $p = 0.004$) and 3 min (NRS: 3.28 (0.27), $p = 0.011$) when compared to initial cuff application.

3.2.2.3 Relationship between physical activity and CPM. There was no significant relationship between baseline IPAQ scores and magnitude of CPM response for any duration of cuff application (all $r < 0.26$, p's > 0.22).

4. Discussion

The aim of this project was to determine whether the pain intensity and/or duration of an occlusion cuff CS influenced the magnitude of CPM, measured using PPT as a test stimulus. Our studies demonstrated that heterotopic pain sensitivity depended on

Table 1 Mean and standard deviation of pressure pain thresholds (PPTs) – at baseline and during each intensity and duration of conditioning stimulus (CS)

Study #	Baseline PPTs Mean (±SD)	CS 2/10 PPTs Mean (±SD) Application	CS 2/10 PPTs Mean (±SD) 1 min	CS 2/10 PPTs Mean (±SD) 2 min	CS 2/10 PPTs Mean (±SD) 3 min	CS 5/10 PPTs Mean (±SD)
1	473 (126)	429 (132)				510 (130)
2	624 (173)	614 (156)	599 (175)	619 (179)	627 (201)	

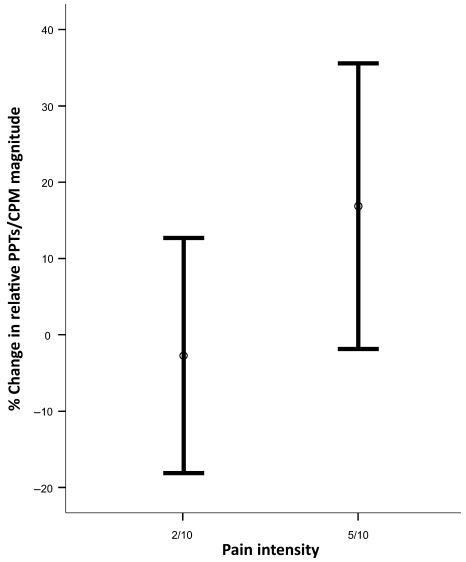

Figure 2 Effect of CS pain intensity on relative PPTs. Bars indicate 95% confidence intervals. PPTs, pressure pain thresholds.

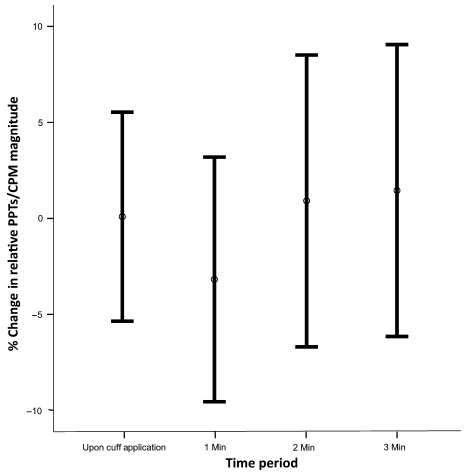

Figure 3 Effect of CS pain duration on relative PPTs. Bars indicate 95% confidence intervals. PPTs, pressure pain thresholds.

the intensity of the CS, but was not affected by the duration of 2/10 CS intensity. A pain intensity of 5/10 occlusion cuff CS applied to the arm resulted in an approximate 20% increase in PPTs over the contralateral lower leg (tibialis anterior), whilst a pain intensity of 2/10 did not result in an increase in PPTs when maintained for a period of 3 min. Our study also demonstrated that there was no relationship between CPM magnitude and physical activity levels.

Our study demonstrated that higher pain intensity of the occlusal cuff CS resulted in increased CPM magnitude in a healthy sample of individuals. This is consistent with the broad literature in healthy individuals indicating that test PPTs increase anywhere from 10 to 38% following application of different CS pain levels. Tuveson et al. (2006) demonstrated an approximate 10% mean increase in PPTs following application of 10 min of 7/10 forearm 'ischaemic pain' induced with application of a cuff inflated to 260 mmHg on the upper arm and combined with approximately 45 repetitive forearm exercises. However, other studies using the same protocol report mean increases of between approximately 17 and 30% (Leffler et al., 2002; Tuveson et al., 2007, 2009). The pair of Kosek studies with an initial CS intensity of 3.5–4.5/10 resulted in PPT increases of 25–35% (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000), whilst Arendt-Nielsen et al. (2010) demonstrated an approximate 15% increase in PPTs following a CS pain intensity of 4/10 CS intensity. More recently, Graven-Nielsen et al. (2017) demonstrated a 15–25% increase in leg PPTs following 60 s of CS pain intensities greater than 5/10 CS intensity in the arm region (Graven-Nielsen et al., 2017). Cathcart et al. (2009) did not measure pain sensitivity, but reported a reduction of mechanically applied repeated test stimuli pain of approximately 1/10 on a visual analogue scale for an occlusion cuff inflated to an individual's 3/10 pain intensity. Our study demonstrated heterotopic PPTs increased by 20% following application of an occlusion cuff of 5/10 local pain intensity. This is consistent with the above study findings. Hence, the above data would suggest that application of an occlusion cuff of ≥3/10 pain results in ≥10% improvement in pain thresholds or reduction in pain levels.

On the other hand, our study was unable to elicit an increase in PPTs for a CS pain intensity of 2/10. It is currently unclear whether a minimum pain intensity of CS is required to elicit endogenous analgesic mechanisms when using an occlusion cuff. The levels of conditioning pain intensity applied in different occlusion cuff studies vary considerably. Cathcart et al. (2009) utilized a pain level of 3/10, Kosek and Hansson (1997) and Kosek and Ordeberg (2000) ≥3/10 and Arendt-Nielsen et al. (2010) 4/10, and Graven-Nielsen et al. (2017) used variable pressures resulting in 3, 5 and 8/10, whilst a series of studies using the modified submaximal effort limit pain to ≤7/10 (Leffler et al., 2002; Tuveson et al., 2006, 2007, 2009; Razavi et al., 2014). Thus, it appears a minimum of 3/10 pain elicited by an occlusal cuff CS is required to significantly increase CPM magnitude.

Our secondary aim investigated the effect of occlusion cuff duration on CPM magnitude measured using PPTs, as it was possible that the observed differences in Study 1 may have resulted from increased duration of CS intensity. Previous research has demonstrated varying time-dependent alterations arising from prolonged CS duration. There is evidence that increased duration of occlusion cuff CS is associated with reduced heat pain sensitivity (Pertovaara et al., 1982). It has also been demonstrated that a reduction in pain intensity induced by CO_2 laser stimulation occurs when a prolonged heat bath CS is applied (Kakigi, 1994). In contrast to these findings, application of prolonged occlusion cuff CS did not cause time-dependent changes in PPTs (Tuveson et al., 2006). Various cuff inflation time periods have been utilized. Similar to our first study, Arendt-Nielsen et al. (2010) immediately deflated the cuff (intensity = 4/10) after application of one test stimulus (PPTs), resulting in increased PPTs of approximately 15% (Arendt-Nielsen et al., 2010), whilst another study demonstrated that 10 min of occlusion cuff inflation time resulted in increased PPTs of approximately 10% (Tuveson et al., 2006). Our cuff pressure was normalized to individualized 2/10 pain levels and was maintained for 3 min and was not associated with a change in heterotopic PPTs. We were surprised with this finding, especially given that strong nonpainful heat stimuli have previously been demonstrated to reduce pain sensitivity (Lautenbacher et al., 2002), whilst low-intensity heat (VAS: 2/10) for 6 min also increased PPTs for the test stimulus outcome measured (Razavi et al., 2014). However, both of these studies utilized a significantly longer duration of heat CS (6–10 min) and one study measured outcomes in terms of heat pain thresholds (Lautenbacher et al., 2002), which may explain the discrepancy in findings. For our study measuring PPTs, and using an occlusion cuff as a CS, it appears that the intensity of CS pain (VAS: 2/10), combined with 3-min duration of application, was not sufficient to increase CPM magnitude. It has previously been demonstrated that forearm pain worsens with time of cuff application when measured using the submaximal effort tourniquet procedure (Sigurdsson and Maixner, 1994). Given that it has been demonstrated that test stimulus outcome is influenced by CS pain intensity, it is reasonable to postulate that test outcomes will also vary across time as the applied CS pain intensity increases. This is pertinent, given that the duration of cuff inflation applied fluctuates between studies to complete the variety of applied test stimuli performed as a result of the different methodologies employed in the respective studies. This has been demonstrated by the Kosek studies, where they demonstrated arm pain increasing from 3.5–4.5/10 to 4.5–6.5/10 upon application of their test stimuli (over a period of 1–3.5 min), with a resultant 25–35% increase in PPTs (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000). Our study resulted in concurrent increased arm pain (3/10 from an initial 2/10) within the first 2 min of application, and 3.3/10 at the 3-min period when the final PPT was measured. Despite the increasing arm pain and maintaining the cuff pressure for 3 min, there was no change in remote PPTs measured. Cathcart et al. (2009) were able to demonstrate that 3/10 CS pain intensity resulted in reduced *pain ratings* to repeated mechanical stimuli. However, it must be noted that various studies have demonstrated that pain ratings and pain sensitivity, although both psychophysical in nature, are not necessarily correlated and may measure different aspects of the human pain experience (Kamper et al., 2010; Siegenthaler et al., 2010). In summary, 3-min duration of cuff inflation at low pain intensity (2/10) did not influence CPM magnitude.

Our study also demonstrated that PPTs, either prior to or during CS, were not related to reported levels of physical activity in healthy individuals. Two prior studies have demonstrated that higher levels of physical activity were associated with greater CPM (Naugle and Riley, 2014; Umeda et al., 2016). These studies included a broad cross section of ages (Naugle and Riley, 2014) and physical activity levels (Naugle and Riley, 2014; Umeda et al., 2016), compared to the high prevalence (64–68%) of highly active individuals our study enrolled. Umeda and colleagues also used accelerometers to measure physical activity levels (Umeda et al., 2016), which would also provide a more accurate representation of physical activity levels, as compared to the self-report measures utilized in our study.

Our project has several limitations that require mention. Our samples included young, physically active individuals and thus may not be representative of the broader community. We also utilized two samples to investigate intensity and duration of the CS. As these phenomena were not investigated in the same sample, we are unable to determine whether our findings resulted due to possible differences in sample characteristics. Although the samples did not differ significantly on baseline variables, repeating this study within one study sample would be warranted. In Study 1, participants were aware that the CS intensity would be 2 or 5/10, which may

have introduced bias to their PPT response upon application of the alternate pressure. However, participants were blinded to PPT values, and aims of the study to limit any such bias. Future studies may wish to add other CS intensities not related to the study aims to reduce any possible bias.

In summary, based on two different studies, it was demonstrated that pain intensity, but not duration of the CS, was associated with increased heterotopic PPTs. A simple and affordable occlusion cuff, which has previously been demonstrated to be reliable, when applied to the upper arm and eliciting 5/10 local arm pain resulted in an approximate 20% increase in tibialis anterior PPTs, whilst intensities of 2/10, even when maintained for 3 min, did not increase CPM magnitude.

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Author contributions

A.S. conceived and designed the study; analysed and interpreted the data; and prepared, revised and approved the manuscript. A.P. conceived and designed the study; analysed and interpreted the data; and prepared and revised the manuscript.

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