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Can offset analgesia magnitude provide additional information about endogenous pain modulation in people with knee osteoarthritis? An experimental study.

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ABSTRACT

Objectives: To investigate the relationship between offset analgesia magnitude and the responsiveness to conditioned pain modulation (CPM), temporal summation of (second) pain (TSP), and clinical pain severity in people with knee osteoarthritis (KOA).

Methods: Electrical stimuli were applied to 88 participants with KOA to measure offset analgesia at the volar forearm of the dominant hand, and CPM and TSP at the most symptomatic knee and ipsilateral volar wrist. Clinical pain severity was assessed using the pain subscale of the Knee injury and Osteoarthritis Outcome Score ($KOOS_{PAIN}$). Linear mixed effects models evaluated pain modulatory effects across all tests, and Spearman's partial correlations assessed associations between offset analgesia, CPM, TSP, and $KOOS_{PAIN}$ while accounting for covariates of interest. Participants unable to validly finish all psychophysical tests were excluded from effect and correlation analyses but were evaluated for predictors of non-valid completion using bivariate Stochastic Search Variable Selection.

Results: Significant pain modulation was observed across all psychophysical tests ($p < 0.05$) and no meaningful predictors of non-valid test completion were found. Offset analgesia magnitude did not significantly correlate with CPM, TSP, or $KOOS_{PAIN}$ ($p \geq 0.05$), with a maximum partial correlation coefficient of $\rho = 0.21$.

Discussion: Offset analgesia was not associated with CPM, TSP, or $KOOS_{PAIN}$ in people with KOA. Despite the lack of case-control studies comparing offset analgesia between people with KOA and

healthy controls, these findings suggest that offset analgesia may provide information about endogenous pain modulation beyond CPM and TSP, though its clinical translation remains uncertain.

KEY WORDS: Endogenous pain modulation; Offset analgesia; Conditioned pain modulation; Temporal summation of second pain; Knee osteoarthritis

1 INTRODUCTION

Knee osteoarthritis (KOA) is one of the world-leading causes of pain and disability (1) and many people with KOA present signs of disrupted endogenous pain modulation (EPM) (2-5). EPM refers to the body's internal mechanisms for pain regulation, including both inhibitory and facilitatory mechanisms (6, 7), and it is commonly quantified using psychophysical tests, such as temporal summation of pain (TSP) and conditioned pain modulation (CPM; (7)). During TSP, exposure to a series of painful stimuli is expected to gradually increase perceived pain intensity (8, 9), whereas CPM reflects a reduced stimulus-evoked pain intensity perceived at one area of the body when another painful conditioning stimulus is applied simultaneously to a remote region (10). Offset analgesia is another common measure of inhibitory EPM (7), characterized by a disproportionately large reduction in perceived pain intensity following a small reduction in stimulus intensity (11). However, only one study has assessed offset analgesia in people with KOA (12), finding it to be both unresponsive to and non-predictive of the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs). Furthermore, no association was found between offset analgesia and clinical pain severity (12). However, offset analgesia has not been found to respond to any pharmacological, pain-modulatory agent (13) and the dissociation between offset analgesia and clinical pain severity is a commonly reported finding in people with pain (14-18). Nevertheless, offset analgesia magnitude has been found to be altered in various chronic pain populations (19), suggesting that it may still provide a valuable marker of EPM (mal)functioning in people with KOA. Moreover, although a few contradictions exist (20, 21), most studies report no

association between offset analgesia and neither CPM nor TSP (14, 22-24), suggesting that it may reveal processes of EPM that are not reflected by other psychophysical test paradigms (25). Using the baseline data of a larger clinical trial (26), this study therefore aims to evaluate the relationship between offset analgesia magnitude and 1) CPM efficiency; 2) TSP effect; and 3) clinical pain severity, in a sample of people with radiographic and painful KOA. Since we believe that offset analgesia magnitude may rely on processes of EPM that are distinct from that of CPM and TSP, we hypothesize that no significant associations will be found between offset analgesia and neither CPM nor TSP. Similar to earlier findings in other pain populations (14-18), we also, however, hypothesize that offset magnitude will not be associated with clinical pain severity in people with KOA.

2 MATERIALS AND METHODS

2.1 Study design and conditions

This study is a secondary analysis of the baseline data collected within a larger, randomized controlled trial with a 12-week intervention and 24-week follow-up in people with KOA. The research protocol for this study was pre-registered on ClinicalTrials.gov (Identifier No. NCT04362618) and can be found in detail elsewhere (26). The protocol was approved by the Ethics Committee of the University Hospital Brussels and Vrije Universiteit Brussel (B.U.N 143201941843) and followed the Declaration of Helsinki. All participants provided written informed consent before their baseline assessment.

2.2 Participants

Ninety middle-aged-to-older adults with radiographic and painful KOA were recruited between January 2020 and April 2023 for the larger clinical trial after expressing an interest in participating. Two of these participants were, however, excluded from the present study due to an inability to perform any pain test because of medical reasons, resulting in a study sample of 88 participants. To be included

in the larger trial, participants had to meet the diagnostic criteria for KOA, as defined by the American College of Rheumatology (27), and display a radiographic grade of ≥2 of tibiofemoral osteoarthritis according to the Kellgren and Lawrence grading system (28)(based on anterior-posterior and mediolateral radiographs of the tibiofemoral joint, plus an axial radiographical view of the patellofemoral joint). The final diagnosis of KOA was made by an orthopaedist or a physical therapist. Additional inclusion criteria were age ≥50 years, and knee pain reported as ≥3 on a 0 to 10 visual analogue scale (0 = no pain; 10 = worst pain imaginable) on most days within the preceding three months. Exclusion criteria were: 1) treatment with prescribed exercise therapy or intra-articular injections (e.g., corticosteroids) in the preceding six months; 2) being on a waiting list for knee replacement; 3) contra-indications for exercise therapy; 4) increased inflammatory state unrelated to osteoarthritis (C-reactive protein >10 mg/L); 5) cognitive impairment inferring an inability to understand the test instructions and/or a score of <23/30 on the Mini-Mental State Examination (29); 6) inability to understand the Dutch language; and 7) disorders (e.g., rheumatoid arthritis, fibromyalgia), and/or regular use of stronger medication (e.g., opioids, immunosuppressants) that influence pain and/or the immune system. If possible, participants were asked to refrain from NSAIDs from inclusion until the last follow-up of the larger clinical trial. For whom a restraint from NSAIDs was not possible, the use of NSAIDs was closely monitored to ensure that any intervention-related effect was not falsely detected as a result of changes in NSAID consumption. However, all participants were instructed to refrain from all types of analgesic drugs 24 hours before their laboratory visits, and the specific use of NSAID was therefore not considered an issue in the present study.

2.3 Procedure

All measurements took place at the Vrije Universiteit Brussel (VUB) and/or the Universitair Ziekenhuis Brussel (UZ Brussel), Belgium. Participants were instructed to refrain from analgesic drugs, alcohol, caffeine, nicotine, and strenuous physical activity (>3 metabolic equivalents) 24 hours before their

laboratory visit. Upon arrival, a computer-based set of standardized questionnaires was completed, followed by individual pain threshold detection and assessment of EPM (*i.e.,* CPM, TSP, and offset analgesia) using electrical stimulation provided by a constant current stimulator (Surpass LT stimulator, EMS biomedical, Korneuburg, Austria). Electrical pain thresholds (EPT) were first determined at the medial peripatellar region of the most symptomatic knee (3 cm medial to the midpoint of the medial edge of the patella) (2), and at the ipsilateral volar wrist along the median nerve (*i.e.,* at the carpal tunnel). Using the EPT values, participants were then first exposed to the TSP, and then the CPM paradigm, with the order of the test regions being randomized across participants. An additional EPT was then assessed at the volar forearm of the dominant hand, 3 cm distal to the elbow joint (30). This was used to individually tailor the stimulation intensities for the following offset analgesia paradigm. During all measurements, participants remained comfortably seated with the test knee placed in 90° and with the hand of the arm being tested resting on the thigh with the palm facing up. A schematic presentation of the assessment procedure is presented in Figure 1.

2.4 Endogenous pain modulation

2.4.1 Electrical pain thresholds

EPTs of the symptomatic medial knee and ipsilateral volar wrist were determined by delivering electrical stimuli in constant rectangular 5-pulse trains (frequency: 250 Hz; pulse duration: 1 ms; (31)). With an interstimulus interval of 5 s, the current was increased in steps of 0.5 mA from a baseline of 0 mA (32) until the participants reported a perception of discomfort. To determine the EPT of the volar forearm, stimuli were delivered in constant rectangular pulse trains of the same pulse duration, but at a lower frequency (100 Hz; (30)). The EPT detection procedure was repeated three times for each test region, interspersed with a 30-s rest period. The mean of the three attempts was calculated for each test region and used during the subsequent EPM assessment.

2.4.2 Temporal summation of (second) pain

Temporal summation of (second) pain (TSP) was assessed by delivering 20 electrical stimuli in constant rectangular 5-pulse trains (frequency: 250 Hz; pulse duration: 1 ms (32)) at the previously determined EPT intensity, and with an interstimulus interval of 0.5 s. The participants were instructed to rate their perceived pain from 0 (no pain) to 10 (worst pain imaginable) using a numeric rating scale (NRS; (32)) after the 1st, 10th, and 20th stimulus. The medial knee and volar wrist were assessed in the same order as during the EPT assessment and three trials, separated by 30 s, were performed at each test region.

2.4.3 Conditioned pain modulation

CPM was assessed with electrical stimuli as the test stimulus, and the cold pressor test (CPT) as the conditioning stimulus (33). Twenty electrical stimuli (33), delivered in 5-pulse trains (frequency: 250 Hz; pulse duration: 1 ms), were applied at a current intensity of 140% EPT during two test runs: one before and one during the CPT. The interstimulus interval varied between 8 to 12 seconds to reduce the risk of stimulus prediction (34). During the CPT, participants were instructed to immerse the hand contralateral to their most symptomatic knee into a bath of cold, distilled, circulating water at 12°C (VersaCoolTM, Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA USA; (35)). The hand was immersed up to the wrist and participants were instructed to keep their fingers spread and not to let any part of their hand touch the walls or bottom of the water container. Following each series of electrical stimuli, the participants were asked to give a NRS score reflecting their average pain experience during all 20 stimuli. The stimulation series was first provided at both the medial knee and volar wrist in the absence of the CPT (same order as EPT detection), and then repeated simultaneously with the CPT. A 15-min break was provided between each test region to let the hand rewarm (Fig 1), and upon completion of the CPM test, a 5-min break was provided before testing offset analgesia.

2.4.4 Offset analgesia

Offset analgesia was assessed using a validated protocol using electrical stimuli (30), in which the stimuli are provided as one train of rectangular pulses (frequency: 100 Hz; pulse duration: 1 ms) lasting for 30 seconds. One test trial and one control trial were performed in a randomized order, with each trial divided into three intervals: T1 (5 s), T2 (5 s), and T3 (20 s). The control trial was performed with a constant stimulus intensity at 150% EPT, whereas the test trial was performed with the following intensities: T1 = 150% EPT; T2 = 180% EPT; T3 = 150% EPT. Participants were instructed to rate their perceived pain intensity using the NRS every 5 s, reflecting the transitions from T1 to T2, from T2 to T3, and four time points with a 5-s interval during T3. As primary afferents adapt during prolonged stimulation (36), a 5-min rest was provided between the test and control trial to minimize carry-over effects on the stimulation site.

2.5 Clinical pain severity and pain-related traits

Before the EPM assessment, all participants completed a number of self-reported questionnaires online via Qualtrics, of which the data of five questionnaires was used in the present study (see (26) for full questionnaire battery). Clinical pain severity was assessed using the Knee injury and Osteoarthritis Outcome Score (KOOS) (37). KOOS is constructed of five subscales, of which the pain subscale is considered a valid and reliable tool for the assessment of pain severity in people with KOA (38). An estimate of clinical pain severity was determined according to the 2012 version of the KOOS scoring manual (39) by computing the mean score of all items and implementing it in the following equation:

$$\mathrm{KOOS_{PAIN}} = 100 - \frac{(\text{mean score} \times 100)}{4}$$

A final score between 0 and 100 was thereby obtained for each participant, with 100 indicating no symptoms (39). To further characterize the study sample, pain catastrophizing and pain hypervigilance

were assessed using the Pain Catastrophizing Scale (PCS; (40)) and Pain Vigilance and Awareness Questionnaire (PVAQ; (41, 42)), respectively. In addition, the Central Sensitization Inventory (CSI; (43)) was used to assess signs of central sensitization, whereas the Brief Illness Perception Questionnaire (IPQ-B; (44)) was used to assess the perception of illness. The scores across all items from the PCS, PVAQ, CSI, and IPQ-B, respectively, were summed and used to describe and evaluate participants who were able to complete the entire EPM test battery versus those who were not (see 2.6.3). All questionnaires were provided in a validated Dutch version (45-49).

2.6 Statistical analysis

All statistical analyses were performed in RStudio running R version 4.4.1, and the significance of all analyses described below was set *a priori* to $p < 0.05$.

2.6.1 Within-subject psychophysical test effects

To evaluate the within-subject effect for offset analgesia, CPM, and TSP, we constructed separate linear mixed-effects models (LMMs) for each test with absolute pain intensity (i.e., NRS score) as the dependent variable. Importantly, only participants who completed all psychophysical tests as outlined above were included in the effect analysis. This choice was made to prevent the possibility of detecting effects in one test that might be influenced by responses of participants excluded from another because of missing and/or invalid data.

For all LMMs, a by-subject random intercept and a by-subject random slope were included for each independent fixed factor to account for the repeated measures design of each test, as well as for inter-individual differences in baseline pain and test responsiveness and/or dynamics. We also included a bivariate covariate across all models controlling for whether the assessor and participant were of the same (= 1) or the opposite sex (= 0). All LMMs were constructed using the *lmer* function from the *lme4* package and assessed in terms of fit by visual inspection of quantile-quantile and residual-versus-fitted

plots of the model residuals. Prior to this visual inspection, the contribution of all random effects to the models was tested using the *ranova* function from the *lmerTest* package, which performs likelihood ratio tests by comparing models with and without each random effect. Likewise, the contribution of the covariate (i.e., assessor-participant sex coherence) was assessed using Type III analysis of variance (ANOVA), applied using the *Anova* function from the *car* package, which tests each fixed effect while controlling for other factors in the model. To avoid overfitting, a random-effects term or covariate was only kept in the final model if it significantly improved the model fit. All random effects significantly improved the model fit across all models, whereas the covariate did not and was therefore excluded from the final models. The results of each final LMM were presented in the more familiar ANOVA table format, generated using marginal Type III ANOVA from the *lmerTest* package (function: *anova*) and with degrees of freedom for the *F*-values calculated using Satterhwaite's method. Post-hoc tests were conducted for significant fixed effects using the *emmeans* function from the *emmeans* package.

2.6.1.1 Offset analgesia magnitude

To evaluate the significance of offset analgesia magnitude (ΔOA), we constructed a LMM with *Condition* (two levels: *Test* or *Control*) and *Time point* (six levels: *5*, *10*, *15*, *20*, *25*, or *30 s*) as fixed factors, with *5 s* set as the reference value for Time point. In most of the previous studies using fixed time points for pain scores during offset analgesia, ΔOA has been defined as the difference in pain intensity between a test and control condition after the first 5 s of T3 (19). However, since it has been recognized that this may not be enough time to capture the true ΔOA in all individuals (19), we considered both the 5- and 10-s pain report of T3 in the evaluation of ΔOA during post-hoc testing of any significant interaction. Since we were only interested in the differences between the Test and Control condition at each time point, we applied Dunnett's post-hoc to reduce the number of pairwise comparisons.

2.6.1.2 Conditioned pain modulation efficiency

CPM efficiency (ΔCPM) was defined as the difference in absolute pain intensity during compared to before CPT (31). To evaluate the significance of ΔCPM, a LMM with *Condition* (two levels: *Before CPT* or *During CPT*) and *Body site* (two levels: *Knee* or *Wrist*) as fixed factors was therefore constructed. Whereas a significant main effect of *Condition* would indicate a significant ΔCPM, Tukey's post-hoc was applied to further investigate any significant interaction.

2.6.1.3 Temporal summation of (second) pain effect

TSP effects (ΔTSP) were defined as the absolute differences in pain intensity between the 1st and the 10th (ΔTSP10; (50)), and between the 1st and 20th stimulus (ΔTSP20). The significance of ΔTSP was thus evaluated by constructing a LMM with *Stimulus* (three levels: *1st*, *10th*, or *20th stimulus*) and *Body site* (two levels: *Knee* or *Wrist*) as fixed factors, with the *1st stimulus* set as the reference value for the Stimulus factor. A significant main effect of *Stimulus* indicated a significant ΔTSP and similar to the evaluation of ΔOA, a significant *Stimulus* effect was further evaluated using Dunnett's post-hoc to compare the 10th and 20th stimulus to the 1st stimulus. A significant interaction was investigated further using Tukey's post-hoc.

2.6.2 Associations with offset analgesia magnitude

Spearman's partial correlation coefficients (Spearman´s rho, *ρ*) were calculated using the *pcor.test* function from the *ppcor* package to evaluate the association between ΔOA and 1) ΔCPM at the knee and wrist, 2) ΔTSP at the knee and wrist, and 3) $KOOS_{PAIN}$. The effect values of each pain test were calculated as described above, and for similar reasons as described in section 2.6.1, only participants with a complete (and valid) pain test battery were included. However, since our LMM for ΔOA showed a significant ΔOA only at 10 s of T3 ($T3_{10s}$), and not at 5 s of T3 ($T3_{5s}$; see Results), only ΔOA at $T3_{10s}$ were used in the partial correlations to reduce the number of comparisons. Holm corrections for multiple comparisons were applied across all correlational analyses using the *p.adjust* function from the *stats* package.

2.6.2.1 Partial correlation covariates

All partial correlations controlled for possible age (51) and sex (20) differences in ΔOA, as well as for body mass index (BMI) to account for possible differences in subcutaneous fat and thereby differences in electrical impedance between test regions and participants. Furthermore, since offset analgesia was tested only at the upper limb in comparison to TSP and CPM, which were assessed at both the upper and lower limb, we also controlled for knee or wrist EPT to reduce the possible bias induced by region-specific differences in peripheral sensitivity (not applicable for $KOOS_{PAIN}$). Likewise, offset analgesia was performed at the forearm of the dominant hand, whereas CPM and TSP were performed at the knee and wrist ipsilateral to the most symptomatic knee. Thus, some participants performed all EPM paradigms at the same body half (*n* = 34), whereas others received the offset analgesia paradigm contralateral to CPM and TSP (*n* = 32). We therefore also controlled for the impact of receiving offset analgesia ipsilateral or contralateral to CPM and TSP by including a binary variable for offset analgesia test site (*i.e., Ipsilateral* or *Contralateral* to CPM and TSP). Finally, a substantial number of participants (*n* = 37) did not adhere to the pre-assessment instructions to refrain from strenuous physical activity (*n* = 6), use of nicotine (*n* = 5) and analgesic drugs (*n* = 9), and intake of alcohol (*n* = 7) and caffeine (*n* = 23) in the 24 hours preceding the laboratory visit. However, since introducing too many covariates into the partial correlations reduces the degrees of freedom, as well as increases the risk of e.g., overfitting and multicollinearity, we decided to control for these pre-assessment variables in a separate exploratory analysis by including them as binary variables (*i.e.,* "*Refrained from*" or "*Not refrained from*"). These analyses did not change the direction of the results (Table S1), and we therefore only present the partial correlations without the pre-assessment variables accounted for in the following sections.

2.6.3 Handling of missing and/or invalid data

As explained in earlier sections, a portion of the study sample (*n* = 22) had missing or invalid data for some of the psychophysical tests and was therefore excluded from the effect and correlation analyses. However, since the reason for missing and/or invalid data was considered likely not at random (see

3.1), this introduces potential selection bias by potentially excluding participants with certain traits. To address this, we analysed two bivariate Stochastic Search Variable Selection (SSVS) models to assess whether self-reported demographic and pain-related factors predicted 1) non-valid completion of at least test (regardless of reason; M1), and 2) participant-initiated cessation of at least one test (excluding non-volitional cases; M2). The predictors tested included age, sex, BMI, $KOOS_{PAIN}$, CSI, PCI, PVAQ, and IPQ-B.

Briefly, SSVS is a Bayesian variable selection method that samples thousands of models to identify key predictors, here based on Marginal Inclusion Probability (MIP) (52, 53), which reflects the frequency of inclusion of predictors across the sampled models. MIP was estimated using Markov Chain Monte Carlo (MCMC) sampling, with a spike-and-slab prior, balancing predictors with no effect (spike at zero) and those with potential effects (non-zero slab). The estimation also considers a prior inclusion probability, reflecting the prior belief about a predictor's importance. Following the structure of Bainter et al. (52, 53), we used the *ssvs* function from the *SSVS* package, running 20,000 iterations with the first 5,000 as burn-in, and with a prior inclusion probability of 0.5. Predictors with a MIP ≥ 0.5 were considered meaningful (54) and further explored for model-averaged regression coefficients (β) and 95% credible intervals.

-

3 RESULTS

3.1 Participant characteristics

Twenty-two participants had incomplete or invalid data in one or more of the psychophysical tests and were therefore excluded from the effect and correlation analyses. Of these participants, five were not able to complete the offset analgesia test at their intended stimulus intensities, whereas three did not perform this test at all because of fainting (n = 2) or self-chosen study withdrawal (n = 1) during or

,following the CPM test. Another 13 participants could not complete the CPM test due to intolerable cold pain during the CPT, and one final participant was excluded after having maximized the capacity of the current stimulator (50 mA) during the EPT detection at the knee, thereby having to stop the trial before the true EPT was reached. The characteristics of all participants, categorized according to whether they could validly complete all psychophysical tests or not, are presented in Table 1, whereas the responsiveness to all psychophysical tests across the participants included in the final effect and correlation analyses is presented in Table 2.

3.2 Endogenous pain modulation observed in people with knee osteoarthritis

A LMM based on each participant's pain intensity scores during the offset analgesia test and control conditions revealed a significant *Time point* by *Condition* interaction (F[5, 454] = 19.89, $p < 0.001$; Fig 2a). Dunnett's post-hoc then showed that participants scored their pain intensity significantly higher during the test compared to the control condition at T2 (t[209] = -5.50, $p < 0.001$), and significantly lower during the test compared to the control condition at 10, 15, and 20 s within T3 ($T3_{10}$: t[209] = -3.26, $p = 0.001$; $T3_{15}$: t[209] = -2.98, $p = 0.003$; $T3_{20}$: t[209] = -3.32, $p = 0.001$), but not at 5 s within T3 (t[209] = -1.15, $p = 0.253$), indicating a significant ΔOA at $T3_{10s}$.

Separate LMMs were also constructed based on the individual pain intensity scores derived from the CPM and TSP paradigms, performed at the knee and wrist, to evaluate the EPM effect of each test. For ΔCPM, we observed a significant main effect of *Condition* (F[1, 109] = 6.35, $p = 0.002$; Fig 2b), indicating a significant ΔCPM. No significant *Condition* by *Body site* interaction was observed (F[1, 65] = 3.93, p = 0.052; Fig 2b), implying that the analgesic effect of the conditioning stimulus was not significantly dependent on the body site at which the test stimuli were delivered.

For ΔTSP, we observed a significant main effect of *Stimulus* (F[2, 107] = 39.99, $p < 0.001$; Fig 2c), for which Dunnett's post-hoc confirmed that there was a significant increase in pain intensity from the 1st to the 10th (t[65] = 7.95, $p < 0.001$) and from the 1st to the 20th stimulus (t[65] = 7.40, $p < 0.001$). This

indicates that ΔTSP was significant over the course of the 20-stimulus train. There was also a significant *Stimulus* by *Body site* interaction (F[2, 195] = 14.74, p < 0.001; Fig 2c), for which Tukey's post-hoc showed that ΔTSP (*i.e.,* the main effect of *Stimulus*) was more prominent for the knee than the wrist between the 1st and 10th stimulus (Fig 2c). Specifically, the significant difference observed between the knee and wrist at the 1st stimulus (t[89] = -4.42, p < 0.001) had diminished at the 10th (t[89] = -1.76, p = 0.495).

3.3 Offset analgesia does not correlate with conditioned pain modulation, temporal summation of (second) pain, and clinical pain severity

Partial correlation analyses (Spearman´s rho, ρ) with ΔOA as the target variable showed no significant correlations with ΔCPM or ΔTSP at neither the most symptomatic knee nor ipsilateral wrist, and no significant correlation between ΔOA and $KOOS_{PAIN}$ (Table 3).

3.4 No certain predictors of psychophysical test completion

Two bivariate SSVS models were constructed to explore if self-reported demographic and/or pain-related factors could predict 1) non-valid completion of any of the psychophysical tests (M1), and/or 2) a participant's decision to prematurely stop at least one test (M2). None of the models identified any important predictors, with the MIP of the strongest predictor (i.e., CSI score) reaching only 0.03 in both models, and with the model-averaged β for this predictor corresponding to -0.01 (95% credible interval: 0.00, 0.00) in both models (Table S2).

4 DISCUSSION

371 The primary aim of the present study was to investigate the relationship between offset analgesia
372 magnitude and the response of CPM and TSP in people with radiographic and painful KOA, while
373 controlling for the impact of relevant covariates (*i.e.,* age, sex, BMI, and test site). Although the sizes
374 of the pain modulatory effects were not defined, we observed a significant EPM effect across all
375 psychophysical tests, indicating a successful test implementation. Some of the participants found the
376 offset analgesia and/or CPM paradigm too painful to complete, but further analysis of self-reported
377 demographic and (psychosocial) pain-related factors revealed no strong predictors of test completion.
378 As hypothesized, offset analgesia magnitude was not significantly associated with neither CPM nor
379 TSP, suggesting that offset analgesia may provide information about EPM in people with KOA that is
380 not captured by the TSP and/or CPM paradigm. Likewise, no significant association between offset
381 analgesia magnitude and $KOOS_{PAIN}$ was found, and this study therefore provides additional support
382 that offset analgesia magnitude does not correlate with clinical pain severity in people with (chronic)
383 pain.

384

385 A dissociation between offset analgesia magnitude and CPM efficiency has been reported in previous
386 studies conducted in both pain-free individuals (23, 24) and people with chronic low back pain (14) and
387 migraine (22). In the present study, our findings show that this dissociation also applies to people with
388 KOA. In contrast, a significant moderate association between offset analgesia and CPM was observed
389 in an early study by Honigman et al. (20). However, this finding was based on a fairly small number of
390 participants (n = 15) and was only observed in pain-free men, and not women (20). Given that later
391 publications, including the present study, have been unable to replicate these results with larger
392 sample sizes (14, 22-24), the current evidence therefore suggests that offset analgesia and CPM are
393 independent from one another. Likewise, there is no previous observation of a significant association
394 between offset analgesia magnitude and TSP effect (14, 22, 24), and the results of this study therefore
395 provide additional support for a dissociation between these psychophysical tests. However, while

agreeing with previous findings, the present study used electrical test stimuli across all psychophysical tests, whereas previous studies evaluating the association between offset analgesia and other psychophysical tests have utilized thermal heat stimuli (14, 22-24). Notably, the present study represents the first to assess and demonstrate a significant offset analgesia magnitude in a pain population using an electrical stimulus-based protocol. However, although not investigated for offset analgesia, the responsiveness to the CPM and TSP paradigm has been found to vary depending on the type of stimulus modality used (55, 56). In line with this, the available validation study of an electrical offset analgesia protocol found "only" a moderate correlation between thermal heat-induced and electrically-induced offset analgesia, with the behaviour of pain ratings across time varying between modalities (30). The offset analgesia magnitude observed in the present study may thus not be directly comparable to the thermal-derived effects reported in previous studies. Yet, it suggests that the dissociative nature of offset analgesia with CPM and TSP applies when electrical stimuli are used too.

One possible explanation for the dissociation between offset analgesia and other EPM paradigms is that it relies on distinct EPM mechanisms. Modulation of offset analgesia magnitude has been implicated at all levels along the neuroaxis, including the peripheral receptors (51, 57-59), spinal dorsal horn (60), brainstem (61, 62), and cortex (23, 62, 63). However, in contrast to TSP and CPM, offset analgesia has not been found to respond to centrally-acting pharmacological agents (13). Whereas TSP has been attenuated by the administration of *N*-methyl-*D*-aspartate receptor antagonists (64, 65), these agents were found to have no effect on offset analgesia magnitude (66), suggesting that offset analgesia does not rely on glutamatergic transmission. Likewise, although exceptions exist (67), many studies have found CPM efficiency to be modulated by the administration of exogenous opioid agonists and antagonists (68, 69), as well as serotonin-norepinephrine reuptake inhibitors (70-72). In contrast, offset analgesia magnitude remains unaltered upon administration of either type of pharmacological agent (13), suggesting that the mechanisms of offset analgesia do not depend on endogenous opioids,

serotonin, and/or norepinephrine. The consistent dissociation observed between offset analgesia and both CPM and TSP in this and previous studies may thus reflect a reliance on different neurotransmitter systems. For example, the impact of dopamine in offset analgesia has not yet been explored, but it was recently shown by Desch et al. that administration of the dopamine precursor levodopa enhanced the pain relief induced by a small intensity reduction of a tonic painful heat stimulus (73). Possibly, the small decrease in stimulus intensity during offset analgesia may act as a reward causing dopamine release and subsequent dopaminergic nociceptive inhibition. Others have suggested that offset analgesia may rely more strongly on cortical mechanisms than other psychophysical tests (25). Offset analgesia does, for example, engage similar cortical regions as placebo analgesia (25), such as the dorsolateral prefrontal cortex (23, 62, 63), periaqueductal gray (PAG), and rostral ventromedial medulla (RVM; (61, 62)). This has led to speculations that the small decrease in stimulus intensity during offset analgesia may serve as a placebo-like cue resulting in a prediction of further intensity reductions (63, 74). Although still to be confirmed, these studies support the notion of offset analgesia potentially relying on EPM processes that are less involved in TSP and CPM.

4.1 Limitations

Although potentially relying on differences in EPM processes, the non-significant associations with offset analgesia magnitude found in our study may also be explained by that offset analgesia was applied to a different body site (volar forearm) as compared to CPM and TSP (volar wrist and medial knee) – a choice made to adhere to the only validated protocol for offset analgesia using electrical stimuli (30). Nevertheless, test site diversity may have had an impact on the strength of the relationship between offset analgesia magnitude and the responsiveness to the CPM and TSP paradigm, possibly (although not significant) explaining the somewhat stronger correlations between offset analgesia magnitude and CPM/TSP responsiveness at the wrist compared to the knee. Furthermore, because of the heavy battery of multiple psychophysical tests (as well as blood samples and

electroencephalography (26)), the participants were not familiarized with the psychophysical tests before the actual assessment, which may have inferred novelty effects interfering with the EPM effects of each test. However, although not eliminated completely, we tried to partly control for these factors by first including the EPT at the knee or wrist as a covariate across all partial correlations between psychophysical tests, aiming to account for differences in peripheral sensitivity between the test regions. Moreover, we kept the test order standardized across participants, as well as ensured that all participants were naïve to the EPM assessment, ensuring equal test conditions across all participants.

However, since we did not hypothesize to find any significant associations, we were unable to perform a valid power calculation. May and Looney recently proposed a required sample size of 47 subjects to detect an association of 0.4 with an 80% power using Spearman´s correlation coefficient (75). This suggests that the number of participants in our study would have been sufficient to detect an association of moderate size, but insufficient to detect small associations of significance. However, since the study of May and Looney concerns a healthy population, in contrast to the more complex pain population of people with KOA included in our study, it is possible that both higher and smaller sample sizes than those mentioned by the authors would have been required to achieve sufficient power.

In addition to the limitations above, the present study constitutes a secondary analysis of data from a larger clinical trial, which inherently infers stringent eligibility criteria and thereby a potentially reduced generalizability of our findings. For instance, individuals with cognitive impairments, as well as people taking stronger pain medications (e.g., opioids) were excluded. The generalisability of our findings may also be affected by the exclusion of participants having missing or invalid psychophysical test data since not missing at random. As mentioned in the beginning of the discussion, no predictors of test completion were identified. Yet, other unobserved factors, such as motivation and/or pain tolerance, may still have influenced test tolerability in certain individuals in our KOA sample. Furthermore, since we were unable to compare the EPM profile of our sample of people with KOA with that of healthy,

pain-free controls, we were unable to make any inferences about whether the psychophysical test responses observed in our study reflect normal or abnormal EPM.

5. CONCLUSION

This study evaluated the association between offset analgesia magnitude and CPM efficiency, TSP effect, and clinical pain severity ($KOOS_{PAIN}$) in people with radiographic and painful KOA. No significant associations were found, suggesting that offset analgesia magnitude may provide information about EPM in people with KOA that is not captured by the CPM and TSP paradigm, but that these processes may not directly relate to clinical pain severity.

AUTHORS CONTRIBUTIONS

D Rice, D Beckwée, D Schiphof, I Bautmans, I Coppieters, and J Nijs provided concept/idea, research design, and obtained funding; D Beckwée, I Bautmans, and J Nijs provided project management and facilities/infrastructure; E Johansson, L Leemans, and S Puts collected the data; E Johansson analysed the data and wrote the first draft of the manuscript; All authors provided critical feedback and approved the final version of the manuscript.

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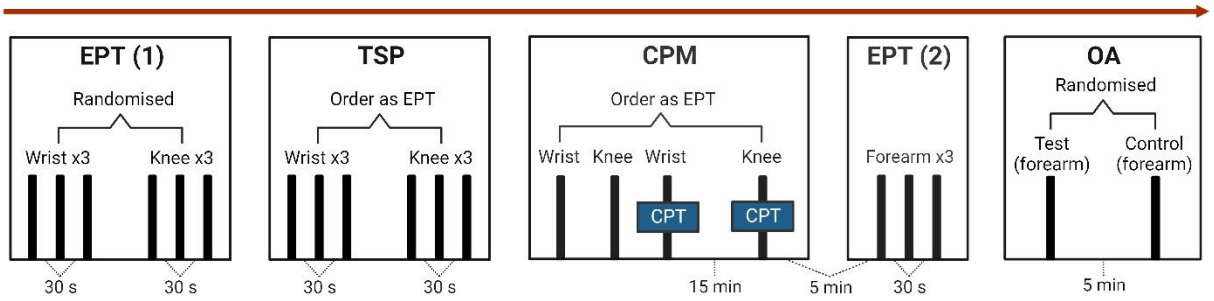

746

747 **Figure 1.** Flowchart of the endogenous pain modulation assessment procedure. *EPT = electrical pain threshold;*

748 *TSP = temporal summation of pain; CPM = conditioned pain modulation; CPT = cold pressure test; OA = offset*

749 *analgesia.*

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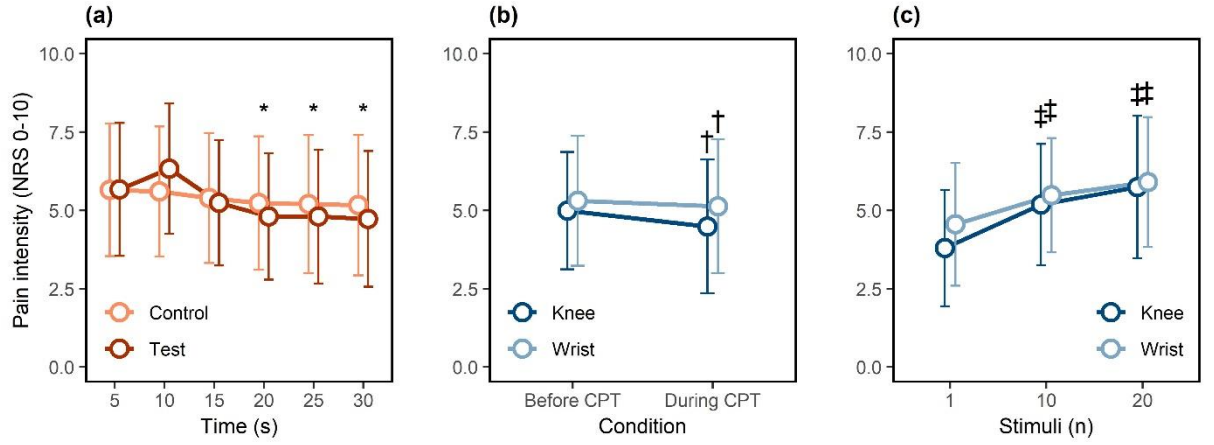

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752 **Figure 2.** Means and standard deviations of pain intensity scores within the (a) offset analgesia, (b) conditioned

753 pain modulation, and (c) temporal summation of pain paradigms. In (a), * indicates a significant difference in

754 pain intensity between the test and control condition of the offset analgesia paradigm. In (b), † indicates a

755 significant reduction in pain intensity "during" compared to "before" the cold pressor test (CPT). In (c), ‡ indicates

756 a significant increase in pain intensity compared to the 1st stimulus in a series of 20 stimuli. Significance is

757 indicated at $p < 0.05$.

758

Table 1. Characteristics of all 88 participants categorized by (valid) completion status of psychophysical test battery				
	Participants completing all psychophysical tests according to instructions (*n* = 66)		Participants unable to complete all psychophysical tests according to instructions (*n* = 22)	
	Mean ± SD	**[Min – Max]**	**Mean ± SD**	**[Min – Max]**
Age (years)	64.80 ± 8.57	[52.00 – 86.00]	66.23 ± 8.42	[54.00 – 81.00]
BMI (kg/m^2)	28.00 ± 3.88	[20.05 – 41.95]	28.99 ± 4.64	[20.82 – 38.54]
Sex (female/male)	53.03% females		15.00% females	
Race/ethnicity (white[a]/Asian)	98.48% white		100% white	
Dominant hand (right/left)	92.42% right		100% right	
Most symptomatic knee (right/left)	50.00% right		50% right	
$KOOS_{PAIN}$	58.36 ± 14.96	[25.00 – 100.00]	58.08 ± 17.06	[30.56 – 86.11]
CSI	30.33 ± 12.19	[3.00 – 63.00]	25.86 ± 9.72	[7.00 – 49.00]
PCS	16.26 ± 11.56	[0.00 – 45.00]	16.09 ± 12.50	[0.00 – 42.00]
PVAQ	30.98 ± 12.19	[4.00 – 63.00]	33.18 ± 11.06	[8.00 – 51.00]
IPQ-B	37.06 ± 8.47	[18.00 – 56.00]	35.32 ± 10.23	[15.00 – 50.00]

[a] "White" refers to European, Middle east, or North American descent.

SD = standard deviation; BMI = body mass index; $KOOS_{PAIN}$ = knee osteoarthritis outcome score, pain subscale; CSI = central sensitization inventory; PCS = pain catastrophizing scale; PVAQ = pain vigilance and awareness questionnaire; IPQ-B = brief illness perception questionnaire

759

Table 2. Psychophysical test responses across the 66 participants with complete test data		
	Mean ± SD	**[Min – Max]**
EPT (mA)		
Medial knee[a]	9.57 ± 5.15	[2.67 – 25.50]
Volar wrist (n. medianus)[a]	4.95 ± 2.93	[1.50 – 16.00]
Volar forearm[b]	8.68 ± 4.09	[3.50 – 20.17]
ΔTSP10 (NRS difference[c])		
Medial knee[a]	1.38 ± 1.36	[-0.33 – 4.67]
Volar wrist (n. medianus)[a]	0.93 ± 1.13	[-1.00 – 4.67]
ΔTSP20 (NRS difference[d])		
Medial knee[a]	1.94 ± 1.99	[-1.00 – 7.00]
Volar wrist (n. medianus)[a]	1.35 ± 1.79	[-1.00 – 6.33]
ΔCPM (NRS difference[e])		
Medial knee[a]	-0.51 ± 1.24	[-4.00 – 3.00]
Volar wrist (n. medianus)[a]	-0.17 ± 1.34	[-5.00 – 3.00]
ΔOA – Volar forearm		
$T3_{5s}$ (NRS difference[f])	-0.15 ± 1.18	[-3.00 – 2.00]
$T3_{10s}$ (NRS difference[g])	-4.43 ± 1.05	[-4.00 – 1.50]

[a] Performed ipsilateral to the most symptomatic knee.
[b] Performed ipsilateral to the dominant hand.
[c] Difference in pain intensity from the 1st to the 10th stimulus in a series of 20 stimuli.
[d] Difference in pain intensity from the 1st to the 20th stimulus in a series of 20 stimuli.
[e] Difference in pain intensity "during" compared to "before" the cold pressor test.
[f] Difference in average pain intensity following the first 5 seconds of T3 between the offset analgesia test and control conditions.
[g] Difference in average pain intensity following the first 5 seconds of T3 between the offset analgesia test and control conditions.

EPT = electrical pain threshold; ΔTSP = temporal summation of pain effect; NRS = numeric rating scale (pain intensity; 0–10); ΔCPM = conditioned pain modulation efficiency; ΔOA = offset analgesia magnitude

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Table 3. Nonsignificant bivariate partial correlations (Spearman´s rho, ρ) between ΔOA and ΔCPM, ΔTSP, and clinical pain severity ($KOOS_{PAIN}$), controlling for the impact of age, sex, BMI, knee/wrist EPT, and test location.

	ΔOA[b]	Unadjusted *p*
ΔCPM_{KNEE}[a]	ρ = -0.02	*p* = 0.905
ΔCPM_{WRIST}[a]	ρ = 0.11	*p* = 0.415
$\Delta TSP10_{KNEE}$[a]	ρ = 0.05	*p* = 0.695
$\Delta TSP20_{KNEE}$[a]	ρ = 0.04	*p* = 0.739
$\Delta TSP10_{WRIST}$[a]	ρ = 0.21	*p* = 0.101
$\Delta TSP20_{WRIST}$[a]	ρ = 0.21	*p* = 0.112
$KOOS_{PAIN}$[c]	ρ = -0.01	*p* = 0.943

[a] Performed ipsilateral to the most symptomatic knee.
[b] Performed ipsilateral to the dominant hand.
[c] Only controlled for the impact of age, sex, and BMI.

ΔOA = offset analgesia magnitude; ΔCPM = conditioned pain modulation efficiency; ΔTSP = temporal summation of pain effect; EPT = electrical pain threshold; $KOOS_{PAIN}$ *= knee injury and osteoarthritis outcome score, pain subscale; BMI = body mass index.*