

1 **Title page**

2 1) **Title:** Psychophysical or spinal reflex measures when assessing conditioned pain modulation?
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4 2) **Running head:** Comparing CPM effects with different test-stimuli
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6 3) **Authors:** M. U. Lie^{1,2}, E. Petriu^{3,4}, D. Matre⁵, P. Hansson^{6,7}, O. K. Andersen⁸, J. A. Zwart^{1,2,4}, K.
7 B. Nilsen^{1,4,9}
8

9 4) **Affiliations:**

- 10 1. Research and Communication Unit for Musculoskeletal Health (FORMI), Oslo University
11 Hospital, Oslo, Norway
- 12 2. Faculty of Medicine, University of Oslo, Oslo, Norway
- 13 3. Faculty of Health Science, Oslo Metropolitan University, Oslo, Norway
- 14 4. Department of Neurology, Oslo University Hospital, Oslo, Norway
- 15 5. Department of Work Psychology and Physiology, National Institute of Occupational
16 Health, Oslo, Norway
- 17 6. Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm,
18 Sweden
- 19 7. Department of Pain Management and Research, Division of Emergencies and Critical
20 Care, Oslo University Hospital, Oslo, Norway
- 21 8. Center for Neuroplasticity and Pain (CNAP), SMI®, Department of Health Science and
22 Technology, Aalborg University, Aalborg, Denmark
- 23 9. Department of Neuromedicine and Movement Science, Norwegian University of Science
24 and Technology, Trondheim, Norway
25

26 5) **Corresponding author:**

27 Marie Udneseter Lie
28 FORMI, Oslo University Hospital
29 Pb. 4956 Nydalen
30 0424 Oslo
31 Norway
32 Tel: +47 22117768
33 E-mail: maulie@ous-hf.no
34

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42

43 9) **Significant statement:** The large difference in CPM effect between the two protocols suggests
44 that the CPM effect relates to pain perception rather than nociception on the spinal level. Due to
45 poor absolute intra-rater reliability, we recommend caution and further research before using any
46 of the investigated CPM protocols in clinical decision making on an individual level.
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1 **Introduction**

2 Assessment of endogenous pain modulatory function may carry a potential for stratification of
3 treatment and follow-up of pain patients. One such measure is conditioned pain modulation (CPM),
4 which assesses an individual's inherent ability to alter the central nervous system processing set up by
5 a nociceptive stimulus (termed test-stimulus) in the presence of another nociceptive stimulus (termed
6 conditioning stimulus) (Yarnitsky et al., 2010). CPM has been shown to be altered in several chronic
7 pain conditions (Lewis et al., 2012) and deficits may predict development of post-operative pain
8 (Wilder-Smith et al., 2010; Yarnitsky et al., 2008) and treatment response (Nahman-Averbuch, Dayan,
9 et al., 2016; Yarnitsky et al., 2012). There is, however, a large variation in applied CPM methodology,
10 which limits the generalization of conclusions for application in daily clinical practice (Pud et al.,
11 2009). Thus, there is a need for standardized and reliable methods to measure CPM (Yarnitsky et al.,
12 2015).

13 CPM is usually assessed with psychophysical outcome measures, i.e., pain intensity ratings of
14 supra-threshold stimuli or pain threshold assessment (Kennedy et al., 2016; Pud et al., 2009), clearly
15 involving subjective interpretation of the stimulus-induced percept. A systematic review suggests that
16 CPM is a reliable measure, but the degree of reliability heavily depends on methodology (Kennedy et
17 al., 2016). In a previous study, we reported large variability when using a protocol involving a thermal
18 test-stimulus (Lie et al., 2017). Assessing CPM with standardized spinal reflex measures such as the
19 nociceptive withdrawal reflex (NWR) elicited by electrical stimulations, may potentially be more
20 reliable since such a measure may be less influenced by cognitive processes than psychophysical
21 measures (Sandrini et al., 2005). One must however keep in mind that the withdrawal to the electrical
22 stimulus is a reflex and not dependent of pain perception. Although not a painful outcome measure, it
23 is commonly used as test-stimulus in CPM studies (Pud et al., 2009). The reliability of neuronal
24 activity induced by an electrical test-stimulus has been investigated (Biurrun Manresa et al., 2014;
25 Jurth et al., 2014), but not compared with more commonly used psychophysical stimuli such as
26 thermal test-stimuli. Therefore, the aim of the present study was to compare the CPM effect and test-
27 retest reliability between a CPM protocol using a thermal test-stimulus and a psychophysical outcome
28 with a CPM protocol with an electrical test-stimulus and a spinal reflex outcome.

29 **Methods**

30 **2.1 Study design**

31 This was an experimental crossover study comparing two CPM protocols with different test-stimuli
32 (thermal vs. electrical) and different outcome measures (psychophysical vs. spinal reflex) but the same
33 conditioning stimulus. A pretest was performed to familiarize subjects with the stimulations and pain
34 intensity rating procedures, before the baseline test-stimulus was applied according to the protocol.
35 After a 5-minute break, the test-stimulus in parallel with a conditioning stimulus was applied. A 30-
36 minute break followed to eliminate carryover effects before the other protocol was carried out with the
37 same procedure contralaterally (Fig. 1). The experiment was repeated with a minimum interval of 7
38 days. The second session was identical to the first session in regards to randomization.

39 A computerized block-randomization for the order of protocol and the test side was conducted
40 prior to the experiments. The subjects were informed of the testing procedure, but were not told
41 whether the conditioning stimulus would influence the test-stimulus and were thus blinded for the
42 study hypothesis. Subjects were also blinded for readouts from the stimulation instruments. A female
43 experimenter (E.P) carried out all experiments. Instructions, placement of instruments, room
44 temperature (21°C–23°C), and the experimenter's clothes were standardized.

45 A written informed consent was obtained prior to participation. The study was approved by the
46 Regional committee for medical and health research ethics (project no. 2010/2927) and conducted in
47 accordance with the Declaration of Helsinki. Subjects received a gift certificate of NOK 500 for
48 participation.

1 2.2 Subjects

2 Men and women self-reported to be healthy and aged 18-45 years were recruited by advertisement at
3 local hospitals and colleges/universities in Oslo, Norway. Exclusion criteria were: chronic pain,
4 somatic or psychiatric disease, headache for more than two days a month, hypertension (>140/90,
5 assessed prior to the experiment after a 5 minute rest), pregnancy (self-reported), breastfeeding,
6 acquaintance with the experimenter and regular use of medication (including non-prescription pain
7 killers) except oral contraceptives. Subjects were requested not to work nightshifts 48 hours before the
8 experiment, not to consume alcohol or pain killers 24 hours before the experiment, or caffeine or
9 tobacco the last hour before the experiment.

10 A priori power analysis based on previous studies from our laboratory (Lie et al., 2017; Nilsen et
11 al., 2014) showed that 20 subjects were needed to detect a 10% difference in the absolute CPM effect
12 in a paired Student t-test between the two protocols with a standard deviation of 1.5 cm on a 10 cm
13 visual analog scale (VAS, left end: 'no pain', right end: 'worst pain imaginable'), assuming a two-
14 sided significance level of 5% and 80% power.

15 1.3 Test-protocol

16 2.3.1 Psychophysical outcome

17 Test-stimulus was contact heat stimulation induced by a 30×30 mm Peltier thermode (Medoc, Ramat
18 Yishai, Israel) (baseline temperature: 32°C, increase rate: 2°C/second, decrease rate: 8°C/second)
19 applied on the proximal volar aspect of the forearm with a constant temperature for 120 seconds (Fig.
20 2a). The subjects continuously rated the pain intensity of the test-stimulus on a computerized 10 cm
21 horizontal VAS by scrolling the wheel on a computer mouse. The stimulation site of the test-stimulus
22 alone and the test-stimulus in parallel with the conditioning stimulus was not overlapping to avoid
23 sensitization or habituation. The temperature of the test-stimulus was aimed to reflect pain intensity
24 equal to approximately 6 cm on 10 cm VAS. In order to find this temperature the following procedure
25 was followed: An average of three tests of heat pain tolerance threshold tested with the methods of
26 limits (baseline: 32°C, increase rate: 1°C/second) minus 2°C was calculated. The estimated
27 temperature was tested with a 30 seconds heat stimulus positioned on the volar aspect of the opposite
28 forearm. If the first 20 seconds was rated outside 4-9/10 cm VAS the temperature was adjusted
29 accordingly.

30 2.3.2 Spinal reflex outcome

31 Subjects were lying at a medical plinth with the back rest inclined 135 degrees relative to the
32 horizontal level, and a pillow under the knees assuring knee flexion of 45 degrees. At stimulation sites
33 existing hair was removed and the skin was lightly abraded and cleaned with sterilizing alcohol.

34 Electrocutaneous stimulation was applied through surface Ag/AgCl-electrode (30x22 mm,
35 type Neuroline 720, Ambu A/S Denmark) placed on the medial aspect on the arch of the foot, and a
36 large surface electrode (5x10 cm, Axelgaard, USA) placed on the dorsum of the foot just proximal to
37 the toes (Fig. 2b). This ensured that the stimulus was perceived in the arch of the foot. The electrodes
38 were repositioned if subjects felt radiating sensation into the toes or on the dorsum of the foot.
39 Recording electrodes were placed on the ipsilateral tibialis anterior muscle by three surface Ag/AgCl-
40 electrodes (30x22 mm, type Neuroline 720, Ambu A/S Denmark, one reference electrode) with an
41 inter-electrode distance of 2 cm. The skin was cleaned and abraded again if high impedance (>5kOhm)
42 occurred.

43 Trains of five 1 ms rectangular pulses (felt as a single stimulus) was delivered at 200 Hz with
44 a 4 ms interpulse interval with Dolosys Paintracker (Dolosys GmbH, Berlin, Germany). Spinal
45 reflexes measures may be difficult to standardize in clinical settings, and we wanted to use a
46 commercial device which could be easy to implement in forthcoming clinical studies. Dolosys
47 Paintracker is a commercial device which is easy to transport and set up and is therefore beneficial to
48 use as a bedside-/point-of-care-test compared to other devices currently used to induce and measure

1 electrical stimuli. The device is specifically developed to determine reflex thresholds continuously
2 over a longer period of time.
3 The intensity of the electrical stimulus was the current needed to evoke a reflex threshold with inter-
4 stimulus intervals randomized between 8-12 seconds to minimize stimulus predictability. The
5 amplitude of the electromyographic (EMG) reflex responses to the electrical stimulations was
6 converted to a peak z-score defined as the baseline-adjusted maximum divided by standard deviation
7 of the EMG amplitudes before stimulation. The NWR threshold was defined as a peak z-score of ≥ 12
8 in the post-stimulus interval of 70-150 ms (France et al., 2009). Electrical stimulations started at 1mA
9 and increased with a rate of 0.5 or 1mA until threshold was detected (minimum 8 values were needed
10 for threshold calculation. After threshold detection, repeated stimulations were given for 120 seconds,
11 resulting in a total of 10 electrical stimulations due to the inter-stimulus interval. Each stimulus was
12 adjusted to be as close to a peak z-score of 12, e.g. if the previous stimulus elicited a large response,
13 the intensity of the next stimulus was decreased. If this resulted in a threshold below a peak z-score of
14 12, the next stimulus was increased. Values of the 3 previous stimulations were used to determine if
15 the intensity changed by 0.5mA or 1.0 mA, which ensures precise threshold determination with the
16 smallest possible steps (Instructions for use Paintracker, Dolosys GmbH). Subjects were told to relax
17 their leg as much as possible, and were reminded to relax if muscle contractions in the leg (high EMG
18 noise) were present between stimulations.

19 The overall level of unpleasantness and pain intensity of the electrical stimulations were rated
20 verbally on a 0-10 numerical rating scale (NRS) (0 = 'no pain' / 'no unpleasantness', 10 = 'worst pain
21 imaginable' / 'worst unpleasantness imaginable') after the test-stimulus was terminated.

22 **2.4 Conditioning stimulus**

23 A 7°C circulating water bath (LAUDA Alpha RA8, LAUDA-Brinkman LP., USA) was used as
24 conditioning stimulus in both protocols at the hand contralateral to the test-stimulus side (Fig. 2c).
25 With water up to the wrist, the hand was held wide open and steady for 120 seconds or until the pain
26 forced the subject to withdraw the hand from the water bath. After 120 seconds, subjects were asked to
27 rate the overall pain intensity of the conditioning stimulus on a 0-10 NRS (0 = 'no pain', 10 = 'worst
28 pain imaginable').

29 **2.5 Data analysis**

30 The CPM effect was defined as the difference in average pain intensity or NWR threshold between the
31 test-stimulus alone and the test-stimulus in parallel with the conditioning stimulus. The CPM effect
32 was also calculated as a percent change (CPM effect/test-stimulus alone x 100). The percent change in
33 CPM effect was used when comparing the two protocols due to different parameters used to calculate
34 the CPM effect. Additionally, subjects were categorized as CPM responders or non-responders.
35 Subjects with decreased pain ratings during conditioning stimulus were defined as CPM responders in
36 the protocol with the thermal test-stimulus, while subjects with increased reflex threshold were defined
37 as CPM responders in the protocol with the electrical test-stimulus.

38 Statistical analyses were conducted using SPSS Statistics v. 21 (IBM, Armonk, NY). Findings
39 with P-values ≤ 0.05 were regarded as significant. The distribution of data was assessed in preliminary
40 analyses by a Shapiro–Wilk test and inspection of descriptive statistics, histograms, boxplots, and Q-Q
41 plots. These analyses did not indicate any extreme values or distributions that would affect the planned
42 parametric analysis.

43 To determine whether a CPM effect was present, pain ratings or NWR threshold during the
44 test-stimulus alone were compared with pain ratings or NWR thresholds during the test-stimulus in
45 parallel with conditioning stimulus in paired sample Student t tests. Differences in CPM effect
46 between the two protocols were estimated with repeated-measures analysis of variance (RM
47 ANOVA), with session (levels: first session vs second session) and protocol (levels: thermal protocol
48 vs electrical protocol) as factors.

49 Intraclass correlation coefficients with a 2-way random-effect model (ICC_{2,1}) and absolute
50 agreement definition for single measures were used to assess relative reliability (0.4: poor reliability;
51 0.4–0.59: fair reliability; 0.6–0.75: good reliability; 0.75: excellent reliability (Shrout et al., 1979).

1 Bland-Altman plot and its related limits of agreement were used to assess the absolute reliability. Bias
2 was calculated as the mean difference between the two sessions by subtracting the mean CPM effect in
3 the first session from the second session, and then evaluated with a 1-sample Student's T test. 95%
4 limits of agreement was calculated as mean difference \pm 1.96 x SDdiff (SDdiff = SD of the mean
5 difference).

6 **Results**

7 Twenty-eight subjects were included in the study. One subject did not participate in the second session
8 for unknown reasons. One subject was excluded when previous participation in a similar study was
9 revealed and one subject was excluded due to missing data because of technical issues. Thus, a total of
10 25 (11 females) were included in the analysis. Sample characteristics are presented in table 1.

11 **3.1 CPM effect**

12 The mean CPM effect for the thermal protocol was -2.2 cm, representing a -46.0% decrease between
13 pain ratings during test-stimulus alone and pain ratings during test-stimulus in parallel with the
14 conditioning stimulus ($p < 0.001$) (Fig. 3a). The mean CPM effect for the electrical protocol was 0.4
15 mA, representing a 4.5% increase between the NWR threshold during test-stimulus alone and NWR
16 threshold during test-stimulus in parallel with conditioning stimulus ($p = 0.216$) (Fig. 3b). The
17 difference in CPM effect between the two protocols was significant ($p < 0.001$) (Fig. 4) with a partial
18 η^2 effect size of 0.7. No significant differences in CPM effect was found between sessions ($p = 0.618$),
19 and no interactions between sessions and protocols ($p = 0.949$). Post hoc analysis (RM ANOVA
20 adjusted for changes in thresholds) showed, in contrast to the NWR thresholds, a significant CPM
21 effect when using pain ratings (-32.5% decrease, $p = 0.002$, partial η^2 effect size 0.4) or
22 unpleasantness ratings (-26.1% decrease, $p < 0.001$, partial η^2 effect size 0.5) of the electrical test-
23 stimulus, comparing ratings during test-stimulus alone with ratings during test-stimulus in parallel
24 with the conditioning stimulus. A mean baseline noise of 0.6 μ V was found with no significant
25 difference between test-stimulus alone and during test-stimulus in parallel with conditioning stimulus,
26 indicating low baseline muscle activity in both conditions.

27 **3.2 Reliability**

28 Detailed reliability values are shown in Table 2 and 3. The ICC values of the CPM effect in both
29 protocols were in the 0.40 – 0.59 range, which suggests fair relative reliability. Regarding absolute
30 reliability, no bias was observed as there was no significant difference in mean difference between
31 sessions in the protocol with thermal test-stimulus ($p = 0.631$) or the protocol with electrical test-
32 stimulus ($p = 0.616$). Large limits of agreement were observed for the CPM effect in both protocols,
33 which indicates large intra-individual differences between sessions (Fig. 5).

34 **Discussion**

35 Our data showed significantly larger CPM effect using a protocol with a psychophysical outcome from
36 using a thermal test-stimulus compared to a spinal reflex outcome using an electrical test-stimulus,
37 where the latter protocol failed to detect a CPM effect. Fair relative reliability was observed for the
38 CPM effect in both protocols. The absolute reliability indices in both protocols displayed good
39 agreement in the mean CPM effect between the two sessions. However, high intra-individual
40 variability was observed for both protocols.

41 **4.1 CPM effect**

42 The large difference in CPM effect between the two protocols (41.5 %) indicate that the perceptual
43 pain experience from a thermal test-stimulus is more prone to modulation during the conditioning
44 stimulation than the nociceptive withdrawal reflex assessed by an EMG response to an electrical test-
45 stimulus. This is somewhat consistent with previous studies. Studies using 120 seconds heat test-

1 stimulus report a CPM effect between -29 – -47% (Lie et al., 2017; Matre et al., 2016; Nilsen et al.,
2 2014; Potvin et al., 2008; Tousignant-Laflamme et al., 2008), while studies using electrical test-
3 stimulus giving rise to a NWR, report a CPM effect between 11.5 – 40% (Biurrun Manresa et al.,
4 2014; Bouhassira et al., 2003; Jurth et al., 2014; Sandrini et al., 2006). The somewhat higher CPM
5 effect in other studies using an electrical test-stimulus in comparison to the result of the present study
6 may be due to different testing sites. The reflex in the present study was elicited from the plantar
7 surface of the foot and the response was measured from the anterior tibial muscle. The comparable
8 studies stimulated the sural nerve trunk and recorded from the biceps femoris muscle. It is argued that
9 sural nerve stimulation often is found intolerable resulting in a large number of failed tests, and that
10 the currently employed set-up is less dependent on exact electrode positioning and demonstrates better
11 test-retest reliability than sural nerve stimulation (Bouhassira et al., 2003; Jensen et al., 2015). Another
12 difference, which could contribute to differences found in the CPM effect between the present study
13 and the comparable studies, is that they did not track the reflex threshold over a longer period of time
14 (120 seconds).

15 In addition to a larger CPM effect, a larger proportion of CPM responders were observed
16 using the protocol with thermal test-stimulus compared to the protocol with electrical test-stimulus. A
17 possible explanation for lower CPM effect and fewer CPM responders when using electrical test-
18 stimulus compared to thermal test-stimulus could be related to differences in the intensity of the test-
19 stimulus between the two protocols in regards to pain intensity, pain quality, and the duration of the
20 stimulus. The NWR threshold has been reported to be correlated with the subjective pain threshold
21 (Sandrini et al., 2005). If this is the case, it is possible that a floor effect for the CPM effect for the
22 electrical test-stimulus is present. The thermal test-stimulus was aimed to reflect a pain intensity of
23 6/10 on a VAS to prevent floor- or ceiling effects. One could argue that a supra-threshold, e.g., a
24 NWR threshold x 1.5 instead of the NWR threshold may have resulted in a larger CPM effect in the
25 protocol with electrical test-stimulus and also have more methodological similarity to the protocol
26 with thermal test-stimulus. However, earlier studies have suggested that the NWR threshold is
27 sufficient to detect a change in test-stimulus evoked by the conditioning stimulus CPM effect and
28 importantly, is more reliable than supra-threshold stimulation (Biurrun Manresa et al., 2014; Jurth et
29 al., 2014). The NWR is commonly considered a proxy for nociception, due to its longer latency and
30 higher threshold than the tactile reflex which first appears after an electrical stimulation (Willer,
31 1977). Still, it is still a possibility that the motor response may be contaminated by innocuous
32 somatosensory processes, such as startle reactions and voluntary movements (although we attempted
33 to reduce such influence by familiarization during pre-tests) or modulated by other types of
34 descending control, e.g. emotions (Rhudy et al., 2008) or attention/distraction (Bjerre et al., 2011).

35 The difference between the outcomes of the protocols may also be a result of different sites of
36 stimulation, which can give rise to activity in different pain modulatory pathways. Two upper limbs
37 are used in the protocol with the thermal test-stimulus, which may possibly reflect a segmental spinal
38 inhibitory effect (although not necessarily limited to that). A combination of a lower limb and an
39 upper limb is used in the protocol with electric test-stimulus, which may be more influenced by an
40 ascending-descending modulatory activity. However, a recent study (Graven-Nielsen et al. (2017) did
41 not find any differences in CPM effect between upper and lower limb stimulation sites when using the
42 same test-stimulus at different locations.

43 The large difference in CPM effect between the two protocols in our study raises questions as
44 to the mechanisms of CPM. Larger CPM effect when the pain percept component is evaluated
45 compared to when reflex processes are measured, suggests that CPM depends more on
46 cognitive/evaluative aspects of the pain percept than on nociception. This theory is supported by our
47 post-hoc analysis where a significant CPM effect was observed when using pain ratings (32.5%) or
48 unpleasantness ratings (26.1%) of the electrical test-stimulus. This result is in contrast to the
49 traditional theory of a more limited neural system interaction, i.e., diffuse noxious inhibitory controls
50 based on animal research, which is considered to rest on a spinal-supraspinal-spinal feedback loop.
51 However, CPM in humans has shown to be highly influenced by supraspinal processes (Nahman-
52 Averbuch, Nir, et al., 2016). Whether the modulation of pain perception found in the present study is
53 influenced by previous pain experiences, expectations, mood, attention or other modulatory influences
54 from the central nervous system have not been embraced in the present study protocol and needs to be
55 addressed in future research.

1 A 7° cold water bath was chosen to induce pain ratings close to tolerance to ensure maximal
2 CPM effect for all subjects, since conditioning stimulus with temperatures inducing higher pain
3 intensity have shown to increase the CPM effect compared to temperatures inducing lower pain
4 intensity or non-painful temperatures (Granot et al., 2008; Tousignant-Laflamme et al., 2008; Willer et
5 al., 1989). However, it is desirable that the temperature and duration is tolerable enough to complete
6 the conditioning stimulus according to protocol.

7 The two protocols have many methodological differences that may affect the CPM effect and
8 make comparison of the outcome of the two protocols difficult. First, the two protocols differ with
9 respect to stimulation parameters such as type of stimulus, duration, stimulus intensity as well as pain
10 intensity. Secondly, when increasing electrical stimulation intensity from 0, there is a range where
11 stimulation is perceived as non-noxious. This means that the scales properties are not directly
12 comparable. When the CPM effect is reported as a percent change for both methods, this may enhance
13 the difference when comparing the CPM effect of the two protocols. However, both thermal and
14 electrical protocols are commonly used to assess CPM and although it is difficult to find measures that
15 are 100% comparable, the comparison of different protocols is important to find a golden standard
16 protocol for CPM assessment.

17 **4.2 Test-retest reliability**

18 Fair relative reliability was found in both protocols. In other studies using thermal test-stimuli, ICC
19 values between 0.21 – 0.62 have been found (Gehling et al., 2016; Granovsky et al., 2015; Imai et al.,
20 2016; Valencia et al., 2013; Wilson et al., 2013). A recent systematic review concludes that
21 differences in reliability heavily depend on stimulation parameters. However, in the present study, the
22 protocol with thermal test-stimulus was identical to a protocol used in a previous study conducted at
23 our laboratory (Lie et al., 2017) which reported good relative reliability (ICC value 0.60). The
24 difference in ICC values between our present (0.40) and our previous study highlights the variation in
25 results despite identical protocols. It also emphasizes the limitations of ICC values as a measure of
26 test-retest reliability. ICC strongly depends on the sample's heterogeneity; ICC values are lower in a
27 homogenous group than in a heterogenous group although the difference in the outcome between
28 sessions are the same in both groups (Atkinson et al., 1998). High ICC values will also occur when
29 subjects maintain their position in the sample across repeated measurements, even though the
30 measurement (i.e., CPM effect) may have changed from session to session. Using ICC alone may lead
31 to false conclusions regarding repeatability and it is therefore recommended to also include measures
32 of absolute reliability in test-retest reliability studies (Atkinson et al., 1998; de Vet et al., 2011;
33 Kennedy et al., 2016). The relative reliability observed in studies using electrical test-stimulus is also
34 conflicting; values between 0.26 (Biurrin Manresa et al., 2014) and 0.61 (Jurth et al., 2014) have been
35 reported. A possible explanation for the poor reliability in our study may be different placement of the
36 electrodes from session to session, even though we tried to prevent this by standardized localization of
37 the stimulation sites. In addition, the two sessions were not conducted at the same time during the day.
38 Time of the day may to a minor degree influence the CPM effect (Aviram et al., 2015).

39 In both protocols, the bias between sessions was close to zero, suggesting absence of learning
40 effects etc. However, large intra-individual variability was observed in both protocols, which indicate
41 that neither of the protocols evokes a reliable CPM effect in healthy adults on an individual level.
42 When it comes to comparing which of the two methods that is most reliable, the different outcome
43 measured challenge the interpretation of the analysis. The level of absolute reliability depends solely
44 on what is acceptable for practical use (Lexell et al., 2005). Considering the average CPM effect of 0.4
45 mA using the electrical test-stimulus, the wide range of limits of agreement (-3.4 – 3.8 mA) seems to
46 constitute a genuine reliability problem. Levels of minimal detectable change when using NRS or
47 VAS at 1-2 NRS points or 1-2 cm VAS is often considered acceptable. In the present, study 7 subjects
48 (28%) showed a CPM difference between sessions of more than 2 cm on the VAS when using the
49 thermal test-stimulus. Considering such a high proportion of subjects with high variability between
50 tests, the implementation of CPM tests employed in the present study is of limited value in clinical
51 practice for stratification or prognostic purposes. However, whether CPM is a fluctuating parameter in
52 healthy controls and a more stable parameter in patients suffering from pain conditions, should be

1 addressed by future research before dismissing the applicability of the testing paradigm in clinical
2 decision making.

3 **Conclusion**

4 The present study demonstrated a variable but fairly pronounced inhibitory CPM effect when the
5 outcome measure is a psychophysical assessment of a thermal test-stimulus. Employing a spinal
6 reflex outcome set up by a point-of-care device with electrical test-stimulus failed to demonstrate a
7 CPM effect. Put together these results raise questions about the mechanisms involved in CPM testing.
8 Fair relative reliability was observed for the CPM effect in both protocols, and poor absolute reliability
9 was found in both protocols due to high intra-individual variability. One should be cautious to
10 extrapolate the results from healthy adults to patients, and the large variability observed in our study
11 calls for extended research in the clinical population before finally concluding on the applicability of
12 CPM methodology in clinical decision making on an individual level.

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16 **Author contributions**

17 All authors contributed to the conception and design or analysis and interpretation of data as well as
18 making intellectual contributions to the manuscript's content. All authors discussed the results and
19 commented on the manuscript.

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