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## USER-INDEPENDENT ASSESSMENT OF CONDITIONING PAIN MODULATION BY CUFF PRESSURE ALGOMETRY

--Manuscript Draft--

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<b>Abstract:</b>	<p><b>Background:</b> The use of conditioning pain modulation (CPM) is hampered by poor reproducibility and lack of user-independent paradigms. This study refined the CPM paradigm by applying user-independent cuff algometry.</p> <p><b>Methods:</b> In 20 subjects, the CPM-effect of conditioning with cuff-stimulation on the arm was investigated by pain test-stimuli on the contralateral leg before and in parallel with different cuff conditionings (10, 30, 60kPa/60s; 30, 60kPa/10s). As test-stimulus, another cuff was inflated (1kPa/s) until the subjects detected the pain tolerance threshold (PTT) during which the pain detection threshold (PDT) and the pressure at a pain intensity of 6 on a 10cm visual analogue scale (PVAS6) were extracted. For comparison, pressure pain thresholds (PPTs) as test-stimuli were recorded by the user-dependent handheld pressure algometry. Combinations of cuff locations for conditioning (pain intensity standardised) and contralateral test-stimuli were additionally evaluated (leg-arm, leg-leg, arm-thigh). The test-retest reliability in two sessions 1 month apart was assessed in five CPM protocols.</p> <p><b>Results:</b> In all protocols the PDT, PVAS6, and PTT increased during conditioning compared with baseline (<math>P&lt;0.05</math>). The CPM-effect (i.e. conditioning minus baseline) for PVAS6, PTT, and PPT increased for increasing conditioning intensities (<math>P&lt;0.05</math>). The CPM-effects were not significantly different for changes in conditioning durations or conditioning/test-stimulus locations. In two sessions, the CPM-effects for PVAS6 and PTT assessed after 60s of conditioning on the leg/thigh showed the highest intra-class correlations (0.47-0.73) where they were 0.04-0.6 for PPTs.</p> <p><b>Conclusions:</b> The user-independent cuff algometry is reliable for CPM assessment and for supra-pain threshold test-stimuli better than the user-dependent technology.</p>

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**Background:** The use of conditioning pain modulation (CPM) is hampered by poor reproducibility and lack of user-independent paradigms. This study refined the CPM paradigm by applying user-independent cuff algometry.

**Methods:** In 20 subjects, the CPM-effect of conditioning with cuff-stimulation on the arm was investigated by pain test-stimuli on the contralateral leg before and in parallel with different cuff conditionings (10, 30, 60kPa/60s; 30, 60kPa/10s). As test-stimulus, another cuff was inflated (1kPa/s) until the subjects detected the pain tolerance threshold (PTT) during which the pain detection threshold (PDT) and the pressure at a pain intensity of 6 on a 10cm visual analogue scale (PVAS6) were extracted. For comparison, pressure pain thresholds (PPTs) as test-stimuli were recorded by the user-dependent handheld pressure algometry. Combinations of cuff locations for conditioning (pain intensity standardised) and contralateral test-stimuli were additionally evaluated (leg-arm, leg-leg, arm-thigh). The test-retest reliability in two sessions 1 month apart was assessed in five CPM protocols.

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# **USER-INDEPENDENT ASSESSMENT OF CONDITIONING PAIN MODULATION BY CUFF PRESSURE ALGOMETRY**

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**Conflict of interest:** Nocitech is partly owned by Aalborg University.

## **What's already known about this topic?**

- Conditioned pain modulation (CPM) is the difference in the response to a painful test-stimulus applied before and during painful conditioning stimulation.
- The clinical use of CPM is hampered by poor reproducibility and lack of user-independent paradigms.

## **What does this study add?**

- A user-independent CPM technique where the conditioning is controlled by one cuff stimulation, and the test-stimulus is provided by another cuff stimulation.
- This study shows that cuff algometry is reliable for CPM assessment.

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## **ABSTRACT**

**Background:** The use of conditioning pain modulation (CPM) is hampered by poor reproducibility and lack of user-independent paradigms. This study refined the CPM paradigm by applying user-independent cuff algometry.

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**Conclusions:** The user-independent cuff algometry is reliable for CPM assessment and for supra-pain threshold test-stimuli better than the user-dependent technology.

**Keywords:** Conditioning pain modulation, cuff algometry, reliability.

## **1. INTRODUCTION**

The descending control of the pain system seems implicated in chronic pain conditions, contributing to the development of widespread hyperalgesia and progression of pain. The phenomenon where a localised tonic nociceptive stimulus modulates acute nociception from extrasegmental sites was originally termed “diffuse noxious inhibitory control (DNIC)” (Le Bars et al. 1979). Conditioned pain modulation (CPM) is the psychophysical protocol exploring DNIC-like effects in humans and is the difference in the response to a painful test-stimulus applied before and during or immediately after painful conditioning stimulation (Yarnitsky et al. 2010). A meta-analysis including 30 studies (778 patients, 664 control subjects) demonstrated less potent CPM in chronic pain patients although most studies lacked assessor blinding, which is a major risk of bias in the assessment (Lewis et al. 2012b) in particular with user-dependent CPM methodologies. The CPM reliability has been reported with mixed results from excellent to good (Cathcart et al. 2009; Arendt-Nielsen et al. 2012) and poor (Wilson et al. 2013). Furthermore, a volunteer could be a CPM responder in one session and a non-responder in another session (Oono et al. 2011a) suggesting a need to improve the reliability.

Despite standardisation has been recommended (Yarnitsky et al. 2015), multiple modalities for conditioning and test-stimuli have been used including cold, heat, electrical, mechanical, chemical, and ischemic stimuli applied to various body regions (Lewis et al. 2012b; Pud et al. 2009). Although not individualised, the cold pressor stimulus seems the most reliable for conditioning (Lewis et al. 2012a). The CPM response detected at a moderate conditioning pain intensity did not increase further by increasing the conditioning intensity (Nir et al. 2011). The conditioning with moderate pain intensity induced, however, significantly higher CPM effects compared with lower conditioning intensities (Oono et al. 2011b). Moreover, conditioning with a moderately painful heat stimulation for 12 minutes

decreased the heat, but not pressure pain sensitivity compared with the pain sensitivity after 6 minutes of conditioning (Razavi et al. 2014).

Pain detection threshold, pain tolerance, pain intensity in response to a supra-pain threshold stimulus, and neurophysiological evaluations have been used as test-stimuli (Biurrun Manresa et al. 2014; Yarnitsky 2015) and often as mechanical modalities (Lewis et al. 2012b) such as pressure (Vaegter et al. 2014) and cuff algometry (Graven-Nielsen et al. 2015). Studies investigating the effect of spatial location of test and conditioning stimuli are few although the general recommendation is to use extrasegmental or contralateral sites (Yarnitsky 2015) such as the upper arm and lower leg (Yarnitsky et al. 2015).

This study explored a novel paradigm for a user-independent CPM technique based on cuff algometry where the conditioning duration, intensity, and location is controlled by a cuff stimulation, and the test-stimulus is provided by another cuff stimulation. It was explored: 1) how the cuff conditioning intensity, duration, and location were associated with the size of the CPM response detected by cuff and pressure algometry, and: 2) if good to excellent test-retest reliability over one month could be obtained with the user-independent cuff algometry in contrast to the user-dependent pressure algometry.

## **2. METHODS**

### *2.1 Subjects*

Based on previously published CPM effects evaluated by cuff algometry (Vaegter et al. 2014) inclusion of at least 16 subjects were estimated (expected effect size of approximately 5 kPa, a power of 0.8, and significance level of 0.05). Twenty healthy pain free subjects (10 females) with no history of musculoskeletal or neurological problems participated (age:  $30 \pm 5$  years, mean  $\pm$  standard deviation). The subjects were recruited by advertisement at the local university, were given detailed written and verbal explanation of the experimental procedures, and signed an informed consent. The study was conducted in accordance with the Helsinki



Declaration and was approved by the local Ethics Committee (N2012-0078).

## *2.2 Experimental procedures*

This experiment included two sessions separated by 1 month. The second session was identical to the first session although a reduced set of measures was obtained. All assessments were performed by the same examiner with subjects lying on a bench in supine position. Experimental tonic pain was induced in the arm or lower leg by cuff-induced pain (conditioning stimulus). At baseline and during the conditioning stimulus, test stimulation with cuff pressure algometry was used to assess the pain sensitivity on the contralateral lower leg, arm, or thigh (Fig. 1). In addition, manual pressure algometry was used as a comparison with a user-dependent methodology. Eight conditioning protocols were evaluated based on different conditioning intensities, durations, and locations. The conditioning stimulus was released immediately after all test-stimuli were completed so in all protocols, the CPM effects were assessed in parallel with the conditioning. The time from the end of each conditioning protocol until the next baseline assessment was 15 minutes to minimize possible carry-over effects of the conditioning stimulus (Reinert et al. 2000). Five minutes were kept between the baseline test stimulation and the conditioning stimulus. Subjects were allocated to a test sequence randomized among three blocks of protocols (intensity, duration, location). Furthermore, allocation of the side for assessment and conditioning were randomized for each subject. The same sequence was used for the follow-up session.

## *2.3 Cuff algometer*

The computer-controlled cuff algometer (NociTech, Denmark, and Aalborg University, Denmark) controlled independently the inflation of two separate cuffs; a 7.5 cm and a 13 cm wide tourniquet (silicone high-pressure cuff, VBM Medizintechnik GmbH, Sulz, Germany). The 13 cm wide cuff had an equal-sized proximal and distal chamber. Further, the system

includes a computer-controlled air compressor, a handheld button to release the inflation, and a 10 cm electronic visual analogue scale (VAS; Aalborg University, Denmark) which signal was sampled at 10 Hz. Zero and 10 cm on the electronic VAS were defined as “no pain” and “maximal pain”, respectively. The cuffs were connected to the compressor and wrapped around the lower leg (at the level of the largest circumference of gastrocnemius muscle), arm (at the level of the largest circumference of biceps muscle), and/or thigh (as proximal as possible, just below inguinal crease). The location of the cuff was marked on the skin to help using same position within the same day (marks from day 1 were not visible on the second test day).

#### *2.4 Cuff test stimulation*

The proximal chamber of the 13 cm wide cuff was inflated with a rate of 1 kPa/s, and the maximal pressure limit was set to 100 kPa. The subjects scored continuously the pressure-induced pain intensity on the electronic VAS. The subjects were instructed to rate the pain intensity continuously on the electronic VAS from the time where the pressure was perceived as pain and to press the hand-held button when the pain was intolerable (pressure pain tolerance: PTT) which released the inflation. In case the subjects did not stop the stimulation before reaching the maximum of 100 kPa, the PTT was defined as 100 kPa. The pressure at VAS equal to 1 cm was defined as the pain detection threshold (PDT) (Graven-Nielsen et al. 2015). The pressure value when the VAS score reached 6 cm was extracted (PVAS6) as a supra-pain threshold measure. Three cuff inflations were done at baseline, and two repetitions were used during conditioning stimulus. Averages of the extracted parameters for the test stimuli at baseline and during conditioning, respectively, were used for analysis.

#### *2.5 Handheld pressure test stimulation*

A handheld algometer (Somedic Sales AB, Sweden) mounted with a 1-cm<sup>2</sup> probe (covered by

a disposable latex sheath) was used to record the pressure pain threshold (PPT) as another test stimulation at the lower leg (m. tibialis anterior; 1 cm distal to the lower rim of the cuff), arm (m. extensor carpi radialis longus; 5 cm distal to the lateral epicondyle of the humerus), or thigh (m. vastus lateralis; 1 cm distal to the lower rim of the cuff) on the assessment side. The manual pressure was increased gradually at a rate of 30 kPa/s until the pain threshold was reached, and the subject pressed a button. The PPT was defined to the subject as “the time point, at which the pressure sensation changed into pain”. Three PPT assessments were completed at baseline and two during conditioning; the averages for test stimuli at baseline and during conditioning, respectively, were used for statistical analysis. An interval of minimum 20 s was kept between each PPT assessment.

## *2.6 Conditioning by cuff stimulation*

For conditioning stimulus, the 7.5-cm-wide cuff was wrapped around the arm or lower leg at the level of the largest circumference of the biceps or gastrocnemius muscle. The computer-controlled cuff algometer (see section 2.3) maintained a constant pressure according to the conditioning protocols (Fig. 1). Cuff pain test-stimuli were applied on the contralateral limb after 10 or 60 seconds with cuff conditioning, and pressure pain test-stimuli were subsequently evaluated during the same conditioning stimulus. The conditioning stimulus was released after the last test-stimuli assessment. After releasing the conditioning cuff, the participants were asked to indicate their conditioning pain intensity using a 10 cm VAS where 0 was “no pain”, and 10 cm was “maximal pain”. The CPM-effect was assessed as the difference in the response between baseline and conditioning test stimuli calculated for cuff PDT, PVAS6, PTT, and manual PPT.

*Effects of conditioning intensity:* The cuff used for test stimuli was wrapped around the lower leg, and the conditioning cuff was applied to the contralateral arm. The cuff and pressure test-stimuli were assessed before and after 60 seconds with conditioning cuff

inflation at the intensity of 10 kPa (protocol 1), 30 kPa (protocol 2), or 60 kPa (protocol 3), respectively.

*Effects of conditioning duration:* These conditioning protocols included test stimuli before and after 10 seconds with conditioning cuff inflation at the intensity of 30 kPa (protocol 4) and 60 kPa (protocol 5). Only the first test-stimulus after 10 seconds with conditioning was included to evaluate pure contributions of the conditioning duration to the CPM effect. These CPM effects were then compared with the same conditionings intensities with duration of 60 s (protocols 2 and 3).

*Effects of location for conditioning and test:* The pairs of the conditioning versus test locations were: Arm-leg (protocol 3), leg-arm (protocol 6), leg-leg (protocol 7), and arm-thigh (protocol 8). The cuff and pressure test stimuli were assessed before and after 60 seconds with conditioning cuff inflation. At baseline, the conditioning stimulus was adjusted to a pain intensity equalling 7 cm on the VAS in protocols 6-8. As a control measure, the subjects scored the obtained pain intensity on a VAS after releasing the conditioning cuff stimulation.

*Test-retest reliability:* The CPM protocols in the second session included a repetition of protocols 3, 5, 6, 7, and 8.

## *2.7 Statistics*

All parameters passed the Kolmogorov-Smirnov test for normality and were presented as mean and standard deviation (SD) or standard error of the mean (SEM) for figures.

Systematic differences in baseline PDT, PVAS6, PTT and PPT across *sequential testing* and *sessions* (day 1 and 2) were analysed with a repeated measure analysis of variance (ANOVA) with factors test number and sessions (data presented in Supporting material, ResultsS1). The PDT, PVAS6, PTT, and PPT were analysed with a 2-way analysis of covariance (ANCOVA) with factors *time* (before and during conditioning) and *protocols* as repeated measure and *sex* (male, female) as covariate (data presented in Supporting material, ResultsS1). The CPM-

responses (i.e. conditioning minus baseline) of the 8 protocols in the first session were analysed for PDT, PVAS6, PTT, and PPT in ANCOVAs with repeated factors of conditioning intensity (*10 kPa, 30 kPa, 60 kPa*), conditioning duration (*10 s, 60 s*), or pairs of conditioning-assessment cuff location (*arm-leg, leg-arm, leg-leg, arm-thigh*) and *sex* as covariate. In the analysis, where inclusion of the two sessions was relevant, a session factor (*session 1 and 2*) was added to the above ANOVAs. Bonferroni tests were used for post-hoc comparisons incorporating correction for multiple comparisons when the ANOVA showed significant factors or interactions. The coefficients of variation (SD/mean) for each parameter and protocol were extracted.

For test-retest reliability assessment, two-way mixed average measures intra-class correlation coefficient (ICC(3,k)) and intra-individual coefficient of variation (CV) were performed between the sessions. According to Fleiss (1986), the ICC values above 0.75 generally mean “excellent” reliability, 0.40–0.75 was fair to good reliability, and less than 0.40 defined poor reliability. The intra-session CV was calculated as  $100 \cdot (\sqrt{\sum d^2 / 2n}) / \chi$  in which  $d$  is the difference between two results obtained from one subject,  $n$  is the number of subjects, and  $\chi$  is the mean of the results obtained from all the subjects. Bland-Altman methods were further used for the analysis of reliability.  $P < 0.05$  was considered significant.

### **3. RESULTS**

Across all protocols and sessions, 97% of the possible PDT and PTT recordings was obtained at baseline, and 96% was achieved during the conditioning. At baseline and conditioning, respectively, 94% and 92% of the possible PVAS6 recordings were obtained. In all baseline and conditioning sessions 3% and 4% of recordings, respectively, reached the maximum stimulation intensity (100 kPa) before subjects identified the PVAS6. For PTT the same frequencies were 1% and 11%. Among all possible PPTs 98% was collected and included in

the analysis.

### *3.1 Effects of conditioning intensity (Session 1: protocol 1, 2, 3)*

The VAS scores of the conditioning arm pain in protocol 1 (10 kPa / 60 s conditioning), 2 (30 kPa / 60 s), and 3 (60 kPa / 60 s) were  $3.2 \pm 2.3$  cm,  $5.6 \pm 1.9$  cm, and  $8.3 \pm 1.4$  cm, respectively, and all significantly different (ANOVA:  $F[2,38] = 134.5$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ).

A one-way ANOVA showed that the CPM-effect for PVAS6 and PPT, respectively, was highest for 60 kPa conditioning and different among all three conditioning intensities (Fig. 2C; ANCOVA:  $F[2,32-34] = 18.11$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.05$ ). Increased CPM-effect for handheld PPT was shown for 60 kPa and 30 kPa conditioning compared with 10 kPa conditioning (Fig. 2D; ANCOVA:  $F[2,36] = 12.50$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.01$ ).

### *3.2 Effects of conditioning duration (Session 1: protocol 2 & 4; Session 1 & 2: protocol 3 & 5)*

The VAS scores of the conditioning arm pain in protocol 4 (30 kPa / 10 s conditioning) and 5 (60 kPa / 10 s) were  $5.6 \pm 1.5$  cm and  $7.9 \pm 1.3$  cm, respectively, and not significantly different from the comparable VAS scores with the same conditioning intensities applied for 60 s (protocols 2 and 3, ANOVA:  $F[1,19] < 3.04$ ,  $P > 0.1$ ).

*Conditioning with 30 kPa in 60 s or 10 s (Session 1: protocols 2 and 4):* An increased CPM-effect for the PDT was found with 60 s conditioning compared with 10 s conditioning (Fig. S3B; ANCOVA:  $F[1,16] = 5.18$ ,  $P < 0.04$ , Bonferroni:  $P < 0.04$ ).

*Conditioning with 60 kPa in 60 s or 10 s (Session 1 and 2: protocols 3 and 5):* For the CPM-effect based on the PDT, an interaction between session, protocol and gender (Fig. S3B; ANCOVA:  $F[1,16] = 6.36$ ,  $P < 0.02$ ) was found showing that males in session 2 had higher CPM-effect for 10 s compared with 60 s conditioning (Bonferroni:  $P < 0.04$ ).

The CPM effects for PVAS6 and PTT were not significantly affected by conditioning time.

### *3.3 Cuff location protocol (Session 1&2: protocols 3, 6, 7, 8)*

The conditioning intensity was adjusted to evoke a VAS score around 7 cm for protocols 6-8. The conditioning intensity was  $36.1 \pm 14.6$  kPa in protocol 6 and 7 (leg) and  $52.2 \pm 14.9$  kPa in protocol 8 (arm) and significantly different (ANOVA:  $F[1,19] = 33.42$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ). In protocol 3 the conditioning stimulation on the arm was higher (60 kPa) with a resulting VAS score of  $8.3 \pm 1.4$  cm.

The CPM effect was not different across protocols for any parameter (Fig. S4C). However, in session 1 the CPM effect for the PDT was higher in males compared with females (ANCOVA:  $F[1,45] = 7.83$ ,  $P < 0.01$ ; Bonferroni:  $P < 0.002$ ). No difference was found in the CPM-effect of PPTs comparing protocols 3, 6, 7, and 8 (Fig. S4D; ANOVA:  $F[3,51] = 0.58$ ,  $P > 0.63$ ).

### *3.4 Variation and efficacy of CPM protocols*

Relating the coefficient of variation (CV) with the percentages CPM-effect for all parameters and protocols illustrates the performance of CPM protocols (Fig. 5). Across all protocols, the median CPM-effect was 22%, 18%, 13%, and 18% for PDT, PVAS6, PTT, and PPT, respectively, and the median coefficients of variation were 1.54, 0.91, 0.91, and 1.11. In protocols 3, 5, 7, and 8, the cuff algometry parameters (PDT, PVAS6, PTT) provide a cluster of test-stimuli with comparably low variation ( $CV < 1.75$ ) and highest CPM-effect ( $> 14\%$ ). The same cluster includes all protocols for handheld pressure algometry (PPT) except for protocol 1.

### *3.5 Test-retest reliability (Session 1&2: protocols 3, 5, 6, 7, 8)*

For cuff algometry test stimulations, the CPM-effect in PDT, PVAS6, and PTT (Table S1), consistently showed high ICCs (with lowest 95% CI) for protocols 7 (leg conditioning/ leg test-stimulus) and 8 (arm/thigh). In protocol 3 (arm/leg), supra-threshold parameters (PTT, PVAS6) showed fair to good ICCs and likewise for PTT in protocol 6 (leg/arm). Non-acceptable ICCs were observed for the CPM-effect in protocol 3 (PDT) and protocol 6 (PDT and PVAS6). The CPM-effect in PPTs presented with good ICC except in protocol 8. Intra-CV values were acceptable, ranging from 25% to 47% where the best was found for PTT and PPT in all protocols. The results from the Bland and Altman analysis showed reasonable agreement for all CPM-difference parameters.

The test-retest analysis for test-stimuli measured at baseline (Table S2) and during conditioning (Table S3) demonstrated mainly excellent ICCs and low intra-CV like the Bland/Altman analysis illustrating fine agreement.

## **4. DISCUSSION**

The present study showed that the user-independent cuff algometry is reliable and can be used as test stimulus to assess CPM-effects evoked by cuff conditioning stimulations. When using the supra-pain thresholds cuff stimulations as test-stimuli, the CPM-effect was positively associated with the conditioning stimulus intensities. The CPM-effect was reliably detected for most protocols although conditioning for 60 s on the arm or leg and test stimulation on the leg provided the best intra-class coefficients (0.47-0.73) and intra-session coefficient (<30 %) of variations.

### *4.1 Effects of conditioning intensity and time*

Using the cold pressor stimulus as conditioning, the test stimuli by cuff algometry and handheld pressure algometry have previously demonstrated CPM-effects (Graven-Nielsen et



al. 2015;Graven-Nielsen et al. 2012). A median CPM increase was found to 29% across all assessment modalities reported in more than 30 studies (Pud et al. 2009). In the present study, the median CPM-effect was between 13% and 22% depending on the test stimulus parameter and conditioning intensity.

Studies relating the conditioning pain intensities to the CPM-effects have revealed mixed results where one study did not demonstrate any relation, and another found a positive relation (Nir et al. 2011;Granot et al. 2008). Using conditioning in the present study with different cuff intensities on the arm, the moderate to high pain intensities were found to evoke the most expressed CPM-effect with test stimuli applied on the leg. The supra-pain threshold test-stimuli (PVAS6 and PTT) efficiently differentiated all three intensity conditionings. This is partly in line with findings by Nir et al. (2011) reporting that a low heat pain conditioning (12 out of 100 on a numerical pain rating scale) did not evoke a significant CPM effect whereas conditioning with mild and moderate pain intensities (32 and 58 out of 100, respectively) caused a CPM-effect, but not different. The three conditioning intensities in the present study were in general higher with mild (3.2 cm VAS), moderate (5.6 cm), and high (8.3 cm) conditioning intensities, and these higher levels may explain the ability to differentiate the CPM effects in the different conditions by cuff algometry. Similar findings were reported by Oono et al. (2011b) where a mechanical conditioning intensity on 5 cm VAS induced a higher CPM-effect compared with conditioning intensities at 3 cm, 1 cm, and 0 on the VAS. In the present study, the handheld pressure algometry as test-stimulus did not show a CPM effect for the mild conditioning intensity, which may be due to the variation introduced by the examiner. However, for the moderate and high conditioning intensities, the CPM-effects between the two intensities were detected with pressure algometry although not different, and this is likely explained with the better discrimination by supra-pain threshold test stimulations as also found for cuff algometry. Based on current data, the conditioning stimulus should obviously be standardized as much as possible.

Applying the cuff conditioning stimulus for 10 s versus 60 s before testing did not change the CPM-effect systematically for supra-pain threshold test-stimuli (PVAS6 and PTT). In accord with the present data, a previous study showed that the CPM responses based on pressure as test stimuli were not affected by different conditioning durations of 6 and 12 minutes (Razavi et al. 2014). Only the first test stimulus (and not repeated twice as in the other protocols) was included for the short conditioning duration to make sure evaluating the immediate effects. Thus, it cannot be excluded that the variability of cuff test stimuli increased when not repeating the measures twice as per standard. This may also explain the variable findings where the pain threshold test stimuli showed better CPM effects for 60 s versus 10 s conditioning with 30 kPa, whereas the opposite effect was found for 60 kPa conditioning in males in the second but not first session. Nonetheless, cuff algometry assessment also with one trial was recently found reliable (Graven-Nielsen et al. 2015). The conditioning stimulus was maintained until end of the test stimulus (threshold determination) and therefore the total duration of the conditioning stimulus was of variable length (i.e. 10 s or 60 s plus the time needed for threshold assessment). Since there was no systematic difference in the CPM effect based on supra-pain thresholds between protocols with 10 s or 60 s conditioning before the test stimulus, it is not likely that the variation in conditioning duration have significantly affected the current results. The design with conditioning and test stimuli in parallel may include distraction as a component of the CPM effect. Distraction by visual cognitive tasks applied during CPM assessment in a parallel design provided an additional effect to the CPM effect (Moont et al. 2010) which may argue that CPM is independent of distraction although this issue is open for future clarification.

#### *4.2 Location for conditioning and assessment*

The previous recommendation on locations for condition and assessment has been limited to remote sites such as separate limbs (Yarnitsky 2015). In the present study, the contralateral

test sites on homotopic and distant limbs were explored although a potential involvement of segmental mechanisms when using homotopic test sites cannot be excluded. For studies assessing e.g. disease laterality, the CPM protocols including both conditioning and test-sites on one side should be considered (Granovsky et al. 2013). Importantly, all combinations of conditioning and test sites (arm, thigh, leg) were found to provide increased algometry parameters (PVAS6 and PTT) during conditioning compared with baseline.

Comparing the test stimulation on the leg/thigh with conditioning on the contralateral arm (protocols 3 and 8) or lower leg (protocol 7) did not result in significantly different CPM-effect. Only protocol 6 included cuff test stimulation on the arm where the PDT and PVAS6 algometry parameters were in general higher compared with the other protocols in line with a recent study (Graven-Nielsen et al. 2015). This also explains why relatively few subjects in this protocol reached the VAS score at 6 cm before reaching the maximum stimulation intensity and as such also why the CPM-effect for the PTT seemed non-significantly reduced in protocol 6 because the PTT was conservatively assumed to be 100 kPa (see methods). The maximum stimulation intensity in the cuff algometry system is a limitation to the generalisation of the current PTT findings. Nonetheless, the general CPM effects detected with the PTT were also found for the PVAS6 where less subjects reach the maximum stimulation intensity and where the missing data was not assumed to be 100 kPa. In line with another study (Graven-Nielsen et al. 2015), the handheld pressure algometry on the arm demonstrated higher pain sensitivity than on the lower extremities which is opposite to the lower cuff pain sensitivity on the arm.

#### *4.3 Reliability of CPM*

Interestingly, the PDT was less stable to demonstrate the CPM effect and influenced by sex differences when evaluating the increase during conditioning compared with baseline.

Generally, the PDT in males showed similar effects as the supra-pain threshold test stimuli for

both females and females. Such variability may also explain the moderate test-retest findings for the CPM effects assessed by the PDT (especially for protocol 6). Although the same CPM protocol was not replicated within one session in the present study, no systematic changes in test stimuli were detected during sequential baseline assessment. When evaluating the CPM-effect with supra-pain threshold cuff test stimulation, the reliability is challenged although fair to good ICC (0.53-0.75) and acceptable intra-session CVs (< 30%) were found for the best protocols (leg/leg, arm/thigh). For handheld pressure algometry as test stimulation, better intra-session CVs, but lower ICCs for the CPM-effect were found compared with cuff algometry. Comparable with the present findings, a previous study reported ICCs of 0.57-0.69 for the CPM-effect in two sessions separated by 1 hour with the cuff pain stimulation as conditioning and manual pressure stimulation as test-stimuli (Cathcart et al. 2009).

Assessing the coefficient of variation and CPM effect for each test stimulus parameter in all protocols, it was evident that especially the short duration conditioning at a medium intensity (protocol 4) or a longer conditioning at a low intensity level (protocol 1) did not perform as well as the other protocols indicated as higher coefficient of variation and low CPM-effect (Fig. 5). Therefore, robust conditioning stimulations (e.g. 60 s and moderately painful) are warranted for successful CPM protocols.

The intra-session variability was higher in protocol 6 during baseline (Table S2) and conditioning (Table S3) test stimulations for the handheld pressure algometry with good ICC values whereas the ICCs for the test stimuli on the lower limbs during baseline and conditioning were excellent. The ICCs for all cuff test stimulation parameters in protocols 7 and 8 were highest compared with the other protocols indicating that the combination of leg conditioning and leg testing or arm conditioning and testing on the thigh provides the best locations among those tested in this study. This concurs with another study reporting the leg as the site for the largest CPM response when applying the painful conditioning to upper extremities (Oono et al. 2011a).

In general, mixed reliability of the CPM-effect has been reported. Heat pain conditioning and heat test stimulations provided ICCs between 0.61-0.82 for the CPM-effect in two sessions with the highest ICCs for males (Valencia et al. 2013). In contrast, for the pressure algometry as test and cold pressor conditioning, an overall ICC of 0.59 was reported, but substantially lower (0.33) in men compared with women (Martel et al. 2013). An even higher ICC of 0.8 was found for the CPM effect on pressure tolerance levels in cold pressor conditioning (Arendt-Nielsen et al. 2012) in line with the present findings with better reliability for supra-pain threshold test measures. Interestingly, the CPM-effect did not vary across phases in the menstrual cycle, but the ICC was modest (0.39) for the CPM-effect evaluated by heat conditioning and heat testing (Wilson et al. 2013). Conditioning with hot water and testing ratings to electrical stimulation and the nociceptive withdrawal reflexes in two sessions provided ICCs of 0.54-0.61 for the CPM-effect (Jurth et al. 2014) and similar ICCs of 0.26-0.44 for cold pressor conditioning (Biurrun Manresa et al. 2014). Cold pressor, but not ischemic exercise as conditioning provided fair-good ICC for the CPM effect based on pressure test stimuli (Lewis et al. 2012a). The generalisation of the present test-retest findings may be limited by factors such as sample variation (sex, age, healthy/patient), menstrual cycle, and randomization of protocol sequence making it difficult to repeat the data collection. The methodological advantages of a user-independent procedure for CPM assessment together with a comparable or better reliability compared with handheld pressure algometry as test stimulation indicate that cuff algometry is appropriate for future CPM studies. Nonetheless, larger clinical studies designed to address important covariates are needed to validate the best protocols from the current study to recommend the golden standard for CPM assessment.

#### *4.4 Conclusion*

This study demonstrated that painful cuff stimulations can be used as conditioning stimulus to

evoke the CPM response with test stimulations by cuff or handheld pressure algometry. The cuff algometry approach for CPM assessment was found robust between different sessions and sensitive to different conditioning intensities especially for supra-pain threshold test stimuli on the leg with conditioning on the arm or leg. The user-independent cuff algometry is reliable for CPM assessment and for supra-pain threshold test stimulation generally better than the user-dependent handheld pressure algometry. The use of a user-independent technology for CPM assessment may improve future experimental and clinical studies as the assessor blinding requirement becomes less influential.

### **Acknowledgement**

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### **Author contributions**

All authors contributed to the conception and design of the study as well as making intellectual contributions to its content. MI, KKP, and TGN contributed to the analysis and interpretation of the data and drafting of the manuscript. All authors discussed the results, commented on and approved the final manuscript.

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## FIGURE LEGENDS

**Figure 1.** Experimental set-up. **(A)** Time course of the conditioning and test stimulation in the CPM protocol. Cuff pressure test stimulation was applied twice on the contralateral limb after 10 s or 60 s with the conditioning cuff inflation, and pressure pain thresholds were consecutively evaluated during the same conditioning stimulus except for protocols 4 and 5 (test stimulus after 10 s) where only 1 cuff test stimulation was included. At baseline, the test stimuli were applied in the same way although without conditioning stimulation. **(B)** Cuff location and conditioning parameters. Intensity of the conditioning stimulus and the conditioning duration before algometry included in the 8 different protocols (P1-P8) are illustrated. The effect of conditioning intensity (P1-P3), duration of conditioning (P2-P4), and location of conditioning and test (P3, P6, P7, and P8) were evaluated.

**Figure 2.** Mean ( $\pm$  SEM, N = 18 - 20) pressure pain test stimulus parameters assessed on the lower leg at baseline and during conditioning are illustrated **(A, B)** together with the CPM-effect (test stimulus conditioning minus baseline) **(C, D)** at three different cuff conditioning intensities (10, 30, 60 kPa; protocols 1-3) delivered to the arm. The pain detection thresholds (PDT), pressure levels where the subjects indicate visual analogue scale scores of 6 cm (PVAS6), and the pressure pain tolerance (PTT) levels were recorded by cuff algometry, and the pressure pain thresholds (PPT) were recorded with handheld pressure algometry. Significantly different compared with baseline values (#,  $P < 0.05$ ; †,  $P < 0.05$  for males only) and significantly different compared with the other conditioning intensities (\*, NK:  $P < 0.05$ ) or the 10 kPa conditioning intensity (α, NK:  $P < 0.05$ ).

**Figure S3.** *Please see supporting figures.*

**Figure S4.** *Please see supporting figures.*

**Figure 5.** Scatter plot relating the coefficient of variation (SD/mean) with the mean CPM-effect (in percentages of baseline test stimulus measure) for all parameters and protocols. The pain detection thresholds (PDT), pressure levels where subjects indicate visual analogue scale scores of 6 cm (PVAS6), and pressure pain tolerance (PTT) levels were recorded by cuff algometry, and the pressure pain thresholds (PPT) were recorded with handheld pressure algometry. The protocol number (1 to 8) is indicated for each parameter. For protocols 3, 5, 6-8 the data is based on pooled data from both sessions.

Figure 1

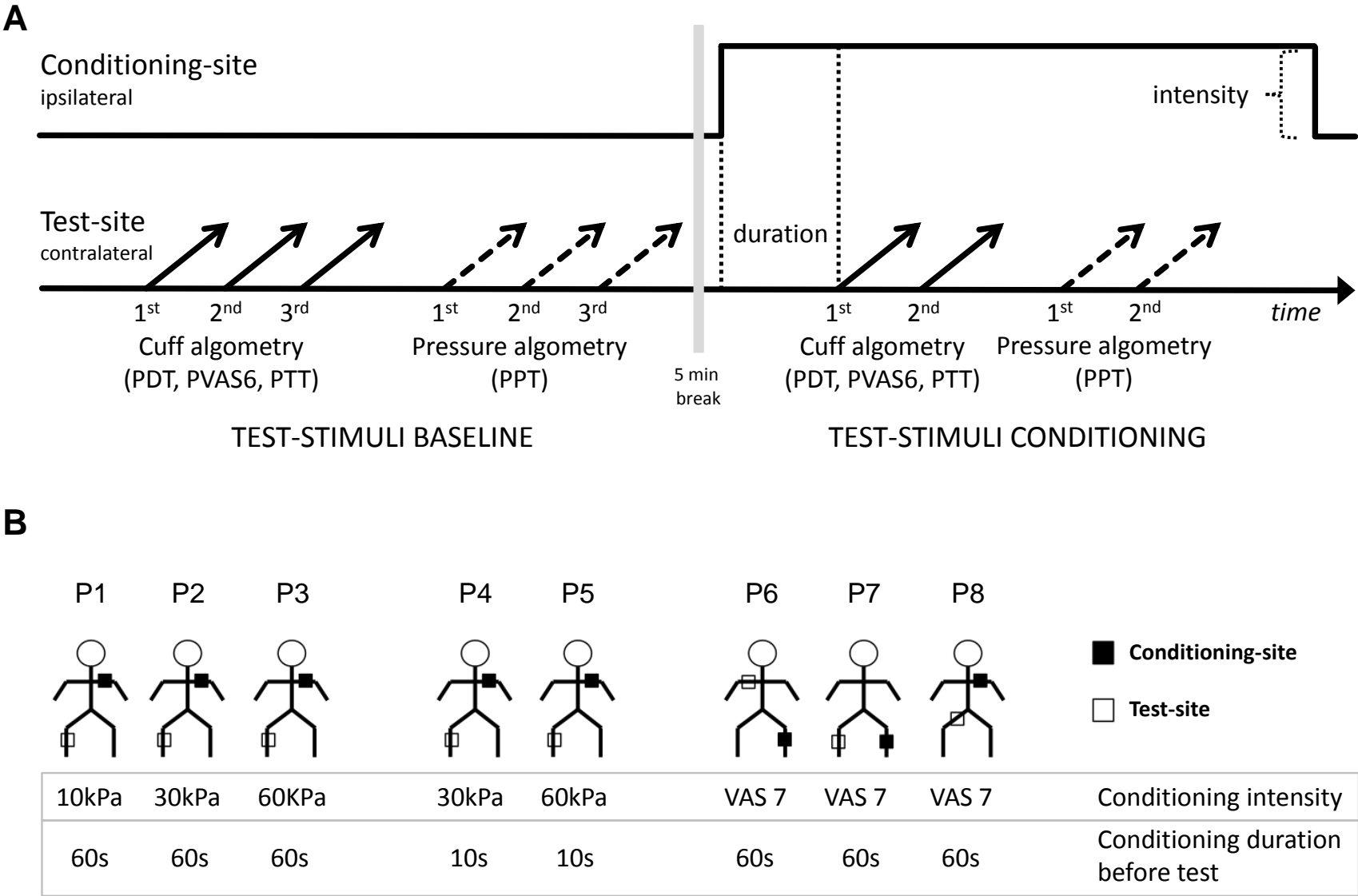


Fig. 1

Figure 2

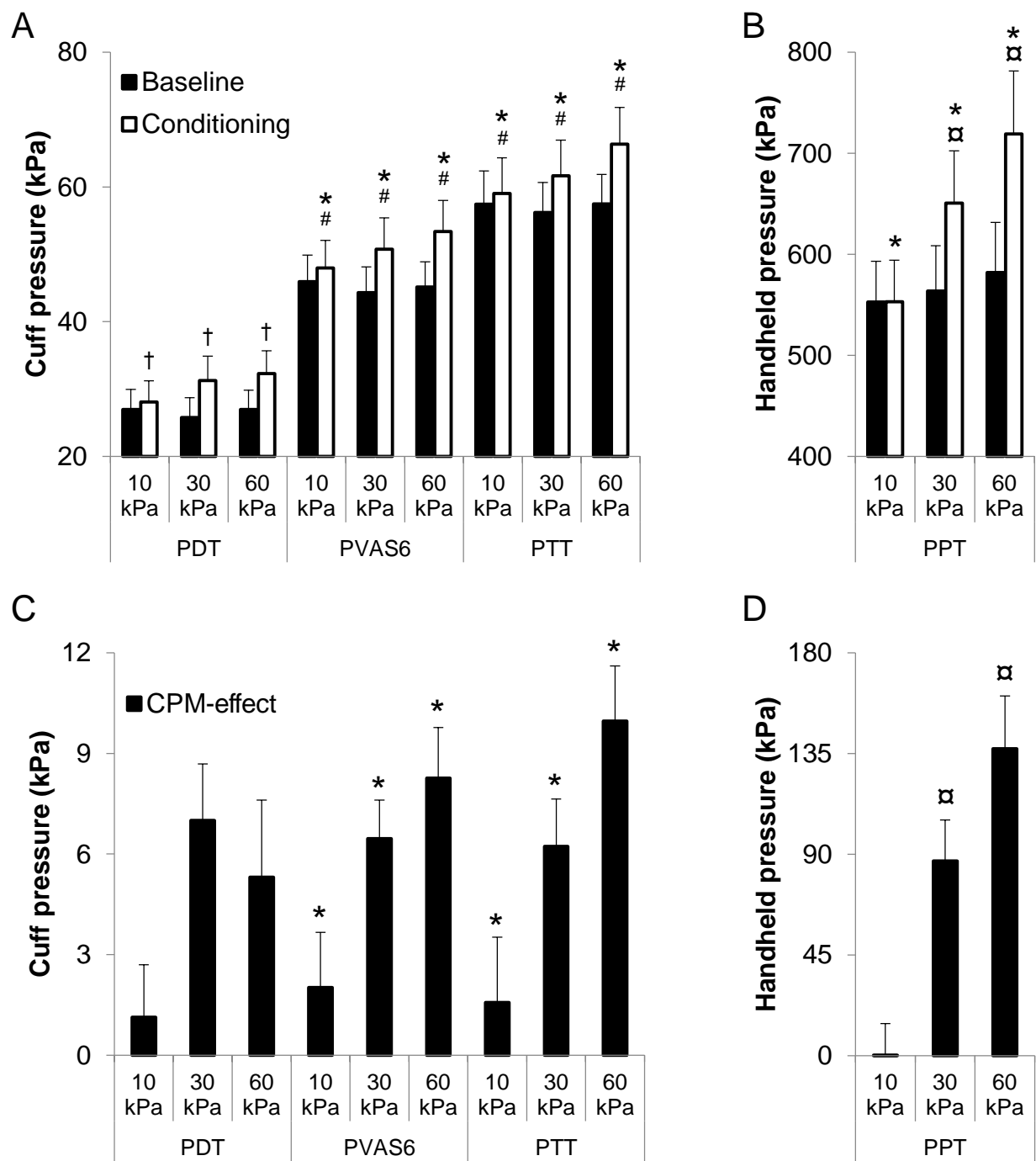


Fig. 2

Figure 5

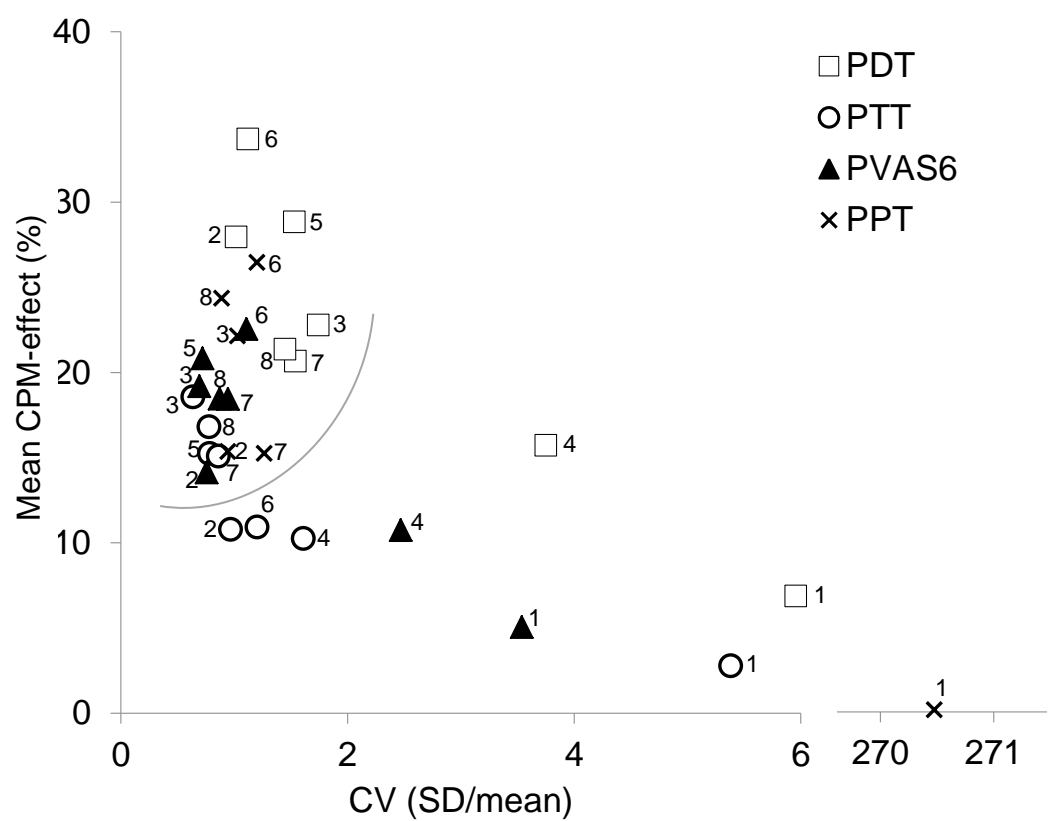


Fig. 5

**Supporting material to**

**USER-INDEPENDENT ASSESSMENT OF CONDITIONING PAIN MODULATION**

**BY CUFF PRESSURE ALGOMETRY**

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## SUPPORTING RESULTS

The Results section presents the CPM-effects based on the difference in test stimulus between during and before conditioning whereas these supporting Results presents the analysis including before and during conditioning test stimuli in the statistical models. Moreover, the supporting Results include analysis of the sequentially recorded baseline test-stimuli.

### *S3.1 Effects of conditioning intensity (Session 1: protocol 1, 2, 3)*

The time factor in the two-way ANOVAs of the PDT, PVAS6, and PTT demonstrated that these were higher during the conditioning stimuli compared with baseline (Fig. 2A; ANCOVA:  $F[1,16-17] > 23.36$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ). An interaction between sex and time (ANCOVA:  $F(1,16) = 6.88$ ,  $P < 0.02$ ) showed higher PDT during conditioning stimuli compared with baseline for males (Bonferroni:  $P < 0.001$ ) but only approaching significance for females (Bonferroni:  $P < 0.055$ ). Moreover, an interaction between time and protocols was found for PVAS6 and PTT where the PVAS6 and PTT were higher during 60 kPa conditioning compared with 30 kPa and 10 kPa conditioning, and higher during 30 kPa conditioning compared with 10 kPa conditioning (ANCOVA:  $F[2,30-32] > 18.12$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.03$ ).

A two-way ANOVA of the handheld PPT demonstrated an interaction between time and protocols with higher PPT during 60 kPa and 30 kPa conditioning compared with baseline (Fig. 2B; ANCOVA:  $F[2,36] = 12.50$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ). Moreover, the PPTs during 60 kPa and 30 kPa conditioning were higher than PPTs during conditioning with 10 kPa (Bonferroni:  $P < 0.002$ ).

### *S3.2 Effects of conditioning duration (Session 1: protocol 2 & 4; Session 1 & 2: protocol 3 & 5)*

*Conditioning with 30 kPa in 60 s or 10 s (Session 1: protocols 2 and 4):* The time factor in the two-way ANOVAs of the PDT, PVAS6, and PTT demonstrated that these were higher during conditioning stimuli compared with baseline (Fig. S3A; ANCOVA:  $F[1,16] > 20.57$ ,  $P < 0.001$ , Bonferroni:  $P < 0.001$ ). Further, an interaction between time and sex (ANCOVA:  $F[1,16] = 4.54$ ,  $P < 0.05$ ) showed



higher PDTs during conditioning stimuli compared with baseline for males (Bonferroni;  $P < 0.001$ ) which only approached significance for females (Bonferroni;  $P < 0.06$ ).

*Conditioning with 60 kPa in 60 s or 10 s (Session 1 and 2: protocols 3 and 5):* The time factor in the three-way ANOVA of the PDT, PVAS6, and PTT demonstrated that these were higher during conditioning stimuli compared with baseline (Fig. S3A; ANCOVA:  $F[1,13-16] > 11.75$ ,  $P < 0.003$ ; Bonferroni:  $P < 0.003$ ). For PDT an interaction between time, session, protocol (60 s or 10 s), and sex (ANCOVA:  $F[1,16] < 6.36$ ,  $P < 0.02$ ) showed that in males higher PDT was found during 60 s conditioning stimulus compared with baseline in session 1 (Bonferroni:  $P < 0.01$ ) and in session 2 males showed increased PDT compared with baseline for the 10 s conditioning (Bonferroni:  $P < 0.01$ ) whereas for females increased PDT compared with baseline was only found in session 2 for 60 s conditioning (Bonferroni:  $P < 0.03$ ).

### *S3.3 Cuff location protocol (Session 1&2: protocols 3, 6, 7, 8)*

The time factor in the two-way ANOVA of the PDT, PVAS6, and PTT demonstrated that parameters in protocol 3, 6, 7, and 8 were significantly higher during conditioning stimuli compared with baseline (Fig. S4A; ANCOVA:  $F[1,10-15] > 30.45$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ). An interaction between time, session, and sex (ANCOVA:  $F[3,45] = 7.83$ ,  $P < 0.01$ ) showed higher PDT during conditioning stimulation compared with baseline for males in both session 1 and 2 (Bonferroni:  $P < 0.001$ ) and for females only in session 2 (Bonferroni:  $P < 0.01$ ). When assessing PDT at baseline it was higher in protocol 6 compared with protocol 3 (Bonferroni:  $P < 0.04$ ) and during conditioning stimulation the PDT was higher in protocol 6 compared with protocols 3, 7, and 8 (ANCOVA:  $F[3,45] = 2.98$ ,  $P < 0.04$ ; Bonferroni:  $P < 0.001$ ). The PDT in males was higher in protocol 6 compared with protocols 3, 7, and 8 (ANCOVA:  $F[3,45] = 3.08$ ,  $P < 0.04$ ; Bonferroni:  $P < 0.008$ ). Interactions between protocol and session (ANCOVA:  $F(3,30-45) > 3.80$ ,  $P < 0.02$ ) showed that the PTT in session 2 was increased in protocol 6 compared with protocols 3, 7, and 8 (Bonferroni:  $P < 0.03$ ) and PVAS6 in session 2 was increased in protocol 6 compared with protocol 3 (Bonferroni:  $P < 0.03$ ); in protocol 3 the PVAS6

and PTT was higher in session 2 compared with session 1 (Bonferroni:  $P < 0.02$ ).

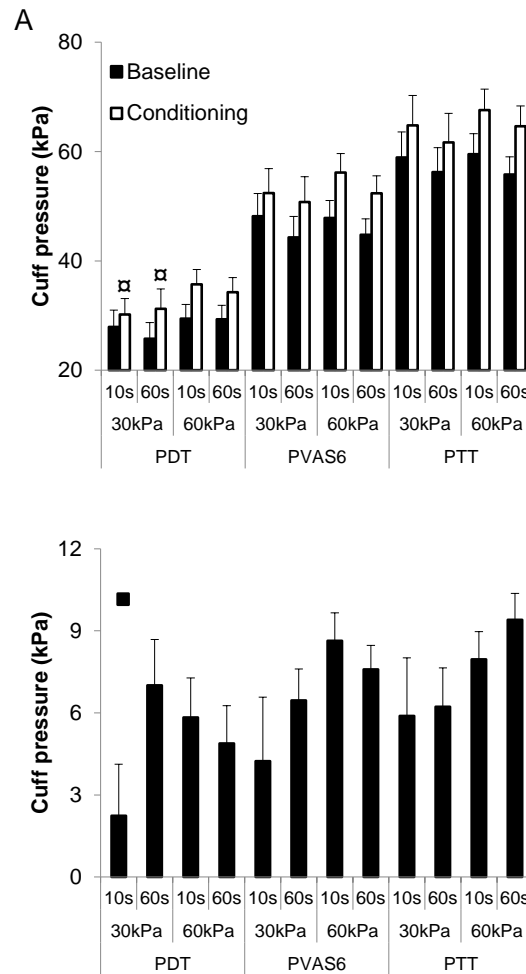
The two-way ANCOVA of the manual PPTs in protocols 3, 6, 7, and 8 showed higher PPTs during conditioning stimuli compared with baseline (Fig. S4B; ANCOVA:  $F[1,17] = 51.18$ ,  $P < 0.001$ ; Bonferroni:  $P < 0.001$ ) and higher PPTs in the second session compared with session 1 (ANCOVA:  $F[1,17] = 6.09$ ,  $P < 0.03$ ; Bonferroni:  $P < 0.03$ ). Lower PPTs (ANCOVA:  $F[3,51] = 56.64$ ,  $P < 0.001$ ) were found in protocol 6 compared with protocols 3, 7, and 8 (Bonferroni:  $P < 0.001$ ), and protocol 8 showed lower PPTs compared with protocol 3 and 7 (Bonferroni:  $P < 0.003$ ).

### *S3.4 Sequential assessment baseline parameters across protocols*

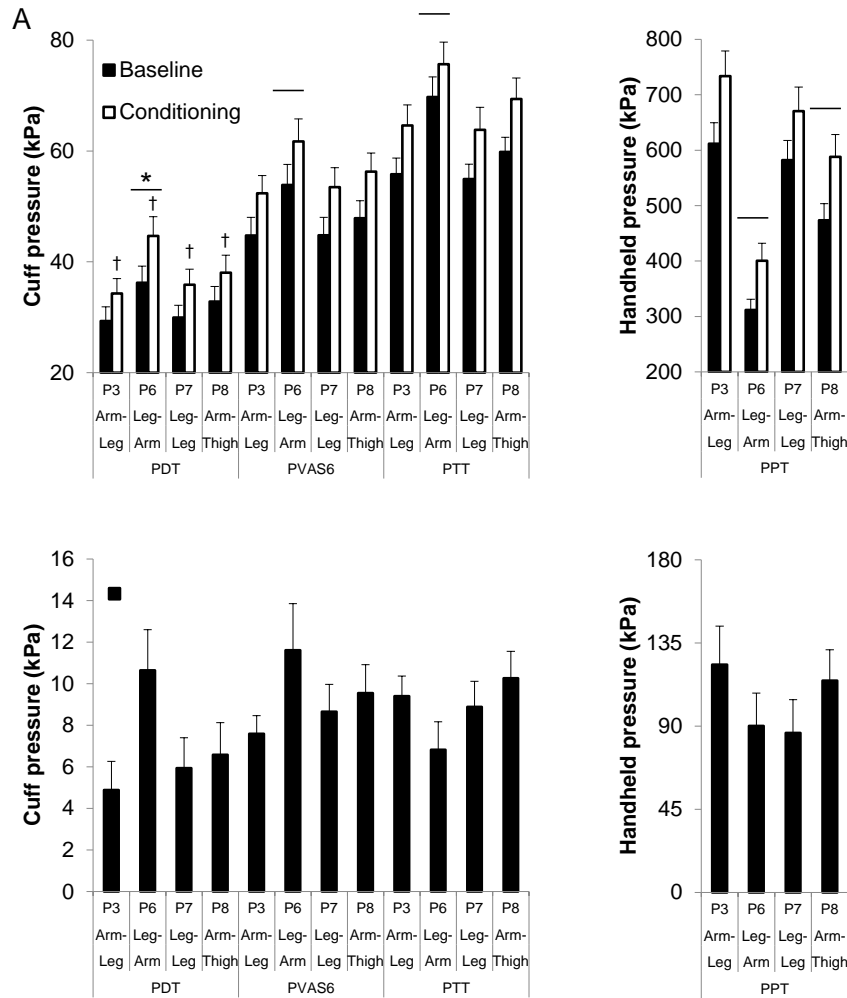
When analysing the six protocols in session 1 with the lower leg as test site, the baseline PDT and PVAS6 were not significantly changed across the sequence of protocols tested (ANOVA:  $F[5,80-85] < 1.19$ ,  $P > 0.3$ ). A sequence effect was found for the PTT (ANOVA:  $F[5,85] = 2.77$ ,  $P < 0.02$ ) although no post-hoc differences could be detected (Bonferroni:  $P > 0.16$ ).

When including baseline data from protocols in both session 1 and 2 with the lower leg as test site it demonstrated higher PDTs in session 2 compared with session 1 (ANOVA:  $F[1,17] = 7.25$ ,  $P < 0.02$ ; Bonferroni:  $P < 0.02$ ). For the PPT, no significant sequence effects was found in session 1 (ANOVA:  $F[3,57] = 2.42$ ,  $P = 0.08$ ) but a significant session factor (ANOVA:  $F[1,18] = 9.70$ ,  $P < 0.006$ ) showed higher PPTs in session 2 compared with session 1 (Bonferroni:  $P < 0.006$ ).

## SUPPORTING FIGURES



**Figure S3.** Mean ( $\pm$  SEM,  $N = 18 - 20$ ) pressure pain test stimulus parameters assessed on the lower leg at baseline and during conditioning are illustrated (**A**) together with the CPM-effect (test stimulus conditioning minus baseline) (**B**) at cuff conditioning intensities of 30 kPa (protocols 2 and 4, session 1) and 60 kPa (protocols 3 and 5, session 1 and 2) delivered to the arm and with durations of 10 s and 60 s before assessment. The pain detection thresholds (PDT), pressure levels where subjects indicate visual analogue scale scores of 6 cm (PVAS6), and pressure pain tolerance (PTT) levels were recorded by cuff algometry. Significantly different compared with baseline value (#,  $P < 0.05$ ;  $\alpha$ , NK:  $P < 0.05$  for males and/or females in session 1 and/or session 2 – see Supporting Results for details) and significantly different compared with the 10 s conditioning at the same intensity (\*, NK:  $P < 0.05$ ;  $\dagger$ ,  $P < 0.05$  for males only in session 2).



**Figure S4.** Mean ( $\pm$  SEM) pressure pain test stimulus parameters assessed at baseline and during conditioning are illustrated (**A, B**) together with the CPM-effect (test stimulus conditioning minus baseline) (**C, D**) in four different combinations of conditioning to the leg or arm and assessment on the leg (protocols 3 and 7, P3 and P7,  $N = 18 - 20$ ), thigh (protocol 8, P8,  $N = 18 - 20$ ), and arm (protocol 6, P6,  $N = 14 - 19$ ) in both sessions. The pain detection thresholds (PDT), pressure levels where subjects indicate visual analogue scale scores of 6 cm (PVAS6), and pressure pain tolerance (PTT) levels were recorded by cuff algometry, and the pressure pain thresholds (PPT) were recorded with handheld pressure algometry. In protocol 6 during conditioning, a VAS score above 6 cm (i.e. PVAS6) was detected in 19 subjects in session 1, but only in 14 subjects in session 2. Significantly different compared with baseline values (#,  $P < 0.05$ ; †, NK:  $P < 0.05$  for males and/or females in session 1 and/or session 2 – see Supporting Results for details). Significantly different for both the baseline and conditioning values compared with other protocols in one or two sessions in males and/or females (\*, NK:  $P < 0.05$ ; see Supporting Results for details), compared with P3 in session 2 (‡, NK:  $P < 0.05$ ), and compared P3, P7 and P8 in session 2 (‡‡, NK:  $P < 0.05$ ).

## SUPPORTING TABLES

**Table S1:** Test-retest statistics on the CPM-effect (i.e. during conditioning minus baseline) between two sessions separated by 1 month (N = 18 - 20).

			Session 1 (mean ± SD)	Session 2 (mean ± SD)	IntraCV (%)	ICC(3.k) (95% CI)	Difference (mean ± SD)	Lower limit (95% limits of agreement)	Upper limit
Protocol 3	Cuff	PDT (kPa)	5.3 ± 10.0	4.5 ± 6.9	47.9	0.21 (-1.25 – 0.71)	1.1 ± 11.8	-22.0	24.2
		PVAS6 (kPa)	8.3 ± 6.4	6.9 ± 3.9	30.0	0.47 (-0.37 – 0.81)	2.0 ± 5.9	-9.5	13.5
		PTT (kPa)	10.0 ± 7.2	8.8 ± 4.6	24.2	0.53 (-0.28 – 0.83)	1.0 ± 7.0	-12.6	14.7
	Manual	PPT (kPa)	137.1 ± 105.5	106.2 ± 141.2	7.5	0.58 (-0.10 – 0.84)	36 ± 145	-247	320
Protocol 5	Cuff	PDT (kPa)	6.1 ± 9.9	5.5 ± 8.1	43.5	0.59 (-0.16 – 0.85)	0.2 ± 10.2	-19.7	20.1
		PVAS6 (kPa)	9.0 ± 6.0	8.3 ± 6.6	27.4	0.40 (-0.78 – 0.79)	1.1 ± 8.6	-15.7	17.9
		PTT (kPa)	7.6 ± 5.6	8.4 ± 7.0	28.0	0.39 (-0.67 – 0.78)	-1.2 ± 7.7	-16.3	13.9
	Manual	PPT (kPa)	-	-	-	-	-	-	-
Protocol 6	Cuff	PDT (kPa)	11.5 ± 12.7	9.7 ± 11.3	32.1	0.22 (-1.22 – 0.71)	1.8 ± 16.3	-30.1	33.6
		PVAS6 (kPa)	13.4 ± 14.5	9.2 ± 10.2*	32.1	-0.11 (-2.51 – 0.64)	6.8 ± 17.9	-28.3	42.0
		PTT (kPa)	8.4 ± 8.7	5.2 ± 7.5	38.4	0.46 (-0.34 – 0.79)	3.3 ± 9.7	-15.7	22.3
	Manual	PPT (kPa)	86.3 ± 115.9	91.2 ± 100.6	10.2	0.61 (-0.06 – 0.85)	0 ± 116	-227	226
Protocol 7	Cuff	PDT (kPa)	4.9 ± 8.5	7.0 ± 9.9	44.7	0.75 (0.37 – 0.90)	-1.8 ± 8.3	-18.1	14.4
		PVAS6 (kPa)	9.5 ± 9.0	7.8 ± 7.4	29.9	0.53 (-0.23 – 0.82)	2.0 ± 9.3	-16.2	20.3
		PTT (kPa)	9.8 ± 8.4	8.0 ± 6.9	27.6	0.63 (0.04 – 0.85)	1.9 ± 8.2	-14.1	17.8
	Manual	PPT (kPa)	105.4 ± 86.8	70.6 ± 124.1	9.8	0.60 (0.02 – 0.84)	38 ± 111	-180	256
Protocol 8	Cuff	PDT (kPa)	6.4 ± 11.9	6.7 ± 6.7	41.5	0.68 (0.12 – 0.88)	-0.3 ± 9.8	-19.6	19.0
		PVAS6 (kPa)	10.7 ± 8.4	8.3 ± 8.3	29.8	0.73 (0.31 – 0.90)	2.9 ± 7.9	-12.7	18.4
		PTT (kPa)	10.7 ± 8.8	9.9 ± 7.3	26.0	0.70 (0.18 – 0.89)	0.2 ± 7.9	-15.3	15.6
	Manual	PPT (kPa)	132.6 ± 112.1	95.7 ± 82.0	7.3	0.04 (-1.58 – 0.64)	42 ± 150	-252	335

“-”: not recorded. \*: N=14. CI: Confidence interval.

**Table S2:** Test-retest statistics on the baseline assessment parameters between two sessions separated by 1 month (N = 19 - 20).

			Session 1 (mean $\pm$ SD)	Session 2 (mean $\pm$ SD)	IntraCV (%)	ICC(3.k) (95% CI)	Difference (mean $\pm$ SD)	Lower limit (95% limits of agreement)	Upper limit
Protocol 3	Cuff	PDT (kPa)	27.0 $\pm$ 12.7	31.8 $\pm$ 18.6	12.3	0.71 (0.28 – 0.89)	-5.3 $\pm$ 14.9	-34.6	24.0
		PVAS6 (kPa)	45.1 $\pm$ 16.0	44.4 $\pm$ 19.3	10.1	0.94 (0.83 – 0.98)	1.3 $\pm$ 9.0	-16.2	18.9
		PTT (kPa)	57.5 $\pm$ 19.6	54.0 $\pm$ 21.3	7.7	0.91 (0.78 – 0.97)	3.1 $\pm$ 11.7	-19.8	26.0
	Manual	PPT (kPa)	582 $\pm$ 221	643 $\pm$ 253	2.2	0.89 (0.65 – 0.96)	-79 $\pm$ 135	-344	186
Protocol 5	Cuff	PDT (kPa)	27.3 $\pm$ 14.5	31.6 $\pm$ 17.5	13.6	0.83 (0.56 – 0.94)	-3.7 $\pm$ 12.1	-27.5	20.1
		PVAS6 (kPa)	48.6 $\pm$ 20.0	47.1 $\pm$ 19.9	9.4	0.92 (0.78 – 0.97)	2.5 $\pm$ 11.3	-19.8	24.7
		PTT (kPa)	61.9 $\pm$ 23.3	57.2 $\pm$ 23.0	8.2	0.94 (0.79 – 0.98)	5.8 $\pm$ 11.1	-15.9	27.6
	Manual	PPT (kPa)	-	-		-	-	-	-
Protocol 6	Cuff	PDT (kPa)	31.9 $\pm$ 13.8	40.7 $\pm$ 22.1	11.0	0.82 (0.38 – 0.94)	-9.2 $\pm$ 12.7	-34.1	15.7
		PVAS6 (kPa)	50.7 $\pm$ 19.9	57.4 $\pm$ 24.1*	9.4	0.90 (0.68 – 0.97)	-6.5 $\pm$ 12.4	30.8	17.7
		PTT (kPa)	66.5 $\pm$ 21.9	73.1 $\pm$ 24.9	6.7	0.91 (0.70 – 0.97)	-7.5 $\pm$ 12.0	-31.1	16.1
	Manual	PPT (kPa)	302 $\pm$ 120	322 $\pm$ 125	3.2	0.66 (0.15 – 0.87)	-31 $\pm$ 118	-263	200
Protocol 7	Cuff	PDT (kPa)	26.9 $\pm$ 13.4	33.2 $\pm$ 14.1	11.9	0.87 (0.43 – 0.96)	-6.4 $\pm$ 7.7	-21.5	8.6
		PVAS6 (kPa)	42.1 $\pm$ 17.1	47.6 $\pm$ 16.7	8.9	0.90 (0.70 – 0.96)	-5.2 $\pm$ 9.4	-23.7	13.3
		PTT (kPa)	52.9 $\pm$ 19.6	57.0 $\pm$ 21.0	7.9	0.93 (0.83 – 0.98)	-3.6 $\pm$ 9.9	-23.0	15.7
	Manual	PPT (kPa)	522 $\pm$ 198	645 $\pm$ 233	2.3	0.76 (0.07 – 0.92)	-142 $\pm$ 151	-438	155
Protocol 8	Cuff	PDT (kPa)	30.0 $\pm$ 17.3	35.8 $\pm$ 16.8	11.2	0.91 (0.68 – 0.97)	-5.7 $\pm$ 8.7	-22.8	11.4
		PVAS6 (kPa)	44.8 $\pm$ 15.0	51.0 $\pm$ 17.5	8.4	0.93 (0.76 – 0.98)	-4.2 $\pm$ 7.1	-18.0	9.7
		PTT (kPa)	59.4 $\pm$ 20.1	60.3 $\pm$ 19.5	7.0	0.96 (0.89 – 0.98)	-1.3 $\pm$ 8.3	-17.6	15.0
	Manual	PPT (kPa)	455 $\pm$ 192	493 $\pm$ 185	2.6	0.81 (0.51 – 0.93)	-63 $\pm$ 130	-317	191

“-”: not recorded. \*: N=17. CI: Confidence interval.

**Table S3:** Test-retest statistics on the assessment parameters during conditioning between two sessions separated by 1 month (N = 18 - 20).

			<b>Session 1</b> (mean $\pm$ SD)	<b>Session 2</b> (mean $\pm$ SD)	<b>IntraCV</b> (%)	<b>ICC(3.k)</b> (95% CI)	<b>Difference</b> (mean $\pm$ SD)	<b>Lower limit</b> (95% limits of agreement)	<b>Upper limit</b>
Protocol 3	Cuff	PDT (kPa)	32.3 $\pm$ 14.8	36.3 $\pm$ 18.2	11.4	0.81 (0.50 – 0.93)	-4.0 $\pm$ 13.5	-30.5	22.4
		PVAS6 (kPa)	53.4 $\pm$ 19.5	51.3 $\pm$ 19.7	9.2	0.95 (0.85 – 0.98)	3.6 $\pm$ 8.8	-13.6	20.9
		PTT (kPa)	66.4 $\pm$ 23.7	62.9 $\pm$ 22.5	7.6	0.93 (0.83 – 0.98)	-0.3 $\pm$ 19.3	-38.1	37.5
	Manual	PPT (kPa)	719 $\pm$ 279	749 $\pm$ 294	2.1	0.89 (0.65 – 0.96)	-79 $\pm$ 135	-394	281
Protocol 5	Cuff	PDT (kPa)	34.3 $\pm$ 14.5	37.2 $\pm$ 19.4	10.8	0.82 (0.54 – 0.93)	-3.4 $\pm$ 13.4	-29.7	23.0
		PVAS6 (kPa)	56.9 $\pm$ 20.6	55.4 $\pm$ 22.2	8.6	0.91 (0.76 – 0.97)	3.0 $\pm$ 12.5	-21.5	27.4
		PTT (kPa)	69.5 $\pm$ 22.8	65.5 $\pm$ 25.7	7.1	0.94 (0.85 – 0.98)	3.6 $\pm$ 11.2	-18.4	25.7
	Manual	PPT (kPa)	-	-	-	-	-	-	-
Protocol 6	Cuff	PDT (kPa)	41.8 $\pm$ 20.7	47.7 $\pm$ 21.9	10.6	0.81 (0.49 – 0.93)	-6.6 $\pm$ 16.9	-39.6	26.5
		PVAS6 (kPa)	64.1 $\pm$ 25.5	58.5 $\pm$ 20.6*	10.0	0.92 (0.75 – 0.98)	-0.2 $\pm$ 13.1	-25.8	25.4
		PTT (kPa)	74.2 $\pm$ 24.5	77.1 $\pm$ 24.9	7.2	0.95 (0.86 – 0.98)	-3.7 $\pm$ 10.6	-24.4	17.0
	Manual	PPT (kPa)	388 $\pm$ 215	413 $\pm$ 181	3.2	0.66 (0.15 – 0.87)	-31 $\pm$ 118	-377	309
Protocol 7	Cuff	PDT (kPa)	31.8 $\pm$ 15.1	40.2 $\pm$ 19.3	11.1	0.79 (0.39 – 0.92)	-8.2 $\pm$ 13.3	-34.2	17.7
		PVAS6 (kPa)	51.6 $\pm$ 21.0	55.4 $\pm$ 23.2	8.5	0.89 (0.72 – 0.96)	-3.4 $\pm$ 14.0	-30.9	24.1
		PTT (kPa)	62.7 $\pm$ 24.8	65.0 $\pm$ 26.7	7.8	0.95 (0.86 – 0.98)	-1.8 $\pm$ 12.0	-25.4	21.9
	Manual	PPT (kPa)	628 $\pm$ 238	715 $\pm$ 301	2.3	0.76 (0.07 – 0.92)	-142 $\pm$ 151	-493	283
Protocol 8	Cuff	PDT (kPa)	33.6 $\pm$ 18.4	42.5 $\pm$ 19.8	11.1	0.86 (0.59 – 0.95)	-6.6 $\pm$ 11.9	-30.0	16.8
		PVAS6 (kPa)	55.5 $\pm$ 20.8	57.1 $\pm$ 20.7	8.5	0.95 (0.87 – 0.98)	-1.9 $\pm$ 9.1	-19.7	15.8
		PTT (kPa)	68.6 $\pm$ 23.6	70.1 $\pm$ 23.7	7.2	0.95 (0.87 – 0.98)	-5.7 $\pm$ 25.0	-54.6	43.2
	Manual	PPT (kPa)	587 $\pm$ 279	589 $\pm$ 227	2.4	0.81 (0.51 – 0.93)	-63 $\pm$ 130	-423	336

“-”: not recorded. \*: N=14. CI: Confidence interval