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THE EFFECT OF STRESS ON REPEATED PAINFUL STIMULI WITH AND WITHOUT PAINFUL CONDITIONING

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Significance: This study did not show any significant effect of experimental stress on pain sensitivity or conditioned pain modulation (CPM), but a correlation was found between changes in conditioned pain sensitivity and cortisol levels. Mechanisms regulating cortisol levels may interact with the effectiveness of a conditioning stimulus, and thus reduces the effectiveness of CPM.

ABSTRACT

Objectives. Stress and pain have been interrelated in clinical widespread pain conditions. Studies indicate that acute, experimental stress in healthy volunteers has a negative effect on the descending inhibitory pain control system and thus the ability to inhibit one painful stimulus with another (conditioned pain modulation, CPM) although without effect on general pain sensitivity. CPM-effects can be assessed immediately after the stress-induction, whereas some physiological stress responses (e.g. cortisol release) are delayed and longer lasting. It is unclear whether CPM may relate to stress-induced increases in cortisol.

Design. Twenty-five healthy men had CPM-effects measured over a period of 10 minutes. Pain detection thresholds (PDT) was assessed by repeated test-stimuli with cuff algometry on one leg, with and without painful cuff-pressure conditioning on the contralateral leg. CPM-effects, assessed as the increase in PDT during conditioning stimulation compared to without, were measured before and after experimental stress and a control condition (Montreal Imaging Stress Task, MIST). Saliva cortisol levels and self-perceived stress were collected.

Results. Participants reported MIST to be more stressful compared with MIST-control but cortisol levels did not change significantly from baseline. In all sessions, PDT increased during conditioning ($P=0.001$) although MIST compared with MIST-control, had no significant effect on PDT or CPM-effects. A negative correlation between changes in cortisol and conditioned PDT was found when applying MIST ($P<0.03$).

Conclusion. No significant effect of stress was found on CPM compared to a matched control-condition. Individual changes in experimental stress and in conditioned pain sensitivity may be linked with cortisol.

INTRODUCTION

People with chronic musculoskeletal pain have increased risk of comorbid diseases[1,2], including stress[3], and impairment of the descending pain modulatory systems[4-6]. Conditioned pain modulation (CPM) paradigms assess the net-effect of descending pain modulation[7,8] and CPM-efficacy is generally reduced in chronic pain patients[7]. CPM-efficacy is measured as the change in pain sensitivity during compared to before a painful conditioning stimulation applied extra-segmentally to the pain assessment site[9]. The user-independent pressure-cuff model is a reliable method to assess CPM[10-12] and studies indicate that repeated assessment with short interval may give a more subtle picture of the descending modulatory system compared to a single test[13,14].

Stress occurs when external demands exceed the adaptive capacity of the individual[15] and stress may lead to analgesia[16] via mechanisms that involve descending modulation[17-19]. Studies have often used arithmetic tasks in combination with negative feedback to induce acute stress and study pain sensitivity in healthy volunteers[20-30]. The majority of studies report no effect of stress on pressure pain sensitivity[20,21,23-25,31-33] but two studies found heat-hyperalgesia after stress[22,28]. Four studies showed a reduction in CPM during mental stress[20-23] and two did not[23,26]. One of these could not analyze data due to carry-over effects between sessions[23] and the other measured CPM as the effect of a conditioning stimulus on temporal summation of pain[26].

In humans, saliva cortisol is a commonly used biomarker for stress[32,34], although the relationship between cortisol and perceived stress is not linear[35]. Increased saliva cortisol levels have been found at different time-points from 10 to 40 minutes after a stressor[36,37]. Yet, most studies measure pain sensitivity and CPM within a few minutes after the stressor[20-29,38] possibly missing a cortisol-induced influence on pain

sensitivity[30]. Pharmacologically suppressed cortisol-levels in twins show an association between cortisol regulation and reduced CPM-effect[33], indicating that dysregulation of cortisol can lead to a reduction in CPM-effectiveness. A commonly used mental stress test, the *Montreal Imaging Stress Task* (MIST), which can reliably induce acute stress in healthy volunteers[20,31,39], has been used to show an association between increases in perceived stress and cortisol during the stress task[20-22].

Previously, pain and cortisol responses during stress have been compared to baseline[20-22,24], quiet rest[20-22,27,38], book reading[23] or to patients[25,26]. However, a recent study compared stress-related changes in PDT in one group to a control condition in a different group and found no differences in pain threshold immediately after the stress and control sessions [30], indicating that previous results could depend on control-conditions.

The present study aimed to explore the effect of a stressful mental task on pressure pain sensitivity and CPM, and to compare these effects to a comparable control-condition. It was hypothesised that pressure-induced CPM was reduced more by stress than a comparable control condition.

METHODS

Subjects

Geva et al (2014) found a reduced CPM-effect during stress of approximately 50% and based on a sample size calculation with a significance level at 0.05 and a statistical power of 70% the total sample size should be at least 25 subjects for a 50% CPM reduction. Twenty-five healthy men between 22 and 72 years participated (average 30.3 years, standard deviation 10.9 years). Exclusion criteria were 1) diagnosis of sleep, neurological, mental or musculoskeletal disorders, 2) pain within the last 48 hours or any use of sleep or

pain medication in this period, 3) any history of chronic stress or chronic pain, or skin lesions in the test-areas (lower legs), 4) less than 6 hours of sleep over the last 24 hours, and 5) smoking, exercise, food or any drinks other than water for the two hours prior to the study. All participants received oral and written information about the experiment and gave their verbal and written consent prior to the study. Exclusion criteria were verbally confirmed after consent was given. The study was approved by the local Ethics Committee (N-20170033) and was performed in accordance with the Helsinki Declaration.

Experimental procedures

The study was conducted in a two-hour session at either 08.30 or 10.30 am to control for diurnal changes in freely available cortisol[40]. At baseline participants gave a saliva sample, completed the pain catastrophizing questionnaire[41], and rated how they perceive their math-skills on a numerical rating scale (NRS) from 0 ('I'm exceptionally bad at math') to 10 ('I'm exceptionally good at math'). Previous studies, randomizing participants to the order of stress and control conditions, find similar results[23,24,38] as those who have not [20-22,25-29]. To avoid expectations of intentional stress during the experiment, participants were informed that they would be exposed to arithmetic tasks during painful stimuli. Consequently, assessment of perceived stress was done during debriefing rather than during the stress task. Four samples of saliva cortisol were obtained in addition to the baseline sample (Fig 1).

Experimental procedures were done with participants positioned in an inclined bed where they also remained during rest. The Montreal Imaging Stress Task (MIST) was used to induce acute, experimental stress[39]. The protocol included a non-stressful training session (60 s), the stress-induction (MIST) consisting of two rounds of arithmetic in combination with social, visual and auditory stressors (2 x 360 s, separated by a three

minute break), and a control (MIST-control) lasting for 360 s. MIST-control was similar in terms of difficulty of the arithmetic tasks but without the stressful context. Pain sensitivity assessment was performed by cuff algometry[12] at baseline, before and after MIST, and before and after the MIST-control (Fig 1).

After the experiment, participants were thoroughly debriefed and informed about the nature of the study. During debriefing, participants were asked to verbally confirm or reject feeling 'very stressed' during the MIST and MIST-control, respectively[23]

The Montreal Imaging Stress Task

The MIST is a software algorithm developed[39] to adjust time and difficulty of a series of arithmetic tasks. The paradigm included a stress-condition (MIST) and a control condition (MIST-control). During the stress task, the program restricted the time for each participant to answer questions when they had three correct answers in a row. Furthermore, social aspects were added, including standardized negative feedback (verbal and visual) and a high, ramping tone. The tone intended to make participants aware how quickly time was running out and thus to give additional pressure onto the participant. The visual feedback was provided via a real-time performance indicator in green (*performing average or above*), yellow (*below average*) and red (*insufficient performance*). Verbal feedback was provided by the investigator in accordance with a written manuscript. The content of the verbal feedback gave the participant the impression that 'red' on the performance indicator was insufficient for the data to be included into the study. In the control-condition, participants were not given any negative (or positive) visual, auditory or verbal feedback although time was still restricted but not reduced by correct answers[39].

Participants had time to familiarize themselves with the software and hardware for 60 s at baseline, which was found to be sufficient during pilot testing. Instructions were

developed by a psychologist, with experience from similar paradigms, to match the context of the study. The training-session included a trial-run at the arithmetic task but did not include any of the stressors. After the training-session, questions or comments from the participant were addressed by the investigator, and the participant was instructed to 'solve as many calculations as possible and to be as correct as possible'.

During MIST and MIST-control the investigator left the room but participants were informed that they would be monitored[39]. The investigator re-entered the room approximately 30 s before the end of the first round of arithmetic tasks. After the first round the investigator briefly revised the results and, as negative feedback, the participant was reminded that he was expected to get 80-90% of the tasks correct (although the algorithm makes it impossible)[39]. Measures of successful MIST was the percentage of correct calculations during MIST (approximately 45%) and MIST-control (approximately 90%), respectively[21,39].

Saliva cortisol

Saliva samples were collected using Cortisol-Salivette® with a citric-acid, which helped to facilitate saliva production (SARSTEDT AG & Co., Nümbrecht, Germany). The participants were instructed to chew on the cotton swap for one minute and then place it back into the Salivette® tube. The samples were put in a thermos-box with ice until saliva was recovered from the cotton swap by centrifugation for 2 minutes at 1000 rpm (no more than four hours after collection). Once saliva was extracted the cotton bud and inner tube was discarded and the saliva sample, was frozen at -80°C until further analysis. The samples were analyzed using a standardized ELISA-kit (SA E-6000, LDN Labor Diagnostika Nord, Germany). Samples were diluted if values were outside the standard curve (0 - 30 ng/ml). Based on manufacturer guidelines the kit has a 95% confidence intervals of 1-11.3 ng/ml

(n = 234) for morning cortisol in otherwise healthy humans[42]. For data-analysis the absolute change in cortisol (Δ-cortisol 1-4) was calculated (e.g. Δ-cortisol 1 = Saliva 1 minus Baseline). Negative values indicate an decrease in cortisol from one measurement to the following.

Pain sensitivity assessment by cuff algometry

Test and conditioning stimulations were delivered by a computer-controlled cuff pressure algometer (NociTech, Denmark), consisting of a computer-controlled air compressor with two independent 7.5 cm tourniquets (silicone high-pressure cuff, VBM Medizintechnik GmbH, Sulz, Germany)[12,43]. The cuff pressure algometer affects nociceptors in deep tissue rather than superficial tissue[44]. The system was connected to an electronic visual analogue scale (VAS, 0-10 cm) and a stop button, which could be used by the participants to terminate inflation of the cuffs (Aalborg University, Denmark). Endpoints of the VAS were defined as 0 being 'no pain' and 10 cm being 'maximal pain'. Cuffs were mounted bilaterally on the most prominent part of the calf and the upper and lower boarders of the cuffs were marked on the skin using a permanent marker. These marks were used to visually confirm that the cuffs did not move between the trials.

For assessment of pain sensitivity, cuff inflation (1 kPa/s) was applied until subjects pressed the stop button to indicate pressure pain tolerance (PTT) or until maximum stimulation intensity (100 kPa). During the cuff inflation subjects rated the cuff-induced pain intensity on the electronic VAS. The pressure equal to 1 cm VAS was considered the pain detection threshold (PDT) whereas pressure-pain tolerance threshold (PTT) was defined as equal to the pressure when subjects stopped the cuff inflation[12]. Pressure pain sensitivity was measured in four trials: pre-MIST, post-MIST, pre-MIST-control, and post-MIST-control. Each trial lasted approximately 15 minutes and trials were separated by

at least 10 minutes. A trial consisted of six painful cuff test stimuli (Fig 1: TS1-6) applied to the dominant lower leg. For each test-stimulus the cuff was inflated (1 kPa/s) and the pain detection threshold (PDT) was extracted. For analysis, the average of unconditioned PDTs in each trial was calculated (PDT_{avg}).

Conditioning pain modulation assessed by cuff algometry

In parallel to the 4th and 6th test stimuli a constant, painful conditioning stimulus on the non-dominant leg was applied. During baseline-testing PDT and PTT were recorded (see above) on the non-dominant leg. The conditioning intensity used throughout the study was 70% of baseline PTT[10] and the duration of the conditioning was maximally 104 seconds (conditioning was terminated a few seconds after the test-stimulus was terminated). Participants verbally reported the perceived pain intensity of the conditioning stimulus at the beginning and immediately after the test-stimulus was terminated. Pain intensity was scored on a NRS (0-10) with 0 defined as 'no pain' and 10 'maximal pain'.

For analysis the average of PDTs during conditioning ($PDT\text{-}CS_{avg}$) was extracted. Moreover, the CPM-effects were calculated based on the change in PDT during conditioning ($PDT\text{-}CS_{avg}$) compared to the average of the four unconditioned PDT recordings (PDT_{avg}). As a consequence, a positive CPM-effect reflects that PDTs increased (reduced pain sensitivity) during conditioning.

Statistics

Unless otherwise specified, results are presented as mean and standard error of the mean (SEM). Q-Q plots were used to confirm normal distribution (IBM® SPSS® Statistics version 23) by visual inspection. Parameters were normally distributed or otherwise log-transformed.

Correct answers during MIST (average of both rounds) and MIST-control were analysed in a two-way analysis of variance (ANOVA) with number of correct *answers* (MIST, MIST-control) as within-subject factor and *math-skills* (high, low) as between-subject factor. A median split based on math-skills would have caused to uneven-sized groups. Thus, for analysis the group was divided by rank into similar-sized groups by score and subsequently by participation number. The change in saliva cortisol levels (Δ-cortisol) between the five measurements were analysed in a two-way repeated-measures ANOVA with the four *samples* as within-subject factor (Δ-cortisol 1 - Δ-cortisol 4) and starting *time* of the trial (08.30 am, 10.30 am) as between-subject factor.

An average of unconditioned PDT (PDT_{avg}) was analysed in a two-way repeated-measures ANOVA with *time* (pre, post) and *session* (MIST, MIST-control) as factors. The effect of a conditioning stimulus (i.e. CPM) was analysed in a three-way repeated-measures ANOVA with within-subject factors *session* (PDT_{avg}, $PDT\text{-}CS_{avg}$) and *time* (preMIST, postMIST, preMIST-control and postMIST-control) and between-subject factor *math-skills* (high, low). The CPM-effects were analysed in a two-way repeated-measures ANOVA with *time* (pre, post) and *session* (MIST, MIST-control) as factors.

Greenhouse-Geisser corrections were applied to all ANOVAs if sphericity was violated. Significant interactions and main effects were adjusted for multiple comparisons with post-hoc Bonferroni (Bon) tests.

Linear regression analysis were used to analyse associations between the relative change in PDT_{avg} (post-MIST minus pre-MIST) and $PDT\text{-}CS_{avg}$ (post-MIST minus pre-MIST), respectively, with Δ-cortisol after MIST (post-MIST minus baseline)[29]. In the regression analysis Δ-cortisol was the predictor (constant variable) and PDT_{avg} or $PDT\text{-}CS_{avg}$, respectively, were dependent variables.

RESULTS

Validation of the stress response

Perceived stress was verbally confirmed by all participants ('very stressed' by the MIST and 'not stressed' by MIST-control, respectively). Participants perceived their *math-skills* to be 7/10 (median). All participants completed MIST and MIST-control. During MIST the mean of correct answers was 45.3 ± 0.6% compared to 90.1 ± 2.1% during MIST-control, indicating a successful implementation of the protocol.

Initial analysis of saliva cortisol found concentrations beyond 30 ng/ml, which were above the standard curve for the ELISA-kit. After samples were diluted and re-analyzed, variability in the results and the baseline cortisol remained higher than expected in some subjects when comparing to manufacturer reference values[42]. The change in cortisol over time (Δ-cortisol 1: - 2.86 ± 2.4 ml/ng, Δ-cortisol 2: - 1.08 ± 2.3 ml/ng, Δ-cortisol 3: - 1.02 ± 1.8 ml/ng and Δ-cortisol 4: - 0.91 ± 1.8 ml/ng) was not significant (ANOVA; $F(3, 69) = 0.164$, $P = 0.85$) and there were no interactions ($P = 0.44$) or indication of difference between the participants tested early or late in the day ($P = 0.22$).

Pain detection thresholds of unconditioned test-stimuli

There were no differences in PDT_{avg} across *time* (Fig 2; ANOVA: $F(1, 24) = 2.92$, $P = 0.1$, $\eta_p^2 = 0.11$) or *session* (ANOVA: $F(1, 24) = 0.87$, $p = 0.36$, $\eta_p^2 = 0.04$), indicating that neither MIST nor MIST-control had any influence on PDT_{avg}.

Conditioned pain modulation

The ANOVA showed a difference between PDT_{avg} and $PDT\text{-}CS_{avg}$ (Fig 2; ANOVA: $F(1, 24) = 15.18$, $P = 0.001$, $\eta_p^2 = 0.40$) without any interactions with *time* or *math-skills*, indicating that there was a significant CPM-effect, which was unaffected by MIST. The mean CPM-

effect was 5.9 ± 1.5 kPa and the CPM-effects did not interact or change significantly over time (ANOVA: $F(1, 24) = 0.18$, $P = 0.68$, $\eta_p^2 = 0.01$) or between sessions (ANOVA: $F(1, 24) = 0,88$, $P = 0.36$, $\eta_p^2 = 0.04$).

Change in cortisol during MIST correlates with change in conditioned PDT

The linear regressions showed that Δ-cortisol 1 (i.e. changes in cortisol from baseline to postMIST) could predict the change in conditioned PDT (PDT-CS_{avg}) during MIST. This correlation explained 19% of the variance (Fig 3a; $R^2 = 0.19$, $F(1, 23) = 5.23$, $P = 0.03$) and unstandardized coefficients ($B = - 0.36 \pm 0.16$, $P = 0.03$) suggest that the change in cortisol was inversely related to the change in PDT-CS_{avg} during MIST (i.e. an increase in cortisol reduced PDT-CS_{avg}). The effect size of this correlation is equal to approximately 0.5 standard deviation and considered 'moderate'[45]. No significant correlations were found for changes in PDT_{avg} versus cortisol during MIST (Fig 3b: $B = - 0.03 \pm 0.28$, $P = 0.9$).

DISCUSSION

In this study stress did not have any significant effect on pressure-induced pain sensitivity or CPM. However, an increase in cortisol during experimental stress was correlated with less efficient pain modulation from a conditioning stimulus. The relationship between cortisol and CPM appears to be multifactorial and cortisol-levels can explain 19% of the variance. Furthermore, this study supports the extensive literature that mental stress has no effect on pressure pain sensitivity.

Experimental stress and pain sensitivity

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is the key mechanism behind a physiological stress response and the release of stress-induced cortisol, however, the interaction between the HPA-axis and pain is highly complex and likely involves the entire neuroaxis[3]. At a system level, stress-induced analgesia is believed primarily to engage endocannabinergic pathways[45,46] whereas stress-induced hyperalgesia seems to be more related to the descending modulation through the rostral ventromedial medulla (RVM)[47]. At a mechanistic level, cortisol-related modulation of nociception is likely to appear directly in the dorsal horn through co-location of glucocorticoid receptors, substance P-receptors and CGRP-receptors[48] as well as through regulation of cannabinoids[3].

In the current study, there was no significant change in pain sensitivity during stress compared to before stress, or compared to the control session. These findings support the majority of the existing literature demonstrating that pressure-induced[26,27], cold-induced[24,29,49] and heat-induced[20,29,30,38] pain sensitivity does not change immediately after experimental stress and thus seems independent of modality. One reason for this may be that the pain threshold is a relatively robust measure[50-52] and that acute pain has saliency even during social stress[53]. Another reason that the pain sensitivity did not change during stress could be that changes in cortisol during experimental stress is either insufficient or unrelated to the pain detection threshold[38,54]. Interestingly, pain sensitivity can be reduced 15 minutes after a combination of mental stress, negative feedback and repeated, painful stimulations[30], but not instantly after the stressful conditions. This could indicate a link between pain sensitivity and cortisol. Indeed, diurnal changes have previously been found to have an effect on heat and cold-induced pain, albeit not on mechanical-induced pain[55]. Including the current study, six studies on

arithmetic-stress tests have taken circadian variations into account[20-22,29,30] and the majority found that time-of-day had no effect on pain sensitivity[20,21,29,30]. This could indicate that diurnal rhythm has a very little effect on PDT.

Experimental stress and CPM

In this study, MIST did not change the CPM-effect after stress compared to before and compared to a matched control condition. This mimics the results of Cathcart et al.[25] and partly those of Nilsen et al.[23]. The latter was able to show an effect of stress on CPM with heat-induced pain as test-stimulus but only a negligible change in pressure-pain threshold from baseline (367 ± 138 kPa) to post-stress (370 ± 101 kPa), indicating that no statistical or clinically relevant changes occurred. The three studies, which showed reduced CPM-efficiency during stress [20-23] have used heat, rather than pressure, to induce pain but neither of them compared CPM during stress to a comparable control-session. Nonetheless, this could support a hypothesis of modality specificity, meaning that although CPM induced by superficial nociceptive signals (heat) is affected during and immediately after mental stress, this does not seem to be the case for deeper tissues. Mechanistic explanations for this are not obvious since nociceptive signals from the deep tissues converge with superficial nociceptors in the dorsal horn[56]. However, dissimilarities do exist and one possible pathway for the different responses of heat and pressure-induced CPM to stress is a subset of muscle primary afferent located in the lateral spinal nucleus of the spinal cord, which respond only to pressure to deep tissue and not to pressure or heat from the skin[57]. It is possible that noxious stimuli transmitted through this pathway is differently affected by descending modulation than the traditional pathways described above.

The present study was done at 08.30 or 10.30 in the morning and time-of-day did not correlate or interact with the CPM effects. In line with this, Aviram and colleagues did not find any diurnal involvement in the effectiveness of CPM[55]. However, two other studies on CPM during stress, which did not restrict data collection to account for diurnal changes, show either no effect of stress on CPM[26] or reduced CPM during stress[23]. In favor of involvement of circadian variations are the studies from Geva and colleagues[20-22] who started data collected at 1 pm on all participants, and found reduction in CPM during stress in all three studies. It is therefore possible that the circadian rhythm (including cortisol levels) may play a role in CPM-effect although no significant association between cortisol levels during stress and the reduction of CPM was reported in any of the studies.

Stress vs control-condition

A recent study on mental stress and pain sensitivity compared results to a control group, which was exposed to a comparable, non-stressful, condition[30]. In the current study, all participants were exposed to a stress-task as well as a comparable control condition[39] and had pain sensitivity and CPM measured before and after both. In disagreement with the hypothesis, there were no significant differences between the stress-condition and the control-condition. These results are also in line with the study by Timmers and colleagues[30], who found no difference in PDT between a ‘control-group’ and a ‘stress-group’ immediately after control or stress condition, respectively. Only studies, which compared pain sensitivity after stress with baseline and recovery[21-23] found reduced CPM-effects after stress. Confounding factors such as distraction[58] could explain some of this inconsistency. Also, it must be considered that this study, as well as the one by Timmers et al.[30], were accomplished early in the day, and the studies by Geva et al.[20-

22] were done at 1 p.m. making a case for possible differences in diurnally related cortisol levels.

Cortisol and CPM

Although there was no significant increase in cortisol during stress in this study, a negative correlation between change in cortisol from baseline to post-MIST and the effectiveness of a conditioning stimulus (i.e. PDT-CS before minus after MIST) was found. This suggests that the ability of a conditioning stimulus to reduce pain sensitivity (i.e. positive CPM-effect) is increasingly compromised with increases in cortisol-levels after mental stress.

Measuring stress-related changes in cortisol levels is not trivial[37,59] and the peak in morning cortisol may counteract with the measurable effects of mental stress and increase the risk of insignificant findings[59]. Geva and colleagues could not show a correlation between cortisol and CPM[20-22] but they found negative correlations between perceived stress and CPM, i.e. inhibition of the CPM-effect during stress, similar to the result in the present study when using cortisol changes as a marker of stress.

Results in the present study and others[22-24,57] imply that cortisol can only account for a small part of the variation in CPM-response and other factors such as melatonin[60] and attention[38] may also interfere with pain modulation and/or cortisol. Therefore, it seems likely that the interaction between mental stress and pain modulation is multifactorial with a high degree of interpersonal variance[23,31,57].

Repeated stimulations after stress

This is the first study to implement repeated test-stimuli into a stress-protocol. It has been suggested that while CPM is considered a 'dynamic' measure of pain[50], it normally only measures CPM in a small temporal window. The present study also intended to analyze

dynamic (CPM) and static (PDT) measures of pain sensitivity for 10 minutes after acute stress in a previously validated paradigm[13]. However, no differences in PDT or CPM were found after MIST or any interactions over time within the 10 minutes (data analysis not shown). No studies have previously looked into the temporal changes in PDT and or CPM after stress. Based on the data in this study, it seems that repeated measures during the expected rise in cortisol after MIST, compliments the findings from single measurements in similar paradigms.

Limitations

This explorative study was conducted only on healthy, male participants and may not be an accurate indicator for CPM or pain sensitivity during stress for healthy female participants. Importantly, it is not likely that experimental, acute stress assimilates real-life stress, which is why the results should be extrapolated to real-life situations or patients suffering from chronic pain. The study was designed to reduce any expectations of stress, which could influence the results, however, at the same time it serves as a limitation that this design did not allow for real-time measurement of perceived stress. There are strengths to the laboratory-based assessments such as the strict control over events during the stressor making comparison between studies possible. This study did not include a rest period before baseline cortisol measurement, which may have contributed to the variability in responses. However, analysis are based upon individual changes so it is unlikely that this has impacted the results. It serves as a strength to this study that the results are compared to a control-condition designed to match the stress-condition, and that both conditions were tested on the same cohort. The included number of participants was based on *a priori* power calculation, however, power calculations are based on an estimate and statistical power was set at 70%, which means that results in the present

study should be viewed in the context of the complete body of evidence in this area, including future studies.

Conclusion

In this study, no significant differences in pain sensitivity and CPM could be measured after a brief episode of experimental stress compared to before or after a control-stress condition. This may be partly modality related since other studies have found a reduction in CPM after mental stress when CPM was induced by thermal stimuli. However, this study showed a correlation between the changes in pain sensitivity during the conditioned test-stimulus and cortisol levels during experimental stress. The effect size of this correlation is moderate, which suggests that cortisol could influence on the effectiveness of the descending pain modulatory system under stress, and thus indicate that cortisol and descending pain modulation rely on overlapping mechanisms.

Conflicts of interest

Nocitech is partly owned by Aalborg University.

Author contributions

TGN and MH contributed in the study design and planning. JNP assisted in planning and analyzing cortisol data. LP contributed in planning the MIST-protocol. All authors contributed to the manuscript.

REFERENCES

1. Gore M, Sadosky A, Stacey BR, Tai K-S, Leslie D. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. Spine. 2012 May 15;37(11):E668–77.

2. Nordstoga AL, Nilsen TIL, Vasseljen O, Unsgaard-Tøndel M, Mork PJ. The influence of multisite pain and psychological comorbidity on prognosis of chronic low back pain: longitudinal data from the Norwegian HUNT Study. BMJ Open. 2017 Jun 6;7(5):e015312.

3. McEwen BS, Kalia M. The role of corticosteroids and stress in chronic pain conditions. Metab Clin Exp. 2010 Oct;59 Suppl 1:S9–15.

4. Potvin S, Marchand S. Pain facilitation and pain inhibition during conditioned pain modulation in fibromyalgia and in healthy controls. PAIN. 2016 Aug;157(8):1704–10.

5. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. PAIN. 2013 Sep;154(9):1588–94.

6. Koelbaek Johansen M, Graven-Nielsen T, Schou Olesen A, Arendt-Nielsen L. Generalised muscular hyperalgesia in chronic whiplash syndrome. 1999 Nov;83(2):229–34.

7. Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. Eur J Pain. 2018 Feb;22(2):216–41.

8. Yarnitsky D, Bouhassira D, Drewes AM, Fillingim RB, Granot M, Hansson P, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. Eur J Pain. 2015 Jul;19(6):805–6.

9. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, et al. Recommendations on terminology and practice of psychophysical DNIC testing. Eur J Pain. 2010 Apr;14(4):339–9.

10. Imai Y, Petersen KK, Mørch CD, Arendt-Nielsen L. Comparing test–retest reliability and magnitude of conditioned pain modulation using different combinations of test and conditioning stimuli. Somatosens Mot Res. 2016 Sep 20;:1–9.

11. Petersen KK, Vaegter HB, Arendt-Nielsen L. An updated view on the reliability of different protocols for the assessment of conditioned pain modulation. PAIN. 2017 May;158(5):988.

12. Graven-Nielsen T, Izumi M, Petersen KK, Arendt-Nielsen L. User-independent assessment of conditioning pain modulation by cuff pressure algometry. Eur J Pain. 2017 Mar;21(3):552–61.

13. Hoegh M, Petersen KK, Graven-Nielsen T. Effects of repeated conditioning pain modulation in healthy volunteers. Eur J Pain. 2018 Nov;22(10):1833–43.

14. Nahman-Averbuch H, Granovsky Y, Coghill RC, Yarnitsky D, Sprecher E, Weissman-Fogel I. Waning of "Conditioned Pain Modulation": A Novel Expression of Subtle Pronociception in Migraine. Headache: The Journal of Head and Face Pain. 2013 Jul 1;53(7):1104–15.

15. Cohen S, Gianaros PJ, Manuck SB. A Stage Model of Stress and Disease. Perspectives on Psychological Science. 2016 Jul 29;11(4):456–63.

16. Butler RK, Finn DP. Stress-induced analgesia. Prog Neurobiol. 2009 Jul;88(3):184–202.

17. Chapman CR, Tuckett RP, Song CW. Pain and Stress in a Systems Perspective: Reciprocal Neural, Endocrine, and Immune Interactions. 2008 Feb;9(2):122–45.

18. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull. 2004 Jul;130(4):601–30.

19. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. J Clin Invest. 2010 Nov 1;120(11):3779–87.

20. Geva N, Pruessner J, Defrin R. Acute psychosocial stress reduces pain modulation capabilities in healthy men. PAIN. 2014 Nov;155(11):2418–25.

21. Geva N, Defrin R. Opposite Effects of Stress on Pain Modulation Depend on the Magnitude of Individual Stress Response. J Pain. Elsevier Inc; 2018 Apr 1;19(4):360–71.

22. Geva N, Pruessner J, Defrin R. Triathletes Lose Their Advantageous Pain Modulation under Acute Psychosocial Stress. Med Sci Sports Exerc. 2016 Sep 23;49(2):1–341.

23. Nilsen KB, Christiansen SE, Holmen LB, Sand T. The effect of a mental stressor on conditioned pain modulation in healthy subjects. Scandinavian Journal of Pain. 2012 Jul;3(3):142–8.

24. Caceres C, Burns JW. Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. PAIN. 1997 Feb;69(3):237–44.

25. Cathcart S, Petkov J, Pritchard D. Effects of induced stress on experimental pain sensitivity in chronic tension-type headache sufferers. Eur J Neurol. 2008 Jun;15(6):552–8.

26. Cathcart S, Winefield AH, Lushington K, Rolan P. Noxious Inhibition of Temporal Summation is Impaired in Chronic Tension-Type Headache. Headache: The Journal of Head and Face Pain. 2010 Mar;50(3):403–12.

27. Bement MH, Weyer A, Keller M, Harkins AL, Hunter SK. Anxiety and stress can predict pain perception following a cognitive stress. Physiol Behav. Elsevier Inc; 2010 Aug 4;101(1):87–92.

28. Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, et al. Stress-Induced Allodynia – Evidence of Increased Pain Sensitivity in Healthy Humans and

Patients with Chronic Pain after Experimentally Induced Psychosocial Stress. Paul F, editor. PLoS ONE. 2013 Aug 7;8(8):e69460.

29. Reinhardt T, Kleindienst N, Treede R-D, Bohus M, Schmahl C. Individual modulation of pain sensitivity under stress. Pain Med. 2013 May;14(5):676–85.

30. Timmers I, Kaas AL, Quaedflieg CWEM, Biggs EE, Smeets T, de Jong JR. Fear of pain and cortisol reactivity predict the strength of stress-induced hypoalgesia. Eur J Pain. Wiley-Blackwell; 2018 Aug 1;22(7):1291–303.

31. Bali A, Jaggi AS. Clinical experimental stress studies: methods and assessment. Reviews in the Neurosciences. 2015 Oct 1;26(5):555–79.

32. Sobas EM, Reinoso R, Cuadrado-Asensio R, Fernández I, Maldonado MJ, Pastor JC. Reliability of Potential Pain Biomarkers in the Saliva of Healthy Subjects: Inter-Individual Differences and Intersession Variability. Nater UM, editor. PLoS ONE. Public Library of Science; 2016;11(12):e0166976.

33. Godfrey KM, Herbert M, Strachan E, Mostoufi S, Crofford LJ, Buchwald D, et al. Dexamethasone-suppressed Salivary Cortisol and Pain Sensitivity in Female Twins. The Clinical journal of Pain. 2016 Jun;:1–8.

34. Dickerson SS, Kemeny ME. Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. Psychol Bull. 2004;130(3):355–91.

35. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009 Feb;34(2):163–71.

36. Dawans von B, Kirschbaum C, Heinrichs M. The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. Psychoneuroendocrinology. 2011 May;36(4):514–22.

37. Kalman BA, Grahn RE. Measuring salivary cortisol in the behavioral neuroscience laboratory. J Undergrad Neurosci Educ. 2004;2(2):A41–9.

38. Gaab J, Jiménez J, Voneschen L, Oschwald D, Meyer AH, Nater UM, et al. Psychosocial Stress-Induced Analgesia: An Examination of Effects on Heat Pain Threshold and Tolerance and of Neuroendocrine Mediation. Neuropsychobiology. 2017 Apr 12;74(2):87–95.

39. Dedovic K, Renwick R, Mahani NK, Engert V, Lupien SJ, Pruessner JC. The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. J Psychiatry Neurosci. 2005 Sep;30(5):319–25.

40. Dmitrieva NO, Almeida DM, Dmitrieva J, Loken E, Pieper CF. A day-centered approach to modeling cortisol: Diurnal cortisol profiles and their associations among U.S. adults. Psychoneuroendocrinology. 2013 Oct;38(10):2354–65.

41. Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. Psychological Assessment. 1995;7(4):524–32.

42. Labor Diagnostika Nord GmbH. Cortisol Saliva ELISA [Internet]. 2018. Available from: http://www.ldn.de/sites/default/files/downloads/SA%20E-6000.pdf.

43. Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L. Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: a reliability study. PAIN. PAIN; 2015 Nov;156(11):2193–202.

44. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry. J Pain. 2002 Feb;3(1):28–37.

45. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, et al. An endocannabinoid mechanism for stress-induced analgesia. Nature. 2005 Jun 23;435(7045):1108–12.

46. Ford GK, Finn DP. Clinical correlates of stress-induced analgesia: Evidence from pharmacological studies. PAIN. 2008 Nov;140(1):3–7.

47. Martenson ME, Cetas JS, Heinricher MM. A possible neural basis for stress-induced hyperalgesia. PAIN. 2009 Apr;142(3):236–44.

48. Pinto-Ribeiro F, Moreira V, Pêgo JM, Leão P, Almeida A, Sousa N. Antinociception induced by chronic glucocorticoid treatment is correlated to local modulation of spinal neurotransmitter content. Mol Pain. SAGE Publications; 2009;5:41.

49. Cathcart S, Winefield AH, Lushington K, Rolan P. Effect of mental stress on cold pain in chronic tension-type headache sufferers. J Headache Pain. 2009 Jun 5;10(5):367–73.

50. Marcuzzi A, Wrigley PJ, Dean CM, Adams R, Hush JM. The long-term reliability of static and dynamic quantitative sensory testing in healthy individuals. PAIN. 2017 Jul;158(7):1217–23.

51. Waller R, Straker L, O'Sullivan P, Sterling M, Smith A. Reliability of pressure pain threshold testing in healthy pain free young adults. Scandinavian Journal of Pain. Elsevier B.V; 2015 Oct 1;9:38–41.

52. Nothnagel H, Puta C, Lehmann T, Baumbach P, Menard M, Gabriel B, et al. How stable are quantitative sensory testing measurements over time? Report on 10-week reliability and agreement of results in healthy volunteers. JPR. 2017;Volume 10:2067–78.

53. Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: a salience detection system for the body. Prog Neurobiol. 2011 Jan;93(1):111–24.

54. al'Absi M, Petersen KL. Blood pressure but not cortisol mediates stress effects on subsequent pain perception in healthy men and women. PAIN. 2003 Dec;106(3):285–95.

55. Aviram J, Shochat T, Pud D. Pain Perception in Healthy Young Men Is Modified by Time-Of-Day and Is Modality Dependent. Pain Medicine. 2015;16(6):1137–44.

56. Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. Scand J Rheumatol Suppl. 2006;122:1–43.

57. Sikandar S, West SJ, McMahon SB, Bennett DL, Dickenson AH. Sensory processing of deep tissue nociception in the rat spinal cord and thalamic ventrobasal complex. Physiol Rep. 2017 Jul 18;5(14):e13323–13.

58. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. “Pain inhibits pain” mechanisms: Is pain modulation simply due to distraction? PAIN. 2010 Jul;150(1):113–20.

59. Atchley R, Ellingson R, Klee D, Memmott T, Oken B. A cognitive stressor for event-related potential studies: the Portland arithmetic stress task. Stress (Amsterdam, Netherlands). Taylor & Francis; 2017 May;20(3):277–84.

60. Wilhelmsen M, Amirian I, Reiter RJ, Rosenberg J, Gögenur I. Analgesic effects of melatonin: a review of current evidence from experimental and clinical studies. Journal of Pineal Research. 2011 May 26;51(3):270–7.

FIGURE LEGENDS

Figure 1. Overview over the study. Saliva was sampled at baseline before questionnaires and cuff pressure test-stimuli (TS) on the dominant leg (blue cuff) and conditioning stimuli (CS) on the non-dominant leg (red cuff). The *Montreal Imaging Stress Task* (MIST) was performed after the first trial of six cuff test-stimuli (pre-stress) and was followed by post-stress test-stimuli. The *Control* was an arithmetic task without a social stress component.

Figure 2. Mean (+SEM, n = 25) pressure detection thresholds (PDT) recorded with and without conditioning on the contralateral leg. The average of unconditioned PDTs (PDT_{avg}) and conditioned PDTs ($PDT\text{-}CS_{avg}$) is presented. There was an increase in $PDT\text{-}CS_{avg}$ compared to the unconditioned PDT_{avg} equal to a significant CPM-effect (*, $p = 0.001$, $\eta_p^2 = 0.40$).

Figure 3. A scatter-plot and linear regression comparing the change in cortisol (Δ-cortisol) after MIST and (a) conditioned pain detection thresholds (Δ-PDT-CS) after MIST or (b) unconditioned pain detection thresholds (Δ-PDT) after MIST. The linear regressions show that increased cortisol during MIST predicts decreased PDT during conditioning (a) but not without conditioning (b).

FIGURES

Figure 1: Timeline

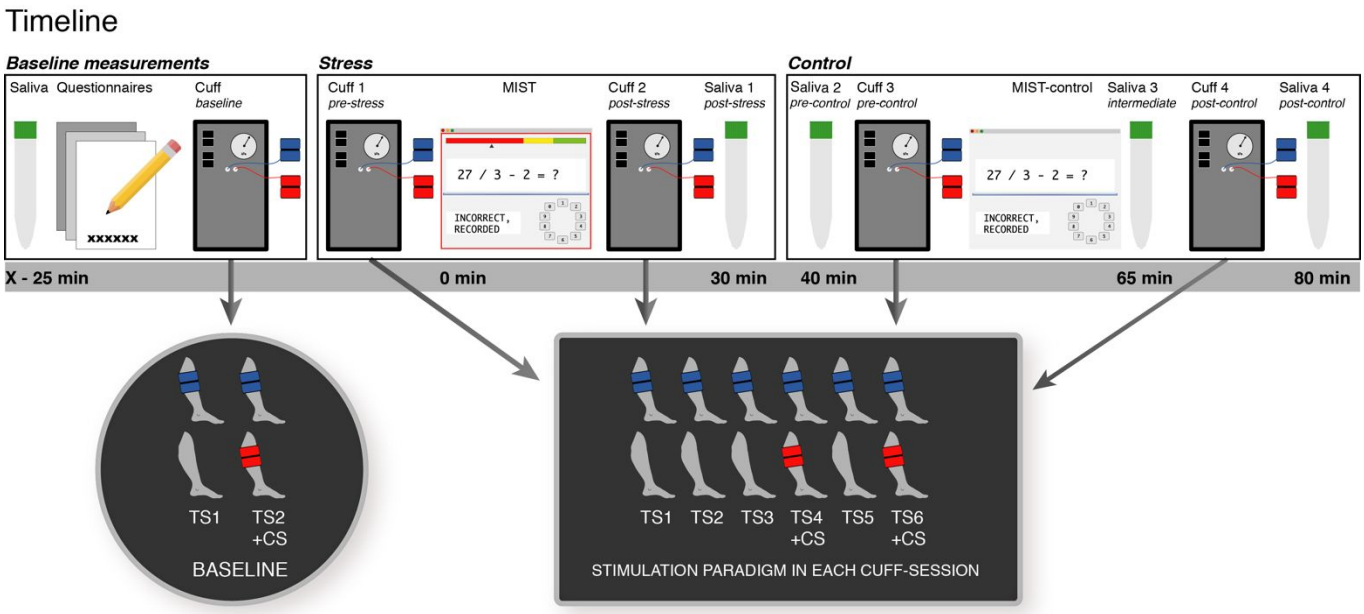


Figure 2: Effect of conditioning

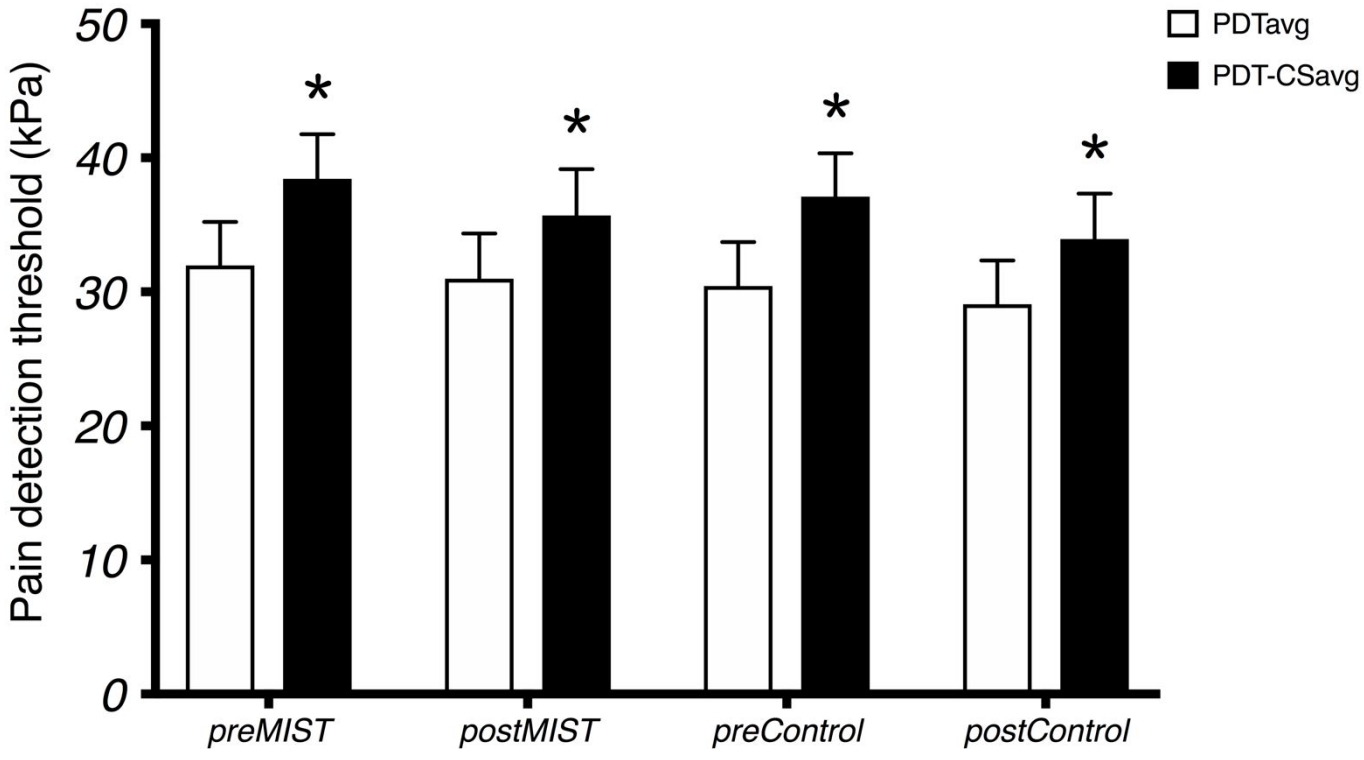


Figure 3: Linear regression

Fig 3a

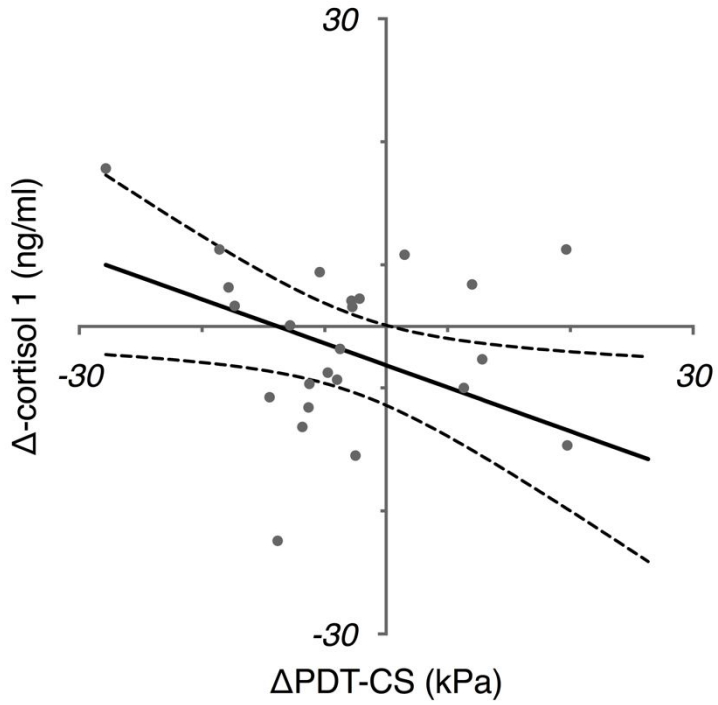
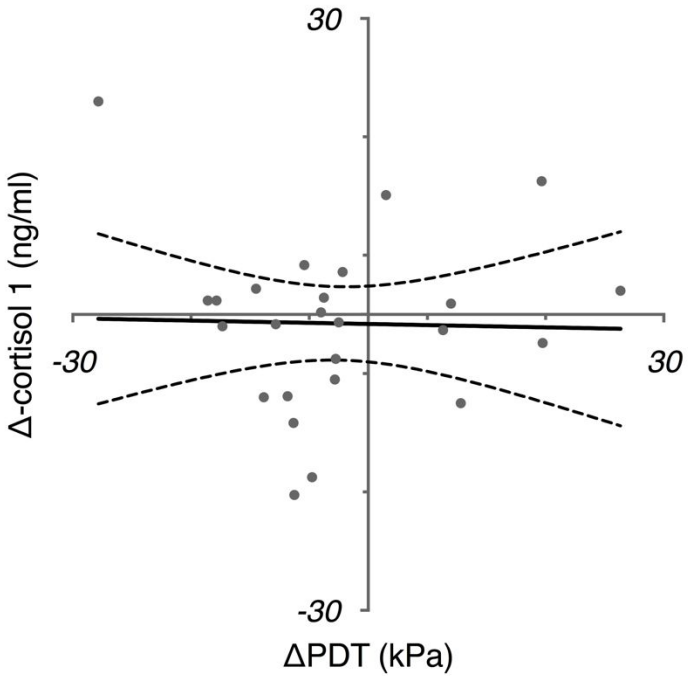
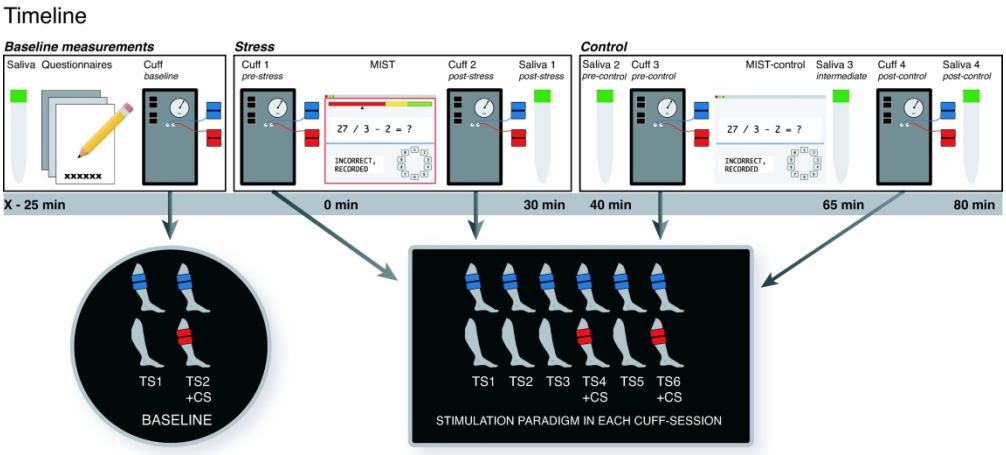


Fig 3b





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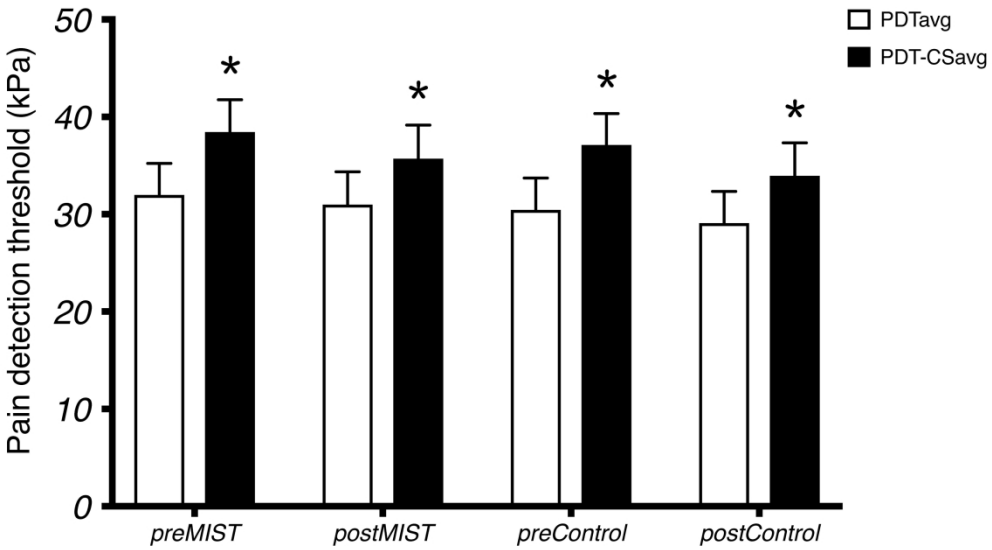

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Fig 3a

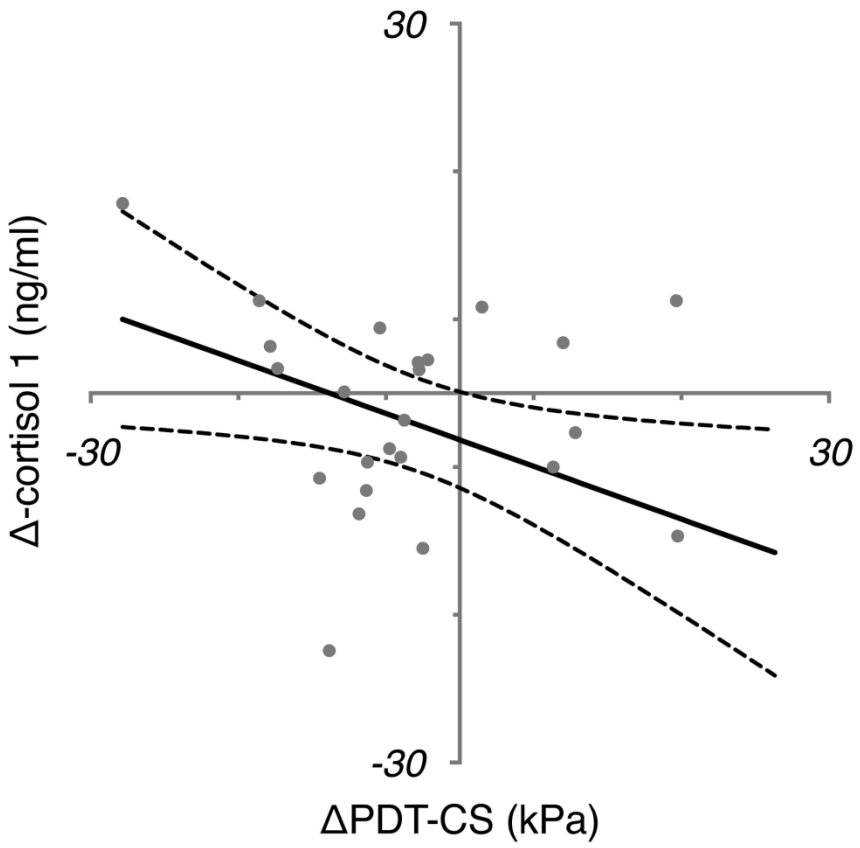

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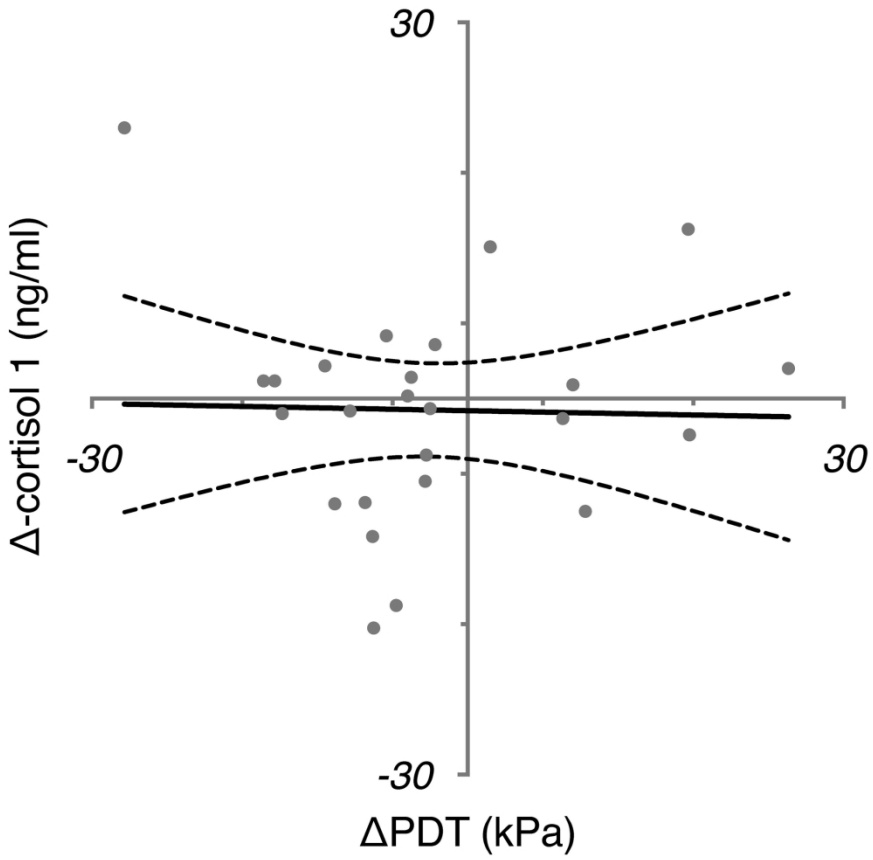
Fig 3b



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197x186mm (300 x 300 DPI)