



## The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning

Høgh, Morten Sebastian; Nørgaard Poulsen, Jeppe; Petrini, Laura; Graven-Nielsen, Thomas

*Published in:*  
Pain Medicine

*DOI (link to publication from Publisher):*  
[10.1093/pm/pnz115](https://doi.org/10.1093/pm/pnz115)

*Publication date:*  
2020

*Document Version*  
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Høgh, M. S., Nørgaard Poulsen, J., Petrini, L., & Graven-Nielsen, T. (2020). The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning. *Pain Medicine*, 21(2), 317-325.  
<https://doi.org/10.1093/pm/pnz115>

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

### Take down policy

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.

**The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning**

Journal:	<i>Pain Medicine</i>
Manuscript ID	PME-ORR-Aug-18-611.R2
Manuscript Type:	Original research
Date Submitted by the Author:	19-Apr-2019
Complete List of Authors:	Hoegh, Morten; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Poulsen, Jeppe; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Petrini, Laura; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Graven-Nielsen, Thomas; Aalborg University, Laboratory for Experimental Pain Research, Centre for Sensory-Motor Interaction, Department of Health Science and Technology
Keywords:	Stress-induced analgesia, Cortisol, Conditioned Pain Modulation (CPM), Endogenous Pain Modulation, Diffuse Noxious Inhibitory Controls (DNIC), Pain mechanisms, Montreal Imaging Stress Test (MIST), Mental stress, Social stress

**SCHOLARONE™**  
 Manuscripts

1  
2  
3 1 **THE EFFECT OF STRESS ON REPEATED PAINFUL STIMULI WITH AND WITHOUT**  
4  
5 2  
6 **PAINFUL CONDITIONING**  
7

8  
9 3 Morten Hoegh, MSc<sup>1</sup>, Jeppe N Poulsen, MSc<sup>1</sup>, Laura Petrini, Ph.D.<sup>1</sup>, Thomas Graven-  
10 4 Nielsen, Ph.D., DMSc.<sup>1\*</sup>  
11

12  
13 5 <sup>1</sup> Center for Neuroplasticity and Pain (CNAP), SMI, Aalborg University, Denmark  
14

15 6 **Original research article: Pain Medicine**  
16

17  
18 7 **Keywords:** Stress-induced analgesia, Cortisol, Conditioned Pain Modulation (CPM),  
19  
20 8 Endogenous Pain Modulation, Diffuse Noxious Inhibitory Controls (DNIC), Pain  
21  
22 9 mechanisms, Montreal Imaging Stress Test (MIST), Mental stress, Social stress.  
23  
24

25  
26 10 **\*Corresponding Author:**

27 11 Prof. Thomas Graven-Nielsen, Ph.D., DMSc.

28 12 Center for Neuroplasticity and Pain (CNAP)

29 13 SMI, Department of Health Science and Technology

30 14 Faculty of Medicine, Aalborg University

31 15 Fredrik Bajers Vej 7 D3, DK-9220 Aalborg, Denmark

32 16 Phone: +45 9940 9832, Fax: +45 9815 4008, E-mail: [tgn@hst.aau.dk](mailto:tgn@hst.aau.dk)  
33  
34  
35  
36  
37  
38 17

39 18 **Funding sources:** Center for Neuroplasticity and Pain (CNAP) is supported by the Danish  
40 19 National Research Foundation (DNRF121).  
41  
42  
43

44 20 **Conflicts of interest:** Nocitech is partly owned by Aalborg University.  
45

46 21 **Significance:** This study did not show any significant effect of experimental stress on pain  
47 22 sensitivity or conditioned pain modulation (CPM), but a correlation was found between  
48 23 changes in conditioned pain sensitivity and cortisol levels. Mechanisms regulating cortisol  
49 24 levels may interact with the effectiveness of a conditioning stimulus, and thus reduces the  
50 25 effectiveness of CPM.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 1 **ABSTRACT**

2 **Objectives.** Stress and pain have been interrelated in clinical widespread pain conditions.  
3  
4 Studies indicate that acute, experimental stress in healthy volunteers has a negative effect  
5  
6 on the descending inhibitory pain control system and thus the ability to inhibit one painful  
7  
8 stimulus with another (conditioned pain modulation, CPM) although without effect on  
9  
10 general pain sensitivity. CPM-effects can be assessed immediately after the stress-  
11  
12 induction, whereas some physiological stress responses (e.g. cortisol release) are delayed  
13  
14 and longer lasting. It is unclear whether CPM may relate to stress-induced increases in  
15  
16 cortisol.  
17  
18  
19  
20  
21  
22  
23

24 **Design.** Twenty-five healthy men had CPM-effects measured over a period of 10 minutes.  
25  
26 Pain detection thresholds (PDT) was assessed by repeated test-stimuli with cuff algometry  
27  
28 on one leg, with and without painful cuff-pressure conditioning on the contralateral leg.  
29  
30 CPM-effects, assessed as the increase in PDT during conditioning stimulation compared  
31  
32 to without, were measured before and after experimental stress and a control condition  
33  
34 (Montreal Imaging Stress Task, MIST). Saliva cortisol levels and self-perceived stress  
35  
36 were collected.  
37  
38  
39

40 **Results.** Participants reported MIST to be more stressful compared with MIST-control but  
41  
42 cortisol levels did not change significantly from baseline. In all sessions, PDT increased  
43  
44 during conditioning ( $P=0.001$ ) although MIST compared with MIST-control, had no  
45  
46 significant effect on PDT or CPM-effects. A negative correlation between changes in  
47  
48 cortisol and conditioned PDT was found when applying MIST ( $P<0.03$ ).  
49  
50

51 **Conclusion.** No significant effect of stress was found on CPM compared to a matched  
52  
53 control-condition. Individual changes in experimental stress and in conditioned pain  
54  
55 sensitivity may be linked with cortisol.  
56  
57  
58  
59  
60

## 1 INTRODUCTION

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

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

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

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

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

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

## 18 **METHODS**

### 19 *Subjects*

20 Geva et al (2014) found a reduced CPM-effect during stress of approximately 50% and  
21 based on a sample size calculation with a significance level at 0.05 and a statistical power  
22 of 70% the total sample size should be at least 25 subjects for a 50% CPM reduction.  
23 Twenty-five healthy men between 22 and 72 years participated (average 30.3 years,  
24 standard deviation 10.9 years). Exclusion criteria were 1) diagnosis of sleep, neurological,  
25 mental or musculoskeletal disorders, 2) pain within the last 48 hours or any use of sleep or  
26

1  
2  
3 1 pain medication in this period, 3) any history of chronic stress or chronic pain, or skin  
4  
5 2 lesions in the test-areas (lower legs), 4) less than 6 hours of sleep over the last 24 hours,  
6  
7  
8 3 and 5) smoking, exercise, food or any drinks other than water for the two hours prior to the  
9  
10 4 study. All participants received oral and written information about the experiment and gave  
11  
12 5 their verbal and written consent prior to the study. Exclusion criteria were verbally  
13  
14 6 confirmed after consent was given. The study was approved by the local Ethics Committee  
15  
16  
17 7 (N-20170033) and was performed in accordance with the Helsinki Declaration.  
18  
19 8

### 9 *Experimental procedures*

10 The study was conducted in a two-hour session at either 08.30 or 10.30 am to control for  
11  
12 11 diurnal changes in freely available cortisol[40]. At baseline participants gave a saliva  
13  
14 12 sample, completed the pain catastrophizing questionnaire[41], and rated how they  
15  
16 13 perceive their math-skills on a numerical rating scale (NRS) from 0 ('I'm exceptionally bad  
17  
18 14 at math') to 10 ('I'm exceptionally good at math'). Previous studies, randomizing  
19  
20 15 participants to the order of stress and control conditions, find similar results[23,24,38] as  
21  
22 16 those who have not [20-22,25-29]. To avoid expectations of intentional stress during the  
23  
24 17 experiment, participants were informed that they would be exposed to arithmetic tasks  
25  
26 18 during painful stimuli. Consequently, assessment of perceived stress was done during  
27  
28 19 debriefing rather than during the stress task. Four samples of saliva cortisol were obtained  
29  
30 20 in addition to the baseline sample (Fig 1).  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48

49 21 Experimental procedures were done with participants positioned in an inclined bed  
50  
51 22 where they also remained during rest. The Montreal Imaging Stress Task (MIST) was used  
52  
53 23 to induce acute, experimental stress[39]. The protocol included a non-stressful training  
54  
55 24 session (60 s), the stress-induction (MIST) consisting of two rounds of arithmetic in  
56  
57 25 combination with social, visual and auditory stressors (2 x 360 s, separated by a three  
58  
59  
60

1 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

1  
2  
3 1 developed by a psychologist, with experience from similar paradigms, to match the context  
4  
5  
6 2 of the study. The training-session included a trial-run at the arithmetic task but did not  
7  
8 3 include any of the stressors. After the training-session, questions or comments from the  
9  
10 4 participant were addressed by the investigator, and the participant was instructed to 'solve  
11  
12 5 as many calculations as possible and to be as correct as possible'.

13  
14  
15 6 During MIST and MIST-control the investigator left the room but participants were  
16  
17 7 informed that they would be monitored[39]. The investigator re-entered the room  
18  
19 8 approximately 30 s before the end of the first round of arithmetic tasks. After the first round  
20  
21 9 the investigator briefly revised the results and, as negative feedback, the participant was  
22  
23 10 reminded that he was expected to get 80-90% of the tasks correct (although the algorithm  
24  
25 11 makes it impossible)[39]. Measures of successful MIST was the percentage of correct  
26  
27 12 calculations during MIST (approximately 45%) and MIST-control (approximately 90%),  
28  
29 13 respectively[21,39].  
30  
31  
32  
33  
34

### 35 15 *Saliva cortisol*

36  
37 16 Saliva samples were collected using Cortisol-Salivette® with a citric-acid, which helped to  
38  
39 17 facilitate saliva production (SARSTEDT AG & Co., Nümbrecht, Germany). The participants  
40  
41 18 were instructed to chew on the cotton swap for one minute and then place it back into  
42  
43 19 the Salivette® tube. The samples were put in a thermos-box with ice until saliva was  
44  
45 20 recovered from the cotton swap by centrifugation for 2 minutes at 1000 rpm (no more than  
46  
47 21 four hours after collection). Once saliva was extracted the cotton bud and inner tube was  
48  
49 22 discarded and the saliva sample, was frozen at -80°C until further analysis. The samples  
50  
51 23 were analyzed using a standardized ELISA-kit (SA E-6000, LDN Labor Diagnostika Nord,  
52  
53 24 Germany). Samples were diluted if values were outside the standard curve (0 - 30 ng/ml).  
54  
55 25 Based on manufacturer guidelines the kit has a 95% confidence intervals of 1-11.3 ng/ml  
56  
57  
58  
59  
60

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

#### 6 *Pain sensitivity assessment by cuff algometry*

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

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

1  
2  
3 1 at least 10 minutes. A trial consisted of six painful cuff test stimuli (Fig 1: TS1-6) applied to  
4  
5 2 the dominant lower leg. For each test-stimulus the cuff was inflated (1 kPa/s) and the pain  
6  
7 3 detection threshold (PDT) was extracted. For analysis, the average of unconditioned PDTs  
8  
9 4 in each trial was calculated ( $PDT_{avg}$ ).  
10  
11  
12  
13 5

### 14 6 *Conditioning pain modulation assessed by cuff algometry*

17 7 In parallel to the 4<sup>th</sup> and 6<sup>th</sup> test stimuli a constant, painful conditioning stimulus on the  
18  
19 8 non-dominant leg was applied. During baseline-testing PDT and PTT were recorded (see  
20  
21 9 above) on the non-dominant leg. The conditioning intensity used throughout the study was  
22  
23 10 70% of baseline PTT[10] and the duration of the conditioning was maximally 104 seconds  
24  
25 11 (conditioning was terminated a few seconds after the test-stimulus was terminated).  
26  
27 12 Participants verbally reported the perceived pain intensity of the conditioning stimulus at  
28  
29 13 the beginning and immediately after the test-stimulus was terminated. Pain intensity was  
30  
31 14 scored on a NRS (0-10) with 0 defined as 'no pain' and 10 'maximal pain'.  
32  
33  
34

35 15 For analysis the average of PDTs during conditioning ( $PDT-CS_{avg}$ ) was extracted.  
36  
37 16 Moreover, the CPM-effects were calculated based on the change in PDT during  
38  
39 17 conditioning ( $PDT-CS_{avg}$ ) compared to the average of the four unconditioned PDT  
40  
41 18 recordings ( $PDT_{avg}$ ). As a consequence, a positive CPM-effect reflects that PDTs  
42  
43 19 increased (reduced pain sensitivity) during conditioning.  
44  
45  
46  
47 20  
48

### 49 21 *Statistics*

51 22 Unless otherwise specified, results are presented as mean and standard error of the mean  
52  
53 23 (SEM). Q-Q plots were used to confirm normal distribution (IBM® SPSS® Statistics  
54  
55 24 version 23) by visual inspection. Parameters were normally distributed or otherwise log-  
56  
57 25 transformed.  
58  
59  
60

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

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

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

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

## 1 RESULTS

### 2 *Validation of the stress response*

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

8 Initial analysis of saliva cortisol found concentrations beyond 30 ng/ml, which were  
9 above the standard curve for the ELISA-kit. After samples were diluted and re-analyzed,  
10 variability in the results and the baseline cortisol remained higher than expected in some  
11 subjects when comparing to manufacturer reference values[42]. The change in cortisol  
12 over time ( $\Delta$ -cortisol 1:  $-2.86 \pm 2.4$  ml/ng,  $\Delta$ -cortisol 2:  $-1.08 \pm 2.3$  ml/ng,  $\Delta$ -cortisol 3:  $-$   
13  $1.02 \pm 1.8$  ml/ng and  $\Delta$ -cortisol 4:  $-0.91 \pm 1.8$  ml/ng) was not significant (ANOVA;  $F(3, 69)$   
14  $= 0.164$ ,  $P = 0.85$ ) and there were no interactions ( $P = 0.44$ ) or indication of difference  
15 between the participants tested early or late in the day ( $P = 0.22$ ).

### 17 *Pain detection thresholds of unconditioned test-stimuli*

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

### 22 *Conditioned pain modulation*

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

1 effect was  $5.9 \pm 1.5$  kPa and the CPM-effects did not interact or change significantly over  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 effect was  $5.9 \pm 1.5$  kPa and the CPM-effects did not interact or change significantly over  
2 time (ANOVA:  $F(1, 24) = 0.18$ ,  $P = 0.68$ ,  $\eta_p^2 = 0.01$ ) or between sessions (ANOVA:  $F(1,$   
3  $24) = 0.88$ ,  $P = 0.36$ ,  $\eta_p^2 = 0.04$ ).

#### 5 *Change in cortisol during MIST correlates with change in conditioned PDT*

6 The linear regressions showed that  $\Delta$ -cortisol 1 (i.e. changes in cortisol from baseline to  
7 postMIST) could predict the change in conditioned PDT (PDT-CS<sub>avg</sub>) during MIST. This  
8 correlation explained 19% of the variance (Fig 3a;  $R^2 = 0.19$ ,  $F(1, 23) = 5.23$ ,  $P = 0.03$ )  
9 and unstandardized coefficients ( $B = -0.36 \pm 0.16$ ,  $P = 0.03$ ) suggest that the change in  
10 cortisol was inversely related to the change in PDT-CS<sub>avg</sub> during MIST (i.e. an increase in  
11 cortisol reduced PDT-CS<sub>avg</sub>). The effect size of this correlation is equal to approximately  
12 0.5 standard deviation and considered 'moderate'[45]. No significant correlations were  
13 found for changes in PDT<sub>avg</sub> versus cortisol during MIST (Fig 3b:  $B = -0.03 \pm 0.28$ ,  $P =$   
14 0.9).

## 16 **DISCUSSION**

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

### 1 *Experimental stress and pain sensitivity*

2  
3  
4  
5  
6 2 Activation of the hypothalamic-pituitary-adrenal (HPA) axis is the key mechanism behind a  
7  
8 3 physiological stress response and the release of stress-induced cortisol, however, the  
9  
10 4 interaction between the HPA-axis and pain is highly complex and likely involves the entire  
11  
12 5 neuroaxis[3]. At a system level, stress-induced analgesia is believed primarily to engage  
13  
14 6 endocannabinergic pathways[45,46] whereas stress-induced hyperalgesia seems to be  
15  
16 7 more related to the descending modulation through the rostral ventromedial medulla  
17  
18 8 (RVM)[47]. At a mechanistic level, cortisol-related modulation of nociception is likely to  
19  
20 9 appear directly in the dorsal horn through co-location of glucocorticoid receptors,  
21  
22 10 substance P-receptors and CGRP-receptors[48] as well as through regulation of  
23  
24 11 cannabinoids[3].

25  
26  
27  
28  
29 12 In the current study, there was no significant change in pain sensitivity during stress  
30  
31 13 compared to before stress, or compared to the control session. These findings support the  
32  
33 14 majority of the existing literature demonstrating that pressure-induced[26,27], cold-  
34  
35 15 induced[24,29,49] and heat-induced[20,29,30,38] pain sensitivity does not change  
36  
37 16 immediately after experimental stress and thus seems independent of modality. One  
38  
39 17 reason for this may be that the pain threshold is a relatively robust measure[50-52] and  
40  
41 18 that acute pain has saliency even during social stress[53]. Another reason that the pain  
42  
43 19 sensitivity did not change during stress could be that changes in cortisol during  
44  
45 20 experimental stress is either insufficient or unrelated to the pain detection threshold[38,54].  
46  
47 21 Interestingly, pain sensitivity can be reduced 15 minutes after a combination of mental  
48  
49 22 stress, negative feedback and repeated, painful stimulations[30], but not instantly after the  
50  
51 23 stressful conditions. This could indicate a link between pain sensitivity and cortisol. Indeed,  
52  
53 24 diurnal changes have previously been found to have an effect on heat and cold-induced  
54  
55 25 pain, albeit not on mechanical-induced pain[55]. Including the current study, six studies on  
56  
57  
58  
59  
60

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

#### 4 5 *Experimental stress and CPM*

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

1  
2  
3 1 The present study was done at 08.30 or 10.30 in the morning and time-of-day did not  
4  
5 2 correlate or interact with the CPM effects. In line with this, Aviram and colleagues did not  
6  
7 3 find any diurnal involvement in the effectiveness of CPM[55]. However, two other studies  
8  
9 4 on CPM during stress, which did not restrict data collection to account for diurnal changes,  
10  
11 5 show either no effect of stress on CPM[26] or reduced CPM during stress[23]. In favor of  
12  
13 6 involvement of circadian variations are the studies from Geva and colleagues[20-22] who  
14  
15 7 started data collected at 1 pm on all participants, and found reduction in CPM during stress  
16  
17 8 in all three studies. It is therefore possible that the circadian rhythm (including cortisol  
18  
19 9 levels) may play a role in CPM-effect although no significant association between cortisol  
20  
21 10 levels during stress and the reduction of CPM was reported in any of the studies.  
22  
23  
24  
25  
26  
27  
28  
29  
30

### 31 12 *Stress vs control-condition*

32  
33 13 A recent study on mental stress and pain sensitivity compared results to a control group,  
34  
35 14 which was exposed to a comparable, non-stressful, condition[30]. In the current study, all  
36  
37 15 participants were exposed to a stress-task as well as a comparable control condition[39]  
38  
39 16 and had pain sensitivity and CPM measured before and after both. In disagreement with  
40  
41 17 the hypothesis, there were no significant differences between the stress-condition and the  
42  
43 18 control-condition. These results are also in line with the study by Timmers and  
44  
45 19 colleagues[30], who found no difference in PDT between a 'control-group' and a 'stress-  
46  
47 20 group' immediately after control or stress condition, respectively. Only studies, which  
48  
49 21 compared pain sensitivity after stress with baseline and recovery[21-23] found reduced  
50  
51 22 CPM-effects after stress. Confounding factors such as distraction[58] could explain some  
52  
53 23 of this inconsistency. Also, it must be considered that this study, as well as the one by  
54  
55 24 Timmers et al.[30], were accomplished early in the day, and the studies by Geva et al.[20-  
56  
57  
58  
59  
60

1  
2  
3 1 22] were done at 1 p.m. making a case for possible differences in diurnally related cortisol  
4  
5 2 levels.  
6  
7  
8 3

#### 10 4 *Cortisol and CPM*

11  
12 5 Although there was no significant increase in cortisol during stress in this study, a negative  
13  
14 6 correlation between change in cortisol from baseline to post-MIST and the effectiveness of  
15  
16 7 a conditioning stimulus (i.e. PDT-CS before minus after MIST) was found. This suggests  
17  
18 8 that the ability of a conditioning stimulus to reduce pain sensitivity (i.e. positive CPM-effect)  
19  
20 9 is increasingly compromised with increases in cortisol-levels after mental stress.  
21  
22  
23

24 10 Measuring stress-related changes in cortisol levels is not trivial[37,59] and the peak  
25  
26 11 in morning cortisol may counteract with the measurable effects of mental stress and  
27  
28 12 increase the risk of insignificant findings[59]. Geva and colleagues could not show a  
29  
30 13 correlation between cortisol and CPM[20-22] but they found negative correlations between  
31  
32 14 perceived stress and CPM, i.e. inhibition of the CPM-effect during stress, similar to the  
33  
34 15 result in the present study when using cortisol changes as a marker of stress.  
35  
36  
37

38 16 Results in the present study and others[22-24,57] imply that cortisol can only account  
39  
40 17 for a small part of the variation in CPM-response and other factors such as melatonin[60]  
41  
42 18 and attention[38] may also interfere with pain modulation and/or cortisol. Therefore, it  
43  
44 19 seems likely that the interaction between mental stress and pain modulation is  
45  
46 20 multifactorial with a high degree of interpersonal variance[23,31,57].  
47  
48  
49  
50  
51

#### 52 22 *Repeated stimulations after stress*

53  
54 23 This is the first study to implement repeated test-stimuli into a stress-protocol. It has been  
55  
56 24 suggested that while CPM is considered a 'dynamic' measure of pain[50], it normally only  
57  
58 25 measures CPM in a small temporal window. The present study also intended to analyze  
59  
60

1  
2  
3 1 dynamic (CPM) and static (PDT) measures of pain sensitivity for 10 minutes after acute  
4  
5 2 stress in a previously validated paradigm[13]. However, no differences in PDT or CPM  
6  
7  
8 3 were found after MIST or any interactions over time within the 10 minutes (data analysis  
9  
10 4 not shown). No studies have previously looked into the temporal changes in PDT and or  
11  
12 5 CPM after stress. Based on the data in this study, it seems that repeated measures during  
13  
14 6 the expected rise in cortisol after MIST, compliments the findings from single  
15  
16 7 measurements in similar paradigms.  
17  
18  
19 8

### 9 *Limitations*

10 This explorative study was conducted only on healthy, male participants and may not be  
11 an accurate indicator for CPM or pain sensitivity during stress for healthy female  
12 participants. Importantly, it is not likely that experimental, acute stress assimilates real-life  
13 stress, which is why the results should be extrapolated to real-life situations or patients  
14 suffering from chronic pain. **The study was designed to reduce any expectations of stress,**  
15 **which could influence the results, however, at the same time it serves as a limitation that**  
16 **this design did not allow for real-time measurement of perceived stress.** There are  
17 strengths to the laboratory-based assessments such as the strict control over events  
18 during the stressor making comparison between studies possible. This study did not  
19 include a rest period before baseline cortisol measurement, which may have contributed to  
20 the variability in responses. However, analysis are based upon individual changes so it is  
21 unlikely that this has impacted the results. It serves as a strength to this study that the  
22 results are compared to a control-condition designed to match the stress-condition, and  
23 that both conditions were tested on the same cohort. The included number of participants  
24 was based on a *a priori* power calculation, however, power calculations are based on an  
25 estimate and statistical power was set at 70%, which means that results in the present  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

### 3 4 *Conclusion*

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

### 14 15 *Conflicts of interest*

16 Nocitech is partly owned by Aalborg University.

### 17 18 *Author contributions*

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

## 1 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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Patients with Chronic Pain after Experimentally Induced Psychosocial Stress. Paul F, editor. PLoS ONE. 2013 Aug 7;8(8):e69460.
- 2
- 3 1
- 4 2
- 5
- 6 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.
- 7 3
- 8 4
- 9
- 10 5 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.
- 11 6
- 12 7
- 13
- 14 8 31. Bali A, Jaggi AS. Clinical experimental stress studies: methods and assessment. *Reviews in the Neurosciences*. 2015 Oct 1;26(5):555–79.
- 15 9
- 16
- 17 10 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.
- 18 10
- 19 11
- 20 12
- 21 13
- 22
- 23 14 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.
- 24 15
- 25 16
- 26
- 27 17 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.
- 28 17
- 29 18
- 30 19
- 31
- 32 20 35. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*. 2009 Feb;34(2):163–71.
- 33 21
- 34
- 35 22 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.
- 36 22
- 37 23
- 38 24
- 39
- 40 25 37. Kalman BA, Grahn RE. Measuring salivary cortisol in the behavioral neuroscience laboratory. *J Undergrad Neurosci Educ*. 2004;2(2):A41–9.
- 41 26
- 42
- 43 27 38. Gaab J, Jiménez J, Voneschen L, Oswald 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.
- 44 28
- 45 29
- 46 30
- 47 30
- 48
- 49 31 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.
- 50 32
- 51 33
- 52 34
- 53
- 54 35 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.
- 55 36
- 56 37
- 57 37
- 58
- 59
- 60

- 1  
2  
3 1 41. Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and  
4 2 validation. *Psychological Assessment*. 1995;7(4):524–32.  
5  
6 3 42. Labor Diagnostika Nord GmbH. Cortisol Saliva ELISA [Internet]. 2018. Available  
7 4 from: <http://www.ldn.de/sites/default/files/downloads/SA%20E-6000.pdf>.  
8  
9 5 43. Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L.  
10 6 Assessment of musculoskeletal pain sensitivity and temporal summation by cuff  
11 7 pressure algometry: a reliability study. *PAIN*. 2015 Nov;156(11):2193–202.  
12  
13 8 44. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in  
14 9 desensitized and hypersensitized muscle and skin assessed by cuff algometry. *J*  
15 10 *Pain*. 2002 Feb;3(1):28–37.  
16  
17 11 45. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, et al. An  
18 12 endocannabinoid mechanism for stress-induced analgesia. *Nature*. 2005 Jun  
19 13 23;435(7045):1108–12.  
20  
21 14 46. Ford GK, Finn DP. Clinical correlates of stress-induced analgesia: Evidence from  
22 15 pharmacological studies. *PAIN*. 2008 Nov;140(1):3–7.  
23  
24 16 47. Martenson ME, Cetas JS, Heinricher MM. A possible neural basis for stress-induced  
25 17 hyperalgesia. *PAIN*. 2009 Apr;142(3):236–44.  
26  
27 18 48. Pinto-Ribeiro F, Moreira V, Pêgo JM, Leão P, Almeida A, Sousa N. Antinociception  
28 19 induced by chronic glucocorticoid treatment is correlated to local modulation of  
29 20 spinal neurotransmitter content. *Mol Pain*. SAGE Publications; 2009;5:41.  
30  
31 21 49. Cathcart S, Winefield AH, Lushington K, Rolan P. Effect of mental stress on cold  
32 22 pain in chronic tension-type headache sufferers. *J Headache Pain*. 2009 Jun  
33 23 5;10(5):367–73.  
34  
35 24 50. Marcuzzi A, Wrigley PJ, Dean CM, Adams R, Hush JM. The long-term reliability of  
36 25 static and dynamic quantitative sensory testing in healthy individuals. *PAIN*. 2017  
37 26 Jul;158(7):1217–23.  
38  
39 27 51. Waller R, Straker L, O’Sullivan P, Sterling M, Smith A. Reliability of pressure pain  
40 28 threshold testing in healthy pain free young adults. *Scandinavian Journal of Pain*.  
41 29 Elsevier B.V; 2015 Oct 1;9:38–41.  
42  
43 30 52. Nothnagel H, Puta C, Lehmann T, Baumbach P, Menard M, Gabriel B, et al. How  
44 31 stable are quantitative sensory testing measurements over time? Report on 10-week  
45 32 reliability and agreement of results in healthy volunteers. *JPR*. 2017;Volume  
46 33 10:2067–78.  
47  
48 34 53. Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: a salience  
49 35 detection system for the body. *Prog Neurobiol*. 2011 Jan;93(1):111–24.  
50  
51 36 54. al’Absi M, Petersen KL. Blood pressure but not cortisol mediates stress effects on  
52 37 subsequent pain perception in healthy men and women. *PAIN*. 2003  
53 38 Dec;106(3):285–95.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 55. Aviram J, Shochat T, Pud D. Pain Perception in Healthy Young Men Is Modified by  
2 Time-Of-Day and Is Modality Dependent. *Pain Medicine*. 2015;16(6):1137–44.
- 3 56. Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue  
4 hyperalgesia. *Scand J Rheumatol Suppl*. 2006;122:1–43.
- 5 57. Sikandar S, West SJ, McMahon SB, Bennett DL, Dickenson AH. Sensory  
6 processing of deep tissue nociception in the rat spinal cord and thalamic ventrobasal  
7 complex. *Physiol Rep*. 2017 Jul 18;5(14):e13323–13.
- 8 58. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. “Pain inhibits pain”  
9 mechanisms: Is pain modulation simply due to distraction? *PAIN*. 2010  
10 Jul;150(1):113–20.
- 11 59. Atchley R, Ellingson R, Klee D, Memmott T, Oken B. A cognitive stressor for event-  
12 related potential studies: the Portland arithmetic stress task. *Stress (Amsterdam,*  
13 *Netherlands)*. Taylor & Francis; 2017 May;20(3):277–84.
- 14 60. Wilhelmsen M, Amirian I, Reiter RJ, Rosenberg J, Gögenur I. Analgesic effects of  
15 melatonin: a review of current evidence from experimental and clinical studies.  
16 *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 ( $PDT-CS_{avg}$ ) is presented. There was an increase in  $PDT-CS_{avg}$  compared to the unconditioned  $PDT_{avg}$  equal to a significant CPM-effect (\*,  $p = 0.001$ ,  $\eta_p^2 = 0.40$ ).

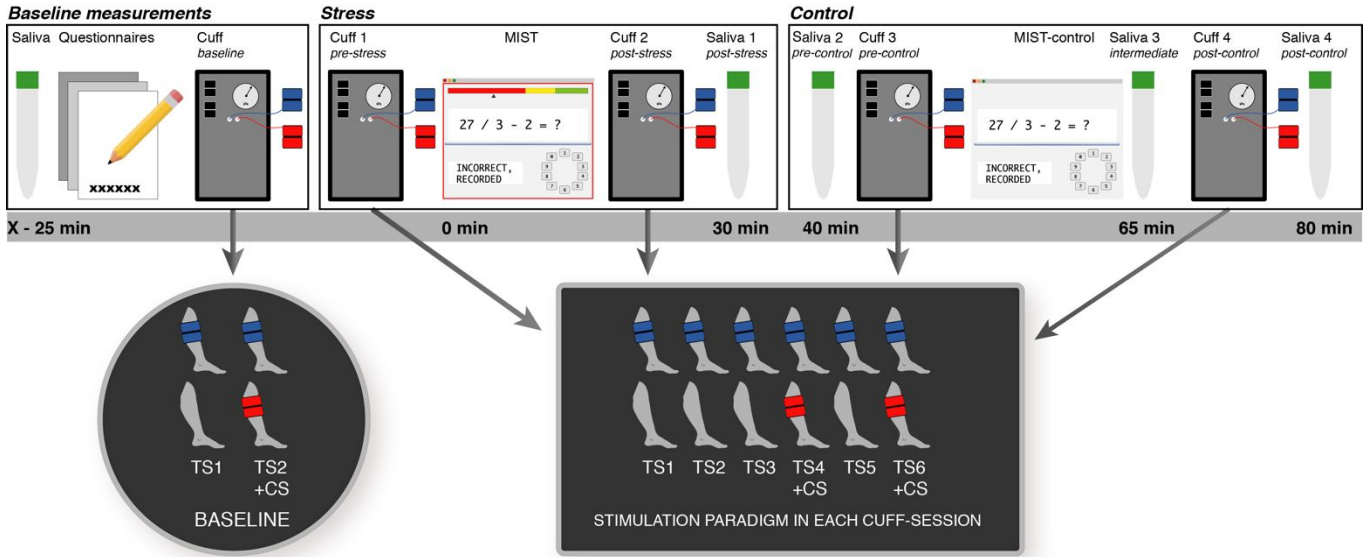
**Figure 3.** A scatter-plot and linear regression comparing the change in cortisol ( $\Delta$ -cortisol) after MIST and (a) conditioned pain detection thresholds ( $\Delta$ -PDT-CS) after MIST or (b) unconditioned pain detection thresholds ( $\Delta$ -PDT) after MIST. The linear regressions show that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

1 **FIGURES**

2 *Figure 1: Timeline*

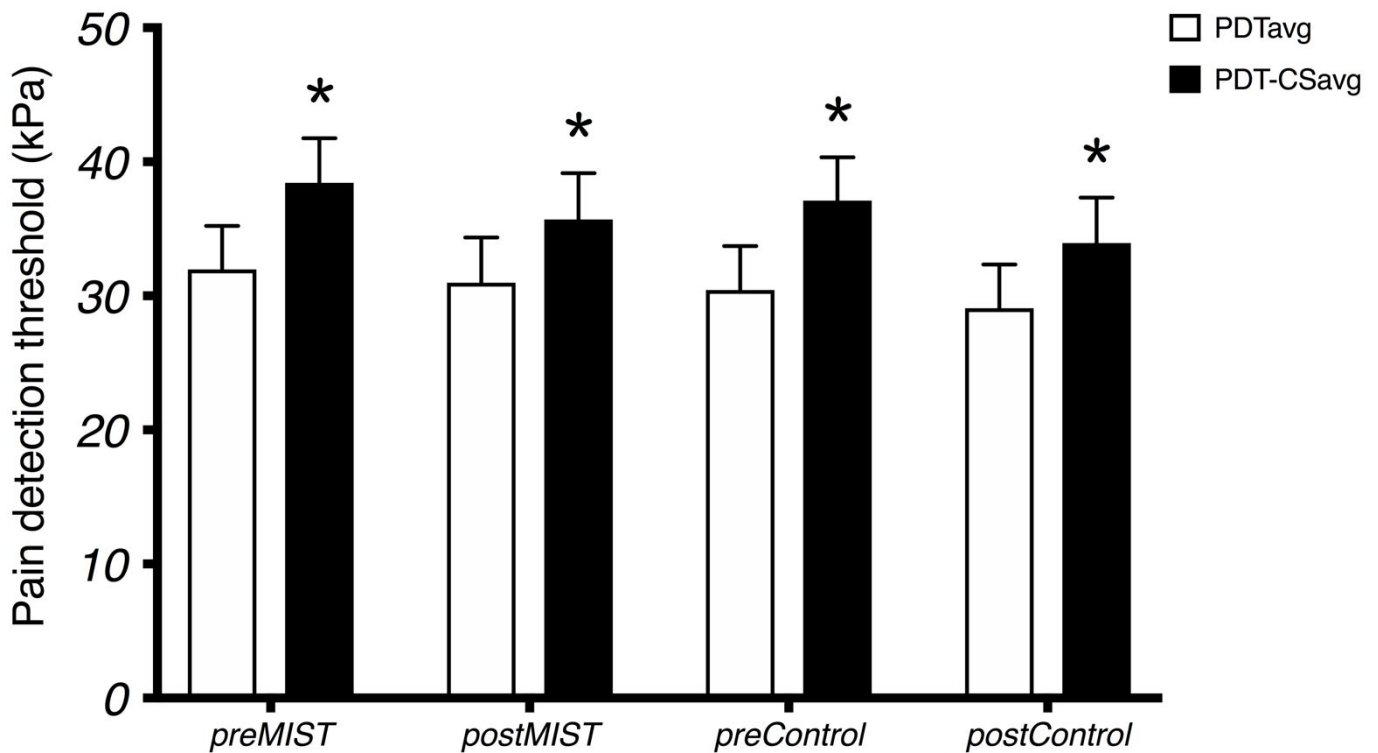
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Timeline



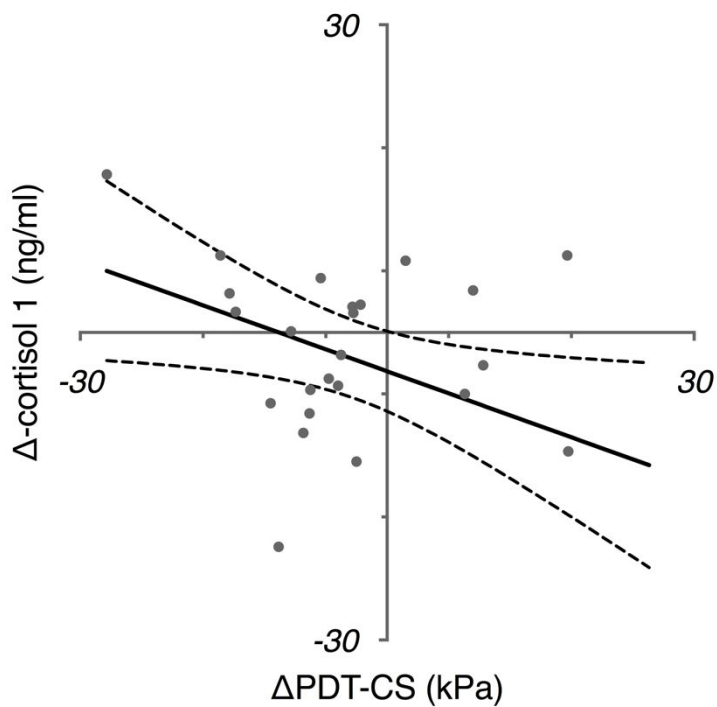
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Figure 2: Effect of conditioning

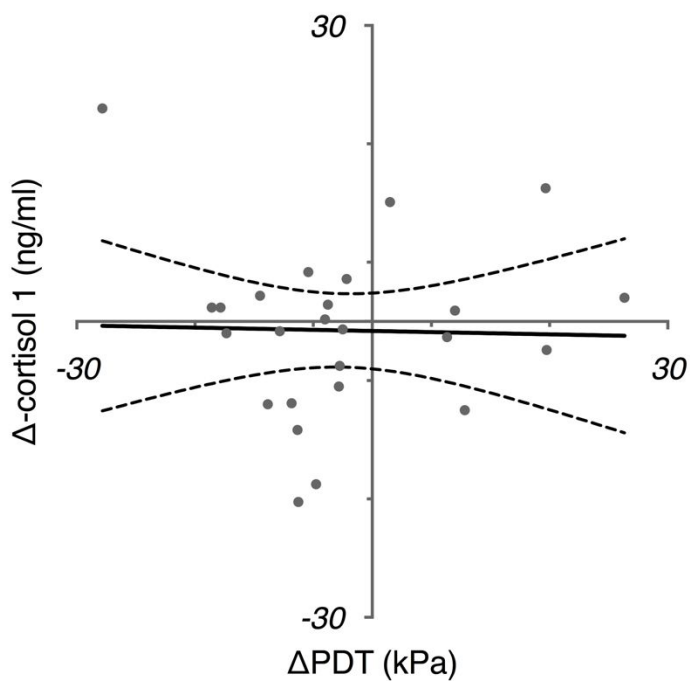


1 Figure 3: Linear regression

2 Fig 3a



31 Fig 3b



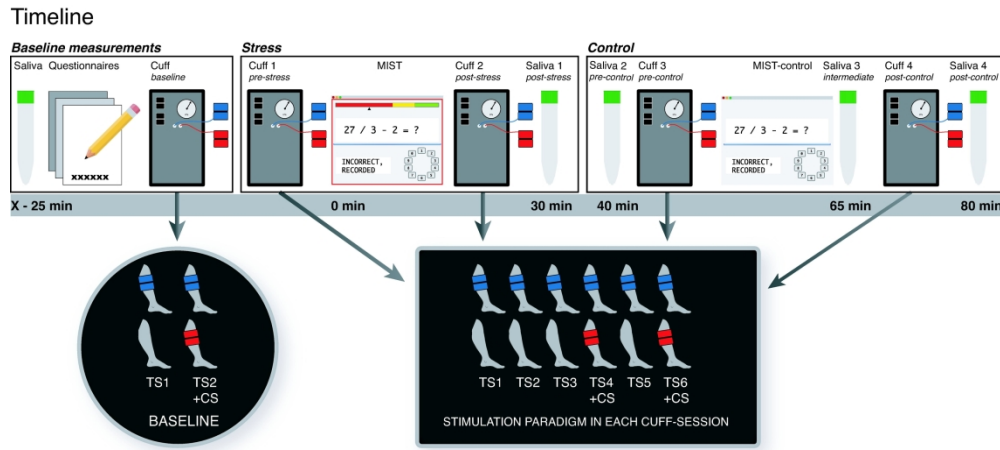


Figure 1. Overview over the study. Saliva was sampled at baseline before questionnaires and cuff pressure test-stimuli (TS) on the dominant leg (blue cuff) and conditioning stimuli (CS) on the non-dominant leg (red cuff). The Montreal Imaging Stress Task (MIST) was performed after the first trial of 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.

269x127mm (300 x 300 DPI)

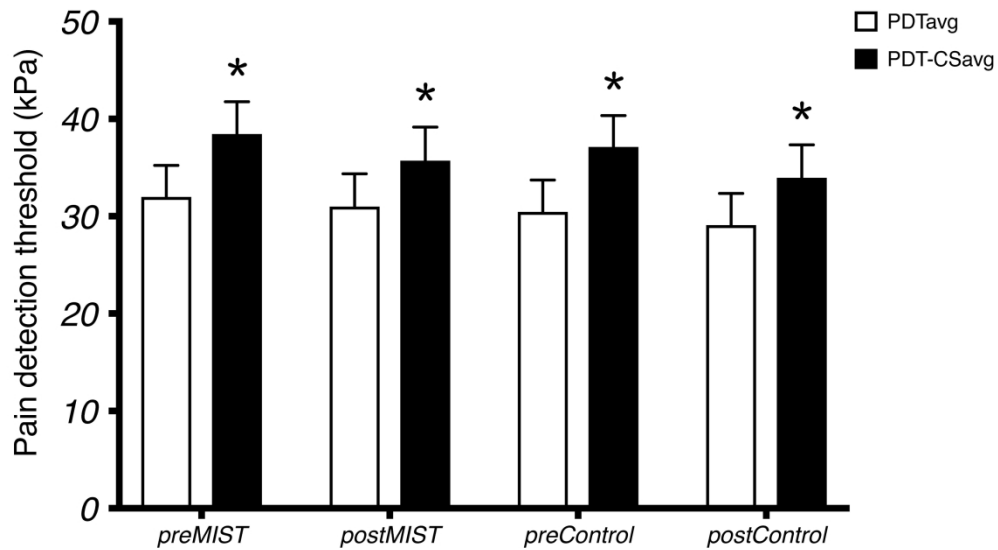


Figure 2. Mean (+SEM,  $n = 25$ ) pressure detection thresholds (PDT) recorded with and without conditioning on the contralateral leg. The average of unconditioned PDTs (PDTavg) and conditioned PDTs (PDT-CSavg) is presented. There was an increase in PDT-CSavg compared to the unconditioned PDTavg equal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

271x154mm (300 x 300 DPI)

Fig 3a

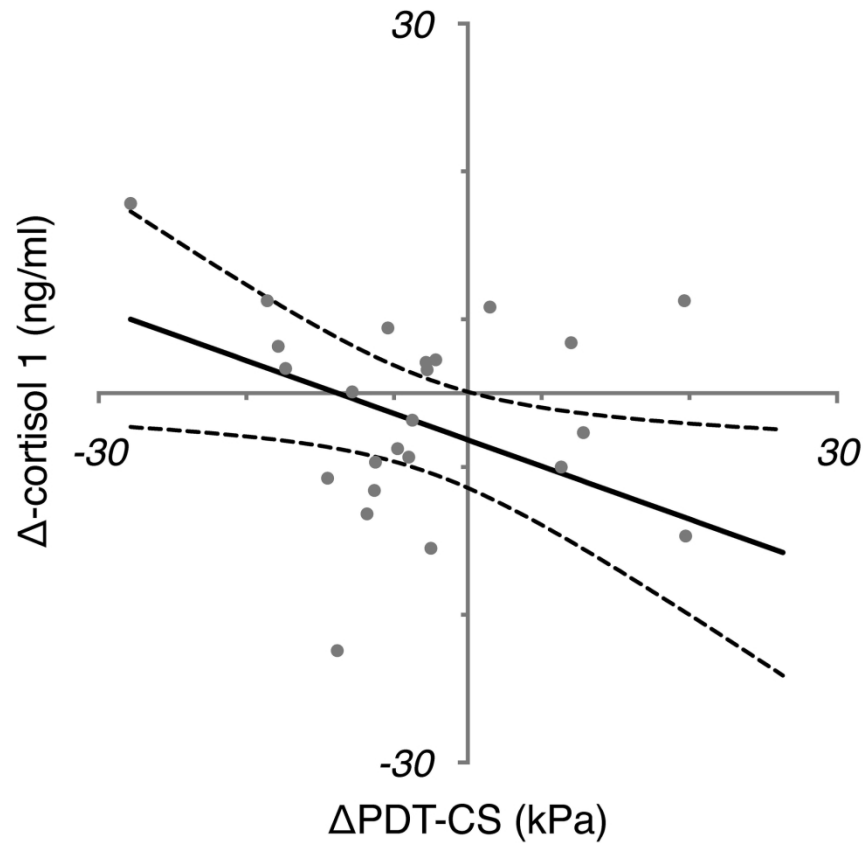


Figure 3. A scatter-plot and linear regression comparing the change in cortisol ( $\Delta\text{-cortisol}$ ) after MIST and (a) conditioned pain detection thresholds ( $\Delta\text{-PDT-CS}$ ) after MIST or (b) unconditioned pain detection thresholds ( $\Delta\text{-PDT}$ ) after MIST. The linear regressions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

200x185mm (300 x 300 DPI)

Fig 3b

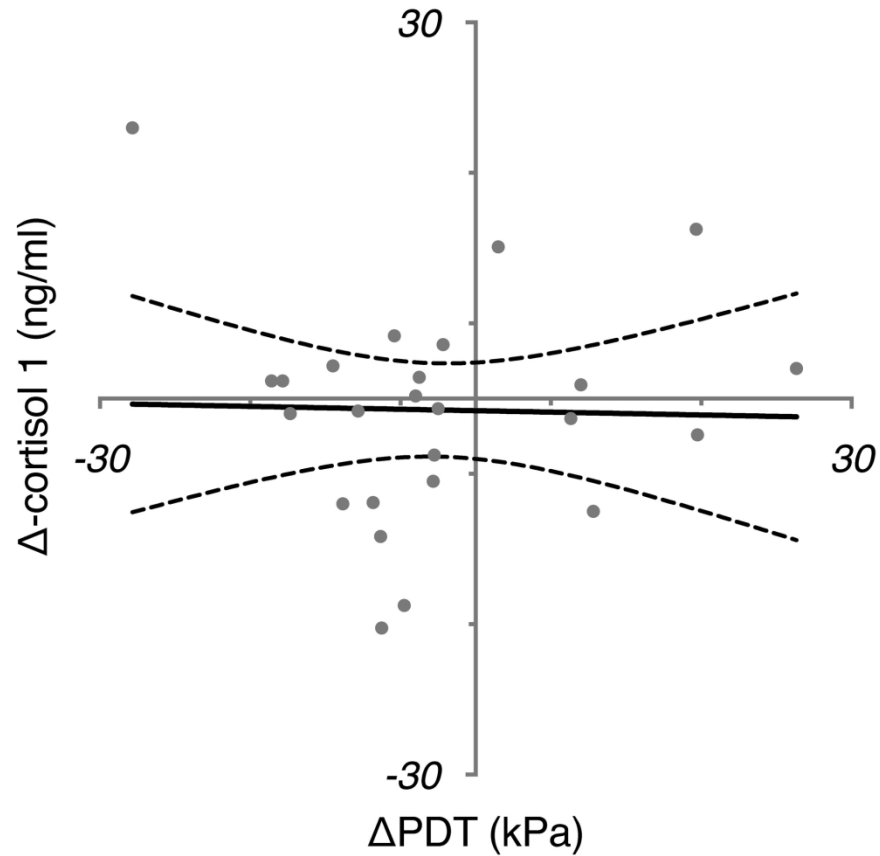


Figure 3. A scatter-plot and linear regression comparing the change in cortisol ( $\Delta\text{-cortisol}$ ) after MIST and (a) conditioned pain detection thresholds ( $\Delta\text{-PDT-CS}$ ) after MIST or (b) unconditioned pain detection thresholds ( $\Delta\text{-PDT}$ ) after MIST. The linear regressions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

197x186mm (300 x 300 DPI)