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## Modulation of itch by conditioning itch and pain stimulation in healthy humans

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1           **Modulation of itch by conditioning itch and pain stimulation in healthy humans**

2

3 **Running head:** Assessing endogenous itch inhibition

4

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30

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32 inhibition

1 **Abstract**

2

3 Little is known about endogenous descending control of itch. In chronic pain, descending pain  
4 inhibition is reduced as signified by lowered conditioned pain modulation (CPM). There are  
5 indications that patients with chronic itch may also exhibit reduced endogenous descending  
6 inhibition of itch and pain. This study aimed to investigate whether and the extent to which itch  
7 can be modulated by conditioning itch and pain stimuli. Twenty-six healthy volunteers  
8 participated. The study consisted of 5 conditions designed to systematically assess endogenous  
9 modulation of itch or pain: 1) itch-induced modulation of contralateral itch, 2) pain-induced  
10 modulation of contralateral itch, 3) pain-induced modulation of ipsilateral itch, 4) pain-induced  
11 modulation of contralateral pain, and 5) itch-induced modulation of contralateral pain.  
12 Conditioning stimuli were cold pressor-induced pain and histamine-evoked itch, while the test  
13 stimuli were electrical stimulation paradigms designed to evoke itch or pain. Pain was  
14 significantly reduced (CPM-effect) by the conditioning pain stimulus ( $p < 0.001$ ), but not by the  
15 conditioning itch stimulus (negative control condition). Itch was significantly reduced (CIM-  
16 effect) by both contra- and ipsilateral applied conditioning pain (both  $p < 0.001$ ), while  
17 conditioning itch stimulation only marginally reduced itch. Endogenous descending itch  
18 inhibition through mechanisms that are independent of segmental gating can be readily evoked  
19 by heterotopic conditioning pain stimulation. However, robust descending inhibition of itch  
20 cannot be evoked with itch conditioning stimulation.

21

22 **Perspective:** The study shows a hierarchical prioritization favouring pain-induced central  
23 descending modulation of both itch and pain in humans. Future studies addressing potential  
24 aberrations in pain-evoked descending modulation of itch in chronic itch patients are warranted.

25

## 1 Introduction

2  
3 Itch is an unpleasant sensory experience, distinct from pain, transmitted by two parallel  
4 nociceptive pathways: a subgroup of mechano-insensitive C-fibers transmit histaminergic itch  
5 and a subgroup of polymodal C-fibers transmit non-histaminergic itch<sup>36,59</sup>. After synapsing in the  
6 superficial spinal dorsal horn signalling is transmitted in the anterolateral tracts to areas  
7 including the thalamus, periaqueductal grey, and the parabrachial area<sup>46</sup>. Itch and pain share  
8 numerous of mechanistic similarities<sup>58</sup>. Notably, as for pain, itch is under strict segmental control  
9 as well as descending endogenous modulation<sup>12,18,48</sup>. In the spinal dorsal horn, inhibitory basic  
10 helix-loop-helix B5-interneurons (Bhlhb5), which are activated by painful stimuli, control  
11 pruriceptive transmission<sup>15,56</sup>. This can be quantified in humans by application of a homotopic  
12 nociceptive stimulus (e.g., scratching) to an itching skin area whereby the itch is transiently  
13 inhibited<sup>6,71</sup>. The dysesthesias that pain and itch are capable of inducing, and for both modalities  
14 thought to reflect central sensitization, are also alike<sup>58</sup>. Specifically, allodynia (itch evoked by a  
15 stimulus not normally evoking itch) and hyperknesis (increased itch in response to stimuli  
16 normally evoking itch) are analogues to allodynia and hyperalgesia, respectively<sup>1,35</sup>.

17  
18 Conditioned pain modulation (CPM) is an endogenous centrally-mediated pain regulatory  
19 phenomenon occurring in humans, considered the perceptual correlate of diffuse noxious  
20 inhibitory controls (DNIC) established in animals<sup>8,67</sup>. In CPM-paradigms the pain evoked by a  
21 test stimulus can be reduced by applying a nociceptive conditioning stimulus to a location remote  
22 (i.e. heterotopically) from that of the test stimulus site<sup>51</sup>. Multiple parallel descending pain  
23 modulatory pathways exist, involving areas such as the medullary reticularis nucleus dorsalis, the  
24 rostral ventromedial medulla and the periaqueductal grey<sup>45,53</sup>. CPM-efficacy has been shown to  
25 be impaired in a multitude of chronic pain conditions such as osteoarthritis, diabetic neuropathy,  
26 and fibromyalgia, when compared to healthy individuals<sup>38</sup>. Decreased CPM-efficacy has also  
27 been shown to predict the development of chronic post-operative pain<sup>69</sup> as well as increased  
28 analgesic responsiveness to certain anti-depressant/convulsive drugs (suggested to restore  
29 endogenous pain inhibition<sup>70</sup>). Moreover, although evidence is limited and/or conflicting there  
30 are indications that individual psychological characteristics such as optimism, catastrophizing,  
31 and negative affectivity may be associated with CPM-efficacy<sup>17,22,34</sup>.

32  
33 Previous studies suggests that deranged endogenous sensory modulation and sensitization may  
34 play a role in maintaining or enhancing chronic itch in patients suffering common chronic itch  
35 conditions e.g., atopic dermatitis or psoriasis<sup>23,25,31-33</sup>. Such findings includes the lack of a good  
36 correlation between objective disease measures and experienced itch<sup>14</sup>, sensitization to itching  
37 and thermal stimuli<sup>25,27,63</sup>, decreased efficacy of homotopic counter-stimuli<sup>27</sup>, and reports of  
38 antipruritic effectiveness of drugs thought to enhance endogenous pain inhibition<sup>50</sup>. However, it  
39 is currently unclear whether a central endogenous modulation system akin to that involved in

1 CPM affects itch processing (i.e. conditioned itch modulation; CIM) and if so, which kinds of  
2 conditioning stimuli are required to activate it<sup>3,32,34</sup>.

3  
4 To examine the organization and efficacy of central pain- and itch-mediated endogenous  
5 descending modulation of itch in humans, this study aimed to investigate the effect of  
6 conditioning itch and conditioning pain stimuli on electrically evoked itch, primarily focusing on  
7 the mean levels of itch and pain and secondarily on the peak levels of itch and pain. In parallel, a  
8 standard CPM-paradigm acting as a positive comparator, and a condition assessing the potential  
9 effect of a conditioning itch stimulus on pain perception were conducted. We hypothesized that  
10 for pruriception; a descending inhibitory system parallel to that of the nociceptive system would  
11 exist. Exploratively, development of mechanical dysesthesias was monitored and individual  
12 characteristics of catastrophizing, optimism, and psychological distress were assessed.

1 **Methods**

2

3 ***Participants***

4 Twenty-eight healthy participants (14 males/14 females, mean age 23.0 years with standard  
5 deviation 2.8, range 18-29) were included. Recruitment took place at the campus of Aalborg  
6 University and via social media, with advertisements clearly displaying the criteria for  
7 participation. All participants gave written informed consent after being provided with written  
8 and verbal study information, and received a monetary compensation for participating. The study  
9 protocol was approved by the local Ethics Committee (N-20160026) and conducted in  
10 accordance with the Helsinki Declaration (World Medical Association, 2013). Inclusion criteria  
11 were being healthy, in the age group 18-65 years, and having a good understanding of English.  
12 Participants would have been rescheduled to a later moment if, in the 24 hours prior to testing,  
13 experiencing itch or pain >3 (on a scale from 0 to 10, ranging from no itch/pain to worst  
14 imaginable itch/pain), they had taken medication that could affect itch or pain sensitivity, e.g.,  
15 antihistamines or analgesics or if they had consumed an excessive amount of alcohol (>5 units)  
16 or illicit drug. No participants had to be rescheduled. Two of the included participants had  
17 consumed medication deemed non-influential; an antibiotic for the treatment of intestinal  
18 parasites and an antidyslipidemic for hypercholesterolemia.

19

20 ***Design***

21 The study had a within-subjects design. There were five randomized conditions; three  
22 investigating CIM and two investigating CPM. In each condition, first a baseline test stimulus  
23 (TS), and subsequently a simultaneous application of a test stimulus and a conditioning stimulus  
24 (TS+CS) was applied. Condition 1 (“CIM-itch”) consisted of an itch TS and a contralateral itch  
25 CS (see Table 1). Condition 2 (“CIM-pain”) consisted of an itch TS and a contralateral pain CS.  
26 Condition 3 (“CIM-pain<sub>ipsi</sub>”) consisted of an itch TS and an ipsilateral pain CS. Condition 4  
27 (“CPM-pain”) consisted of a pain TS and a contralateral pain CS. Condition 5 (“CPM-itch”)  
28 consisted of a pain TS and a contralateral itch CS. All stimuli were applied on the forearms and  
29 hands of the participants and a testing session lasted approximately 2 hours and 20 min per  
30 participant (See Figure 1). In a subsequent additional experiment, a CIM-itch<sub>sequential</sub> condition,  
31 i.e., with the TS applied after the CS, was tested based on findings of the first 5 conditions (see  
32 *Additional experiment*). Tests were conducted by a male (HHA) or female (AIMvL)  
33 experimenter in a laboratory at the Center for Sensory-Motor Interaction (SMI) of Aalborg  
34 University.

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36

37 ***<Insert Table 1 about here>***

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41 ***<Insert Figure 1 about here>***

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***Somatosensory stimuli and psychophysics***

***Electrical test stimuli:*** All electrical stimuli were delivered by a constant current stimulator (Isolated Bipolar Constant Current Stimulator DS5, Digitimer, Hertfordshire, UK), controlled by a laptop via a data acquisition system (NI USB-6221 or NI-DAQmx, National Instruments, Austin, Tx, USA). The participants' arms were prepared for electrical stimulation included scrubbing with NuPrep skin prep gel (Weaver and company, Aurora, Co, USA) and application of conductive gel (Spectra 360 electrode gel, Parker Laboratories Inc., Fairfield, NJ, USA). Tape (Transpore surgical tape 3M, St. Paul, MN, USA) was used to attach the electrodes. Electrical stimulation was chosen as test stimuli over other more physiological methods, such as cowhage or histamine provocations, because it permits: 1) accurate temporal control and 2) assessment of stimulus-response<sup>1</sup>.

***Electrically evoked itch:*** For itch induction, two surface electrodes (disk electrode of 1 cm and reference electrode of 2 cm diameter, VCM Medical, Leusden, the Netherlands) were attached to the central volar surface of the forearm halfway the total forearm length (see Figure 2). In accordance to previous studies<sup>9,34</sup>, stimuli were applied at the volar side, at 50 Hz with a pulse duration of 100  $\mu$ s, and at a continuously increasing current intensity of 0.05 mA/s. The current intensity of each itch stimulus started at 0.4 mA and ended at 6.4 mA, resulting in a  $\approx$ 2 min duration per stimulus ramp.

***Electrically evoked pain:*** For pain induction, two surface electrodes (two disk electrodes of  $\varnothing$  1 cm, VCM Medical, Leusden, the Netherlands) were attached to the central dorsal surface of the forearm halfway the total forearm length (see Figure 2). According to favourable pain-induction results from a previous study (van Laarhoven et al., unpublished), stimuli were applied at the dorsal side, at 50 Hz with a pulse duration of 400  $\mu$ s. The stimulus intensity increased using a step-up paradigm, from 0.4 mA to 7.0 mA in 11 steps of 6 s each with 0.60 mA increment and a 2 s interval in-between the steps, thus also resulting in a  $\approx$ 2 min duration per stimulus step-up.

***Assessing electrically evoked itch and pain:*** Ratings of electrically induced itch and pain test stimuli were obtained during the electrical stimuli by using two electronic visual analogue scales (VASs) on a tablet (Galaxy Tab S2, Samsung, Seoul, South Korea) using a VAS application (Aalborg University, Aalborg, Denmark). A VAS for itch was displayed on top and a VAS for pain below, with anchors at the left end indicating no itch/pain (representing 0) and at the right end indicating worst imaginable itch/pain (representing 100). During all itch and pain test stimuli VAS ratings on both the itch and pain scale were continuously conducted and sampled once every 5 seconds.

***<Insert Figure 2 about here>***

1  
2 **Conditioning itch stimulation:** As itch CS, histamine (as dihydrochloride in a concentration of  
3 10 mg/ml, i.e. 1% EEAACI recommended positive control)<sup>4,16</sup> was applied with 1-mm weight-  
4 calibrated skin prick test lancets (SPT) using 120 g weight<sup>4</sup>. The SPTs were performed at the  
5 central volar forearms, 5 cm proximally from the cease of the wrist. A small drop of histamine  
6 was placed on the skin and percutaneously introduced with the SPT lancet<sup>11,20,72</sup>.

7 **Conditioning pain stimulation:** To elicit pain as CS, a cold pressor task (CPT) was used with  
8 water of ca. 8 °C (mean  $7.9 \pm 0.1$  and  $8.3 \pm 0.1$  before and after CPT, respectively) in an 8 liter  
9 plastic box isolated by styrofoam. The water was circulated by an Anova Precision Cooker  
10 (Anova, San Francisco, California, USA) at a rate of 8 L/min. Participants were instructed to  
11 immerse their hand up to the level of their wrist into the water for the duration of the TS (i.e.  $\approx 2$   
12 min).

13  
14 **Assessing conditioning itch and pain intensity:** The perceived average intensity of evoked itch  
15 and pain by conditioning itch and pain stimulation were reported using two numeric rating scales  
16 (NRSs) from 0 (no itch or pain) to 10 (worst imaginable itch or pain). Ratings of the conditioning  
17 stimuli were conducted immediately after the 2 min test stimulus.

18  
19 **Mechanical itch sensitivity:** In between the two itch electrodes sensitivity to touch evoked itch  
20 (STI) was assessed using von Frey monofilaments (Stoelting, North Coast Medical, Gilroy,  
21 California, USA). Three monofilaments were applied: 4.08 mN, 4.17 mN, and 4.31 mN in  
22 consecutive order as previous described<sup>2,6</sup>. The monofilaments were applied by pressing them  
23 onto the skin perpendicularly for 1 s until the filament bowed, after which the filament was  
24 gently lifted from the skin. Each filament was applied as triplicate. The participants indicated the  
25 average intensity of itch experienced following each of the triplicate stimulus application using  
26 the NRS from 0 to 10 for itch.

27  
28 **Mechanical pain sensitivity:** In between the two pain electrodes, mechanical pain sensitivity  
29 (MPS) was assessed using weight-calibrated pinprick stimulators with blunt tips (The PinPrick,  
30 MRC Systems GmbH, Heidelberg, Germany). Based on previous research indicating a  
31 mechanical pain threshold of  $\approx 71$  mN in healthy adult volunteers<sup>55</sup>, the pins of 128 mN, 256  
32 mN, and 512 mN were selected to probe supra-threshold pain mechanical pain sensitivity. These  
33 pinprick stimulators were applied perpendicularly to the skin for 2s each in consecutive order,  
34 which procedure was repeated thrice, resulting in nine MPS assessments in each round. The  
35 participants indicated the average intensity of pain evoked by each pin-prick stimulus using the  
36 NRS from 0 to 10 for pain.

### 37 **Self-report questionnaires**

38 Based on previous research<sup>22</sup> the following self-report questionnaires were administered in  
39 English. To keep the procedure across participants as comparable as possible and to allow  
40



1 recruitment of non-Danish speakers, the experimental procedures were conducted in English  
2 regardless of whether HHA (Danish) or AIMvL (Dutch) was the active investigator. The Pain  
3 Catastrophizing Scale (PCS)<sup>64</sup> was administered to assess catastrophizing of pain experienced in  
4 daily life. This questionnaire consists of 13 items, each rated on a Likert scale from 0 (not at all)  
5 to 4 (all the time). The total score was obtained by summing the scores for all items, with a  
6 theoretical range from 0 to 52. The Cronbach's alpha in the present study was 0.88. The PCS  
7 was also administered in a for itch adjusted version (PCS-Itch), in which only the word "pain"  
8 for all items was substituted by the word "itch". The Cronbach's alpha of the PCS-Itch in the  
9 present study was 0.85. Dispositional optimism was measured with the revised Life Orientation  
10 Test (LOT-R)<sup>57</sup>, consisting of 3 positive, 3 negative, and 4 filler items which were rated on a  
11 Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The total score can range  
12 from 0 to 24, with higher scores indicating higher optimism. Cronbach's alpha was 0.75. The  
13 Hospital Anxiety and Depression Scale (HADS)<sup>73</sup> was administered to assess psychological  
14 distress. This questionnaire consists of a subscale for depression (7 items; Cronbach's alpha in  
15 the present study 0.62) and a subscale for anxiety (7 items Cronbach's alpha in the present study  
16 0.61). Items were rated on a scale from 0 to 3, and the total scores for both subscales (each  
17 potentially ranging from 0 to 21) were obtained by summing the respective items with total  
18 scores.

19

## 20 ***Procedure***

21 Upon arrival at the lab, participants were informed about the tests and fulfilment of inclusion and  
22 exclusion criteria were checked (see Figure 1 for the time line). Participants filled out the self-  
23 report questionnaires before initiating the itch and pain inductions. Based on the hand dominance  
24 of the participant, the side (dominant or non-dominant) of the itch and pain stimulation  
25 (contralaterally attached) was randomized using the randomly permuted block method  
26 (randomization.com) with separate lists for males and females. Before applying electrical  
27 stimuli, baseline mechanical sensitivity assessments were conducted, starting with either the  
28 assessments for itch (STI) or pain (MPS), determined by balanced randomization. Hereafter, in  
29 accordance to the order of the mechanical assessments, an electrical familiarization stimulus for  
30 either itch or pain was given, which participants perceived without rating the sensations. Then a  
31 similar electrical stimulus followed during which participants continuously rated the itch and  
32 pain levels using the electronic VAS. This procedure was then repeated for the other modality  
33 (e.g., when started with pain, itch stimuli were applied hereafter). Based on these stimuli, it was  
34 assessed whether the participant responded with adequate levels of itch or pain (pre-defined as a  
35 peak score of  $\geq 20$  on the intended modality). Further testing was terminated if participants were  
36 determined as non-responders for both stimulation modalities (i.e. peak VAS  $< 20$ ), which was  
37 the case for two participants, so the experiment was continued in 26 participants. CIM (1. CIM-  
38 itch, 2. CIM-pain 3. CIM-pain<sub>ipsi</sub>) and CPM (4. CPM-pain, 5. CPM-itch) conditions were applied  
39 in a random order. Within each condition, a TS was applied first (i.e. baseline TS) followed by

1 the TS+CS. After each itch stimulus, STI was assessed and after each pain stimulus, MPS was  
2 assessed.

#### 3 4 ***Additional sequential experiment***

5 An additional experiment was conducted in 20 participants (mean age 22.7, standard deviation  
6 3.0 years; 11 females) all non-selectively derived from the main cohort of participants to assess  
7 whether timing of the CS with respect to the TS influences CIM-itch response. In this experiment  
8 the exact same stimuli of the CIM-itch condition were applied, but an intermission was held  
9 between the itch CS and the second TS so as to make the paradigm sequential (CIM-itch<sub>sequential</sub>)  
10 rather than simultaneous. Other procedures of the main experiment, e.g., familiarization  
11 procedures and STI assessment were also conducted to obtain as much similarity as possible.  
12 Timing of the second TS was based on the participant's CS itch score, rated on the same  
13 electronic VAS as previously described. The investigator checked the VAS every 30 s after the  
14 histamine SPT and the TS was re-applied when the VAS itch was <10 (/100), after a minimum  
15 of 4 minutes and before a maximum of 15 minutes.

#### 16 17 ***Statistical analyses***

18 Descriptive characteristics were calculated in Excel (Microsoft Office 2013, Redmond, WA,  
19 USA) and analyses were conducted in SPSS version 24 (IBM Corporation, Armonk, NY, USA).  
20 The sample size calculation was performed based on previously obtained test-retest reliability  
21 data for the TS<sup>34</sup>. A  $\alpha$ -level of 0.05, a power of 0.8 and smallest relevant difference of 30% were  
22 applied using methodology for paired study designs previously outlined<sup>28,41</sup>. Calculation of the  
23 variables included averaging the continuous VAS scores within each TS. For mechanical  
24 stimulation, a mean NRS score was calculated for itch by averaging the three STI assessments  
25 and for pain by averaging the 9 MPS assessments at each time point (i.e. at baseline and after  
26 each TS). In addition, a grand average was calculated for all NRS scores after the baseline TS for  
27 itch (STI) and pain (MPS) separately. As measure of CIM and CPM efficacy, for each CS the  
28 itch and pain reduction was calculated by the formula CIM/CPM-efficacy = MEAN VAS<sub>TS+CS</sub> –  
29 MEAN VAS<sub>TS</sub>. When VAS scores were missing for a baseline TS (n=1, for one condition only),  
30 the average VASs of the TSs in the same modality was taken according to the last-observation  
31 carried forward method. Other missing data (e.g., for mechanical stimuli) were handled by  
32 pairwise deletion. In addition to the two non-responders, one participant was unable to complete  
33 any of the CPTs (immersion times were all <30 s). Therefore all data of this participant were  
34 omitted from the statistical analyses, which were performed on 25 participants. Data variables  
35 were checked for normal distribution by standardized skewness and kurtosis values and potential  
36 outliers (> 3 standard deviations from the group mean<sup>39</sup>). Some variables were not normally  
37 distributed because of an outlier (i.e. mean VAS pain during the CPM-itch condition, the peak  
38 VAS itch during the CIM-itch condition, CPM-efficacy by itch CS). Excluding the outlier  
39 resulted in normal distribution and the analyses were rerun. However, for the variable peak pain  
40 for CPM-itch both an LN-transformation and removing an outlier were necessary to obtain

1 normal distribution. Data that includes the outliers were reported since outliers did not change  
2 the outcome and interpretation in term of the levels of statistical significance ( $p < 0.05$ ).

3  
4 Four repeated measures analyses of variance (RM-ANOVAs) were conducted, two for itch  
5 (primary outcome: mean VAS scores and secondary outcome peak VAS scores) and two for pain  
6 (mean and peak VAS scores). For itch, the RM-ANOVA was constructed with the factors  
7 *conditioning stimulation* (TS and TS + CS) x *condition* (CIM-itch, CIM-pain, and CIM-pain<sub>ipsi</sub>),  
8 while for pain, the test was constructed with *conditioning stimulation* (TS and TS + CS) x  
9 *condition* (CPM-pain and CPM-itch). Moreover, as reliability measure for the TSs before the CS,  
10 two-way random consistency model (2,1) intra-class coefficients (ICCs) were calculated for the  
11 mean VAS itch for the three baseline TSs for itch and for the mean VAS pain for the two  
12 baseline TSs for pain (i.e. intra-individual variability of the primary outcome parameter). Post  
13 hoc paired t-tests were performed for the additional CIM-itch<sub>sequential</sub> condition in which the mean  
14 and peak VAS itch for the baseline TS compared with the TS after CS were compared. For  
15 exploring the effect of the conditioning stimuli on mechanical dysesthesia, comparable RM-  
16 ANOVAs were carried out with the NRS itch evoked with *conditioning stimulation* (STI/MPS  
17 applied after the TS and after the TS + CS) x *condition* (all CIM/CPM conditions, respectively).  
18 Additionally, in order to explore the effect of electrical stimulation on mechanical dysesthesia  
19 (irrespective of conditioning stimulation), two additional RM-ANOVAs, one for itch and one for  
20 pain, were conducted comparing baseline NRS with NRS after the baseline TSs. Mauchly's test  
21 of sphericity was applied for the analyses and in cases where sphericity was violated the  
22 Greenhouse-Geisser corrected p-values were used. The Sidak-Holm correction was applied for  
23 all RM-ANOVAs when performing post hoc tests. While the study was not designed to detect  
24 gender differences an exploratory RM-ANOVA with *gender* as a between-subjects-variable was  
25 conducted for the outcomes of CIM and CPM efficacy. To address the possibility of biases  
26 related the randomized order of which tests were conducted (i.e. carry-over inhibitory or  
27 facilitatory effects from test or conditioning stimuli), two methods were applied: 1) The orders of  
28 the five conditions were dichotomized by arranging subjects by those whom were subjected to  
29 the itch conditions (1, 2 and 3) first and the pain conditions (4 and 5, see Table 1) secondly, as  
30 well as vice versa. This was then added as a between-subjects factor in the main RM-ANOVA  
31 (two levels: "itch first" or "pain first"), 2) All VAS data in response to electrical stimuli  
32 conducted first were pooled as well as those conducted secondly etc., and thereafter compared  
33 with a one-way ANOVA. This approach would detect potential adaptation or sensitization to the  
34 stimuli *per se*. Pearson correlation coefficients were calculated across all CIM and CPM efficacy  
35 measures (uncorrected). Moreover, total scores of the self-report questionnaires were  
36 exploratively correlated with the CIM- and CPM-efficacy measures in each of the five conditions  
37 separately using Pearson's correlation coefficients (uncorrected). Unless stated otherwise, data is  
38 presented as arithmetic means  $\pm$  standard error of the mean (SEM). A p-value  $< 0.05$  was  
39 considered statistically significant.

## 40 **Results**

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**Validation of applied test and conditioning stimuli**

The baseline electrical itch test stimuli of the three CIM conditions (Fig. 3a) induced, on average, moderate levels of itch with significantly less concurrent pain ( $t(24) = -8.70, p < 0.001$ ), while the baseline electrical pain test stimuli of the two CPM-conditions (Fig. 3b) produced, on average, more pain than itch ( $t(24) = 3.59, p = 0.001$ ). The electrical itch test stimuli predominantly induced itch, while concomitant levels of pain were low ( $\leq$  VAS 5/100) in 19/26 participants. The overall induced “itch vs. pain”-percentage was 87% (i.e. 87% of the mean ratings were perceived as itch and 23% as pain). The electrical pain stimuli predominantly induced pain with the “pain vs. itch”-percentage being 68%. Similarly, the CS (Fig. 3c) for itch (histamine) induced more itch than pain ( $t(24) = 27.31, p < 0.001$ ), while the CS for pain (CPT) induced more pain than itch ( $t(24) = 4.08, p < 0.001$ ).

<Insert Figure 3 about here>

**Conditioned modulation of itch**

**Mean itch scores:** For mean VAS itch evoked by the TSs in the CIM conditions (see Fig. 4a, b, c, and f), the RM-ANOVA showed a significant *conditioning stimulation x condition* interaction ( $F(2,48) = 20.56, p < 0.001$ ). Post hoc Sidak corrected tests showed significantly lower VAS itch for TSs during CS than for the baseline TS in the CIM-pain ( $14.1 \pm 1.7$  versus  $6.4 \pm 1.1$ ;  $p < 0.001$ ) and the CIM-pain<sub>ipsi</sub> condition ( $14.8 \pm 1.9$  versus  $4.8 \pm 0.9$ ;  $p < 0.001$ ). There was no significant modulation effect in the CIM-itch condition, although an insignificant trend was observed ( $13.8 \pm 1.7$  versus  $12.1 \pm 1.5$ ;  $p = 0.063$ ). There were no significant differences in mean VAS itch (primary outcome parameter) between the baseline TSs (all  $p > 0.661$ ) and the ICC (2,1) for the three VAS itch TSs ratings at baseline was 0.81. VAS itch for the TSs applied during CS was significantly lower in both the CIM-pain and CIM-pain<sub>ipsi</sub> conditions when compared to the CIM-itch condition (mean difference  $-5.7 \pm 1.0, p < 0.001$  and  $-7.3 \pm 1.1, p < 0.001$ , respectively). The CIM-pain and CIM-pain<sub>ipsi</sub> condition did not significantly differ, although there was a tendency towards lower VAS itch for the TSs applied during CS in the latter than in the former condition (mean difference  $-1.6 \pm 0.7, p < 0.096$ ).

**Peak itch scores:** The peak VAS itch evoked by the TSs in the CIM conditions, RM-ANOVA also showed a significant *conditioning stimulation x condition* interaction ( $F(2,48) = 17.66, p < 0.001$ ). Post hoc Sidak corrected tests showed significantly lower peak VAS itch for TSs during CS than for the baseline TS in the CIM-itch ( $33.8 \pm 4.0$  versus  $30.1 \pm 3.9$ ;  $p = 0.048$ ), the CIM-pain ( $33.7 \pm 4.0$  versus  $17.9 \pm 2.9$ ;  $p < 0.001$ ), and the CIM-pain<sub>ipsi</sub> ( $36.1 \pm 4.4$  versus  $15.1 \pm 2.7$ ;  $p < 0.001$ ) condition. There were no significant differences in peak VAS itch between the baseline TSs (all  $p > 0.440$ ). Peak VAS itch for the TSs applied during CS was significantly lower in both the CIM-pain and CIM-pain<sub>ipsi</sub> conditions when compared to the CIM-itch

1 condition (mean difference  $-12.2 \pm 2.5$ ,  $p < 0.001$  and  $-15.0 \pm 2.9$ ,  $p < 0.001$ , respectively). The  
2 CIM-pain and CIM-pain<sub>ipsi</sub> condition did not significantly differ ( $p = 0.409$ ).

### 3 4 5 ***Conditioned modulation of pain***

6  
7 ***Mean pain scores:*** For mean VAS pain evoked by the TSs in the CPM conditions (see Fig. 4d, e  
8 and g), the RM-ANOVA showed a significant *conditioning stimulation x condition* interaction  
9 ( $F(1,24) = 6.16$ ,  $p < 0.020$ ). Post hoc Sidak tests showed significantly lower VAS pain scores for  
10 the TSs during CS than for the baseline TSs in the CPM-pain condition ( $21.0 \pm 2.3$  versus  $14.8 \pm$   
11  $2.3$ ;  $p < 0.001$ ), and there was no significant CPM effect in the CPM-itch condition, although an  
12 insignificant tendency was observed ( $19.4 \pm 2.1$  versus  $17.5 \pm 2.0$ ;  $p = 0.056$ ). The VAS pain  
13 evoked by the baseline TSs did not significantly differ ( $p = 0.161$ ) and the ICC (2,1) between the  
14 two baseline TSs was 0.92. VAS pain for the TSs applied during CS was not significantly  
15 different between both CPM conditions, although an insignificant tendency was observed for  
16 lower VAS pain for the TSs applied during CS in the CPM-pain than in the CPM-itch condition  
17 (mean difference  $-2.6 \pm 1.5$ ,  $p = 0.097$ ).

18  
19 ***Peak pain scores:*** For peak VAS pain evoked by the TSs in the CPM conditions, the RM-  
20 ANOVA also showed a significant *conditioning stimulation x condition* interaction ( $F(1,24) =$   
21  $11.28$ ,  $p = 0.003$ ). Post hoc Sidak corrected tests showed significantly lower VAS pain scores for  
22 the TSs during CS than for the baseline TSs in the CPM-pain condition ( $54.9 \pm 4.9$  versus  $42.8 \pm$   
23  $5.2$ ;  $p < 0.001$ ), and there was no significant CPM effect in the CPM-itch condition ( $52.0 \pm 5.1$   
24 versus  $50.8 \pm 5.0$ ;  $p = 0.626$ ). Peak VAS pain evoked by the baseline TSs did not significantly  
25 differ ( $p = 0.161$ ). Peak VAS pain for the TSs applied during CS was significantly lower in the  
26 CPM-pain than the CPM-itch condition (mean difference  $-8.0 \pm 3.0$ ,  $p = 0.015$ ).

27  
28  
29 ***<Insert Figure 4 about here>***

### 30 31 32 ***Mechanical itch stimulation***

33 STI exhibited a significant increase from  $1.2 \pm 0.2$  (NRS<sub>0-10</sub>) at baseline to an average of  $1.8 \pm$   
34  $0.3$  following the baseline electrical test stimuli for itch ( $t(23) = 2.15$   $p = 0.042$ ), signifying that  
35 electrical itch stimulation evoked punctuate hyperknesis (Fig. 5a). The RM-ANOVA showed a  
36 main effect of *condition* ( $F(2,46) = 6.02$ ,  $p = 0.005$ ), with the post hoc test showing average STI-  
37 scores to be significantly higher in the CIM-itch ( $1.8 \pm 0.3$ ) than in the CIM-pain<sub>ipsi</sub> condition  
38 ( $1.4 \pm 0.2$ ,  $p = 0.017$ ). A main effect of *conditioning stimulation* was also present ( $F(1,23) =$   
39  $11.78$ ,  $p = 0.002$ ) and post hoc tests showed STI during TS + CS to be significantly lower than  
40 during baseline TS ( $p = 0.002$ ), signifying that the conditioning stimuli reduced development of

1 punctuate hyperknesis. The interaction term *conditioning stimulation x condition* was not  
2 significant, although a trend was observed ( $F(2,46) = 3.02, p = 0.058$ ).

### 3 4 ***Mechanical pain stimulation***

5 MPS did not significantly increase following the baseline electrical test stimuli for pain ( $t(23) =$   
6  $1.10, p = 0.284$ ), indicating that the applied electrical stimuli ramps did not evoke cutaneous  
7 mechanical hyperalgesia (Fig. 5b). The RM-ANOVA did not show a significant main effect for  
8 *condition* ( $p = 0.263$ ), and only a insignificant trend was observed for *conditioning stimulation*  
9 ( $F(1,23) = 3.66, p = 0.068$ ) with average MPS being decreased when comparing scores following  
10 baseline TS ( $1.9 \pm 0.2$ ) to those following application of TS + CS ( $1.7 \pm 0.2$ ). The *condition x*  
11 *conditioning stimulation* interaction was not significant ( $F(1,23) = 0.22, p = 0.642$ ).

### 12 13 ***Correlational analyses***

14 The intercorrelations for the CIM and CPM efficacy (uncorrected) were significant between  
15 CIM-itch and CIM-pain ( $r = 0.52, p = 0.008$ ), between CIM-pain<sub>ipsi</sub> and CIM-pain ( $r = 0.65, p <$   
16  $0.001$ ) as well as CPM-pain ( $r = 0.42, p = 0.039$ ), and between CIM-pain and CPM-pain ( $r = 0.44,$   
17  $p = 0.028$ ). The remaining correlation coefficients (uncorrected) were not significant. The self-  
18 report questionnaire outcomes for catastrophizing (PCS:  $16.5 \pm 1.7$  and PCS-Itch:  $17.2 \pm 1.5$ ),  
19 psychological distress (HADS-anxiety:  $14.8 \pm 0.6$  and HADS-depression:  $17.8 \pm 0.5$ ), and  
20 optimism (LOT-r:  $16.5 \pm 0.8$ ) were not significantly correlated with CIM- and CPM-efficacy in  
21 any of the conditions, except for one significant correlation between more optimism and higher  
22 CPM-efficacy by itch CS ( $r = 0.47, p = 0.021$ ; after removing the outlier in the CPM-itch  
23 condition).

### 24 25 ***Carry-over effects between conditions and gender differences of CIM and CPM efficacy***

26 For both applied carry-over analysis methods outlined in *statistical analyses* no significant biases  
27 were detected in relation to the order of which the five different paradigms were performed ( $p \geq$   
28  $0.315$ ). The exploratory RM-ANOVA conducted to detect potential gender differences in the  
29 CIM and CPM efficacy did not show any significant gender-related differences neither as an  
30 overall main effect of *gender* ( $F(1,23) = 2.81, p = 0.107$ ) nor as a significant *condition x gender*  
31 interaction ( $F(4,92) = 0.98, p = 0.425$ ).

### 32 33 ***Additional sequential condition with histamine CS***

34 In the sequential CIM-itch condition, the NRS itch evoked by histamine was on average  $4.5 \pm$   
35  $2.0$  over a mean time of  $9.4 \pm 3.0$  min (range 4.2 – 15.0). The mean VASs itch evoked by the TS  
36 before and after the CS were  $11.7 \pm 1.7$  and  $15.2 \pm 2.8$  respectively, and were not significantly  
37 different ( $t(19) = 1.61, p = 0.123$ ). The peak VASs itch were on average  $31.8 \pm 4.1$  and  $35.2 \pm$   
38  $5.4$  respectively, and did not significantly differ ( $t(19) = 1.18, p = 0.254$ ).

### 39 40 **Discussion**

1  
2 This study showed that itch could be decreased significantly by simultaneous application of a  
3 heterotopically located painful stimulus similarly to what has been shown for the standard CPM-  
4 paradigm (pain-inhibits-pain). Conversely, no itch-inhibitory effect of conditioning heterotopic  
5 itch (CIM-itch) as reflected on the mean itch ratings, was observed. The peak itch intensity only  
6 displayed a comparatively small itch-inhibitory effect by heterotopically applied itch. An  
7 additional experiment where the CIM-itch paradigm was conducted in a sequential manner (in  
8 accordance with two previous studies<sup>33,34</sup>) also failed to detect a significant CIM-effect from an  
9 itch CS.

### 10 11 ***Conditioning pain and itch stimuli***

12 The CPT using 8°C circulating water effectively and consistently produced moderate to high  
13 intensities of pain, and barely any to no itch at all, in accordance with several previous studies  
14 applying and validating this test<sup>67,68</sup>. Similarly, 1% histamine introduced by skin pricks reliably  
15 produced itch to a moderate extent and low levels of pain, which is in accordance with several  
16 papers utilizing and validating this method<sup>7,13</sup>. This indicates highly specific induction of pain  
17 and itch by the conditioning stimuli. No adverse reactions were associated with neither the CPT  
18 nor the SPT procedure. Only a single subject was unable to endure the 2-minute CPT.

### 19 20 ***Modality specificity and validation of applied experimental test stimuli***

21 The electrical pain and itch stimuli predominantly induced the intended sensation as reflected by  
22 the “pain vs. itch” and “itch vs. pain” percentages. Mild itch was particularly observed prior to  
23 the onset of pain (at  $\geq 2.9$  mA pain became dominant), indicating that preferential stimulation of  
24 pruriceptive nociceptors occur at a sub-painful level (Fig. 3b). This is in line with previous  
25 observations of a significant positive correlation between itch and pain induced by cowhage  
26 spicules<sup>37,62</sup> indicating that the sensations are not unconditionally mutually exclusive. While peak  
27 itch scores were slightly lower than those achieved for electrically induced pain, they were on  
28 par with both previous studies using a similar stimulation methodology, although these studies  
29 assessed the evoked itch intensity retrospectively and ramped up only to 5 mA<sup>9,34</sup>. Notably the  
30 achieved peak itch intensity and the itch purity were comparable to most standardized chemical  
31 itch provocations, e.g., histamine iontophoresis/SPT or cowhage application<sup>5,7,34</sup>. In this relation,  
32 the present study did screen out participants not responsive to both the itch and pain induction  
33 paradigms (2 participants were excluded). Lastly, it is conceivable that slightly higher electrical  
34 currents could have evoked more intense itch, but as indicated by stimulus-response curve  
35 flattening at high intensities (e.g., Fig. 3a), itch would likely increase only marginally with  
36 concomitant increases in pain as shown in earlier studies on electrically induced itch<sup>10,24,60,65</sup>.  
37 This highlights the sparsely articulated conundrum of the contrasting outcomes resulting from  
38 increasing pain and itch stimulation intensities (of various modalities), i.e. resulting in reaching  
39 the tolerance threshold level for pain, while itch generally reaches a ceiling at a moderate level.

### 1 ***Conditioned modulation of itch and pain***

2 The magnitude of the decrease found in the standard CPM-paradigm (pain inhibits pain)  
3 conducted as a “positive control” is in line with results from numerous previous studies using the  
4 CPT or deep somatic stimulation as the CS<sup>26,66,68,70</sup>. The paradigm in which a conditioning itch  
5 stimulus was applied together with a contralateral pain TS (CPM-itch) conducted as a “negative  
6 control”, did not produce a significant decrease, although a trend towards a small decrease was  
7 evident, perhaps related to itch-induced distraction. Statistical analyses of a potential order or  
8 “carry-over” effect between the conditions did not yield significant findings indicating that 10-  
9 minute breaks were sufficient to avoid significant carry-over interference as also suggested by a  
10 previous study<sup>26</sup>.

11 Itch levels were on average not significantly affected by conditioning itch stimulation (CIM-itch,  
12 Fig. 4a), although a tendency towards a small decrease was observed. Notably, a comparatively  
13 small ( $\approx 9\%$  reduction from baseline) but significant decrease following CIM-itch was observed  
14 for the peak itch indicating that there could be minor inhibitory effect in the higher range of the  
15 TS ramp. These findings are in opposition to two previous studies detecting a significant itch-  
16 evoked CIM-effect<sup>32,34</sup>. These studies both used iontophoretically delivered histamine as the CS  
17 and electrically induced itch as TS conducted with a comparable stimulation paradigm, although  
18 the intensity was tailored to each individual participant, resulting in an overall lower electrical  
19 current being applied. In these previous studies, the test and conditioning stimuli were delivered  
20 in a sequential manner,<sup>32,34</sup> opposed to the simultaneous approach used in the main experiment of  
21 the present study. However, the present additional experiment with a sequential design also  
22 failed to detect a significant modulation of itch following conditioned itch stimulation. The  
23 explanation for the discrepancy in results may lie in differential methodology, but as the present  
24 study used both more intense conditioning and test stimuli than both previous studies, larger  
25 decreases were expected in line with what is known from CPM studies<sup>21,43</sup>. Whether, and the  
26 extent to which, there is a modulation effect of itch by conditioning itch stimulation should be  
27 investigated further. In contrast, itch levels were prominently reduced by conditioning pain  
28 stimuli (Fig. 4b and 4c). The itch-inhibitory effect of distantly located pain stimulation has  
29 previously been assessed thrice using the CPT or deep-somatic cuff-algometric stimulation as  
30 conditioning stimuli<sup>3,34,42</sup>. The findings of the present study, i.e. a reduction of itch during contra-  
31 /ipsilateral conditioning pain stimulation, are well in line with previous findings of pain  
32 modulation when applying the CPT<sup>21,43</sup>. However, deep somatic conditioning pain stimulation  
33 has previously been found not to modulate histaminergic itch<sup>3</sup>. This discrepancy is likely related  
34 to tissue-preferentiality of the CPM-effect, i.e., several studies found no effect of deep somatic  
35 conditioning pain on painful cutaneous test stimuli, while it was highly effective in reducing pain  
36 arising from musculoskeletal test stimuli.<sup>26,29,44</sup> The exploratory analysis of gender-related  
37 effects did not reveal any significant differences for CIM and CPM efficacy between males and  
38 females. Previous studies suggest a fairly consistent pattern of results, with females exhibiting  
39 enhanced pain sensitivity, increased pain facilitation as well as reduced endogenous pain  
40 inhibition compared with males.<sup>30,52</sup> However, meta analyses of studies on gender differences in



1 pain sensitivity has repeatedly voiced concerns regarding underpowered studies.<sup>19,54</sup> The present  
2 study was not *a priori* powered nor designed to detect gender differences, so this exploratory  
3 analysis should be interpreted with caution.

4 While previous studies suggest C-fibers as the probable substrate for electrically induced itch  
5 <sup>24,40</sup> it is unclear whether the applied test stimuli neurophysiologically mimics itch observed in,  
6 patients with chronic itch and as such extrapolation should be made with care. However, a  
7 previous study using cowhage as test stimulus, in combination with the CPT, found similar  
8 reductions of itch to those we observed, indicating that the itch test-stimulus is not highly  
9 modality-dependent <sup>42</sup>. Along these lines, histamine as a conditioning itch stimulus has only been  
10 incidentally tested <sup>32,34</sup> and since cowhage is generally capable of inducing significantly more  
11 severe itch than histamine <sup>2,5,47</sup>, it could potentially be better suited as conditioning stimulation.  
12 Here however it should be noted that cowhage is a less “pure” pruritogen than histamine in that it  
13 also evokes more coinciding pricking/stinging pain <sup>2,61,62</sup>. This could potentially make it unclear  
14 whether a potential modulatory effect is in fact related to the evoked itch or the evoked pain.

15 Collectively, these findings may suggest that pain and itch, as somatosensory modalities, are  
16 under hierarchical prioritization in a manner promoting perception of pain whenever both  
17 sensations are heterotopically presented in a simultaneous manner. This has previously been  
18 established and mechanistically explored for homotopically applied itch and pain, where painful  
19 counter-stimuli generally prominently inhibit itch,<sup>6,27,71</sup> but this study is the first to systematically  
20 elucidate such sensory prioritization for heterotopically applied itch and pain stimuli. In this  
21 context it should be highlighted that although a high CPT temperature (8°C) was used, higher  
22 effective conditioning intensity scores were achieved for pain conditioning than for itch. While  
23 this could lead to a relative overestimation of the efficacy of pain-induced descending inhibition,  
24 a previous study suggests that a ceiling effect in the CPM-effect is reached when increasing the  
25 conditioning pain intensity from mild, i.e. VAS  $\approx$  30 (well below the herein achieved  
26 conditioning itch intensity), to moderate, i.e. VAS  $\approx$  60 (VAS<sub>0-100</sub>) <sup>21,43</sup>. This is in line with  
27 recent CPM consensus recommendations<sup>68</sup> and would infer that in terms of achieved intensity,  
28 the presently applied itch and pain conditioning stimuli have comparable inhibitory capacity.  
29 Moreover, while no studies assessing the affect of the intensity of conditioning itch stimulation  
30 in CIM paradigms exist, previous findings suggest that a CIM-effect is achievable a lower itch  
31 intensities than herein applied <sup>32,34</sup>.

### 32 33 ***Role of individual characteristics***

34 Catastrophizing, optimism and psychological distress did, in contrast to some previous  
35 indications for pain <sup>17,22</sup>, not seem to play a significant role (only one correlation coefficient out  
36 of multiple, uncorrected tests was significant) in the itch and pain modulation in healthy humans.  
37 Although internal consistency was good, non-significant associations of the catastrophizing scale  
38 modified for itch should be interpreted with caution since this adjusted scale has not previously  
39 been validated.

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***Elicitation and modulation of dysesthesias***

STI significantly increased following electrical itch stimuli, indicative primarily of hyperknesis rather than alloknesis, because the majority of participants reported itch in response to the von Frey probing at baseline and because of the punctuate character of the applied stimuli. However, the modest absolute increases observed for mechanically evoked itch combined with substantial variability limit clinical relevance for the applied induction and/or assessment technique. Hyperknesis, as well as alloknesis, have previously been reported following electrically evoked itch<sup>24</sup> and following chemical itch provocations when using the same assessment technique as the present study<sup>2</sup>. Mechanistically, hyperknesis is thought to perceptually reflect centrally-mediated sensitization of A $\delta$ - or polymodal C-nociceptor<sup>2,24,35</sup>, enabling increased itch in response to pricking and mildly itching mechanical stimuli. Conditioning stimulation caused a significant decrease in STI, signifying that this sensitization was partly inhibited by concomitant conditioning itch or pain stimuli. Notably, an insignificant trend was observed on the interaction term indicating that STI was reduced most prominently in the CIM-pain and CIM-pain<sub>ipsi</sub> conditions. MPS was not significantly altered by the painful electrical stimulation, indicating that the paradigm did not cause cutaneous mechanical hyperalgesia. Contralateral pain and itch conditioning stimulation did not alter MPS, which is partly in line with a previous study assessing the effect of conditioning pain stimulation of pinprick-evoked pain sensitivity<sup>44</sup>.

***Conclusion***

In summary the present study showed that while conditioning pain stimulation effectively induce inhibition of both concurrent pain as well as itch, conditioning itch stimulation does not elicit a reduction of concurrent pain nor itch, although a small decrease was observed when specifically assessing peak itch responses. Conditioning itch stimulation is likely insufficient to evoke robust descending inhibition.

***Perspective***

Provided that an itch-evoked CIM-effect exists, its physiological role, and consequently its clinical relevance seem limited, given that it cannot be robustly elicited under controlled conditions. On the other hand, the endogenous inhibitory system activated by painful stimulation inhibited itch to at least the same extent as painful test stimuli. The significant associations between itch modulation by itch and pain conditioning as well as CPM efficacy by painful conditioning stimulation indicates involvement of a shared inhibitory system, which is most effective with painful conditioning stimulation. As such, pain-induced endogenous modulation of itch seems to be a better measure of one's itch modulatory capacity than itch-evoked CIM. Aligned with previous studies showing reduced CPM-efficacy in patients with chronic itch<sup>28,49</sup>, less efficacious itch modulation by pain may be involved in the pathophysiology of chronic itch. This is supported by previous findings that patients with chronic itch require more intense homotopic noxious counter-stimulation to achieve itch-relief compared to healthy controls<sup>27</sup>. As



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**Figure/table legends**

**Table 1:** Overview of the different conditioned itch modulation (CIM) and conditioned pain modulation (CPM) conditions as well as assessed mechanical dysesthesias pertaining to each condition.

**Figure 1: Time line of the experiment.** IC: informed consent; CIM: conditioned itch modulation; CPM: conditioned pain modulation; TS: test stimulus; CS: conditioning stimulus. Note: within each CIM/CPM condition, also mechanical stimulation was applied after each TS.

**Figure 2: Application sites for somatosensory stimuli.** The side (left/right) on which the electrically evoked itch and pain test stimuli were applied was randomized. Consequently, the conditioning stimuli for itch (histamine skin prick) and pain (cold pressor task) as well as the dysesthesia assessments for itch (Von Frey monofilaments) and pain (mechanical pinprick stimulators) were applied accordingly. SPT: skin prick test.

**Figure 3: Selectivity and intensity of itch and pain induced by test- and conditioning stimulation.** **a)** Average itch and pain induced by all itch test stimuli, i.e. average itch and pain ratings for the test stimuli within the experimental itch and pain conditions, **b)** Average itch and pain induced by all pain test stimuli. Notice that for **a** and **b** the electrical test stimulus paradigms quite selectively induced itch and pain, respectively, **c)** Average itch and pain induced by histamine provocations and cold pressor tasks. CIM: conditioned itch modulation; CPM: conditioned pain modulation, CS: conditioning stimulus; mA: miliAmpère; NRS: Numeric rating scale; VAS: Visual analogue scale. \*\* =  $p < 0.01$ .

**Figure 4: Modulation of electrically induced itch and pain by conditioning itch and pain stimulation displayed as stimulus response curves (a-e) and with the itch and pain ratings averaged over the stimulation period (f-g).** Specifically displayed in **a)** Itch during itch as CS – 1. “CIM-itch”, **b)** Itch during pain as CS (contralaterally) – 2. “CIM-pain”, **c)** Itch during pain as CS (ipsilaterally) – 3. “CIM-pain<sub>ipsi</sub>”, **d)** Pain during pain as CS (contralaterally) – 4. “CPM-pain”, **e)** Pain during pain as CS (contralaterally) – 5. “CPM-itch”, **f)** Mean itch intensities within each condition **g)** Mean pain intensities within each condition. CIM: Conditioned itch modulation; CPM: Conditioned pain modulation, CS: Conditioning stimulus; mA: miliAmpère; NRS: Numeric rating scale; TS: Test stimulus; VAS: Visual analogue scale. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

**Figure 5: Assessment of mechanical hyperknesis and hyperalgesia.** **a)** Sensitivity to touch evoked itch (STI) before TS (dotted line), after TS (white bars), and after TS and CS (grey bars) for each CIM condition, **b)** Mechanical pain sensitivity (MPS) before (dotted line), after TS, and after TS and CS for each CPM condition. CIM: Conditioned itch modulation; CPM: Conditioned



- 1 pain modulation, CS: Conditioning stimulus; NRS: Numeric rating scale; TS: Test stimulus. \*/# =
- 2  $p < 0.05$ , \*\* =  $p < 0.01$ , \* indicates difference from baseline STI, # indicates difference for the
- 3 main effect of *condition*.

**Figure 1**  
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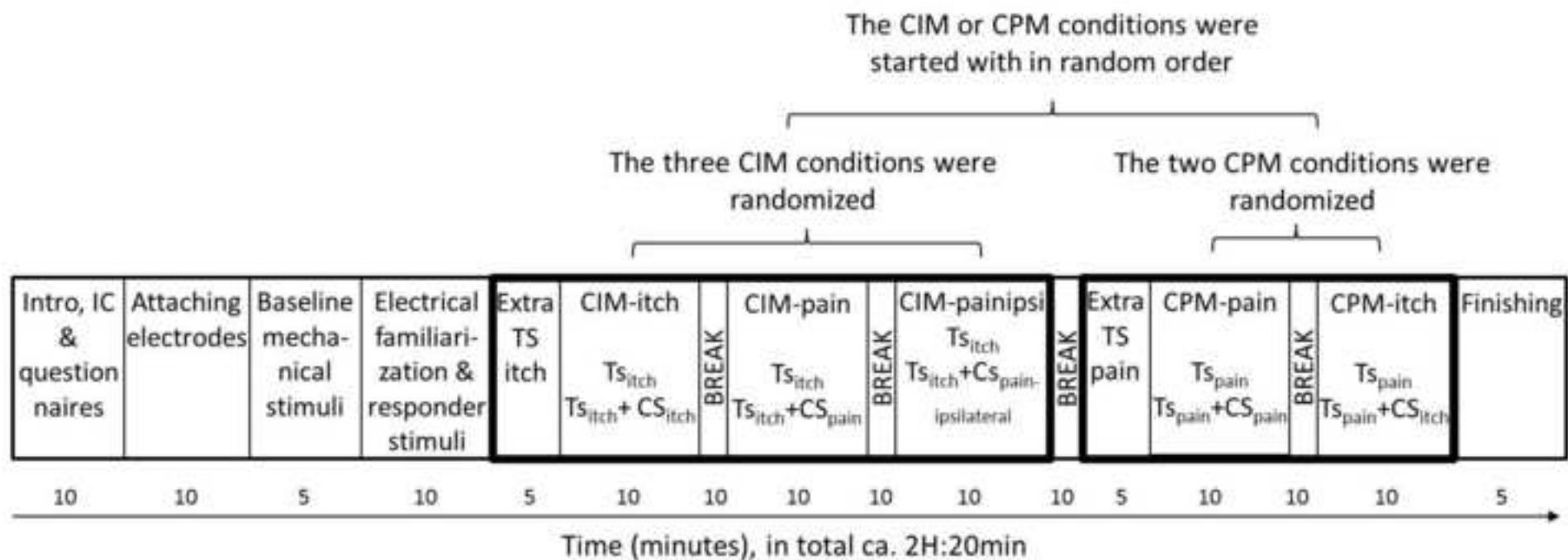


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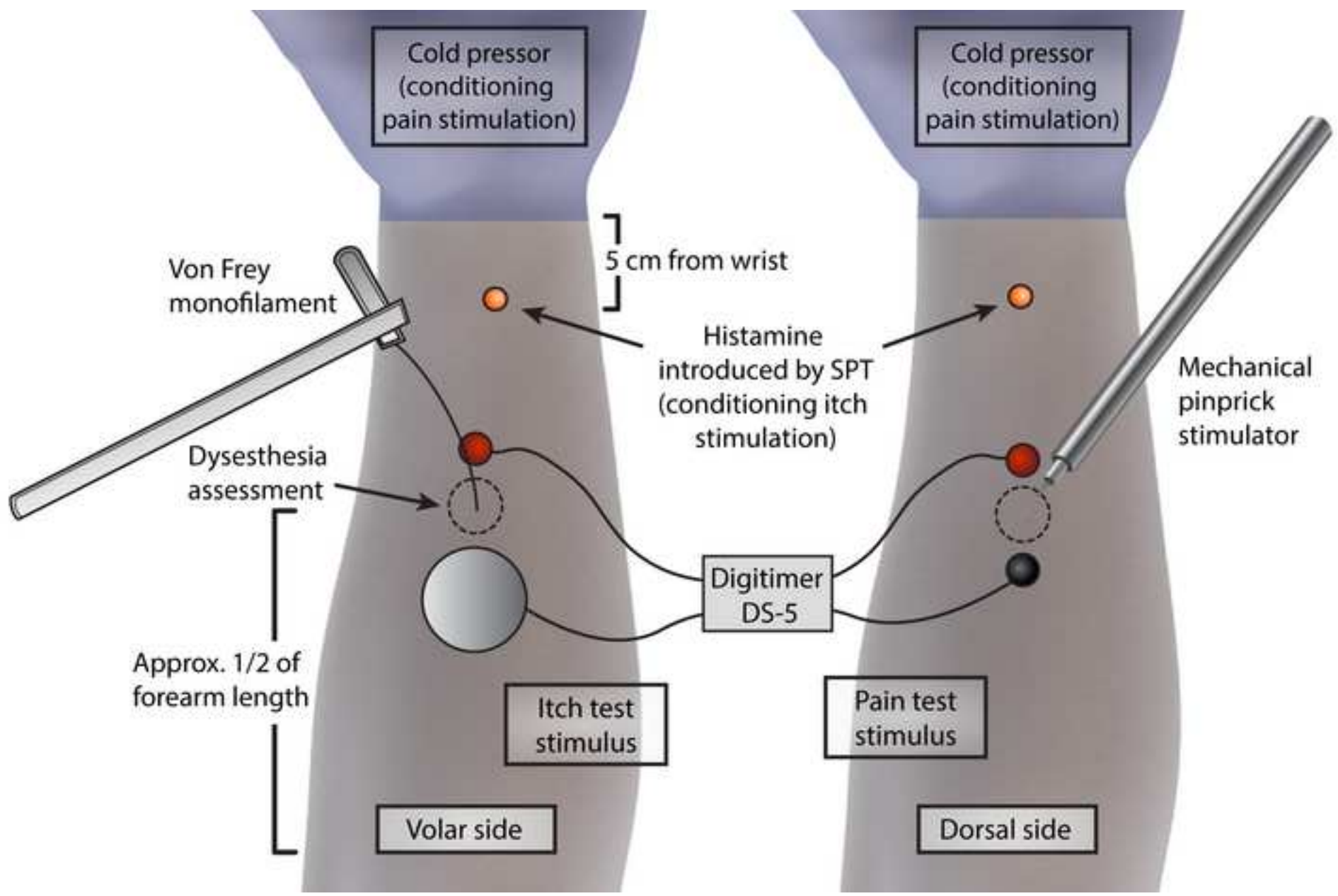
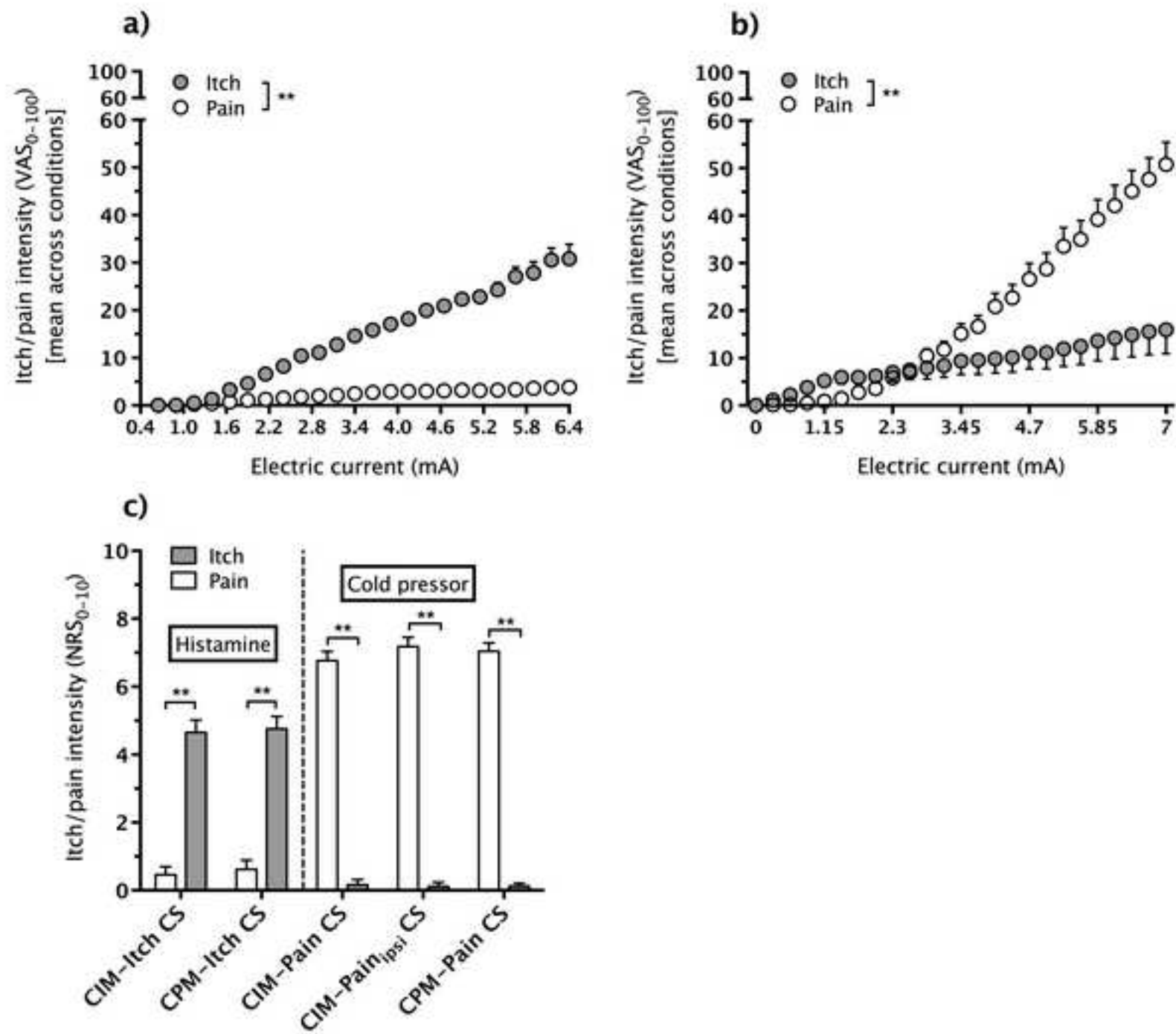


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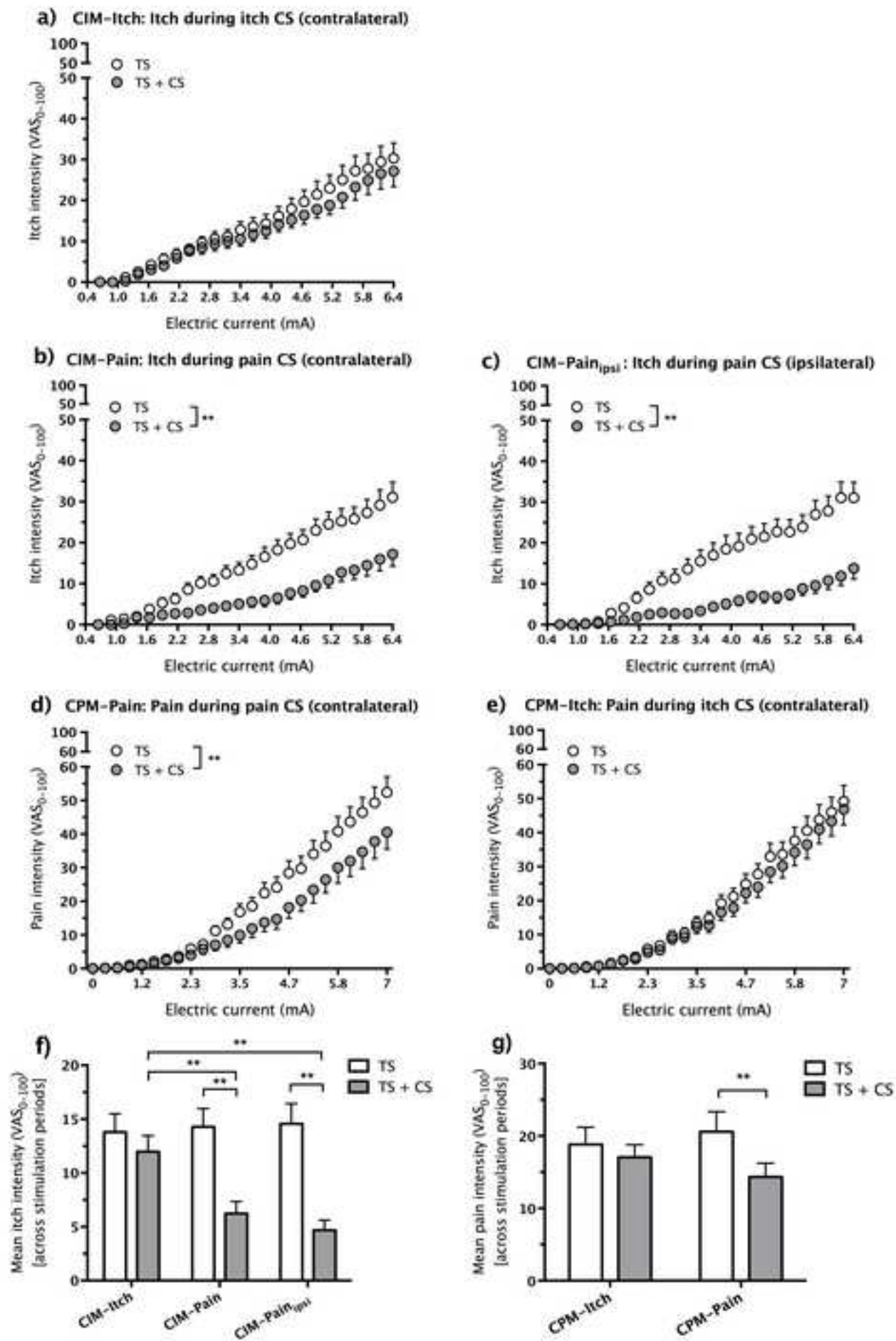


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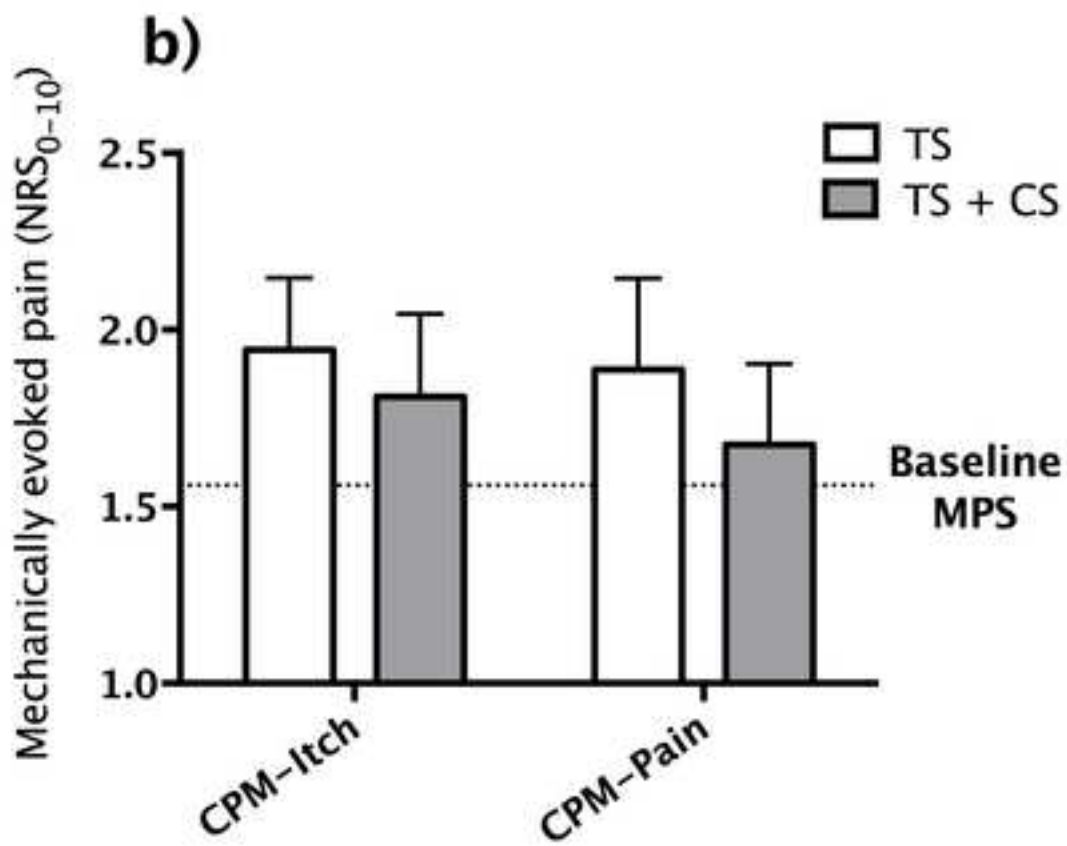
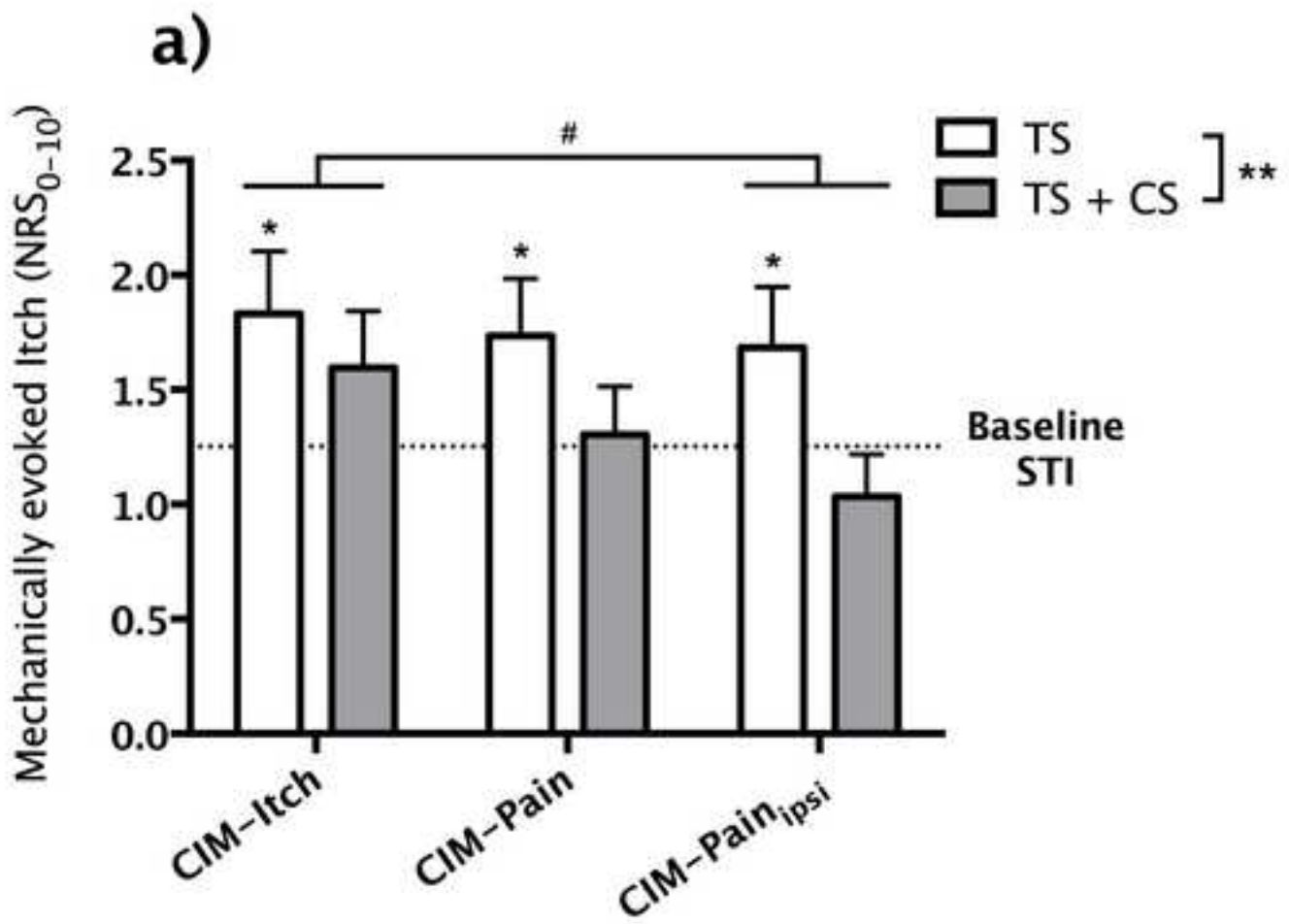


Table 1

<b>Condition</b>	<b>Test stimuli</b>	<b>Conditioning stimulation</b>	<b>Assessment of dysesthesia</b>
1. CIM-itch	Electrical itch test stimuli	Contralateral conditioning itch stimulation with histamine	Sensitivity to touch evoked itch with von Frey monofilaments
2. CIM-pain	Electrical itch test stimuli	Contralateral conditioning pain stimulation with the cold pressor task	Sensitivity to touch evoked itch with von Frey monofilaments
3. CIM-pain <sub>ipsi</sub>	Electrical itch test stimuli	Ipsilateral conditioning pain stimulation with the cold pressor task	Sensitivity to touch evoked itch with von Frey monofilaments
4. CPM-pain	Electrical pain test stimuli	Contralateral conditioning pain stimulation with the cold pressor task	Mechanical pain sensitivity using pinprick stimulators
5. CPM-itch	Electrical pain test stimuli	Contralateral conditioning itch stimulation with histamine	Mechanical pain sensitivity using pinprick stimulators