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Published in:
Journal of Pain

DOI (link to publication from Publisher):
10.1016/j.jpain.2017.07.002

Publication date:
2017

Document Version
Accepted author manuscript, peer reviewed version

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Running head: Assessing endogenous itch inhibition

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Article category: Original manuscript

Disclosures: No conflicts of interests to declare

Conflict of interest: None to declare

Number of pages: 24 (including figure legends and references)

Number of figures/tables: 5 figures, 1 table

Key words: Itch, pain, conditioned pain modulation, conditioned itch modulation, descending inhibition

Abstract

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

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

Introduction

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

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

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

CPM affects itch processing (i.e. conditioned itch modulation; CIM) and if so, which kinds of conditioning stimuli are required to activate it[3,32,34].

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

Methods

Participants

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

Design

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

<Insert Table 1 about here>

<Insert Figure 1 about here>

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 [1].

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[9,34], stimuli were applied at the volar side, at 50 Hz with a pulse duration of 100 µs, 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 ≈2 min duration per stimulus ramp.

Electrically evoked pain: For pain induction, two surface electrodes (two disk electrodes of ø 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 µs. 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 ≈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>

Conditioning itch stimulation: As itch CS, histamine (as dihydrochloride in a concentration of 10 mg/ml, i.e. 1% EEAACI recommended positive control)[4,16] was applied with 1-mm weight-calibrated skin prick test lancets (SPT) using 120 g weight[4]. The SPTs were performed at the central volar forearms, 5 cm proximally from the cease of the wrist. A small drop of histamine was placed on the skin and percutaneously introduced with the SPT lancet[11,20,72].
Conditioning pain stimulation: To elicit pain as CS, a cold pressor task (CPT) was used with water of ca. 8 °C (mean 7.9 ± 0.1 and 8.3 ± 0.1 before and after CPT, respectively) in an 8 liter plastic box isolated by styrofoam. The water was circulated by an Anova Precision Cooker (Anova, San Francisco, California, USA) at a rate of 8 L/min. Participants were instructed to immerse their hand up to the level of their wrist into the water for the duration of the TS (i.e. ≈2 min).

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

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

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

Self-report questionnaires

Based on previous research[22] the following self-report questionnaires were administered in English. To keep the procedure across participants as comparable as possible and to allow recruitment of non-Danish speakers, the experimental procedures were conducted in English regardless of whether HHA (Danish) or AIMvL (Dutch) was the active investigator. The Pain Catastrophizing Scale (PCS)[64] was administered to assess catastrophizing of pain experienced in daily life. This questionnaire consists of 13 items, each rated on a Likert scale from 0 (not at all) to 4 (all the time). The total score was obtained by summing the scores for all items, with a theorectical range from 0 to 52. The Cronbach's alpha in the present study was 0.88. The PCS was also administered in a for itch adjusted version (PCS-Itch), in which only the word "pain" for all items was substituted by the word "itch". The Cronbach's alpha of the PCS-Itch in the present study was 0.85. Dispositional optimism was measured with the revised Life Orientation Test (LOT-R)[57], consisting of 3 positive, 3 negative, and 4 filler items which were rated on a Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The total score can range from 0 to 24, with higher scores indicating higher optimism. Cronbach's alpha was 0.75. The Hospital Anxiety and Depression Scale (HADS)[73] was administered to assess psychological distress. This questionnaire consists of a subscale for depression (7 items; Cronbach's alpha in the present study 0.62) and a subscale for anxiety (7 items Cronbach's alpha in the present study 0.61). Items were rated on a scale from 0 to 3, and the total scores for both subscales (each potentially ranging from 0 to 21) were obtained by summing the respective items with total scores.

Procedure

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

Additional sequential experiment

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

Statistical analyses

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

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

Results

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 (≤ 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 ± 1.7 versus 6.4 ± 1.1; $p < 0.001$) and the CIM-pain$_{ipsi}$ condition (14.8 ± 1.9 versus 4.8 ± 0.9; $p < 0.001$). There was no significant modulation effect in the CIM-itch condition, although an insignificant trend was observed (13.8 ± 1.7 versus 12.1 ± 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$_{ipsi}$ conditions when compared to the CIM-itch condition (mean difference -5.7 ± 1.0, $p < 0.001$ and -7.3 ± 1.1, $p < 0.001$, respectively). The CIM-pain and CIM-pain$_{ipsi}$ 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 ± 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 ± 4.0 versus 30.1 ± 3.9; $p = 0.048$), the CIM-pain (33.7 ± 4.0 versus 17.9 ± 2.9; $p < 0.001$), and the CIM-pain$_{ipsi}$ (36.1 ± 4.4 versus 15.1 ± 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$_{ipsi}$ conditions when compared to the CIM-itch condition (mean difference -12.2 ± 2.5, $p < 0.001$ and -15.0 ± 2.9, $p < 0.001$, respectively). The CIM-pain and CIM-pain$_{ipsi}$ condition did not significantly differ ($p = 0.409$).

Conditioned modulation of pain

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

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

<Insert Figure 4 about here>

Mechanical itch stimulation

STI exhibited a significant increase from 1. 2 ± 0.2 ($NRS_{0\text{-}10}$) at baseline to an average of 1.8 ± 0.3 following the baseline electrical test stimuli for itch ($t(23) = 2.15$ $p = 0.042$), signifying that electrical itch stimulation evoked punctuate hyperknesis (Fig. 5a). The RM-ANOVA showed a main effect of *condition* ($F(2,46) = 6.02$, $p = 0.005$), with the post hoc test showing average STI-scores to be significantly higher in the CIM-itch (1.8 ± 0.3) than in the CIM-pain$_{ipsi}$ condition (1.4 ± 0.2, $p = 0.017$). A main effect of *conditioning stimulation* was also present ($F(1,23) = 11.78$, $p = 0.002$) and post hoc tests showed STI during TS + CS to be significantly lower than during baseline TS ($p = 0.002$), signifying that the conditioning stimuli reduced development of punctuate hyperknesis. The interaction term *conditioning stimulation x condition* was not significant, although a trend was observed (F(2,46) = 3.02, p = 0.058).

Mechanical pain stimulation

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

Correlational analyses

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

Carry-over effects between conditions and gender differences of CIM and CPM efficacy

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

Additional sequential condition with histamine CS

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

Discussion

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

Conditioning pain and itch stimuli

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

Modality specificity and validation of applied experimental test stimuli

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

Conditioned modulation of itch and pain

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

Itch levels were on average not significantly affected by conditioning itch stimulation (CIM-itch, Fig. 4a), although a tendency towards a small decrease was observed. Notably, a comparatively small (≈ 9% reduction from baseline) but significant decrease following CIM-itch was observed for the peak itch indicating that there could be minor inhibitory effect in the higher range of the TS ramp. These findings are in opposition to two previous studies detecting a significant itch-evoked CIM-effect[32,34]. These studies both used iontophoretically delivered histamine as the CS and electrically induced itch as TS conducted with a comparable stimulation paradigm, although the intensity was tailored to each individual participant, resulting in an overall lower electrical current being applied. In these previous studies, the test and conditioning stimuli were delivered in a sequential manner,[32,34] opposed to the simultaneous approach used in the main experiment of the present study. However, the present additional experiment with a sequential design also failed to detect a significant modulation of itch following conditioned itch stimulation. The explanation for the discrepancy in results may lie in differential methodology, but as the present study used both more intense conditioning and test stimuli than both previous studies, larger decreases were expected in line with what is known from CPM studies[21,43]. Whether, and the extent to which, there is a modulation effect of itch by conditioning itch stimulation should be investigated further. In contrast, itch levels were prominently reduced by conditioning pain stimuli (Fig. 4b and 4c). The itch-inhibitory effect of distantly located pain stimulation has previously been assessed thrice using the CPT or deep-somatic cuff-algometric stimulation as conditioning stimuli[3,34,42]. The findings of the present study, i.e. a reduction of itch during contra-/ipsilateral conditioning pain stimulation, are well in line with previous findings of pain modulation when applying the CPT[21,43]. However, deep somatic conditioning pain stimulation has previously been found not to modulate histaminergic itch[3]. This discrepancy is likely related to tissue-preferentiality of the CPM-effect, i.e., several studies found no effect of deep somatic conditioning pain on painful cutaneous test stimuli, while it was highly effective in reducing pain arising from musculoskeletal test stimuli.[26,29,44] The exploratory analysis of gender-related effects did not reveal any significant differences for CIM and CPM efficacy between males and females. Previous studies suggest a fairly consistent pattern of results, with females exhibiting enhanced pain sensitivity, increased pain facilitation as well as reduced endogenous pain inhibition compared with males.[30,52] However, meta analyses of studies on gender differences in pain sensitivity has repeatedly voiced concerns regarding underpowered studies.[19,54] The present study was not *a priori* powered nor designed to detect gender differences, so this exploratory analysis should be interpreted with caution.

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

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

Role of individual characteristics

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

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[24] and following chemical itch provocations when using the same assessment technique as the present study[2]. Mechanistically, hyperknesis is thought to perceptually reflect centrally-mediated sensitization of Aδ- or polymodal C-nociceptor[2,24,35], 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$_{ipsi}$ 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[44].

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[28,49], 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[27]. As such, future research on the inhibitory efficacy of conditioning pain stimuli on both pain and itch test stimuli in chronic itch patients is warranted.

Acknowledgments

This research is supported by an Innovation Scheme (Veni) Grant (451-15-019) of the Netherlands Organization for Scientific Research (NWO), granted to A.I.M. van Laarhoven. HHA acknowledges support from the *EliteForsk Rejse-Stipendiat 2016* granted by the Danish Ministry of Higher Education.

References

1. Andersen HH, Elberling J, Arendt-Nielsen L: Human Surrogate Models of Histaminergic and Non-histaminergic Itch. Acta Derm Venereol 95:771–7, 2015.
2. Andersen HH, Elberling J, Lo Vecchio S, Arendt-Nielsen L: Topography of itch: evidence of distinct coding for pruriception in the trigeminal nerve. Itch 1:1–10, 2016.
3. Andersen HH, Imai Y, Petersen KK, Koenig J, Elberling J, Arendt-Nielsen L: Conditioning pain stimulation does not affect itch induced by intra-epidermal histamine pricks but aggravates neurogenic inflammation in healthy volunteers. Somatosens Mot Res 33:49–60, 2016.
4. Andersen HH, Lundgaard AC, Petersen AS, Hauberg LE, Sharma N, Hansen SD, Elberling J, Arendt-Nielsen L: The Lancet Weight Determines Wheal Diameter in Response to Skin Prick Testing with Histamine. Wright NT, editor. PLoS One 11:e0156211, 2016.
5. Andersen HH, Marker JB, Hoeck EA, Elberling J, Arendt-Nielsen L: Antipruritic effect of pretreatment with 8% topical capsaicin on histamine- and cowhage-evoked itch in healthy volunteers - a randomized, vehicle-controlled, proof-of-concept trial. Br J Dermatol 38:42–9, 2017.
6. Andersen HH, Melholt C, Hilborg SD, Jerwiarz A, Randers A, Simoni A, Elberling J, Arendt-Nielsen L: Antipruritic Effect of Cold-induced and Transient Receptor Potential-agonist-induced Counter-irritation on Histaminergic Itch in Humans. Acta Derm Venereol 97:63–70, 2017.
7. Andersen HH, Sørensen A-KR, Nielsen GAR, Mølgaard MS, Stilling P, Boudreau SA, Elberling J, Arendt-Nielsen L: A Test-Retest Reliability Study of Human Experimental Models of Histaminergic and Non-histaminergic Itch. Acta Derm Venereol 97:198–207, 2017.
8. Le Bars D, Dickenson AH, Besson J-M: Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain 6:283–304, 1979.
9. Bartels DJP, Van Laarhoven AIM, Haverkamp EA, Wilder-Smith OH, Donders ART, Van Middendorp H, Van De Kerkhof PCM, Evers AWM: Role of conditioning and verbal suggestion in placebo and nocebo effects on itch. PLoS One 9:e91727, 2014.
10. Bishop GH: Responses to electrical stimulation of single sensory units of skin. J Neurophysiol 6:361–82, 1943.
11. Bjerring P, Arendt-Nielsen L: Inhibition of histamine skin flare reaction following repeated topical applications of capsaicin. Allergy 45:121–5, 1990.
12. Bromma B, Scharein E, Darsow U, Ring J: Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci Lett 187:157–60, 1995.
13. Darsow U, Ring J, Scharein E, Bromm B: Correlations between histamine-induced wheal, flare and itch. Arch Dermatol Res 288:436–41, 1996.
14. Darsow U, Scharein E, Simon D, Walter G, Bromm B, Ring J: New aspects of itch pathophysiology: Component analysis of atopic itch using the 'Eppendorf Itch Questionnaire'. Int Arch Allergy Immunol 124:326–31, 2001.
15. Dhand A, Aminoff MJ: The neurology of itch. Brain 137:313–22, 2013.
16. Dreborg ES, Frew A, Frew A: Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. Allergy 48:48–82, 1993.
17. Edwards RR, Dolman AJ, Michna E, Katz JN, Nedeljkovic SS, Janfaza D, Isaac Z, Martel MO, Jamison RN, Wasan AD: Changes in Pain Sensitivity and Pain Modulation During Oral Opioid Treatment: The Impact of Negative Affect. Pain Med 17:1882–91, 2016.

18. Ernst E, Fialka V: Ice freezes pain? A review of the clinical effectiveness of analgesic cold therapy. J Pain Symptom Manage 9:56–9, 1994.

19. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL: Sex, Gender, and Pain: A Review of Recent Clinical and Experimental Findings. J Pain Elsevier Ltd; 10:447–85, 2009.

20. Gazerani P, Pedersen NS, Drewes AM, Arendt-Nielsen L: Botulinum toxin type A reduces histamine-induced itch and vasomotor responses in human skin. Br J Dermatol 161:737–45, 2009.

21. Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D: Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? Pain 136:142–9, 2008.

22. Hermans L, Van Oosterwijck J, Goubert D, Goudman L, Crombez G, Calders P, Meeus M: Inventory of Personal Factors Influencing Conditioned Pain Modulation in Healthy People: A Systematic Literature Review. Pain Pract 16:758–69, 2016.

23. Hosogi M, Schmelz M, Miyachi Y, Ikoma A: Bradykinin is a potent pruritogen in atopic dermatitis: a switch from pain to itch. Pain 126:16–23, 2006.

24. Ikoma A, Handwerker H, Miyachi Y, Schmelz M: Electrically evoked itch in humans. Pain 113:148–54, 2005.

25. Ikoma A, Rukwied R, Ständer S, Steinhoff M, Miyachi Y, Schmelz M: Neuronal sensitization for histamine-induced itch in lesional skin of patients with atopic dermatitis. Arch Dermatol 139:1455–8, 2003.

26. 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 33:169–77, 2016.

27. Ishiuji Y, Coghill RC, Patel TS, Dawn A, Fountain J, Oshiro Y, Yosipovitch G: Repetitive scratching and noxious heat do not inhibit histamine-induced itch in atopic dermatitis. Br J Dermatol 158:78–83, 2008.

28. Julious SA: Tutorial in biostatistics: Sample sizes for clinical trials with Normal data. Stat Med 23:1921–86, 2004.

29. Kosek E, Hansson P: Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. Pain 70:41–51, 1997.

30. Kvachadze I, Tsagareli MG, Dumbadze Z: An overview of ethnic and gender differences in pain sensation. Georgian Med News :102–8, 2015.

31. van Laarhoven AIM, Kraaimaat FW, Wilder-Smith OH, van de Kerkhof PCM, Cats H, van Riel PLCM, Evers AWM: Generalized and symptom-specific sensitization of chronic itch and pain. J Eur Acad Dermatology Venereol 21:1187–92, 2007.

32. van Laarhoven AIM, Kraaimaat FW, Wilder-Smith OH, van de Kerkhof PCM, Evers AWM: Heterotopic pruritic conditioning and itch--analogous to DNIC in pain? Pain 149:332–7, 2010.

33. van Laarhoven AIM, Kraaimaat FW, Wilder-Smith OH, van Riel PLCM, van de Kerkhof PCM, Evers AWM: Sensitivity to itch and pain in patients with psoriasis and rheumatoid arthritis. Exp Dermatol 22:530–4, 2013.

34. van Laarhoven AIM, Ulrich DJO, Wilder-Smith OH, van Loey NEE, Nieuwenhuis M, van der Wee NJA, Evers AWM: Psychophysiological Processing of Itch in Patients with Chronic Post-burn Itch: An Exploratory Study. Acta Derm Venereol 96:613–8, 2016.

35. LaMotte RH: Encyclopedia of Pain - Allodynia and Alloknesis. Gebhart GF, Schmidt RF, editors. Encycl pain. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013.

36. LaMotte RH, Dong X, Ringkamp M: Sensory neurons and circuits mediating itch. Nat Rev Neurosci Nature Publishing Group; 15:19–31, 2014.

37. LaMotte RH, Shimada SG, Green BG, Zelterman D: Pruritic and Nociceptive Sensations and Dysesthesias From a Spicule of Cowhage. J Neurophysiol 101:1430–43, 2009.

38. Lewis GN, Rice DA, McNair PJ: Conditioned Pain Modulation in Populations With Chronic Pain: A Systematic Review and Meta-Analysis. J Pain 13:936–44, 2012.

39. McGill R, Tukey JW, Larsen WA: Variations of Box Plots. Am Stat 32:12, 1978.

40. Mochizuki H, Inui K, Yamashiro K, Ootsuru N, Kakigi R: Itching-related somatosensory evoked potentials. Pain 138:598–603, 2008.

41. Mørch CD, Gazerani P, Nielsen TA, Arendt-Nielsen L: The UVB cutaneous inflammatory pain model: A reproducibility study in healthy volunteers. Int J Physiol Pathophysiol Pharmacol 5:203–15, 2013.

42. Murray FS, Weaver MM: Effects of ipsilateral and contralateral counterirritation on experimentally produced itch in human beings. J Comp Physiol Psychol 89:819–26, 1975.

43. Nir R-R, Granovsky Y, Yarnitsky D, Sprecher E, Granot M: A psychophysical study of endogenous analgesia: the role of the conditioning pain in the induction and magnitude of conditioned pain modulation. Eur J Pain 15:491–7, 2011.

44. Oono Y, Baad-Hansen L, Wang K, Arendt-Nielsen L, Svensson P: Effect of conditioned pain modulation on trigeminal somatosensory function evaluated by quantitative sensory testing. Pain; 154:2684–90, 2013.

45. Ossipov MH, Morimura K, Porreca F: Descending pain modulation and chronification of pain. Curr Opin Support Palliat Care 8:143–51, 2014.

46. Papoiu ADP, Coghill RC, Kraft RA, Wang H, Yosipovitch G: A tale of two itches. Common features and notable differences in brain activation evoked by cowhage and histamine induced itch. Neuroimage Elsevier Inc.; 59:3611–23, 2012.

47. Papoiu ADP, Tey HL, Coghill RC, Wang H, Yosipovitch G: Cowhage-induced itch as an experimental model for pruritus. A comparative study with histamine-induced itch. PLoS One 6:e17786, 2011.

48. Parks DJ, Parsons WH, Colburn RW, Meegalla SK, Ballentine SK, Illig CR, Qin N, Liu Y, Hutchinson TL, Lubin M Lou, Stone DJ, Baker JF, Schneider CR, Ma J, Damiano BP, Flores CM, Player MR: Design and optimization of benzimidazole-containing transient receptor potential melastatin 8 (TRPM8) antagonists. J Med Chem 54:233–47, 2011.

49. Pereira M, Lotts T, Dreyer T, Cremer A, Englbrecht J, Ringkamp M, Ständer S, Pogatzki-Zahn E: Somatosensory Dysfunctions in Patients with Chronic Pruritus. Abstr Eur Pain Fed P060:3, 2015.

50. Pongcharoen P, Fleischer ABB: An evidence-based review of systemic treatments for itch. Eur J Pain 20:24–31, 2016.

51. Pud D, Granovsky Y, Yarnitsky D: The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain; 144:16–9, 2009.

52. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choiniere M, Choinière M: A systematic literature review of 10 years of research on sex/gender and experimental pain perception - part 1: are there really differences between women and men? Pain 153:602–18, 2012.

53. de Resende MA, Silva LFS, Sato K, Arendt-Nielsen L, Sluka KA: Blockade of Opioid Receptors in the Medullary Reticularis Nucleus Dorsalis, but not the Rostral Ventromedial Medulla, Prevents Analgesia Produced by Diffuse Noxious Inhibitory Control in Rats With Muscle Inflammation. J Pain 12:687–97, 2011.

54. Riley JL, Robinson ME, Wise EA, Myers CD, Fillingim RB: Sex differences in the perception of noxious

experimental stimuli: A meta-analysis. Pain 74:181–7, 1998.

55. Rolke R, Baron R, Maier C, Tölle TR, Treede - D. R., Beyer A, Binder A, Birbaumer N, Birklein F, Bötefür IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihöfner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B: Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. Pain 123:231–43, 2006.

56. Ross SE, Mardinly AR, McCord AE, Zurawski J, Cohen S, Jung C, Hu L, Mok SI, Shah A, Savner EM, Tolias C, Corfas R, Chen S, Inquimbert P, Xu Y, McInnes RR, Rice FL, Corfas G, Ma Q, Woolf CJ, Greenberg ME: Loss of Inhibitory Interneurons in the Dorsal Spinal Cord and Elevated Itch in Bhlhb5 Mutant Mice. Neuron Elsevier Ltd; 65:886–98, 2010.

57. Scheier MF, Carver CS, Bridges MW: Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. J Pers Soc Psychol 67:1063–78, 1994.

58. Schmelz M: Itch and pain differences and commonalities. Handb Exp Pharmacol 227:286–301, 2015.

59. Schmelz M, Schmidt R, Bickel a, Handwerker HO, Torebjörk HE: Specific C-receptors for itch in human skin. J Neurosci 17:8003–8, 1997.

60. Shelley WB, Arthur RP: The Neurohistology and Neurophysiology of the Itch Sensation in Man. Arch Dermatol 76:296, 1957.

61. Sikand P, Shimada SG, Green BG, LaMotte RH: Sensory responses to injection and punctate application of capsaicin and histamine to the skin. Pain; 152:2485–94, 2011.

62. Sikand P, Shimada SG, Green BG, LaMotte RH: Similar itch and nociceptive sensations evoked by punctate cutaneous application of capsaicin, histamine and cowhage. Pain; 144:66–75, 2009.

63. Ständer S, Schmelz M: Chronic itch and pain--similarities and differences. Eur J Pain 10:473–8, 2006.

64. Sullivan M, Bishop S, Pivik J: The pain catastrophizing scale: development and validation. Psychol Assess 7:524–32, 1995.

65. Tuckett RP: Itch evoked by electrical stimulation of the skin. J Invest Dermatol 79:368–73, 1982.

66. Vaegter HB, Handberg G, Graven-Nielsen T: Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. Pain; 155:158–67, 2014.

67. Yarnitsky D: Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. Curr Opin Anaesthesiol 23:611–5, 2010.

68. Yarnitsky D, Bouhassira D, Drewes AM, Fillingim RB, Granot M, Hansson P, Landau R, Marchand S, Matre D, Nilsen KB, Stubhaug A, Treede RD, Wilder-Smith OH: Recommendations on practice of conditioned pain modulation (CPM) testing. Eur J Pain 19:805–6, 2015.

69. Yarnitsky D, Crispel Y, Eisenberg E, Granovsky Y, Ben-Nun A, Sprecher E, Best L-AA, Granot M: Prediction of chronic post-operative pain: Pre-operative DNIC testing identifies patients at risk. Pain 138:22–8, 2008.

70. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y: Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 153:1193–8, 2012.

71. Yosipovitch G, Duque MI, Fast K, Dawn AG, Coghill RC: Scratching and noxious heat stimuli inhibit itch in humans: a psychophysical study. Br J Dermatol 156:629–34, 2007.

72. Zachariae R, Bjerring P: The effect of hypnotically induced analgesia on flare reaction of the cutaneous histamine prick test. Arch Dermatol Res 282:539–43, 1990.

73. Zigmond AS, Snaith RP: The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 67:361–70, 1983.

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$_{ipsi}$", **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

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

Figure 1
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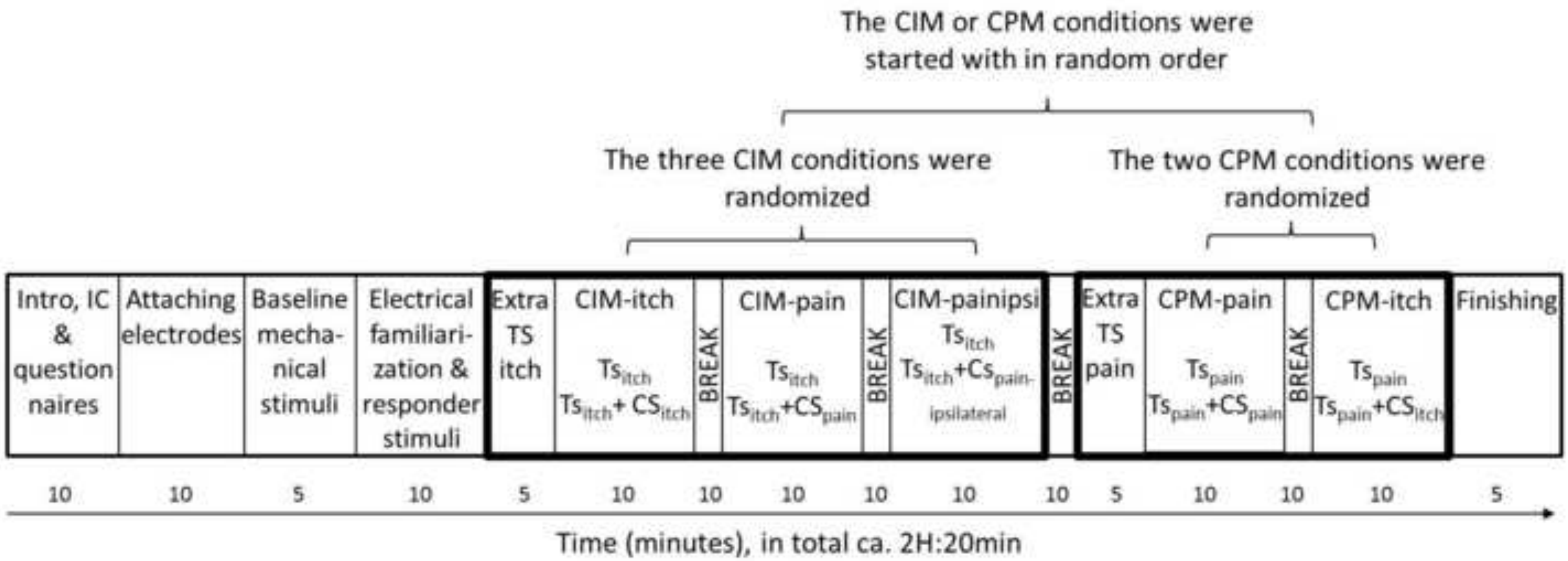

Figure 2

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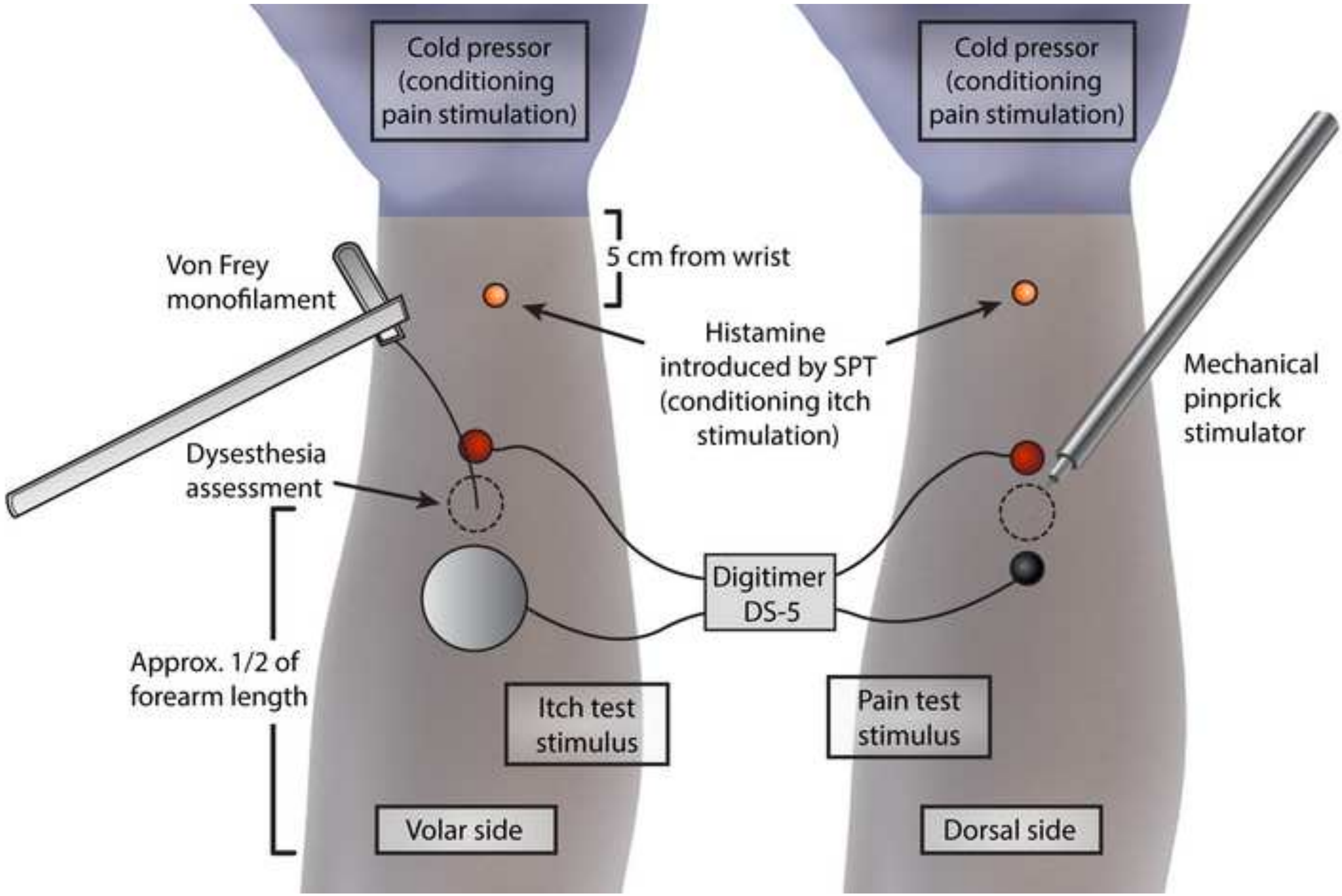

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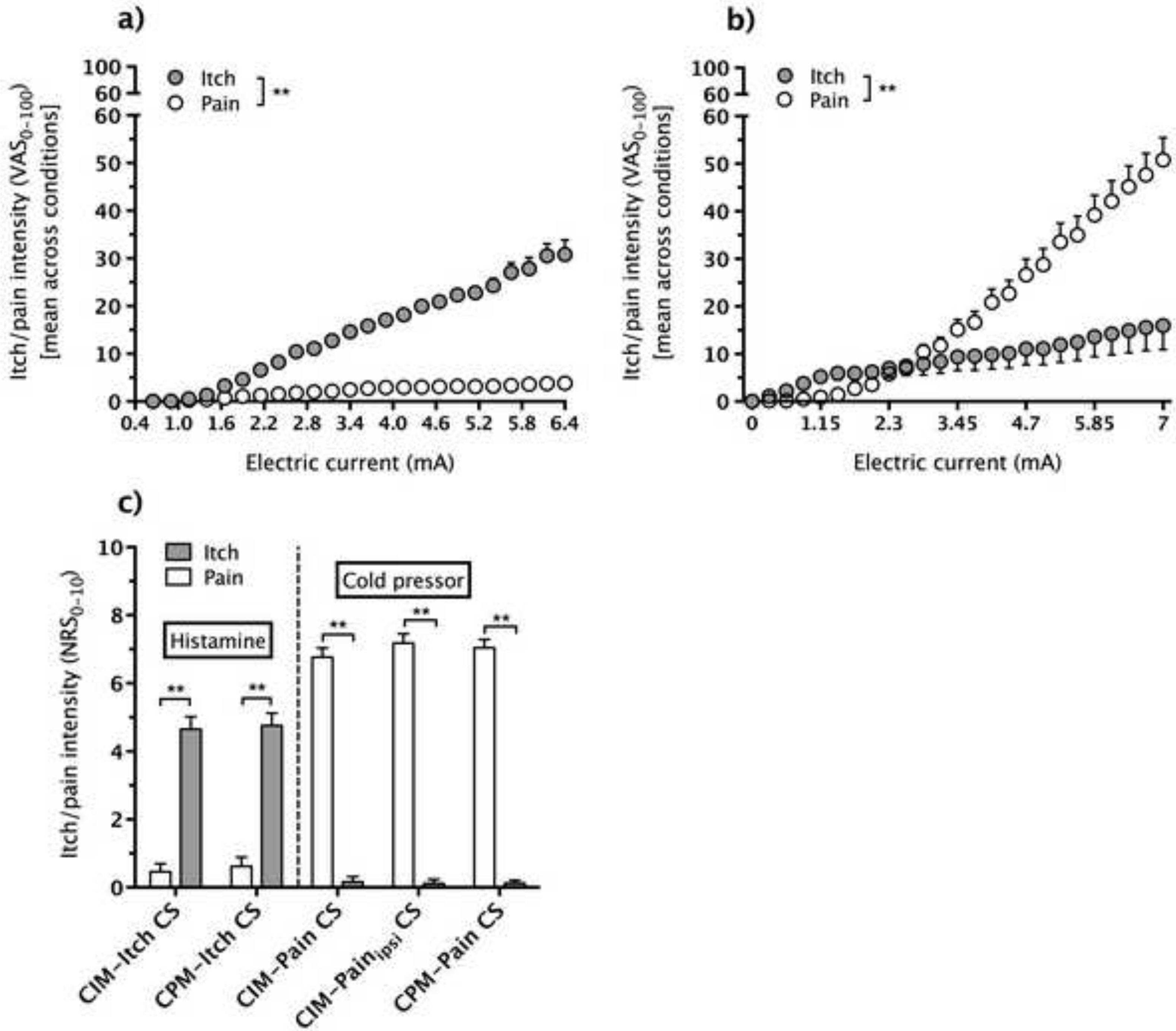

Figure 4

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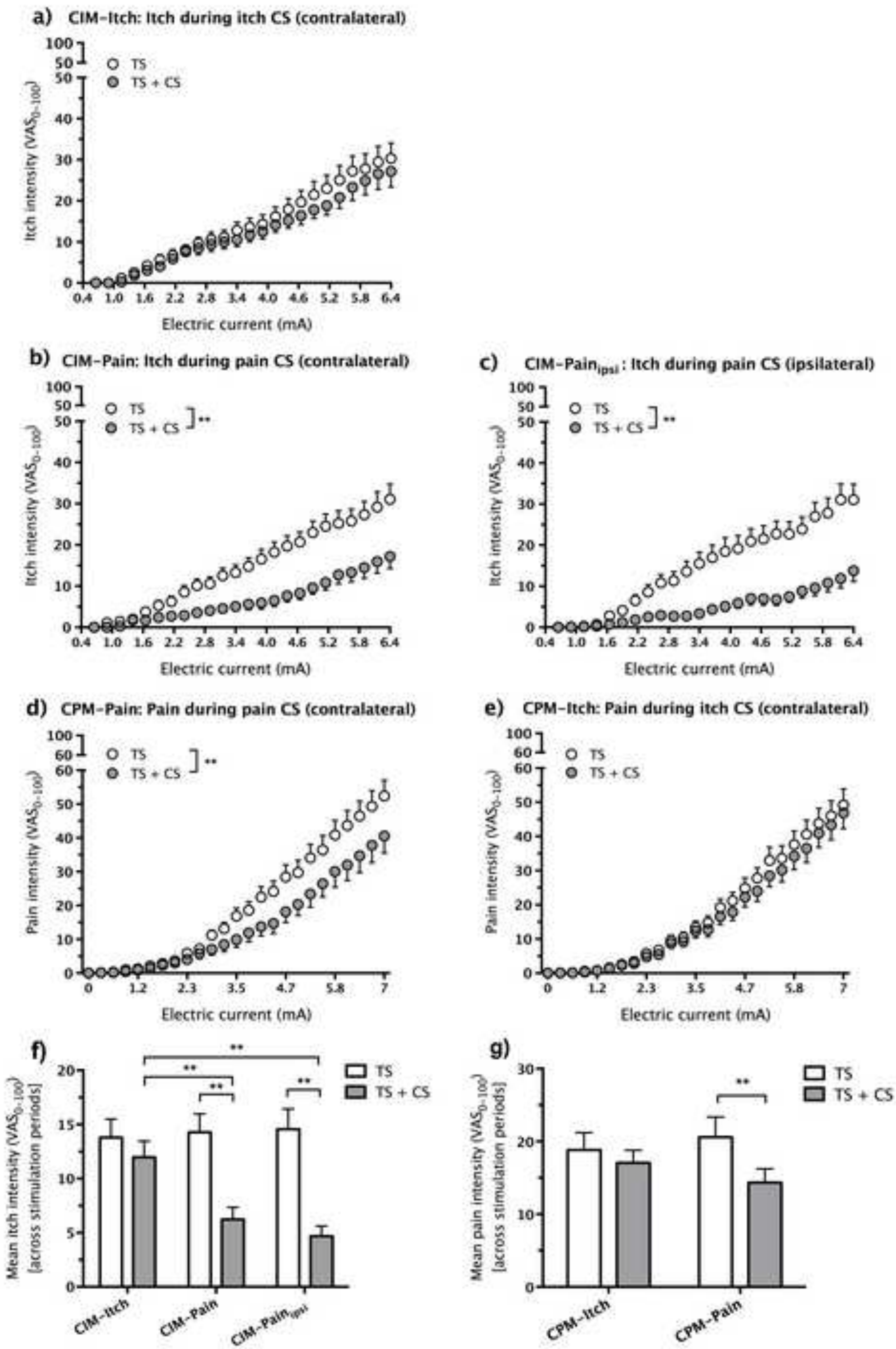

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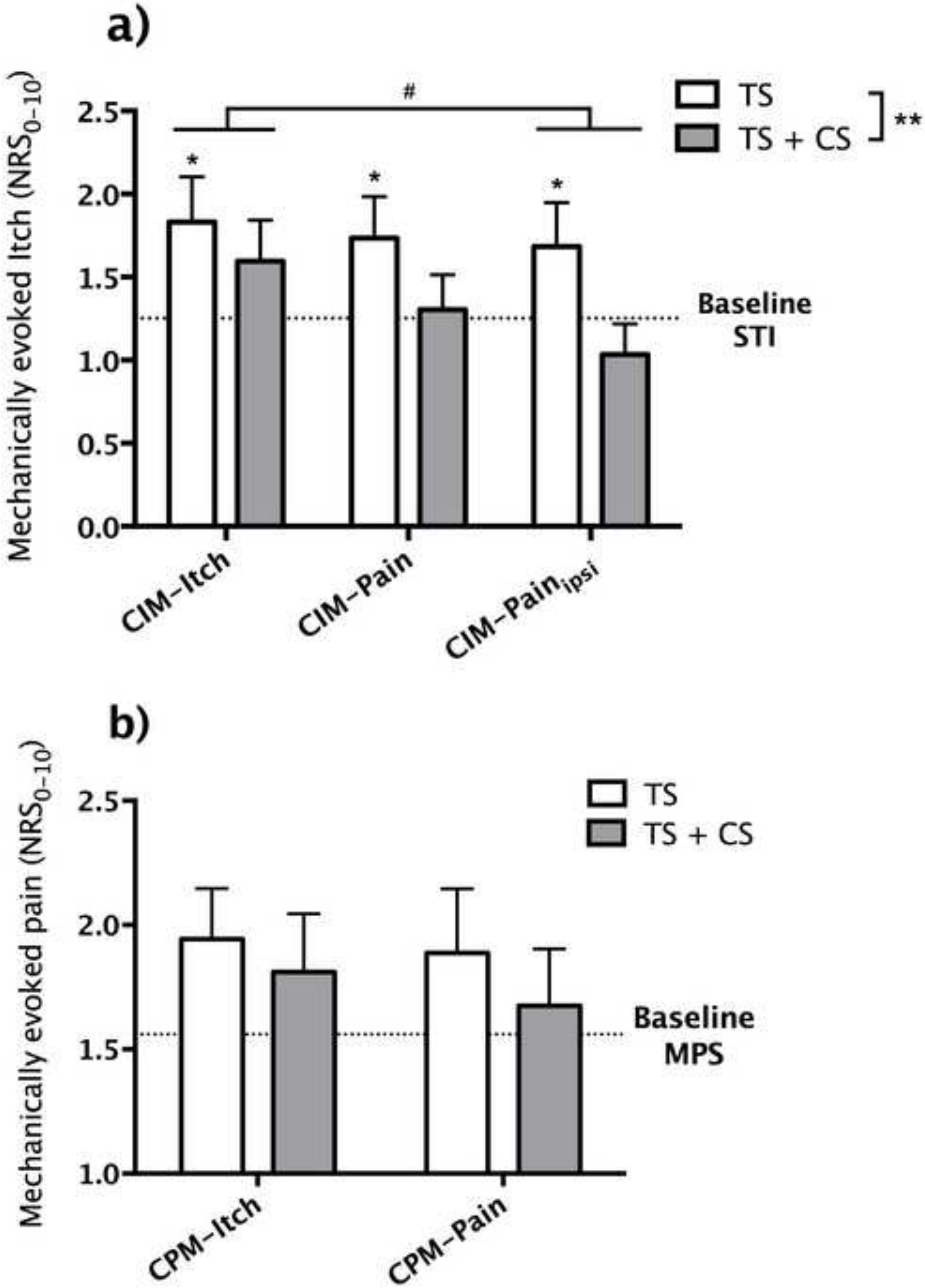

Table 1

Table 1

Condition	Test stimuli	Conditioning stimulation	Assessment of dysesthesia
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-$pain_{ipsi}$	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