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# Effects of short-term temperature change in the innocuous range on histaminergic and non-histaminergic acute itch

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## Abstract

While temperatures in the noxious range are well-known to inhibit acute itch, the impact of temperature in the innocuous temperature range is less well understood. We investigated the effect of alternating short-term temperature changes in the innocuous range on histamine and cowhage-induced acute itch, taking into account individual differences in baseline skin temperature and sensory thresholds. Results indicate that cooling the skin to the cold threshold causes a temporary increase in the intensity of histamine-induced itch, in line with previous findings. Skin warming increased cowhage-induced itch intensity. Potential mechanisms of this interaction between thermosensation and pruritoception could involve the TRPM8 receptor at the peripheral level in the case of histamine. The rapid modulation of cowhage-related itch induced by skin warming, reported here for the first time, enables statistically powerful neuroimaging studies, to further elucidate the cortical network underpinning cowhage-induced itch, as well as comparisons of cowhage and histaminergic itch.

# Introduction

It is well documented that thermal counter-stimulation in the noxious range (i.e., skin temperatures below 15 °C or above 45 °C) reduces the intensity of acute itch (1-4). However, the effect of brief, less extreme changes of temperature on itch, either by slightly increasing or decreasing the skin temperature from its baseline temperature of about 32 °C, is less well understood. Determining this relationship is not only of clinical interest, but also relevant for basic research on itch in humans, which often requires an experimental itch model where itch intensity can be quickly modulated (5).

Several studies have investigated the effect of innocuous warmth on experimentally induced acute itch. Ward et al. (2) induced itch using histamine iontophoresis and found that warming to the skin directly adjacent to the itch induction site to 38 °C using a thermode did not influence itch intensity. Yosipovitch et al. (4) also used histamine iontophoresis in combination with a thermode applied 3cm distal to the itch induction site, and again found that repeatedly warming the skin to 41 °C did not modulate itch intensity. Fruhstorfer and colleagues (1) used a slightly different approach in that the thermode was placed directly on the itching skin site. Skin temperature was then slowly increased from baseline at a rate of 0.5 °C/sec until heat pain was reported. They observed large inter-individual differences in the effect of warmth. The majority of participants (N=23) reported a decrease in itch intensity as temperature increased. Notably this effect was not restricted to the noxious range, but also evident at sub-noxious temperatures below 40 °C. A smaller subset of participants (N=7) reported the opposite pattern, with itch increasing as temperature increased. A final subset (N=4) showed no influence of warmth on itch.

In summary, findings about a potential effect of innocuous warmth on itch are mixed. The evidence suggests that directly warming the affected skin site (1), as opposed to an adjacent skin site (2, 4), may be a more sensitive approach. Another limitation of existing studies is that inter-individual differences in baseline skin temperatures and individual warm thresholds have so far not been taken into account (6).

Regarding the effects of cooling on acute itch, findings are, at first glance, contradictory. Yosipovitch et al. (4) observed that repeatedly cooling skin adjacent to the itch site to 15 °C (i.e., at the threshold from innocuous to noxious cold) does not influence itch intensity. Fruhstorfer and colleagues (1) observed that slowly cooling the skin, from baseline at a rate 0.5 °C/sec until cold pain was perceived, led to a decrease in itch intensity. This effect was consistent across subjects and began to manifest itself already in the innocuous temperature range below 25 °C. Andersen et al. (7) observed significant reductions of itch intensity when skin was cooled to temperatures of 22 °C, 12 °C and 4 °C. In contrast, Pfab et al. (5) observed that fast, short-term cooling of the skin, with temperature alternating between 32 °C and 25 °C every 20 seconds, lead to a significant increase in itch intensity during cool periods. These opposing effects of skin cooling on histaminergic itch - with slow cooling having an inhibitory, but fast cooling an excitatory influence on acute itch - may be related to adaptation effects. Unlike receptors in the noxious temperature range, innocuous thermoreceptors are fast adapting (8). TRPM8, a major thermoreceptor for innocuous cold, shows a rapid increase in firing rate upon moderate cooling, but also rapid adaptation within the first 8 seconds of a cold ramp (9).

The present study aims to extend existing knowledge in several ways. First, we will explore the effects of rapid cooling and warming of the skin on histamine-induced itch, taking into account individual differences in baseline skin temperature and individual warm and cold thresholds as determined via quantitative sensory testing. Second, we will also characterize, for the first time, the influence of temperature on non-histaminergic itch. The cowhage provocation paradigm has recently been established as an alternative experimental itch model (10) and is taken to be more characteristic of itch occurring in chronic pruritic diseases such as atopic dermatitis (11). Investigating the potential modulatory influence of innocuous temperature changes is not only potentially of clinical benefit, but also important for basic psychophysiological studies, which often require that itch intensity can be modulated within a matter of seconds (5, 12).

# Experiment 1: Histamine-induced itch

## Method

### Participants

Seventeen healthy volunteers (14 females), aged between 18 and 34 years (mean age = 21.8) took part in this study. All participants were right-handed students of the University of Hull. Exclusion criteria included a history of allergy, acute or chronic skin conditions, vascular disease, low blood pressure, asthma, histamine intolerance, sensitive skin, hypersensitivity to certain food types and any intake of drugs. The full exclusion criteria are detailed in the protocol approved by the University of Hull ethics committee in accordance with the

Declaration of Helsinki. All participants were naïve as to the purposes of the experiment and gave full written informed consent.

## Procedure

The experimental session started with a familiarization trial of the itch stimulus and rating scale, as recommend by Phan et al. (13), followed by determination of individual warm and cold thresholds (WT and CT, respectively), and two temperature trials. During these temperature trials, an initial itch stimulus was either accompanied by temperature alternating between baseline and WT (warm trial) or temperature alternating between baseline and CT (cold trial). The order of warm and cold trials was counterbalanced across participants. After the end of each itch rating period, the qualitative experience of itch was additionally measured using a questionnaire (see below), and the size of the skin reaction (wheal and flare) was evaluated.

## Itch Stimulus

In Experiment 1, itch was induced using the histamine skin prick test. One drop of 1% histamine dihydrochloride in aqueous solution was applied to the volar aspect of the forearm and then the skin superficially punctured with a special lancet (ALK, Sweden). Care was taken to ensure epidermal histamine delivery. Had any bleeding occurred the test session would have been halted and rescheduled. All skin pricks were done by the same female investigator to minimize variability in the application technique. After an interval of at least 5 minutes, during which the participant reported no further itching, a second trial of itch stimulation was performed on the alternate arm. Stimulation side was counterbalanced across participants.

## Quantitative assessment of itch

Starting with the histamine prick, participants rated itch intensity using the general version of the Labelled Magnitude Scale (gLMS) (14, 15), which has been recently found been found to yield more reliable assessments of acute itch intensity (16). The gLMS consists of a vertical line presented on a computer screen with quasi-logarithmically placed labels of "no sensation" at 0, "barely detectable" at 1, "weak" at 6, "moderate" at 17, "strong" at 35, "very strong" at 53 and "strongest imaginable sensation" at 100. Participants were asked to continuously rate the itch sensation experienced for 540 seconds beginning immediately following itch application, by using the scroll wheel on a computer mouse to move a red line up or down the vertical line on the screen to indicate the required rating. Ratings were sampled at a frequency of 1Hz. The mouse was operated face down using the non-stimulated hand as both forearms were in a palm up position throughout all conditions.

## Thermal Modulation of Itch

After the familiarization, baseline skin temperature of the stimulation area was taken, followed by a determination of individual warm and cold thresholds (WT and CT) using the method of limits (Mean baseline ±SD: 31.2 °C ± 0.83, CT = 25.3 °C ± 1.03, WT = 34.7 °C ± 0.99). Immediately following itch application, skin temperature was modulated using a Medoc Pathway Cheps (27mm diameter) thermode, placed directly onto the test area. To minimize adaptation effects, participants completed two trials (innocuous cold and innocuous warmth) each lasting 540 seconds which alternated a neutral block with a stimulation block rather than one run alternating innocuous cold blocks with innocuous warmth blocks. Each trial consisted of thirteen cycles. Each cycle began with a neutral block (at baseline

temperature) followed by either a cold block (at CT) or a warm block (at WT) depending on the condition. Each block lasted 20 seconds, with temperature change occurring at the end of each block at a rate of 5°C per second. The first temperature block began 20 seconds after itch application (see also Fig. 1).

## Qualitative assessment of itch

After each trial, a shortened version of the Eppendorf Itch Questionnaire (EIQ) (17), adapted for qualitative and quantitative assessment of pruritus in healthy volunteers, was completed by each participant. The 23 statements are designed to measure and identify different sensory qualities of the itch sensation. Each statement is rated on a five-point scale from 0 (not appropriate) to 4 (absolutely appropriate).

## Assessment of Skin Reaction

At the end of each itch trial, but before completion of the EIQ, the magnitude of the histamine-induced skin reaction was evaluated by measuring the size of the wheal (raised, red, itchy bump) and flare (reddening of the skin due to leaking capillaries surrounding the prick site). Wheal and flare response from the application of histamine were photographed, alongside a measurement scale for post-test analysis. This analysis was undertaken by a third party who was blind to the experimental condition to which the photograph pertained. Wheal and flare measurements could not be taken from 3 participants due to technical problems.

## Statistical Analysis

The analysis of continuous computerised data was simplified by using the itch intensity rating at the mid-point of each 20 second block, a point in time by which the target temperature was always fully established. This provided 13 ratings each for neutral and cold in the innocuous

cold trial and 13 ratings for neutral and warm each in the innocuous warmth trial. Statistical analysis was restricted to the descending flank of the itch response (i.e., the first 100 seconds of itch ratings were excluded, see Fig. 1).

Psychophysical ratings of itch were then analysed using a two-factor (Time and Temperature) repeated measures ANOVA to determine changes in itch sensation due to change in temperature and the passage of time (for a similar approach, see 4). There were two levels of the Temperature factor (baseline vs innocuous warmth or innocuous cold) and 11 levels of time. A significant main effect of either the Temperature factor or a significant interaction between the Temperature and Time factors indicate that the perception of itch is significantly influenced by the presence of the temperature stimulus. Greenhouse-Geisser correction was applied where necessary.

## Results

### Effects of temperature on intensity of histamine-induced itch

As can be seen in Figure 1, histamine elicited the typical itch response, peaking about 100 s after the onset of stimulation, followed by a slow decay. Descriptively, there is a trend that warming the skin temporarily decreases itch, followed by a rebound when temperature returns to baseline levels. Cooling the skin seems to have the opposite effect on histamine-induced itch, with an increase in itch observed during cool periods, followed by increase when temperature has returned to baseline.

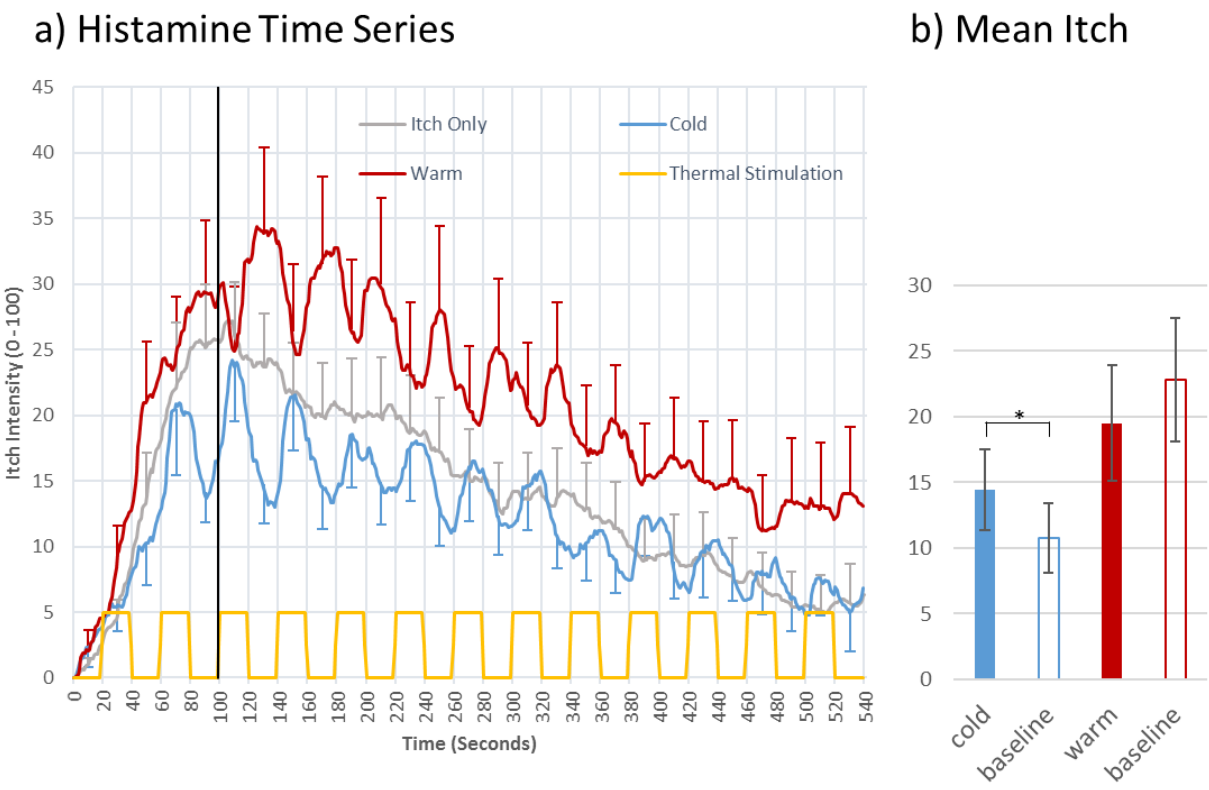


Figure 1. **a)** *Mean itch intensity ratings (n=17) over 540 seconds for innocuous cold, innocuous warmth and the familiarisation procedure (Itch-Only). The pattern of thermal modulation for 13 temperature cycles is shown at the bottom. Time 0 represents the onset of the histamine prick. The thick vertical represents the beginning of the segments used for statistical analysis. The standard error of the mean is provided for the mid-cycle ratings that were entered into the ANOVA (cycles 3 to 13).* ***b)*** *Illustration of the main effect of Temperature (altered temperature vs. baseline, as determined in the ANOVA), separately for cold and warm conditions. Significant effects ($p < .05$) are marked by *.*

The ANOVA for the cold condition showed a significant effect of Temperature, ($F$ (1,16) = 4.79, $p$ = .044), indicating that cooling the skin elicited higher itch ratings (M=14.47, SE=3.08) relative to when skin temperature was at baseline (M=10.77, SE=2.68). There was also a significant main effect of Time, ($F$ (1.95,31.21) = 9.31, $p$ = .001), but no significant interaction ($F$ (2.76,44.08) = 1.846, $p$ = .157) suggesting that the itch enhancing effect of cooling the skin is maintained throughout the descending flank of the itch response.

In contrast, the ANOVA for the warm condition only revealed a significant main effect of Time ($F$ (1.66, 26.57) = 8.65, $p$ =.002) but no main effect of Temperature ($F$ (1,16) = 1.46, $p$=.245) and no significant interaction, ($F$ (1.63, 26.00) = 0.90, $p$ = .401) suggesting that a short-term increase in temperature does not systematically affect subjective itch ratings. Visual inspection of single subject timecourses indicated that only a subset of participants (N=4) reported strong experiences of itch relief when skin was warmed to the WT (which is also reflected in the mean response shown in Figure 1), but this response pattern was not systematic enough across participants to reach statistical significance.

## Effects of temperature on sensory quality of itch sensation

The qualitative experience of the histamine prick test, as indicated by the abbreviated Eppendorf Itch questionnaire, can be seen in Figure 2. Relative to the cold condition, the itch during warm trials was perceived as more sharp (p = .017), pulsating (p = .029), stinging (p = .004), warm (p = .016) and burning (p = .018).

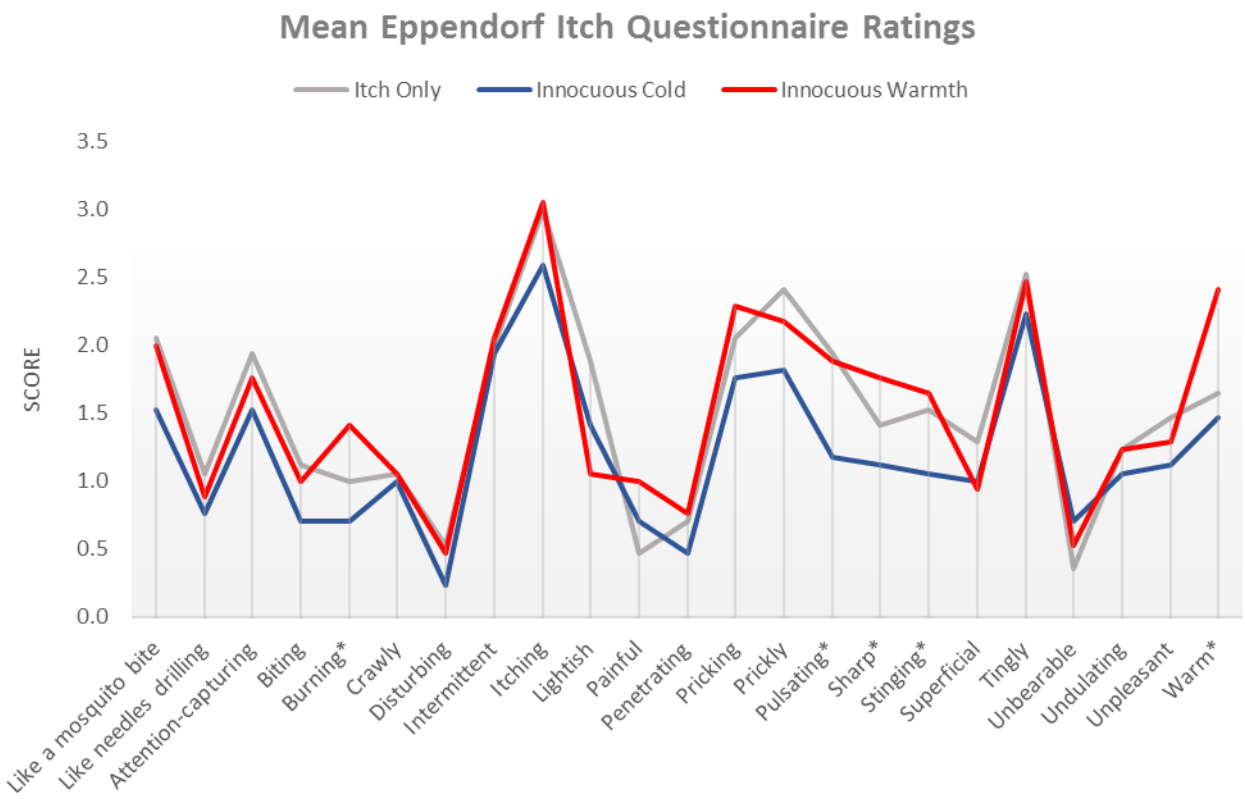


*Fig.2. Mean rating for items of the adapted-Eppendorf Itch Questionnaire (17). Rating scale ranged from 0 (not appropriate) to 4 (absolutely appropriate). Items were ratings significantly differed ($p < 0.05$) between the cold and warm condition are marked by *.*

### Skin Reaction

We also analyzed whether thermal stimulation affected the size of the skin reaction typically elicited by a histamine prick, i.e. wheal and flare size (see Table 2). The corresponding paired t-tests indicated that cooling the skin, relative to warming, significantly reduced the flare size ($t(13) = 2.23$, $p = .044$). Wheal size was not affected by temperature ($t(13) = 1.01$, $p = .332$).

*Table 2 Mean wheal and flare size (SD) for experimental conditions in $cm^2$*

| | *Condition* | | |
|---|---|---|---|
| | *Itch-only* | *cold* | *Warm* |
| Wheal | 0.13 (0.08) | 0.11 (0.05) | 0.13 (0.07) |
| Flare | 2.35 (1.33) | 2.26 (1.66) | 3.34 (1.92) |

*N=14. Wheal and flare measurements from 3 participants were missing due to technical problems.*

## Experiment 2 – Cowhage Induced Itch

### Method

### Participants and procedure

14 healthy volunteers were recruited for Experiment 2. Data from one participant was excluded due to failure to comply with the itch rating procedure. Ages ranged from 18 to 35 years (mean age = 21) with 3 males and 11 females. The participants were all right-handed students of the University of Hull, England.

## Itch Stimulus

In Experiment 2, itch was induced using cowhage spicules as an exogenous route to stimulate PAR-2 receptors in the epidermis. The cowhage stimulus has been shown to ensure a relatively long-lasting, stable, and intense itch sensation, with no induction of pain nor any other sensation than itch (11). A number of 40 cowhage spicules were counted under a magnifying glass, picked-up by microtweezer and applied within a 4cm squared area on the skin. The spicules were gently rubbed for 45 seconds onto the volar aspect of the forearm, 3 cm proximal of the distal wrist crease, with a circular motion to facilitate contact. Scotch tape was used to demarcate the area of the forearm to prevent any stray spicules from stimulating surrounding skin.

Each experimental trial lasted 540 seconds after which the spicules were removed using scotch tape. All itch applications were carried out by the same male investigator to minimize variability in the application technique. After an interval of at least 5 minutes during which the participant reported no further itching, a second trial of itch stimulation was performed on the alternate arm. Stimulation side was counterbalanced across participants. Since there is usually no wheal and only a minimal flare in reaction to cowhage-induced itch (10, 17), no skin reaction measurements were taken in Experiment 2. The modified EIQ was not administered in Experiment 2.

## Thermal Modulation of Itch

Base skin temperature of the stimulation area was taken, followed by a determination of individual warm and cold thresholds (WT and CT) using the method of limits (Mean baseline

± SD: 31.4 °C ± 1.09, CT = 24.5 °C ± 0.73, WT = 35.3 °C ± 1.16). As cowhage spicules were applied during a 40 second initial rub-in period, the first stimulation cycle was initiated 60 seconds after itch application compared with 20 seconds in Experiment 1 resulting in 12 equal cycles. All other aspects of the thermal modulation procedure were identical to Experiment 1.

## Statistical Analysis

Due to the time taken for the itch sensation to be fully experienced, the first 140 seconds of itch ratings were excluded from the ANOVA (i.e. the first 2 cycles). Thus, the statistical analysis included 10 cycles rather than 11 cycles in Experiment 1. All other aspects of the statistical analyses were identical to Experiment 1.

## Results

### Thermal Modulation of Cowhage-induced Itch

As can be seen in Figure 3, cowhage elicited a typical itch response, peaking around 140 seconds after the onset of itch induction. Descriptively, warming the skin seems to temporarily increase cowhage-induced itch, whereas no systematic effect for cooling is discernible.

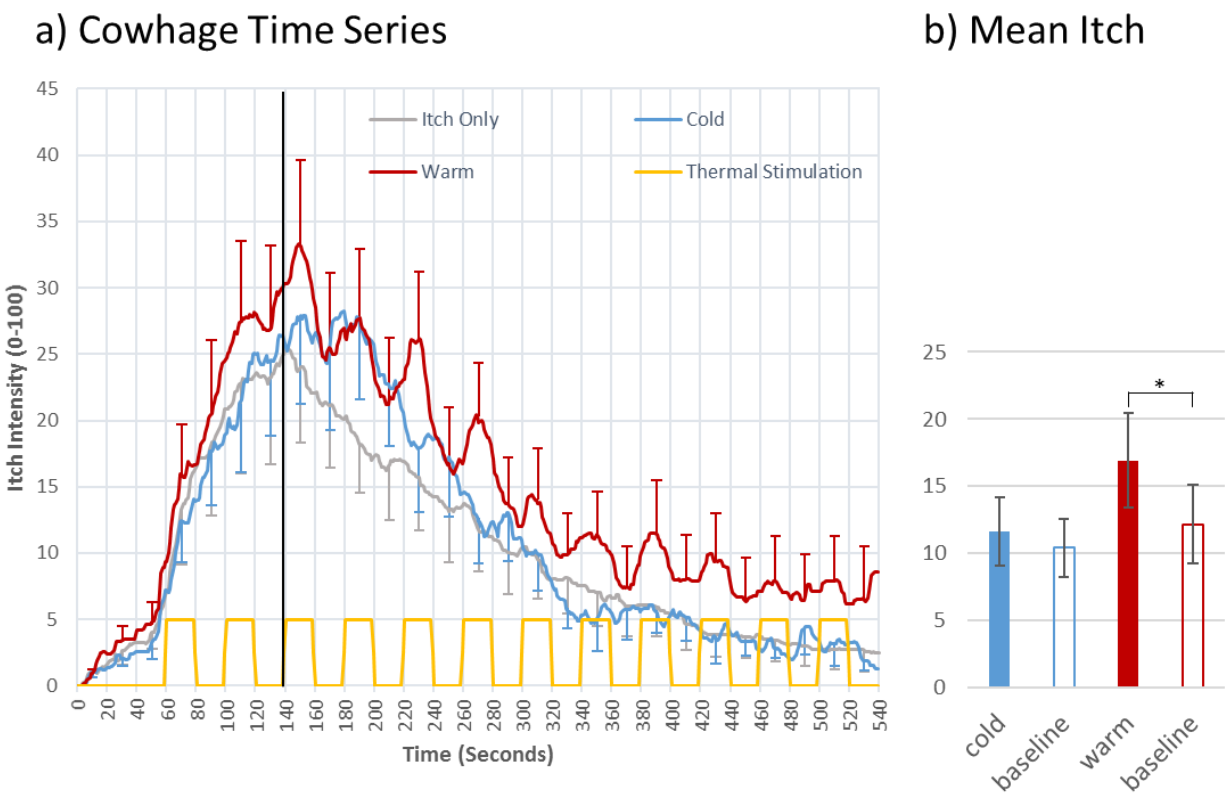


Fig.3. *Mean itch intensity ratings (n=13) of cowhage-induced itch for the familiarization session (Itch Only) and the two experimental trials. Time 0 represents the beginning of itch induction. Only ratings obtained during the descending flank of the itch response (i.e., to the right of the thick black vertical line) were included in the statistical analysis .* ***b)*** *Illustration of the main effect of Temperature (altered temperature vs. baseline, as determined in the ANOVA), separately for cold and warm conditions. Significant effects ($p < .05$) are marked by *.*

The corresponding ANOVA for the warm trials revealed a significant main effect of Temperature ($F(1, 12) = 7.97$, $p = .015$), indicating that warming the skin significantly enhanced cowhage-induced itch (M = 16.91, SE = 3.53) relative to when skin temperature was at baseline (M = 12.17, SE = 2.91). Additionally, there was a significant main effect of Time ($F(1.28, 15.31) = 8.93$, $p = .006$). The interaction was not significant ($F(2.78, 33.35) = 2.73$, $p = .063$).

In contrast, the ANOVA for the cold trials only yielded a significant main effect of Time ($F$ (1.43, 15.73) = 12.76, $p$ = .001). The main effect of Temperature ($F$ (1, 11) = 1.57, $p$ =.236) as well as the interaction between Time and Temperature ($F$ (2.17, 23.87) = 1.04, $p$ = .374) were not significant, suggesting a *reduction* in temperature does not have a systematic effect on cowhage-induced itch.

## Discussion

The aim of the present study was to investigate the effect of alternating short-term temperature changes in the innocuous temperature range on histamine and cowhage-induced acute itch, taking into account individual differences in baseline skin temperature and sensory thresholds. Results provided clear evidence that cooling the skin to the cold threshold causes a temporary increase in the intensity of histamine-induced itch, in line with previous findings. Warming the skin did not consistently influence histaminergic itch. An opposite pattern was observed for cowhage-induced itch, where cooling had no systematic effect, but warming increased itch intensity.

A novel finding of the present study is that we demonstrate, for the first time, the effect of innocuous temperature change on cowhage-induced itch. We found that a short-term increase of the skin temperature to the individual warm threshold is accompanied by an increase in the intensity of cowhage-induced itch. This raises the possibility that itch intensity can be rapidly modulated, within a matter of seconds, which is a requirement for many psychophysiological studies, including functional magnetic resonance imaging. Existing studies on the cortical network underpinning cowhage-induced itch are sparse (18). The findings of the present

study raise the possibility that a statistically powerful paradigm involving temperature modulation of itch intensity, as it has been developed for histaminergic itch (5), can now be developed for cowhage as well.

Our finding that short-term cooling elicits a temporary increase in histamine-induced itch is in line with previous findings by Pfab and colleagues (5). They observed that repeatedly cooling the skin from 32 °C to 25 °C reliably modulates itch, a finding that has since been replicated several times (12, 19-21). Our results show that the same effect can also be elicited when the skin cooled down to the individual cold threshold.

Regarding the effect of warming on histaminergic itch, there is, descriptively, a tendency towards an anti-pruritic effect of warming, however, this is not reliable across participants. Inspection of individual timecourses indicated that 24% of participants (N=4) reported a decrease of histaminergic itch upon skin warming, whereas others (N=13) showed no systematic effect. Fruhstorfer et al. (1) observed a similar pattern, with 68% of participants showing an antipruritic effect of warming, but the remainder reporting the opposite (20%) or no systematic effect (12%). One possible explanation for these individual differences might be genetic variation of thermoreceptors in the innocuous warmth range. For example, TRPV3 is a receptor responding to temperature changes of 33 °C and above (22). TRPV3 is not directly expressed in sensory neurons, but in keratinocytes, but may communicate with sensory neurons via second messenger systems involving diffusible ATP or NO (8). However, recently Huang et al. (23) demonstrated that a contribution of TRPV3 to thermosensation of innocuous warmth is limited to particular genetic strains of mice.

Another possible explanation for the lack of a consistent effect of warming on histaminergic itch could that the warm temperatures in our study, although determined via individual threshold testing, were not optimally tuned to the response curves of innocuous warmth thermoreceptors. Warm-sensitive fibres show a maximum response between 40 – 43 °C (8, 24), whereas the mean warm threshold in our study was 34.7 °C (Experiment 1) and 35.5 °C (Experiment 2).

The underlying mechanisms of the effects of short-term temperature changes on acute itch are yet to be determined. One possibility is that such effects are merely due to demand characteristics (i.e., participants forming an interpretation of the experiment's purpose and subconsciously changing their behaviour to fit that interpretation), non-specific masking or distraction (25). However, these explanations cannot account for the present findings because opposing temperature effects were observed for histamine and cowhage. We are not aware of a mechanism having been proposed for the itch-enhancing of short term cooling when histamine is used as pruritogen. Most likely this is due to an interaction between thermoreception and pruritoception, either at the peripheral or spinal level.

A potential peripheral mechanism for the cooling effect on histaminergic itch could involve the TRPM8 receptor. Cooling to the skin to about 25 °C, as in the present study, results in activation of the TRPM8 receptor, the main thermoreceptor for innocuous cold (9). Interestingly, long-lasting chemical activation of this receptor using menthol reduces both acute histaminergic (25) as well as chronic itch (26), whereas short-term activation via skin cooling increases acute histaminergic itch. One possible explanation of these opposing effects

could be differential involvement of A-delta fibres. TRPM8 is expressed in both unmyelinated C-fibres as well as myelinated A-delta fibres (9). However, the TRPM8 expressing A-delta fibres have a much lower activation threshold of about 7 °C, where the C-fibres are already activated during innocuous skin cooling (9). Thus, selectively activating TRPM8 expressing C-fibres via skin cooling may increase histaminergic itch, but activating the complete set of TRPM8 neurons (both A-delta and C-fibres), either chemically or via noxious cold temperature (25), may decrease it.

Regarding a potential mechanism underlying the itch-enhancing effect of skin warming for cowhage, as demonstrated in the present study, there are several possibilities. For example, vasodilatation associated with skin warming may generally increase release of pruritogenic mediators. However, this explanation is less likely since no comparable effect of skin warming was observed for histamine. Another possibility, though again not very likely given the lack of a corresponding effect for histamine, is that warming the skin leads to a general increase in membrane excitability. Hensel and Iggo (24) found that warm-sensitive C-fibres do not respond to mechanical deformation. In contrast, the C-fibres activated by cowhage are mechanosensitive (27), suggesting that an interaction between thermosensation and cowhage-related pruritoception at the peripheral level is also not the likely cause. We therefore suggest that a higher level interaction between thermal and pruriceptive neural fibres may underlie the observed increase in cowhage-induced skin during skin warming. This interaction could be instantiated at the spinal and/or the cortical level, but more research is needed to better understand the neural mechanism.

In conclusion, the present study replicated previous findings of an itch-enhancing effect of short term skin cooling for histaminergic itch, and provided new evidence for an itch-enhancing effect of skin warming cowhage-induced itch. This rapid modulation of cowhage-related itch induced by skin warming enables statistically powerful neuroimaging studies, to further elucidate the cortical network underpinning cowhage-induced itch, as well as comparisons of cowhage and histaminergic itch.

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