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Antipruritic effects of transient heat-stimulation on histaminergic and non-histaminergic itch

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What's already known about this topic?

1. Long-lasting noxious heat stimuli are able to inhibit experimentally evoked histaminergic itch.
2. To date the mechanisms behind these antipruritic effects in humans are unclear and there is no suitable way to implement counter-stimuli in a clinical setting.

What does this study add?

3. Very transient noxious heat inhibits both histaminergic and non-histaminergic itch by up to 76% and 43%, respectively.
4. The inhibitory effect is stimulus-dependent; at higher temperatures, increased itch suppression is reached.
5. The inhibitory effect of a transient stimulus is present only when applied homotopically relative to the itch provocation, but not heterotopically.

What is the translational message?

6. This study introduces and tests a simple psychophysical paradigm which engages and measures the efficacy of endogenous itch-inhibition.
7. The endogenous itch-inhibitory system engaged by counter-stimuli could constitute a new target substrate for the development of antipruritic therapeutic strategies.

Abstract

Background: Chronic itch is notoriously difficult to treat. Counter-stimuli are able to inhibit itch, but this principle is difficult to apply in clinical practice, and the mechanisms behind counter-stimulation-induced itch suppression in humans are unclear.

Objectives: 1) To analyse the stimulus-response effects of transient heat stimuli on histaminergic and non-histaminergic itch; 2) to investigate whether the antipruritic effect depends on homotopic (peripheral mediation) versus heterotopic (central mediation) counter-stimulation relative to the itch provocation site.

Methods: 18 healthy subjects (8 females, 25.7±0.8 y.o.) participated. Itch was evoked on pre-marked areas of the volar forearms, by either histamine (1% solution), or cowhage (35-40 spicules). In addition to the itch provocations (Experiment 1), 5-seconds homotopic heat stimuli of 32, 40, 45 or 50°C were applied. In Experiment 2, heat stimuli were applied either homotopically, intra-segmentally (next to the provocation site), or extra-segmentally (dorsal forearm). Itch intensity was evaluated throughout the procedures using a digital Visual Analog Scale.

Results: Homotopic counter-stimuli inhibited histaminergic itch by 41.27% at 45°C ($p<0.01$), and by 76.66% at 50°C ($p<0.0001$). Cowhage-induced itch was less prone to counter-stimulation and was only significantly diminished at 50°C by 43.60% ($p=0.009$). Counter-stimulations applied heterotopically were not able to significantly inhibit itch.

Conclusions: Itch pathway-specific effects of counter-stimuli were observed between homo- and heterotopic stimulation. Histaminergic itch was robustly inhibited by short-term homotopic noxious heat stimuli for up to 10 minutes. Non-histaminergic itch was only weakly inhibited. The inhibitory effects exerted by the short-term heat stimuli only occurred following homotopical counter-stimulation.

Introduction

By definition itch is considered the unpleasant sensation that causes the desire to scratch. Chronic itch severely impedes patients' quality-of-life and affects an estimated 8-16% of the western population. This places chronic itch amongst the most burdensome aspects of dermatological diseases.[1–4] Therefore, effort is needed to find new potential targets to be exploited in the development of new therapies. The urge to scratch aims at removing the source of itch, like insects, plants or other forms of irritation, at the same time acting as counter stimulus is able to reduce the itch sensation. It is known that various types of counter-stimuli are remarkably effective in providing

transient itch relieve, at least in the healthy somatosensory system.[5,6] Interestingly, even transient noxious counter-stimuli will produce a homotopic antipruritic effect which far outlasts the counter-stimulus itself.[7,8] This indicates that itch signalling is highly liable to profound endogenous modulation. However, little is known about the mechanisms underlying this endogenous itch-inhibitory system and currently no widely implemented treatment strategies are designed to engage it. Several preclinical studies have focused on understanding the mechanisms of this inhibitory signalling in experimental histaminergic and non-histaminergic itch models, but studies in humans are relatively scarce and rely on counter-stimuli producing cutaneous hyperalgesia. Notably, studies in rodent itch models indicate that the lack of spinal itch inhibition can act as a causative or a contributing factor in driving and maintaining chronic itch.[9–11] In humans, the proportional role of spinal versus peripheral contributions to the antipruritic effects of noxious counter-stimulation are unclear. Moreover, very transient counter-stimuli have not been tested.

The aims of this study were to 1) evaluate the itch-inhibitory effect of transient homotopic heat stimuli, using both innocuous and noxious stimuli, on histaminergic and non-histaminergic human models of itch and 2) to explore whether the inhibitory effect relies on homotopic inhibitory processing or is conserved when the counter-stimulus is delivered to heterotopic sites (intra-segmental or extra-segmental relative to the itch provocation).

Materials and Methods

Experimental design

18 healthy subjects were included (10 male, 8 female, 25.67±0.80 y.o.) and two experiments were conducted. All participants underwent all of the procedures explained below.

Experiment 1 explored the effect of transient heat stimuli applied homotopically (Fig.1A, C) relative to histaminergic and non-histaminergic itch provocations. It was carried out in two sessions each lasting approximately 75-90 minutes. Itch was evoked by histamine in one session and cowhage in

the other (randomized order; see below for details; Fig. 1A). Five stimulation areas were marked on the volar aspect of the participants' forearms (3 on one forearm, 2 on the other). These areas were 3x3 cm large and placed 4 cm apart (Fig. 1A).

Experiment 2 explored whether non-homotopical (intra-segmental and extra-segmental; Fig. 1B, C) stimuli were able to exert an antipruritic effect, i.e. whether the antipruritic effect of counter-stimulation is maintain when the itch provocation and the counter-stimulus are delivered to distinct skin areas. For this purpose, 6 stimulation areas (3 on each forearm) were marked. Itch was evoked as described below using histamine on one arm and cowhage on the other (anatomical placement and order of stimuli were randomized; Fig. 1B). The thermal stimulation was applied either on top (homotopic), next to the itch provocation (intra-segmental), or on the dorsal forearm (extra-segmental; see Fig. 1C). The study was approved by the regional ethical committee (N-20180035). The participants were all healthy, without previous neurological, allergic or dermatological diseases, consented to the study after written as well as oral information, and had the possibility to stop the experiment at any time.

Itch provocations (Experiment 1 and 2)

Histaminergic itch was evoked by a 1% histamine solution (Diagenics, UK) using 1 mm shouldered skin prick test (SPT) lancets (Diagenics, UK). The lancet was pierced through a droplet of histamine solution using a custom-made weight device providing 120g of pressure. Non-histaminergic itch was evoked by the use of cowhage spicules (35-40 spicules per itch induction). These were counted using a stereomicroscope, placed at the centre of the marked area and then gently rubbed by the experimenter with the use of the index fingertip. This procedure was performed for approximately 10 seconds and confirmed by the participants who were prompted to report the sensation of several tiny pricks (caused by the immediate insertion and indicating that the spicules were successfully

penetrating the epidermis). This method of cowhage administration have been used in several previous studies.[12,13] The itch elicitation techniques were identical for both experiments.

Thermal counter-stimulation (Experiment 1 and 2)

The thermal stimulation was conducted using a CHEPS thermal probe (3 cm diameter) attached to a Medoc Pathway thermal stimulator (Medoc Ltd, Ramat Yishay, Israel). This probe was always set to 32°C at baseline. In Experiment 1, 5-second stimuli were delivered exactly on top of the itch provocation (homotopically, see Fig.1A, C) and the temperature was either maintained at 32°C or increased rapidly to 40, 45 or 50°C. In one of the five marked areas no counter-stimulus was delivered (i.e., No Counter Stimulation, 'NCS'). In Experiment 2, the stimulation temperature was 48°C for all delivered stimulations (5-second stimuli) and was delivered in a randomized order either homotopically or heterotopically (intrasegmental and extrasegmental; Fig.1B, C). This temperature was chosen because it provided approximately a 50% decrease in itch intensity based on data from Experiment 1. All thermal stimuli were delivered using an ascending linear ramp of 70°C/s and a descending ramp of 40°C/s. The stimuli were delivered in a randomized, balanced manner with respect to their order and anatomical location.

Itch and pain assessment (Experiment 1 and 2)

Immediately following the itch provocations and 10 minutes hereafter, participants were instructed to continuously rate the itch intensity using a digital Visual Analog Scale (eVAS Software; Aalborg University) 0-100, where 0 was labelled "no itch" and 100 "worst itch imaginable" (0.2 Hz rating frequency). Continuous rating using a VAS was conducted to achieve accurate temporal profiles of the evoked itch. After 2 minutes, the thermal counter-stimuli were applied. Immediately after the thermal stimulations, the participants orally rated the evoked pain on a numerical rating scale (NRS) from 0-10 where 0 was labelled "no pain" and 10 "worst pain imaginable". This scale was applied to

obtain a single pain ratings of the pain evoked by counter-stimulus and to prevent the subjects from having to consider two VASs simultaneously. The assessment of the counter-stimulation-evoked pain was identical for Experiment 1 and 2.

Statistical analysis (Experiment 1 and 2)

Data was compiled in Excel (Microsoft, Redmond, Washington, USA). All the statistical analyses were performed in SPSS v. 25 (IBM, Armonk, New York, USA) or GraphPad Prism (GraphPad Software, San Diego, California, USA). For the itch intensity data, the area under the curve (AUC) was calculated from each temporal profile starting from 130 seconds after the itch provocation (the first time point after the end of the thermal counter-stimulation) following the trapezoidal rule as explained by Bailer [14]. Normality was ensured using visual inspection of Q-Q plots. Statistical comparisons were conducted using RM-ANOVAs and post-hoc tests with Bonferroni corrections. Within-subject normalization was conducted using each subjects' own control value (NCS and extra-segmental). The delta AUC (ΔAUC), was calculated for every test stimulus by subtracting the AUC of the within-control condition. For the relative fold-change calculation, the base 2 logarithm of the ratio between the AUC of every test stimulus and the within-control condition was calculated.

Finally, the immediate itch-suppression caused by the counter-stimuli represented as the Δ pre vs post was calculated by subtracting the rating given at 120 seconds after the itch provocation (last rating before the thermal stimulation occurred), and the rating given at 155 seconds after the itch provocation (30 seconds after the thermal counter-stimulation ended).

A p-value <0.05 was considered significant and are indicated as follow: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. All values are provided as means and standard error of the mean (SEM).

Results

Pain evoked by thermal counter-stimuli

Experiment 1: The chosen thermal homotopic counter-stimuli provoked stimulus-response-dependent pain (Fig. S1A-B). For counter-stimuli applied during histaminergic itch, the pain ratings for 32, 40, 45 and 50°C were: 0.33±0.11, 2.61±0.37, 5.08±0.48, and 7.44±0.37, respectively ($NRS_{0\text{-}10}$), while pain ratings for counter-stimuli applied during non-histaminergic itch, were: 0.39±0.14, 2.86±0.47, 5.64±0.35, and 7.81±0.34, respectively. No significant differences were observed between pain intensities when comparing stimuli delivered following histamine versus cowhage provocations.

Experiment 2: Homotopic heat stimuli were slightly more painful compared with extra-segmental stimuli (Fig. S1C-D). Histaminergic itch counter-stimulation: 7.44±0.38 for homotopic, 7.42±0.44 for intra-segmental, and 6.00±0.53 for extra-segmental (p=0.0262, compared to homotopical stimulation). Non-histaminergic itch: 7.75±0.42 for homotopic, 7.36±0.52 for intra-segmental, and 6.06±0.55 for extra-segmental (p=0.0225 compared to homotopical stimulation).

Painful heat stimuli inhibit histaminergic itch more robustly than non-histaminergic itch

Experiment 1: Histaminergic itch was significantly inhibited by 45 and 50°C homotopic counter-stimuli (Fig. 2A). The observed inhibition was stimulus-dependent (32°C -12.70%±15.36, p=0.99; 40°C -21.25%±14.32, p=0.79; 45°C -41.27%±10.52, p<0.01; 50°C -76.66%±5.20, p<0.0001). The inhibition achieved with 50°C was found statistically different compared to all other stimuli (32°C, $p < 0.0001$; 40°C, $p = 0.0002$; and 45°C $p = 0.042$; Fig. 2A).

On the contrary, cowhage-induced itch was only significantly inhibited by the homotopic counter-stimulation at 50°C (Fig. 2B; 32°C -15.74%±22.80, p=0.99; 40°C -9.93%±16.19, p=0.99; 45°C -18.57%±20.03, p=0.99; 50°C -43.58%±11.03, p= 0.011).

Analysis of the ΔAUC of the itch intensity showed that the inhibition achieved with 50°C was statistically different between the histamine and the cowhage (Fig. 3A; p=0.0482). An analogous finding was observed with the analysis of the relative fold change (Fig. 3B; p=0.0005). Finally, the inhibition observed with homotopic stimuli at 45 and 50°C was already established 30 seconds after the thermal stimulation in the histaminergic itch (Fig. 4A; NCS -2.50±1.29; 32°C -0.33±1.18, p=0.99; 40°C 3.79±1.32, p=0.73; 45°C 9.88±2.98, p=0.0045; 50°C 8.83±3.91, p=0.0125; all expressed as $\Delta VAS_{0\text{-}100}$). Whereas, for the non-histaminergic itch such antipruritic effect was only evoked by the 50°C stimulus (Fig. 4B; NCS 2.89±1.64; 32°C 3.78±1.32, p=0.99; 40°C 4.61±1.51, p=0.99; 45°C 9.06±2.48, p=0.69; 50°C 13.33±3.91, p=0.0251; all expressed as $\Delta VAS_{0\text{-}100}$).

Only homotopic counter-stimulation is effective in the inhibition of the itch sensation

Experiment 2: Histaminergic itch was significantly inhibited only when the stimulus was applied homotopically (Fig. 5A; homotopical -65.67%±8.32, p=0.0048; intra-segmental -8.03%±18.85, p=0.99; compared with extra-segmental). Similarly, non-histaminergic itch was only inhibited by the homotopical stimulation (Fig. 5C; homotopical -41.02%±13.29, p=0.0303; intra-segmental -9.07%±21.99, p=0.99; compared with extra-segmental). In addition, the same results were observed with the analysis of the relative fold change (Fig. 5B, D).

Discussion

The main purpose of this study was to explore the effects of transient heat counter-stimuli, using different temperatures, on both experimentally induced histaminergic and non-histaminergic itch. Our findings showed that homotopic counter-stimuli were able to inhibit histaminergic itch at 45 and 50°C, whereas non-histaminergic itch was only inhibited at 50°C and with reduced efficacy. Importantly, we observed that transient heat counter-stimuli did not induce significant inhibition of itch intensity when applied in a heterotopic position relative to the itch provocation.

Evoked heat pain following itch provocation (Experiment 1 and 2)

No differences were observed in the pain evoked by the counter-stimuli during histaminergic versus non-histaminergic itch. However, pain intensity was lower for the extra-segmental compared with the homotopical stimulus. This finding could be linked to pruritogen-induced heat hyperalgesia, but it more likely represents an anatomical difference in heat sensitivity considering the uniform results in heat-evoked pain between intra- versus homotopical stimulation. [15–17]

Differential inhibitory effects on histaminergic and non-histaminergic itch (Experiment 1)

This study showed that a short-term intense homotopic heat stimulation is able to robustly inhibit histaminergic itch in a stimulus-response-dependent manner, up to 8 minutes after the counter-stimulation. On the other hand, for the non-histaminergic itch provocation (cowhage), the itch-inhibitory amplitude was lower and shorter lasting.

As expected, the homotopic heat stimuli evoked neurogenic inflammation constituting an increase in local cutaneous blood flow, which could potentially lead to increased tissue clearance.[18,19] Such changes could facilitate removal of histamine more efficiently than cowhage given that histamine is a smaller and more diffusible molecule than mucunain (the pruritogenic proteinase responsible for the effect of cowhage spicules).[20] However, for both itch provocations, inhibition, although of distinct magnitudes, was established almost immediately following the homotopical 50°C counter-stimulation. It is thus difficult to reconcile such rapid inhibitory effects with a mechanism based on differential increased tissue clearance. Moreover, the histaminergic itch provocation itself establishes neurogenic inflammation increased blood flow in the area, that has been showed to not differ when heat pain is applied.[21]

Transient Receptor Potential Vanilloid receptor-1 (TRPV1) plays an important role in itch transduction[22–26] as well as in pain,[27–33] specifically heat pain. TRPV1 channel is activated by temperatures in the noxious range (>42°C).[34] Histaminergic itch is known to be processed by

activation of the histamine receptor 1 (H1R) which in turn co-opts TRPV1-signalling.[35,36] This occurs in C-mechano-insensitive fibers. Cowhage-induced itch is evoked by activation of protease-activated receptor 2 (PAR2) and/or MrgprX1/2[37] which co-opts Transient receptor potential ankyrin receptor-1 (TRPA1)-signalling,[38,39] a process occurring in C-mechano-sensitive fibers.[35,36] Notably, the TRPA1-channel is less robustly activated by suprathreshold noxious heat stimuli than the TRPV1-channel.[40–42] Given the two different heat-evoked inhibition patterns observed in this study, it can be speculated that heat-evoked TRPV1 activation produces a state in which subsequently H1R-recruited intracellular mediators cannot effectively activate TRPV1 to elicit pruriceptive signalling. This would, in accordance to the present findings, manifest in a much stronger inhibition of histaminergic itch compared to TRPA1-dependent non-histaminergic itch. Lastly, it has previously been shown that a heterotopic mechanical counter-stimulus, such as scratching, more robustly decreases histaminergic itch compared to cowhage-induced itch.[43] Thus, the segmental endogenous itch-inhibitory system may be more effective towards reducing histaminergic itch. The underlying mechanisms behind the observed differential effect warrants further investigations of the possibilities outlined above.[44–46]

Central versus peripheral contributions to heat-evoked itch inhibition (Experiment 2)

This study is the first psychophysical study which directly compares the inhibitory effect of heat on itch at homotopic, heterotopic intra-segmental and extra-segmental sites. Endogenous antipruritic mechanisms can generally occur on three levels; 1) reduced transduction or transmission in peripheral pruriceptive fibers, 2) in the spinal dorsal horn by segmental gating, or 3) through central modulatory mechanisms in both spinal and supraspinal compartments.[35,47,48]

Surprisingly, given that pain-evoked itch inhibition is thought to be predominantly mediated in the spinal dorsal horn,[9,47] no itch-inhibitory effects were observed when counter-stimuli were delivered to a heterotopic intra- or extra-segmental site relative to the itch provocation. In terms of mechanisms, this can be interpreted in two ways. Either the antipruritic effect of the heat counter-

stimulus is predominantly exerted at the level of the primary pruriceptive afferents (option 1 above) or the potential involvement of central inhibitory mechanisms (option 2 or 3 above) requires the counter-stimulatory input to be very close topographically to the activated pruriceptive afferents, as opposed to simply in the same spinal segment. Previous reports partly contradict our findings. Bickford[49] produced an intra-segmental antipruritic state by prolonged noxious heat and Yosipovitch *et al.*[21,50,51] also observed robust itch reduction when counter-stimulating 3 cm distally to a histaminergic itch provocation. However, important methodological differences sets these previous findings apart from the present data. In previous studies on heat counter-stimulation of experimentally evoked itch, much longer and more intense thermal stimuli were applied (49°C for 3 minutes[49] and 50°C for 30 seconds[21,50]). Prolonged noxious heat stimuli produces secondary hyperalgesia [52,53]. Skin, wherein secondary hyperalgesia is present, is known to be resistant to experimentally evoked itch (both histaminergic and non-histaminergic).[54,55] In the present study, the provided heat stimuli (48°C in Experiment 2) were deliberately kept so brief that no sensory sensitization was present in the secondary area.[56] In line with the present findings, Ward *et al.*[57], delivered 48°C heat counter-stimuli immediately adjacent to the itch provocation and observed inhibitory results approximately in-between the present effects of homotopic and heterotopic intra-segmental counter-stimulation.

Interestingly, in a recent preclinical study, central neuronal firing levels evoked by intradermal pruritogen injection (serotonin) were not reduced by transient noxious heat stimuli (45 and 50°C for 5 seconds).[58] Whereas, another study showed that neuronal activation (following dry skin induction) in the superficial dorsal horn was reduced by a longer noxious heat stimuli (48-56°C for 20 seconds). [59] These findings could suggest that with transient counter-stimuli, the upper level of itch transduction remains unchanged, but the inhibitory effect observed in this study rely on peripheral mechanisms. Nonetheless, further studies are needed to clarify these potential mechanisms.

The achieved antipruritic effects for the homotopic and intra-segmental counter-stimuli were evaluated in comparison to the effect of the extra-segmental counter-stimulus. This was done to

eliminate the potential bias of distraction and pain-evoked descending itch-inhibition, which is known to occur in humans when heterotopic itch and pain-stimuli are presented simultaneously[60,61] (the perceptual correlate of 'diffuse noxious inhibitory controls' as described in rodents).[62,63] Such a control condition has to our best knowledge not been used in any previous studies.[21,49–51] Conditioned itch modulation by pain-recruited descending inhibition is generated supraspinally and manifests systemically.[60] Surprisingly, no conditioned itch modulation were observed in the present study for the heterotopic extrasegmental condition. This is conceivably related to either the very transient counter-stimuli not being sufficient to recruit descending modulation[64] or because the control condition and the heterotopic extrasegmental condition were conducted in different sessions.

The hypoalgesic effect derived from a heterotopical pain stimulation has been shown to long-last up to one hour. [65,66] The duration of the itch inhibitory effect of homotopical counter stimulation is not well established but studies suggest that under the right conditions itch inhibition could last from 30 minutes up to 4 hours.[57,67]

The brief stimuli presently applied were insufficient in terms of eliciting an antipruritic state in adjacent skin. It is concluded that, as opposed to more prolonged noxious heat stimuli, the antipruritic effect of transient (5 second, 48°C) heat stimuli rely either on altered signalling in primary pruriceptive afferents and/or spinal gating which is dependent on immediate counter-stimuli adjacency. Based on available preclinical evidence, as well as human studies on mechanical counter-stimulation of itch, the latter option appears most likely.[9,47] However, one has to consider that scratch- and heat-evoked itch inhibition may not be mediated through analogous mechanisms. Certainly, scratching would activate a distinct and more diverse set of peripheral fibres than transient heat stimuli.

Limitations

In Experiment 1, two different homotopical noxious heat stimuli were used (i.e. 45 and 50°C). By investigating intermediate temperatures, a more detailed dose-response inhibition could be defined. Moreover, the effect of transient cold stimuli was not investigated in this study due to technical limitation. However, numerous studies suggests that homotopic innocuous and noxious cooling or cooling compounds is capable of suppressing itch, and patients with chronic itch frequently report that cooling relieves their itch.[21,50,68,69] This warrants further investigation. In Experiment 2, itch intensity without any counter-stimuli was not assessed. Despite the fact that the same subjects participated in the two experiments, it is not possible to make direct comparisons between the two sessions because of intra-individual variations of itch sensitivity.[70,71] This study raises further questions that need answers. 1) To what spatial extent transient noxious heat stimuli are able to inhibit itch. 2) What is the relation between the duration of the stimulation and the degree of the inhibition. 3) How effectively would transient counter-stimuli suppress itch in chronic itch patients. The present data as well as previous data on scratch-induced itch inhibition (ref) indicates that counter-stimulation mediated itch suppression might depend on the subtype of evoked itch.

Conclusion

In conclusion, transient homotopic heat counter-stimuli are able to profoundly inhibit itch. The inhibitory effects were significantly lower for non-histaminergic itch than for histaminergic itch. Homotopic counter stimulation was far more efficient than heterotopic counter stimulation. The present results suggests that actions in the peripheral nervous system is the predominant mediator in relation to itch inhibition evoked by transient heat stimuli. Further investigations are needed in order to fully understand the mechanisms underlying the antipruritic effect of counter-stimuli in humans and to develop counter-stimulatory approaches which could feasibly be utilized in clinical practice.

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Figure legends

Figure 1. Schematic representation of experiment 1 (A) and experiment 2 (B). All itch provocations were randomized for placement and order. The order of the applied counter-stimuli was also randomized. C) Schematic representation of the localization of itch provocation site and counter-stimulus area. NRS=Numeric Rating Scale; eVAS= digital Visual Analog Scale.

Figure2. Temporal profiles of itch ratings on a continuous digital Visual Analog Scale (0-100, 0=no itch, 100=worst itch imaginable) rated starting from the moment after the itch provocation up to 10 minutes after. The dotted line represents the moment when the heat counter-stimuli occurred, at 120 seconds and lasting for 5 seconds. A) Temporal profiles of the histamine-induced itch. B) Temporal profiles of the cowhage-induced itch. Asterisks: * = $p<0.05$; ** = $p<0.01$. NCS = No Counter Stimulation, VAS = Visual Analog Scale

Figure 3. Differences in AUC between the NCS curve and the various heat stimuli applied, starting from the time point following the stimulus. A) Graph showing ΔAUC with respect to NCS AUC. B) Graph showing the relative fold change normalized on the NCS. Asterisks: * $p<0.05$ histamine vs cowhage; NCS = No Counter Stimulation; ΔAUC = delta AUC. Significant differences are only indicated for histamine vs. cowhage conditions. See Fig. 3 for differences between the itch-inhibitory effects of the different heat stimuli.

Figure 4. Immediate effects of counter-stimulation. Differences in ratings between the timepoint prior to the counter-stimuli (120 second after itch provocation) and the timepoint 30 seconds after (155 seconds after the itch provocation). A) Pre vs post counter-stimuli for histamine-induced itch. B) Pre vs post counter-stimuli cowhage-induced itch. Indicated statistics are based on Δpre-vs-post calculations. * $p<0.05$; ** $p<0.01$.

Figure 5. Temporal profiles of itch ratings starting from the moment after the itch provocation up to 10 minutes after. The dotted line represents the moment when the heat stimuli occurred, at 120 seconds and last for 5 seconds. A) Temporal profiles of the histamine-induced itch. B) Graph showing the relative fold change of histamine-induced itch normalized on the extra-segmental AUC. C) Temporal profiles of the cowhage-induced itch. D) Graph showing the relative fold change of cowhage-induced itch normalized on the extra-segmental AUC.* $p<0.05$; ** $p<0.01$.

Figure S1. Pain ratings ($NRS_{0\text{-}10}$, 0=no pain, 10=worst pain imaginable) in response to the administered counter-stimuli. Pain ratings of stimuli applied homotopically relative to histamine (A) and cowhage (B) itch provocations (Experiment 1). Pain ratings of stimuli applied homotopically,

heterotopically intra-segmental and heterotopically extra-segmental to histamine (C) and cowhage (D) itch provocations (Experiment 2). All counter-stimuli in this experiment were 48°C. Individual data, means and SEMs are shown. Asterisks: * p<0.05; *** = p<0.001