Placebo and nocebo effects in itch : from conditioning to psychophysiological effects

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Chapter 3

Antipruritic placebo effects by conditioning H1-antihistamine

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ABSTRACT

Objective. Allergic rhinitis symptoms can be reduced by behaviorally conditioning antihistamine. It is unclear whether these findings extend to histamine-induced itch or work when participants are informed about the conditioning procedure (open-label conditioning). The current study aims to investigate the efficacy of (open-label) antipruritic behavioral conditioning for histamine-induced itch.

Methods. Healthy participants (n = 92; 84% female) were randomized to I) an open-label conditioned, II) closed-label conditioned, III) conditioned-not-evoked control, or IV) nonconditioned control group. A two-phase conditioning paradigm was used. During acquisition, a conditioned stimulus (CS; distinctively tasting beverage) was repeatedly paired with the H1-antihistamine levocetirizine (groups I–III). During evocation, the CS was paired with placebo (I, II), or instead of the CS, water was paired with placebo (III). The nonconditioned control group (IV) received CS with placebo in both phases. Itch after histamine iontophoresis and physiological data (i.e., spirometry, heart rate, skin conductance) were assessed. Combined conditioned and combined control groups were first compared, and analyses were repeated for separate groups.

Results. Marginally lower itch was reported in the combined conditioned compared with the control groups ($F(1,88) = 2.10$, $p = .076$, $\eta^2_{partial} = 0.02$); no differences between separate groups were found. No effects on physiological data were found, except for heart rate, which reduced significantly and consistently for control groups, and less consistently for conditioned groups (group by time interaction: $F(7,80) = 2.35$, $p = .031$, $\eta^2_{partial} = 0.17$).

Conclusion. Limited support was found for the efficacy of antipruritic behavioral conditioning, regardless of whether participants were informed about the conditioning procedure. The application of open-label conditioning in patient populations should be further researched. **Trial registration.** www.trialregister.nl; ID NTR5544.

INTRODUCTION

Placebo effects are beneficial effects that cannot be attributed to active treatment ingredients [1,2]. Instead, these effects are ascribed to expectancy mechanisms, with expectations of benefit resulting in improvement of somatic symptoms (e.g., itch and pain; [3-6]). The opposite has also been demonstrated, with expectations of deterioration resulting in exacerbation of symptoms or increased adverse effects (i.e., nocebo effects; [3,7]). Current evidence shows that placebo and nocebo effects can be induced through multiple pathways, for example, by providing positive or negative information regarding treatments, or through associative learning processes such as conditioning [8-10]. In behavioral conditioning, repeated pairing of an initially neutral stimulus (to-be conditioned stimulus [CS]) with an unconditioned stimulus (UCS), which elicits a certain innate response, may lead to the CS eliciting a similar response (conditioned response), even when the UCS is not presented [9,10].

There is evidence that conditioning of allergens to a CS can exacerbate allergic symptoms, upregulate histamine release in animal models of allergy (which has been linked to exacerbation of allergic responses), and adversely influence itch [11-20]. Moreover, studies indicate that conditioning can also potentially alleviate allergic symptoms by repeatedly pairing a CS (e.g., a novel-tasting beverage) with an H1-antihistamine (e.g., desloratadine) as UCS [21,22]. This has previously resulted in a conditioned basophil response to dust mite allergens [21]. However, findings for subjective symptoms were less clear, as these also tended to decrease in the control groups [21,22]. Moreover, no study to date has investigated whether conditioning of H1-antihistamine may influence histamine-induced itch specifically. Because histamine is a modulator of itch not only in allergic conditions but also in other inflammatory conditions such as atopic dermatitis [23,24], demonstrating these effects may provide a basis for new therapeutic approaches aimed at enhancement of placebo responses, reduction of medication use, and minimization of adverse effects [25,26].

Traditionally, a blinded study protocol is used for behavioral conditioning, in which participants do not know whether they receive medication or inert pills [27]. This makes direct translation of these effects to clinical practice difficult, as it insinuates that deception is needed to elicit placebo effects, and patients in clinical practice need to be fully informed about treatment [27]. However, there is accumulating evidence that placebo effects may also occur when it is known that an inert substance is given (i.e., open-label). Symptoms of

allergic rhinitis, irritable bowel syndrome, and chronic low back pain can be reduced when placebo pills are given together with a rationale explaining the placebo effect [28-34]. The efficacy of open-label conditioning (i.e., explaining the learning procedure from the beginning) for reduction of symptoms such as itch has not yet been demonstrated.

The current study investigated whether behavioral conditioning of the antihistaminergic properties of levocetirizine could reduce itch in response to a short-term histamine challenge. Effects of behavioral conditioning on other clinical, physiological, and psychological responses were explored. Moreover, the study aimed to explore the effects of open- versus closed-label conditioning.

MATERIAL AND METHODS

Study design

Detailed methodology is described in the Methods section in the **Supplementary Material**. This study was a block-randomized (1:1:1:1), placebo-controlled crossover study (Dutch Trial Registry ID: NTR5544, registration on October 6, 2015) that was approved by the Medical Ethical Committee at the Leiden University Medical Center, the Netherlands (ID NL52687.058.15) and conducted in concordance with the Declaration of Helsinki [35]. All participants provided written informed consent. Data for the study were collected between October 2015 and October 2017.

Conditioning paradigm and blinding

In line with previous studies [21,22,36-39], a two-phase conditioning paradigm was applied that consisted of an acquisition phase, in which a distinctively tasting beverage (to-be CS) was combined with a UCS (a capsule containing 5 mg levocetirizine diHCl, an H1-antihistamine) or an identically looking placebo capsule, and an evocation phase, in which the CS was combined with a placebo capsule. Both phases had three sessions on three consecutive days, and were separated by a 4-day drug washout period. Participants were allocated to I) an open-label conditioned group (acquisition: CS + UCS with an explanation of conditioning and its expected effects; evocation: CS + placebo); II) a closed-label conditioned group (acquisition: CS + UCS; evocation: CS + placebo); III) a conditioned-

not-evoked control group (acquisition: CS + UCS; evocation: water + placebo), which was added to control for carry-over effects of the conditioning procedure; or IV) a nonconditioned control group (acquisition: CS + placebo; evocation: CS + placebo), which was added to control for the effects of CS only. Block randomization was used to generate a randomization sequence and was managed by an independent party (the Leiden University Medical Center pharmacy that distributed the UCS and placebo capsules). The study was conducted double blinded for the closed-label conditioned group and nonconditioned control group, single blinded for the conditioned-not-evoked group, and nonblinded for the open-label conditioned group. In the conditioned-not-evoked group, the CS was not administered during evocation, and the acquisition phase was conducted by a different experimenter in a different laboratory setting (e.g., location and lighting), to prevent conditioning to the environment. In the open-label conditioned group, the experimenter provided participants with information regarding the conditioning procedure at the start of acquisition (see the **Supplementary Material** for further details). Notification of allocation to these two groups by the pharmacy was given to the experimenter after inclusion.

Participants

Healthy male and female volunteers aged between 18 and 35 years were recruited for this study. Inclusion criteria consisted of a good understanding of written and spoken Dutch, and absence of allergic rhinitis or allergic conjunctivitis within 3 months before enrolment in the study. Potential participants were excluded in case of somatic or psychological morbidities that may interfere with the study protocol or participants' safety; allergic rhinitis or conjunctivitis within 3 months before participation; any allergic condition presenting symptoms other than rhinitis or conjunctivitis; recent use of analgesics, antibiotics, antihistamines, or anti-inflammatory medication; recent vaccinations; (intended) pregnancy; or intolerance for any substances used in the study.

Procedure and study outcomes

An overview of the study protocol is provided in **Figure 1**. The study took place at Leiden University and was advertised as a study on the influence of psychological factors on anti-allergic medication. Participants were invited for a screening session, and upon inclusion, psychological factors and expected itch were assessed. Well-being was measured through

questionnaires (*measurement set A*; i.e., Positive and Negative Affect Schedule [40], State Trait Anxiety Index-State Anxiety [41], and Numeric Rating Scales (NRS) for general wellbeing items). Next, spirometry (forced vital capacity, $FVC_{\%predicted}$; forced expiratory volume in 1 second, $FEV_{1\%predicted}$) was assessed, and 5-minute measures of heart rate (HR) and skin conductance level (SCL) were taken (*measurement set B*). Itch was induced experimentally through 2.5 minutes of transdermal iontophoresis with a 0.6% diphosphate histamine solution on the volar side of the nondominant forearm. Itch was assessed verbally every 30 seconds during iontophoresis, and the self-rated and clinical skin response to histamine was measured (*measurement set C*). Finally, participants indicated how much itch they expected to experience during the final evocation session, and blood samples were taken to assess eosinophil profile and immunoglobulin E response to aeroallergens. In the next week, participants were invited for the acquisition sessions. For each of the three acquisition sessions, measurement set A was assessed before the CS was administered with the UCS or placebo pill. After a 4-day drug washout, participants were invited for the evocation sessions. During evocation, measurement sets A + B were assessed pre-CS, and +30 and +60 minutes post-CS administration, with an additional +90-minute post-CS assessment for the final session. Measurement set C (histamine iontophoresis) was reassessed in the final session between +60 and +90 minutes post-CS. At the start of the final session, expected itch, remembered itch, and expected medication efficacy were assessed. Finally, participants filled in a closing questionnaire in which they indicated whether they suspected to have received placebo or active medication, and compared the itch experienced during both tests. Participants rated the pleasantness of the CS taste in each session on an NRS. Participation was reimbursed by €150. An overview of the measurement schedule is provided in **Figure 2.**

Power calculation and statistical analysis

A detailed description of the statistical analyses can be found in the Methods section in the Supplementary Material. An a priori power calculation using 1000 simulated datasets at a power level of β=0.85, an alpha level of α=.05, and an assumed effect size of Δ/σ = 1/1, indicated that 92 participants were needed to find differences between the four groups. All analyses were performed using SPSS 23.0 for Windows (IBM SPSS Inc., Chicago, Illinois, US). As described in the a priori plan for the statistical analyses, differences in mean itch during iontophoresis in the evocation phase between the combined open- and closed-label

conditioned groups and the combined control groups were assessed using a one-sided general linear model (GLM) analysis of covariance (ANCOVA), including baseline itch as covariate. Secondarily, a GLM ANCOVA was conducted two-sided to explore effects between the separate groups. In case of significant group effects, Bonferroni post hoc tests were conducted. These analyses were repeated for the secondary parameters itch expectation and other iontophoresis-related outcomes (measurement set C). For well-being and physiological outcomes (measurement sets A + B), mixed between-within-subject repeated-measures analysis of variance (RMAs) were conducted. In case of significant effects, within-subjects RMAs were conducted post-hoc to assess changes from baseline for individual groups. The groups were compared on the closing questionnaire items by χ^2 tests. Relations between suspected medication intake and the primary outcome of itch were assessed by GLM ANCOVAs. Because the open-label group received information on medication administration, analyses for the closing questionnaire items were repeated without this group. Assumptions were checked before analyses, and all analyses were conducted with $\alpha = .05$. As an effect size, $\eta^2_{partial}$ was calculated for each analysis. All values in the Results section represent mean (standard deviation, or M [SD]), unless stated otherwise.

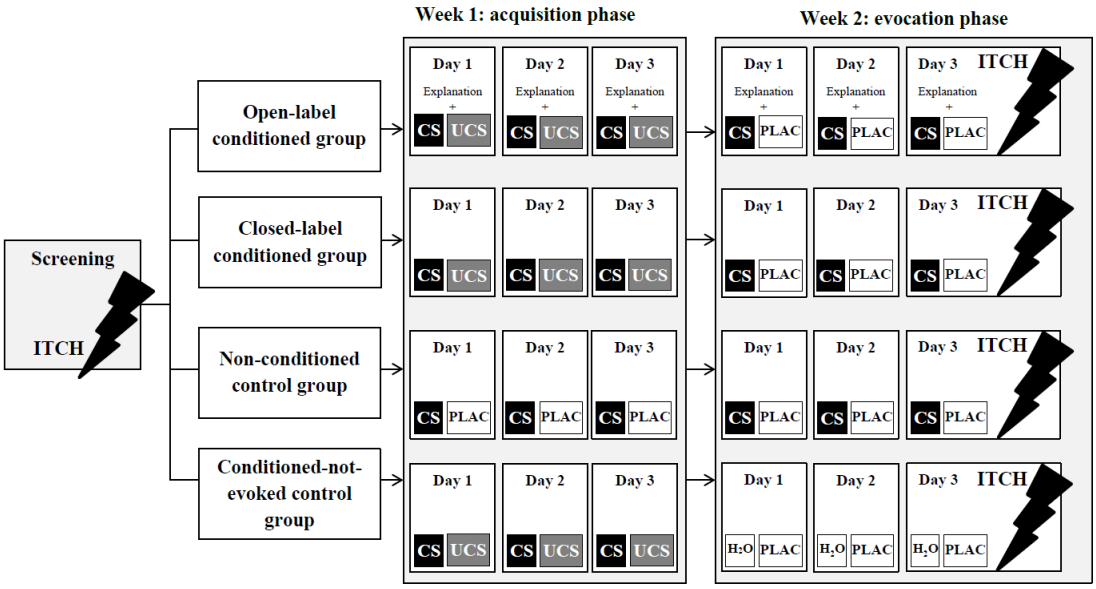


Figure 1. Overview of the study protocol. A conditioned stimulus (CS; distinctively tasting drink) was combined with an unconditioned stimulus (UCS; levocetrizine) or placebo capsule (PLAC) during acquisition. During evocation, the CS was combined with PLAC, and for the conditioned-not-evoked group, PLAC was provided with water (H2O). Histamine iontophoresis (ITCH) was conducted at baseline and in the final evocation session.

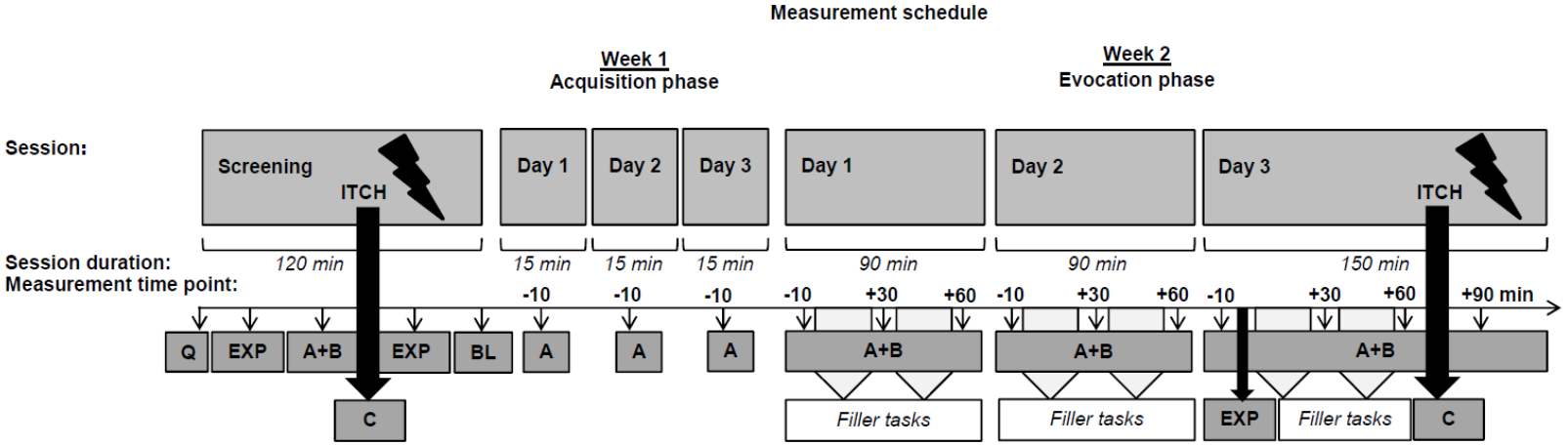


Figure 2. Overview of the measurement schedule. Numbers on the timeline are deducted from CS administration, with −10 representing pre-CS, and +30, +60, and +90 representing post-CS measurements. Personality questionnaires (Q); expected itch (EXP); measurement sets for well-being (A); spirometry, heart rate, and skin conductance (B); and histamine iontophoresis (e.g., itch; C) and blood samples (BL) were taken. Filler tasks consisted of neutral magazines, Sudokus, and puzzles.

RESULTS

Participants

Ninety-nine participants were included in the study, of whom 7 dropped out of the study after inclusion for various reasons. For a complete overview of participants' flow see **Supplementary Figure S1**. The final sample consisted of 92 participants (M_{age} [SD], 22.1 [2.5] years, 84% female) randomized to the open-label conditioned group (n=23), the closed-label conditioned group (n=24), the conditioned-not-evoked control group (n=23) or the non-conditioned control group (n=22). Participants did not differ significantly between groups on demographic factors (see **Table 1,** combined groups; and **Supplementary Table S1,** separate groups).

Group differences at baseline and during the acquisition phase

Participants randomized to the combined open- and closed-label conditioned groups showed a larger wheal area after baseline histamine iontophoresis (M [SD], 12.3 [3.1]) compared with the combined control groups (M [SD], 10.6 [3.6]; $F(1,88) = 6.14$, $p = .015$, $\eta^2_{partial} = .07$). A marginal overall difference between the separate groups was found for positive affect on the second acquisition day ($F(3,88) = 2.61$, $p = .057$, $\eta^2_{partial} = 0.08$; Bonferroni post hoc tests: $p > .31$). No other differences were found between groups at baseline, or at the pre-CS measurements during the acquisition and evocation sessions (all, $p > .09$). Groups did not differ in their rating of the pleasantness of the taste of the CS (all, $p > .20$), which was generally rated as unpleasant (M_{rating} [SD], 3.8 [1.5]).

Expected itch

No differences in expected itch, remembered itch, or expected medication efficacy were found between the combined conditioned groups and the control groups (all, $p > .11$). When effects of separate groups were explored, a medium-sized effect on expected itch was demonstrated ($F(3,86) = 2.96$, $p = .037$, $\eta^2_{partial} = 0.09$), with post hoc Bonferroni tests illustrating that the open-label conditioned group expected borderline significantly less itch (M [SD], 3.2 [2.2]) compared with the conditioned-not-evoked group (M [SD], 4.6 [1.6]; $p = .050$; **Figure 3** and **Supplementary Table S1**).

Mean self-reported itch

As illustrated in **Figure 4,** a marginal small-sized conditioned effect was demonstrated for mean itch ($F(1,88)=2.10$, $p=.076$, $\eta^2_{partial}=.02$), with the combined conditioned groups reporting lower itch compared to the combined control groups in response to iontophoresis during evocation ($M_{difference}=-0.34$, $SE=0.24$). A non-significant difference in itch was found when analyses were repeated for the separate groups; $F(3,86)=1.47$, $p=.23$, $\eta^2_{partial}=.05$.

Self-rated and clinical skin response to histamine iontophoresis

No effects on self-rated skin response to iontophoresis were demonstrated for both the combined ($F(1,88) = 0.47$, $p = .25$, $\eta^2_{partial} = 0.01$) and separate group analyses ($F(3,86) = 0.53$, $p = .66$, $\eta^2_{partial} = 0.02$). Moreover, no effects were detected for the clinical skin response parameters (all, $p > .21$, see also **Table 1** [combined groups] and **Supplementary Table S1** [separate groups]).

Spirometry

No significant group by time interactions were found for $FVC_{\%predicted}$ or $FEV_{1\%predicted}$ during the course of the evocation sessions for both the combined and separate group analyses (all, $p > .32$), indicating that conditioning did not evoke changes in spirometry over time. In addition, no main effect of group on spirometry parameters was found (all, $p > .13$; see **Supplementary Tables S2 and S3**).

Table 1. Analyses of (co)variance results, means, and standard deviations for the combined conditioned groups vs the combined control groups

	Combined open- and closed-label conditioned groups (n=46)	Combined conditioned-not-evoked and non-conditioned control groups (n=45)	ANCOVA results: effects of group on outcome parameter	
			p-value	$\eta^2_{partial}$
Demographic factors				
Age [A]	22.59 ± 3.00	21.44 ± 1.80	.15	
Body Mass Index [B]	23.53 ± 3.29	22.90 ± 3.35	.37	
Sex [male]: n(%)	9 (19.6)	6 (13.3)	.42	
Ethnicity [Caucasian]: n(%) [C]	41 (93.2)	41 (95.3)	.51	
Allergy – anamnesis [yes]: n(%)	14 (30.4)	14 (31.1)	.94	
Allergy – IgE response [positive]: n(%) [D]	16 (65.2)	18 (41.9)	.49	
Eosinophilic profile [within normal range]: n(%)	42 (93.3)	45 (97.8)	.39	
History of antihistamine use [E]	12 (26.1)	8 (17.8)	.34	
Pre-conditioning histamine iontophoresis (baseline)				
Process measure				
Expected itch pre-iontophoresis	4.27 ± 2.06	4.17 ± 2.04	.83	< .01
Expected itch post-iontophoresis	3.79 ± 1.87	3.92 ± 1.93	.75	< .01
Primary outcome measure				
Mean self-reported itch	3.66 ± 1.94	3.39 ± 1.66	.48	< .01
Secondary outcome measures				
Subjective skin response	24.19 ± 14.22	24.62 ± 11.79	.88	< .01
Wheal area (cm^2) [F]	12.33 ± 3.05	10.63 ± 3.55	.02	.07
Flare area (cm^2) [F]	47.98 ± 12.46	46.90 ± 10.63	.66	< .01
Skin temperature change (°C) [G]	1.66 ± 1.57	1.64 ± 1.83	.96	< .01
Post-conditioning histamine iontophoresis (evocation)				
Process measure				
Expected itch [H]	3.79 ± 2.25	4.25 ± 1.71	.15	.02
Remembered itch from baseline	3.96 ± 2.12	3.90 ± 1.99	.90	< .01
Expected medication efficacy	4.60 ± 2.33	3.81 ± 2.40	.11	.03
Primary outcome measure				
Mean self-reported itch [H]	2.88 ± 1.96	3.02 ± 1.54	.08	.02
Secondary outcome measures				
Subjective skin response [H]	23.81 ± 14.28	25.39 ± 11.37	.50	< .01
Wheal area (cm^2) [I]	11.03 ± 3.09	10.00 ± 3.41	.66	< .01
Flare area (cm^2) [I]	45.29 ± 12.82	45.31 ± 12.18	.45	< .01
Skin temperature change (°C) [G]	1.33 ± 1.71	1.06 ± 1.47	.42	< .01

Note (**Table 1**). [A] As tested by non-parametric Mann Whitney test (ANOVA assumptions were violated). [B] n=1 missing. [C] n=4 missing. [D] n=2 missing. [E] Not within past 2 months and an extensive history of levocetirizine use was considered ground for exclusion [F] Analysis corrected for the amount of time passed between histamine iontophoresis and measurement of the variable. [G] Calculated as post-histamine iontophoresis skin temperature – control. [H] Analysis corrected for pre-conditioning (baseline) variable. [I] Analysis corrected for pre-conditioning (baseline) variable, as well as for the amount of time passed between histamine iontophoresis and measurement of the variable.

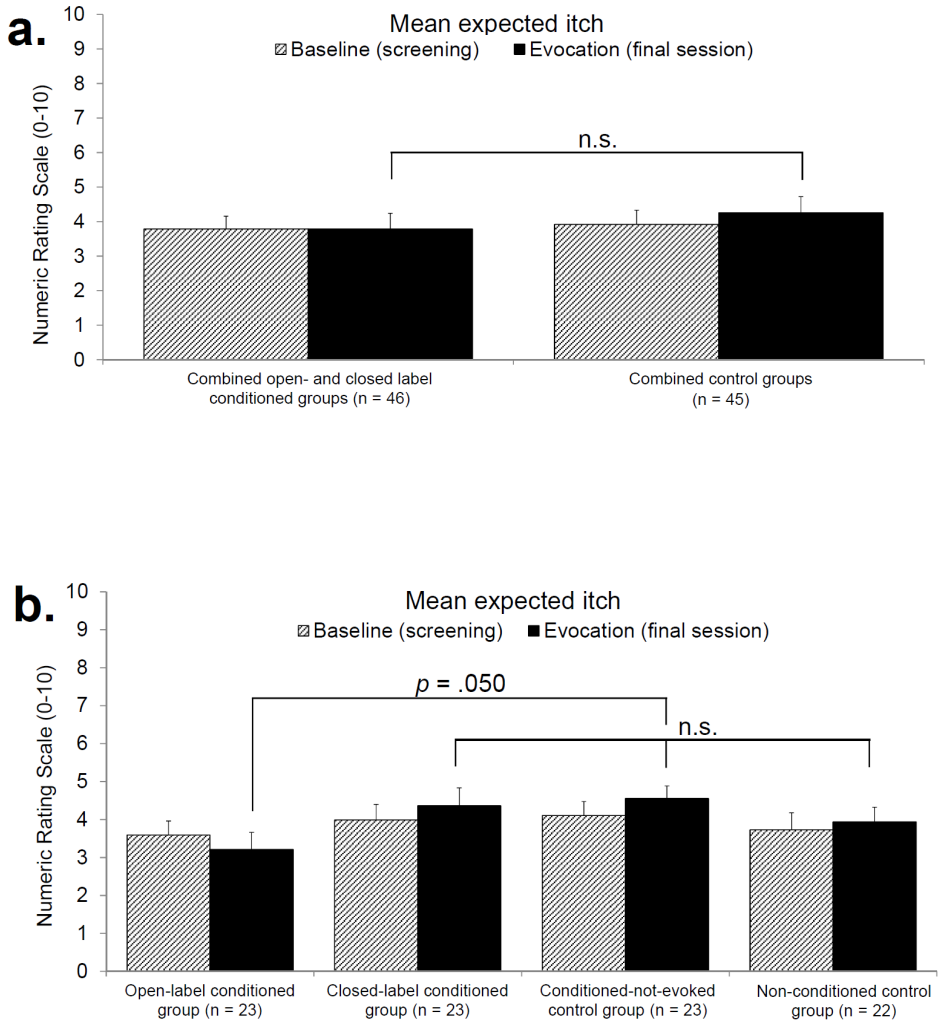
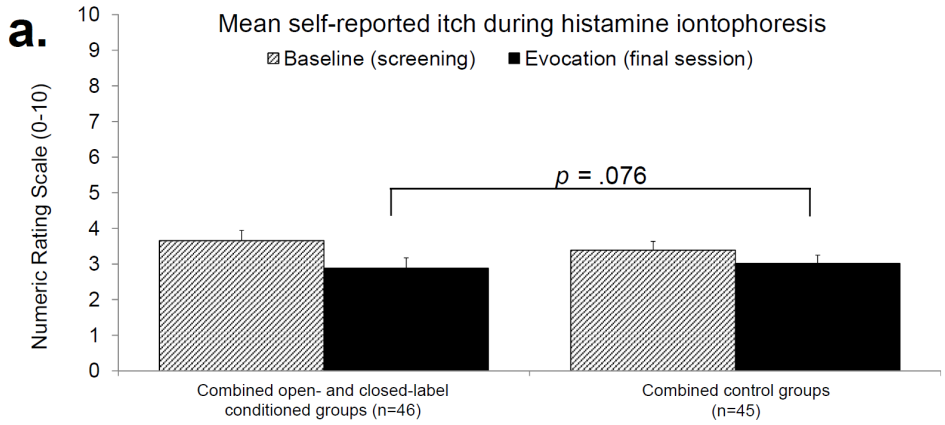


Figure 3. Means and standard errors of expected itch, with (A) the effects of the combined conditioned groups and the combined control groups on expected itch, controlled for baseline expected itch as measured post-iontophoresis during the screening, and (B) the effects of the separate groups on expected itch.



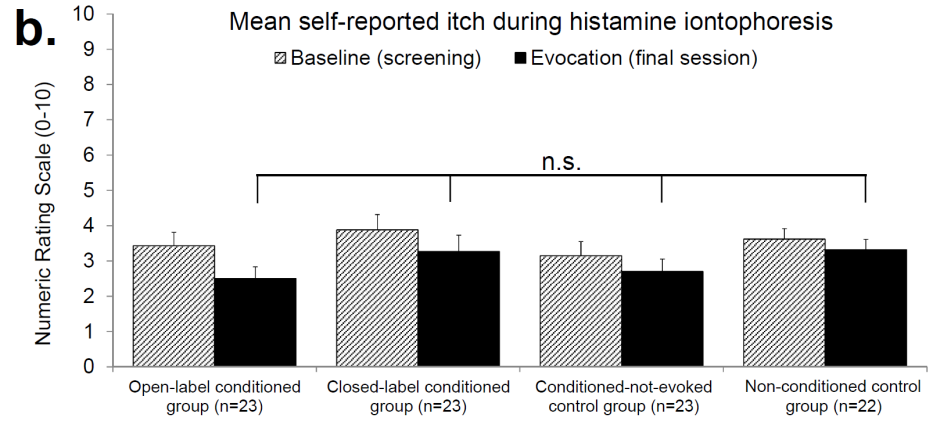


Figure 4. Means and standard errors of the mean for itch during iontophoresis in the final evocation session, with (A) mean itch for the combined conditioned and the combined control groups, and (B) mean itch for the separate groups.

HR and SCL

A medium-sized significant group by time interaction was demonstrated in the combined groups for HR (Wilk $\lambda = 0.83$, $F(7,80) = 2.35$, $p = .031$, $\eta^2_{partial} = 0.17$). Separate-group RMAs demonstrated an overall reduction in HR compared with baseline for both conditioned and control groups (both, Wilk $\lambda > 0.25$; both, $p < .001$). Post hoc comparisons over time demonstrated that in the combined conditioned groups, HR was significantly reduced compared with baseline for only three of seven post-CS measures ($p \leq .001$). In the combined control groups, HR was significantly reduced compared with baseline for six of seven post-CS measures ($p \leq .001$) and marginally reduced for the other (1/7) post-CS measure ($p = .075$). When analyses were repeated for the four (noncombined) groups, a similar medium-sized group by time interaction was found (Wilk $\lambda = 0.64$, $F(21,225) = 1.79$, $p = .021$, $\eta^2_{partial} = 0.14$). Post hoc separate-group RMAs and pairwise comparisons demonstrated significant HR reduction in line with the patterns for the combined groups. No group by time interactions (both, $p > .44$) or main effects of group (both, $p > .43$) were found for SCL in analyses with combined or separate groups. An overview is provided in **Supplementary Tables S2 and S3**.

Wellbeing

No group by time interactions (all, $p > .23$) or main effects of group (all, $p > .11$) were demonstrated for the Positive and Negative Affect Schedule positive affect, State Trait Anxiety Index–State Anxiety, or NRS general well-being measures for both the combined and separate group analyses (see **Supplementary Table S2 and S3**).

Closing questionnaire: suspected medication intake and its association with mean itch and other iontophoresis-related outcomes

No differences between groups were found when participants compared baseline and evocation itch in the closing questionnaire (all, $p > .15$). The groups differed marginally to significantly in suspected medication intake for all sessions (all, $p < .066$), except for the first evocation session. When the open-label conditioned group was excluded from the analysis, no differences were found (all, $p > .11$). Participants who suspected taking active medication during the final evocation session had reported less itch during iontophoresis as

compared with those who suspected taking placebo (open-label conditioned group included: $F(1,88) = 3.82$, $p = .054$, $\eta^2_{partial} = 0.04$; open-label conditioned group excluded: $F(1,65) = 6.09$, $p = .016$, $\eta^2_{partial} = 0.09$) and also reported lower subjective skin response (open-label conditioned group included: $F(1,88) = 5.95$, $p = .017$, $\eta^2_{partial} = 0.06$; open-label conditioned group excluded: $F(1,65) = 4.92$, $p = .030$, $\eta^2_{partial} = 0.07$; **Supplementary Table S4 and S5**).

DISCUSSION

The current study investigated whether behavioral conditioning of the antihistaminergic properties of levocetirizine could reduce itch and other clinical, physiological, and psychological responses to histamine, under both open-label (i.e., with participants knowing about the conditioning procedure) and closed-label conditions. Conditioning was found to be marginally effective in reducing itch when the combined conditioned groups were compared with the combined control groups. However, no effects of conditioning were found for self-rated or clinical skin responses to histamine. Marginal antipruritic effects occurred regardless of whether participants were informed about the procedure, implying that, if further optimized, open-label behavioral conditioning might be suitable for future applications in clinical practice.

These findings show that conditioning, albeit only marginally, influenced self-reported itch, which is in line with previous findings that show that associative learning mechanisms can influence itch and allergic symptoms [11,14,21,22]. Most studies have investigated conditioned exacerbation of allergic responses, whereas evidence for alleviation of itch through associative learning mechanisms is more limited and has only so far been examined in allergic patients [21,22]. In patients, it may be especially difficult to ascribe findings exclusively to behavioral conditioning because external influences on learning may also be relevant. For example, natural fluctuations in symptom severity during acquisition of the conditioned response may affect conscious expectancy, due to these fluctuations being interpreted as medication effects. This in turn could influence symptom reporting within both the conditioned and control groups. Resultantly, to reduce the influence of such external factors on conditioning, the current study sought to investigate whether antipruritic effects could be conditioned in healthy volunteers.

Goebel and colleagues [21] had previously found a unique conditioned response for basophil activation in allergic patients, but symptoms reduced regardless of group allocation. Vits and colleagues [22] confirmed these findings and demonstrated symptom reduction for the conditioned and sham-conditioned (placebo) patient groups, compared with a natural history group. This led them to conclude that other cognitive processes, for example, patients' expectations of benefit, may be relevant. Likewise, the current study provides only limited evidence for the role of conditioning in reducing histamine-induced itch. Some differences between the current study and previous studies can be noted. In the studies of Goebel and colleagues [21] and Vits and colleagues [22] patients reported symptoms at the time of enrolment in the study. In the current study, the sample consisted of nonallergic participants, or allergic participants who had not experienced symptoms for some time before enrollment. Potentially, this may have elicited smaller conditioned responses, as the pharmacological effects of levocetirizine during acquisition may not have been clearly perceived as much as they would be when allergic symptoms were present. Moreover, itch was induced in the final evocation session, to prevent that histamine iontophoresis—which entails the introduction of a foreign chemical substance to the skin [42]—interfered with measurements of conditioned responses for other study outcomes. Although literature indicates that conditioned immunological responses can persist for multiple—potentially even up to fourteen—evocation moments [39,43,44], it may be possible that some extinction in the conditioned response was already present in the second and third evocation sessions. Future research could investigate whether conditioned effects for itch are stronger at earlier evocation moments, for example, when participants are for the first time reexposed to the CS after the acquisition phase. Alternatively, it may be possible that the antipruritic effects of levocetirizine were too small for experimental histamine-induced itch to be effectively conditioned. Indeed, in the current study, itch reduced from baseline in general, with only marginal differences between the conditioned and control groups (21.3% reduction of itch from baseline in the conditioned groups versus 10.9% reduction in the control groups). Previous evidence dispels the notion that this small difference between groups may be due to failure of the UCS to suppress itch though, because it is demonstrated that levocetirizine has a suppression rate for itch that lies between 62% and 94% [45-47]. A similar suppression rate would be expected for levocetirizine in the current study. Future research, however, may want to include a drug control group to confirm this notion and to be able to directly compare conditioned with nonconditioned responses.

Speculatively, the marginal antipruritic conditioned effect in the current study could have emerged through peripheral neurobiological mechanisms, for example, immune-mediated inhibition of pruriceptor neurons [48-50]. Such mechanisms have been proposed to underlie systemic behaviorally conditioned immunosuppression [8,51]. Alternatively, effects may have emerged through top-down central nervous system antipruritic mechanisms, for example, in case of itch with a neuropathic and psychogenic origin [23,52,53]. As an example of central nervous system–mediated itch, itch has been found to be socially contagious in both patients and healthy volunteers [54-56]. Future research may aim to clarify through which pathways antipruritic conditioned effects are established.

No conditioning effects were found for spirometry parameters. Literature indicates that pulmonary conditions such as asthma are sensitive to placebo responding [57,58], and antihistamines have been found to have bronchodilatory properties, as shown by their impact on spirometry parameters such as FEV_1 [59-62]. As such, we explored whether conditioning of antihistamines could affect these parameters as well. The missing data rate in the current study likely affected the findings, however, and the study may have been underpowered for small effects. Moreover, as the sample consisted of healthy volunteers, conditioned responses may be very small because lung function may have already been optimal for a large number of participants. It may be interesting for future research to test the effects of conditioning with antihistamines by experimentally inducing bronchoconstriction, for example, through embedding a histamine bronchial provocation test. No conditioned responses were found for the secondary parameter SCL. HR reduced significantly during evocation for the combined control groups. The time that participants spent sitting in the laboratory was relatively inactive, which likely explains the decrease in HR. For the conditioned groups, HR did not decrease as much in the second and final evocation sessions. Levocetirizine is considered safe for use, and studies show no effects on cardiac safety parameters [63], however, subclinical cardiac effects are often not reported. Moreover, H_1-antihistamines—including cetirizine, from which levocetirizine is derived—have been associated with tachycardia and other cardiac adverse effects [64-66]. As such, the difference in HR change over time between the conditioned and control groups might speculatively be the result of a conditioned response, although this should be further investigated. In addition, future research may aim to investigate how to enhance the learning process exclusively for the itch-suppressive effects of antihistamines, while avoiding conditioning of adverse effects.

Following the open-label rationale, significantly lower itch was expected during evocation in the open-label group compared with the conditioned-not-evoked group. However, although findings were in the expected direction, itch expectations in the open-label group did not significantly differ from those in the closed-label conditioned and nonconditioned groups. That an open-label rationale may potentially influence expectancy is in line with studies that found that inert pills combined with an open-label rationale can reliably induce placebo effects [28-34]. It has also been shown that an open-label rationale regarding the role of expectations in eliciting placebo effects for itch can, in an experimental setting, result in lower expected itch even without providing inert pills [67]. The current study extends these findings by preliminary showing an effect of an open-label rationale for a conditioning framework. Potentially, these expectations may help strengthen placebo effects induced by conditioning, although this needs to be investigated more extensively. Demonstrating the efficacy of open-label conditioning could lead toward new therapeutic possibilities and help facilitate utilization of placebo effect mechanisms in clinical practice. It should be noted, though, that the open-label rationale in the current study consisted of multiple components (e.g., an explanation of the conditioning procedure, a suggestion that effects may be as large as the effects of the medication, and a suggestion of reduced itch). Future research may clarify which of these components are essential for inducing expectations of reduced itch, and investigate what other factors help optimize these effects. For example, higher likability and competence of a health care provider have been shown to enhance placebo effects for allergic responses [68]. It may be worthwhile to investigate to which extent factors such as likability and competence may influence the efficacy of an open-label rationale as well.

Some limitations of the current study should be considered. Because participants were mostly women, a sex bias cannot be excluded. The experimenter was blinded to group allocation only for the closed-label conditioned and the nonconditioned groups, but not for the open-label conditioned and conditioned-not-evoked groups, because of the differences in the protocol for these latter two groups. Future research may consider having a second, blinded experimenter performing measurements, to prevent that the experimenters' own expectations influence measurement of the outcome parameters. Second, participants underwent histamine iontophoresis only twice, to prevent interference of histamine application on the conditioned response. As a result, it was not possible to assess conditioned effects for itch on the first and second evocation days, or to assess whether extinction may have taken place. In addition, no drug control group was included in the

current study. Moreover, effects of antihistamine administration were not assessed in the acquisition phase because this could influence participants' conscious expectancy and thus the conditioning procedure. Because the efficacy of levocetirizine for inhibiting the response to histamine has been described in previous literature [45-47,63], we did not directly compare the magnitude of conditioned effects with those of levocetirizine. Future research may consider measuring the response to histamine on multiple testing days and including a drug control group. Finally, all groups received some form of intervention (either conditioning or placebo throughout the study). This may complicate an estimation of a true placebo response, as the idea of receiving an intervention may already influence study outcomes. Moreover, itch was induced twice. Although unlikely to have largely affected study findings—given that the itch stimulus was of short duration and inductions were spaced over 2 weeks apart—habituation cannot be ruled out. Future research may also consider adding a natural history group to control for this.

In conclusion, the current study provides preliminary support for behavioral conditioning of antipruritic effects. In addition, the findings suggest that conditioning may be effective when it is known that a learning paradigm is used. Future research may aim to clarify under which circumstances and on which evocation moments conditioning can be successful in reducing itch. Demonstrating the efficacy of (open-label) conditioning of antipruritic effects may lead toward new therapeutic possibilities. Moreover, further investigation of the content of the open-label rationale may help facilitate utilization of placebo effect mechanisms in clinical practice.

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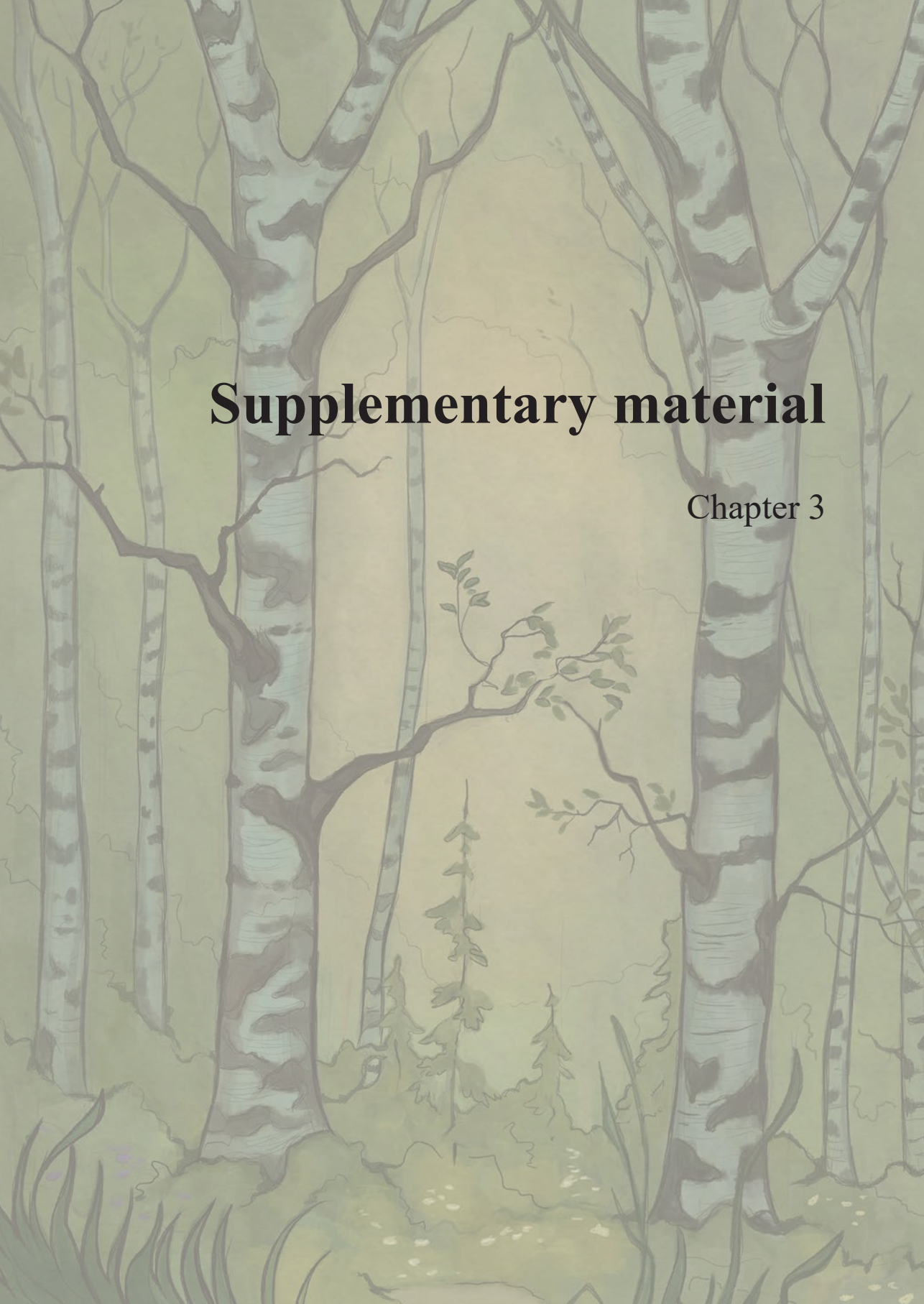


Supplementary material

Chapter 3

SUPPLEMENTARY METHODS

Elaboration on the participant group

Healthy male and female volunteers, aged between 18 and 35 years, were recruited for this study through advertisements at locations of Leiden University, the Leiden University Medical Center (LUMC), the University of Amsterdam, and the University of Delft, and through social media (e.g., Facebook). Inclusion criteria consisted of a good understanding of written and spoken Dutch, and absence of allergic rhinitis or allergic conjunctivitis within the three months prior to enrolment in the study. Participants were excluded in case of any (severe) allergic condition that presented symptoms other than rhinitis or conjunctivitis (e.g., food allergy); sensitivity to levocetirizine diHCl or other substances used in the study; lactose intolerance; somatic morbidity that could interfere with the participant's safety or with the study protocol (e.g., histamine intolerance, asthma); current Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) psychiatric diagnoses; recent (within past 2 months) use of antihistamines, antibiotics, or anti-inflammatory medication; recent vaccinations; and pregnancy. Participants were asked to refrain from consuming heavy meals, caffeine, or smoking 2 hours, exercise 12 hours, and alcohol and drugs 24 hours prior to the sessions. Adherence to these lifestyle guidelines, as well as any significant changes in health status during the course of the study (e.g., illness or other changes in physical health, or occurrences of highly stressful events) were monitored at the start of each session.

Elaboration on the conditioning paradigm

The CS was a distinctively-tasting green beverage that has been used as a CS in previous conditioning studies [1-6]. The beverage consisted of 150 mL of commercially available strawberry milk, which was coloured green by adding the coloring powders Quinoline Yellow (E104, 80 mg/L) and Patent Blue V (E131, 20 mg/L) and flavoured with lavender oil (0.6 mL/L)[1]. As unconditioned stimulus (UCS), 5 mg of levocetirizine diHCl was capsuled by the LUMC pharmacy. Identically-looking placebo capsules were also prepared by the pharmacy. Presentation of the CS and UCS or placebo in both the acquisition and

[1] Three participants (1 in the open-label conditioned group, 2 in the conditioned-not-evoked group) received a beverage containing 160 mg/L of Quinoline Yellow and 40 mg/L of Patent Blue due to administrative error. Sub-analyses of the total sample without these participants indicated no differences in the main results.

evocation sessions was accompanied by a brief instruction that emphasized: 1) that it was important that the beverage and capsule were taken simultaneously, and 2) that the experimenter did not know whether the capsule contained active medication or an inert substance (for the open-label conditioned group, a different instruction was used, see '*Open-label instructions*').

Elaboration on materials and measures

1. Open-label instructions

At the start of the acquisition phase, participants in the open-label conditioned group were provided with scripted instructions regarding five points: 1) that part of the effects of anti-allergic medication can be learned through the principle of conditioning, 2) that an example of conditioning is the experiment of Pavlov, in which a dog was taught to respond to the ringing of a bell with salivating, by pairing this sound with food, 3) that this learning paradigm can be utilized for medication use by, for example, pairing medication with a beverage, 4) that these effects may be large, and potentially just as large as the effects of the medication itself, and 5) that effects may be noticed in the evocation phase, for example, as improved performance on the spirometry tests and reduced itch during iontophoresis in the final session. During each session, administration of the beverage and capsule was accompanied by instructions that consisted of a brief repetition of points 1 and 4. In addition, point 5 was briefly repeated at the start of the final session.

2. Histamine iontophoresis

Itch was evoked experimentally by transdermal histamine iontophoresis (Chattanooga Group, Hixson, TN, USA) at baseline and during the final evocation session. Histamine iontophoresis has been previously used as a reliable method to induce itch in healthy participants [7-10]. An electrode with an active surface of 11.7 cm^2 (Iogel, Iomed, DJO Global, Hannover, Germany) was treated with 2.5 ml of a 0.6% diphosphate histamine solution (prepared in distilled water with propylene glycol and Hypromellose 4000 mPa; equivalent to 1% histamine dihydrochloride). The prepared electrode was placed on the volar side of the non-dominant forearm. A reference electrode was placed on the volar

surface of the upper arm. Histamine iontophoresis was conducted for 2.5 minutes with the current level set at 0.4 mA.

3. Primary outcome measure: self-reported itch

During iontophoresis, itch was assessed verbally every 30 seconds on a Numeric Rating Scale (NRS) ranging from 0 (*'no itch'*) to 10 (*'worst itch ever experienced'*). Directly following iontophoresis, mean self-reported itch during the test was assessed using the same NRS. Between 1 and 4 minutes after iontophoresis, itch was again assessed every 30 seconds as a follow-up period to the test. Mean self-reported itch during iontophoresis assessed directly following iontophoresis was used as the primary outcome measure, and correlations with other itch measures taken during iontophoresis were calculated in order to validate the reliability of the main outcome measure.

4. Secondary outcome measures

4.1. Expectations regarding histamine iontophoresis

Participants rated the amount of itch they expected to experience during iontophoresis on the same NRS as used for the itch assessments. Measures of expectations were taken at the start of both the screening session and the final evocation session. Moreover, participants rated the amount of itch they expected to experience during the final evocation session at the end of the screening session (following the first iontophoresis test). Finally, using the same NRS, participants rated, prior to histamine iontophoresis in the final evocation session, how much itch they remembered experiencing at baseline (screening session), as well as the expected efficacy of the administered capsules (0 *'not effective*', 10 *'very effective'*).

4.2. Self-rated skin response

Self-rated skin response was measured using an adjusted version of the Sensitive Scale-10 (SS-10; [11]). This questionnaire assesses a variety of skin symptoms that are either subjectively experienced (e.g., itch, tingling, burning, pain), or visibly rateable (e.g., redness of the skin). Symptoms are rated on a 0 ('zero intensity') to 10 ('intolerable

intensity') scale. Total scores are calculated by summing across items. For the purpose of the current study, the timeframe for which the symptoms were rated was tailored to histamine iontophoresis (i.e., *'during the histamine test'*, rather than the original *'during the past three days'*). As a baseline measurement, participants also filled in the original questionnaire. Cronbach's alpha was .58 for the original questionnaire in the current study. For the adjusted SS-10 following histamine iontophoresis at baseline and during evocation, Cronbach's alpha was .88 and .89, respectively.

4.3. Clinical skin response

A 1 cm^2 gridded, transparent sheet was used to trace the wheal and flare area in response to histamine iontophoresis. The outer edges of the drawn areas were retraced in ImageJ [12], after which the areas of the wheal and flare response were calculated in cm^2. Skin temperature following iontophoresis was measured using a handheld infrared thermometer (accuracy ±2.0 °C, resolution 0.1 °C, BaseTech, Conrad Electronic Benelux B.V., Hirschau, Germany). Measurements were taken with the thermometer held approximately 1 cm above the centre of the wheal. A similar measurement was taken on the same area of skin on the opposite arm, to control for individual differences in skin temperature. Increase in skin temperature as a result of iontophoresis was calculated by subtracting temperature of the control area from temperature of the wheal area, with positive values indicating a higher skin temperature increase following iontophoresis.

4.4. Spirometry

Spirometry was performed in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force guidelines on the Standardisation of Lung Function Testing [13]. The experimenters were trained in spirometry by certified technicians at the LUMC. Tests were performed using a mounted, non-heated Lilly type pneumotachograph and SentrySuite software package Version 2.7 (Carefusion, Hoechberg, Germany). For FVC and FEV_1, percentages of the predicted scores were calculated using the standard DE#GLI 2012 reference values [14]. Tests that did not meet the acceptability and repeatability criteria were excluded from analyses.

4.5. Heart rate and skin conductance level

Heart rate (HR; in beats per minute, BPM) and skin conductance level (SCL) were measured during the screening session and during the sessions of the evocation phase. Measurements were taken using an MP150 system and Acqknowledge software, version 4.4 (BIOPAC Systems Inc., Goleta, CA, USA). As has been done previously by our research group [15], the skin was abraded with Nuprep scrub (Weaver and Company, Aurora, CO, USA) in preparation of the HR measurements, after which two disposable electrodes were placed (Ø 38 mm; Kendall 200 Foam Electrode, Covidien, Mansfield, MA, USA) on the sternum and on the participant's left side below the ribs. An ECG100C amplifier at 100 Hz with a gain of 100, a 0.5-Hz high pass and a 35-Hz low pass filter, and a 50-Hz notch filter measured the electrocardiography signals. The skin was cleaned with water in preparation of the SCL measurements, after which two disposable Ag/AgCl electrodes (Ø 32 mm; DBF3D77, Multi Bio Sensors Inc., El Paso, TX, USA) were placed on the medial phalanges of the index and middle finger of the non-dominant hand. A GSR100C amplifier at 1000 Hz with a gain of 10 μmho/V and a 1.0-Hz low pass filter recorded SCL. Five-minute HR and SCL resting state measurements were taken, once in the screening session, and at various time points during evocation (i.e., prior to, and every 30 minutes post-CS administration). Visual inspection of the data and calculation of mean HR and SCL were done using the Physio Data Toolbox Version 0.1 [16], a standalone MATLAB-based application (MATLAB Release 2016a, The MathWorks, Inc., Natick, MA, USA) that was written at the Faculty of Social and Behavioural Sciences at Leiden University.

4.6. Self-rated wellbeing

Self-rated wellbeing was measured throughout the study by means of questionnaires. To measure positive affect (PA) and negative affect (NA), the 20-item Positive and Negative Affect Schedule (PANAS; [17]) was administered. Cronbach's alpha ranged from .88 to .93 for PA in the current study. As the scores for NA were only within the lower range of the scale for all participants, NA data were not analysed. A short 6-item version of the State Trait Anxiety Index – State Anxiety (STAI-S-s; [18]) was administered to assess state anxiety. Cronbach's alpha ranged from .66 to .81. In addition, participants were asked to rate seven psychological states (relaxed, nervous, calm, well, tense, concerned, stressed) on Numeric Rating Scales (NRS) ranging from 0 (*'not at all'*) to 10 (*'very much so'*). The four

negative items were recoded and all NRS were summed and divided by seven to calculate a general wellbeing score, for which Cronbach's alpha ranged from .81 to .91.

4.7. Taste of the Conditioned Stimulus (CS)

Following each administration of the CS in the acquisition and evocation phase, participants rated the taste of the beverage on a 9-point Likert scale (1 *'very unpleasant'* to 9 *'very pleasant'*). For the conditioned-not-evoked group, the CS was not administered during the evocation phase. Instead, the capsule was administered with water and, to standardise procedures over all groups, participants were asked to rate the taste of the water. The ratings of water during the evocation phase for the conditioned-not-evoked group were not analysed.

5. Additional measures: potential predictors of conditioned effects

5.1. Atopic constitution and allergy

To assess whether participants were allergic or had a tendency towards allergic or overly sensitive responses (atopic constitution), participants were asked during the screening to indicate whether they had ever experienced any allergic responses to food, animals or pollen. In case of severe allergic responses, e.g., throat swelling, or in case of recent allergic responses, participants were excluded. In addition, blood samples were taken at the LUMC, to assess eosinophil profile and to conduct an allergy test using the blood Immunoglobulin-E (IgE) response to inhalant allergens. Blood samples were treated with a mixture of various aeroallergens (i.e., dust mite, grass pollen, animals, birch, mugwort) and the IgE response was measured and divided into semiquantitative classes to determine sensitization level [19]. Data were collected in order to assess – in the event of significant effects of conditioning on the outcome parameters – whether these effects may potentially differentiate between subgroups of participants. Of all participants, 27 (31%) indicated being allergic to either food products or aeroallergens, and 34 (37%) responded positively on the aeroallergen IgE test.

5.2. Individual characteristics

Individual characteristics and personality factors were assessed during the screening session. Participants filled in the following questionnaires: a multidimensional measure of general health status, the RAND SF-36 Health Status Inventory (RAND-36 [20]), the Behavioural Inhibition System / Behavioural Approach System scales (BIS/BAS scales [21]), the Eysenck Personality Questionnaire short version – subscales extraversion and neuroticism (EPQ-RSS-EN [22]), the Hospital Anxiety and Depression Scale (HADS [23]), the Life Orientation Test – revised (LOT-R [24]), the Perceived Stress Scale (PSS [25]), and the Penn State Worry Questionnaire (PSWQ [26]). Potential moderating effects of individual characteristics were tested and are described in the supplementary material (see section 7.5.).

Elaboration on the general procedure

1. Pre-enrolment procedures and additional details on the screening session

Prior to the study, potential participants were briefly screened for the in- and exclusion criteria by telephone, and subsequently, potentially eligible participants were invited to the laboratory for a first (screening) session. An interview was used to further assess whether participants met the inclusion criteria (e.g., presence of any psychological diagnoses according to the DSM-IV criteria). Afterwards, questionnaires assessing individual characteristics and personality factors were filled in, and measurement sets A, B and C were assessed. At the end of the screening session, blood samples were collected at the LUMC to assess eosinophil profile and immunoglobulin-E (IgE) response to aeroallergens for potential subgroup analyses, as well as potential analyses of baseline cytokine levels.

2. Acquisition and evocation phase

The acquisition and evocation phases were scheduled within the same 30-minutes time frame in the next two weeks. Within each phase, all sessions started at the same time on three consecutive days. At the start of each session, participants were given an overview of the procedures of that day, and a brief interview was conducted (e.g., to verify adherence to lifestyle guidelines). Within the evocation phase, participants completed several neutral filler tasks (e.g., reading neutral magazines, and filling out Sudoku and word search

puzzles) for the purpose of standardising the time that participants had to spend waiting between measurements. At the end of the final evocation session, participants filled out a closing questionnaire, in which they were asked, for example, whether they believed to have received active medication, and were debriefed about the study purpose. Finally, participants were asked to provide a saliva sample in order to test associations between genotype and the conditioned response (the results of which will be described elsewhere), and a second blood sample was taken at the LUMC to potentially assess blood cytokine levels.

Elaboration on statistical analysis

1. Pre-analyses checks of data and assumptions

Prior to analyses, variables were checked for normal distribution and outliers, and underlying assumptions for each analysis were checked. To detect differences in demographics and baseline measures of the study outcome parameters, χ^2 tests and general linear model (GLM) analyses of variance (ANOVAs) were used. For wellbeing during the acquisition phase, and taste ratings for the CS throughout the study, GLM ANOVAs were also performed.

2. Reliability of primary outcome measure

The primary outcome measure of mean self-reported itch at evocation correlated highly with the calculated average of the itch measures taken during histamine iontophoresis at evocation (r = .96, p < .001), supporting the reliability of the primary outcome measure used for itch.

3. Covariates included in the analyses of the primary and secondary outcomes

All GLM analyses of covariance (ANCOVAs) conducted for expected itch, self-reported mean itch, and the self-rated and clinical skin response were controlled for baseline values (screening session). Expected itch was assessed twice during the screening session: once prior to baseline histamine iontophoresis, and once following baseline iontophoresis (as a measure assessing the amount of itch participants expected to experience during the final

evocation session). The latter was included as a covariate in the ANCOVA. For remembered itch and expected efficacy of the capsules, no covariates were included. For the clinical skin response measures of wheal and flare area an additional covariate was included, which consisted of the amount of time between the end of iontophoresis and the drawing of the affected skin areas onto the transparent sheet, in order to control for changes in skin response over time.

4. Missing data

Due to technical issues with the equipment for histamine iontophoresis, data of one participant was excluded for the analyses of outcome parameters related to histamine iontophoresis (i.e., expected itch, measurement set C). Due to technical issues and the occurrence of artefacts (e.g., a significant number of extra systoles in HR data), HR and SCL data were not reliable for 4 participants. Subsequently, these participants were excluded from the analyses. For spirometry, only data of participants who performed well on all MEFV curves assessed during evocation (i.e., all 10 tests taken during evocation meeting the ATS/ERS criteria for acceptability and repeatability, to prevent that the group composition changed for each time point in the study) were included in subsequent analyses, resulting in loss of data of 45 participants. Since conditioning only marginally influenced the primary outcome of itch, no further subgroup analyses based on allergic constitution were conducted, nor were the blood samples analysed for cytokine levels.

5. Testing the moderating role of individual characteristics and personality in conditioning the effects of antihistamines for itch

To assess whether individual characteristics would influence conditioning effects on the main outcome of self-reported itch during iontophoresis, controlled for baseline, moderation analyses were conducted according to the Preacher and Hayes moderation regression method PROCESS 3.3. [27]. For each individual characteristic (predictor of the conditioned response), a separate moderation model was tested two-sided with an alpha level of .05. Analyses were first conducted for the combined conditioned versus the combined control groups, and then repeated to assess effects for the separate four groups. Bootstrap was set at 5000 samples in PROCESS, and conditional effects were probed at -1SD, the mean, and +1SD. Prior to analyses, group differences in individual characteristics

were assessed by one-way ANOVA, and the assumptions of regression were checked. In addition, the predictors were centered, and the group variables were dummy coded prior to moderation analyses (with the non-conditioned control group serving as the reference group). For some predictors (i.e., the RAND-36, the EPQ-RSS-EN, and the HADS subscales), there was very low variance in scores between individuals, and scores were non-normally distributed. For these factors, moderation analyses were not conducted.

SUPPLEMENTARY RESULTS

Group differences on individual characteristics and personality

No significant differences between the combined conditioned groups and the combined control groups were found for individual characteristics (all $p>.13$), with the exception of optimism (LOT-R; $F(1,89)=6.07$, $p=.016$). Participants in the conditioned groups scored higher on optimism ($M=18.33\pm2.72$) compared to the control groups ($M=16.93\pm2.67$). Repetition of these analyses for the separate groups showed that factors did not significantly differ between groups ($p\geq.072$). An overview of individual characteristics of the study sample is provided in **Supplementary Table S6.**

Moderating role of individual characteristics and personality in conditioning the effects of antihistamine for itch: the combined conditioned and combined control groups.

No significant moderation of the effect of the combined conditioned and the combined control groups on mean itch in response to iontophoresis during evocation was found for optimism, perceived stress, worrying, behavioural activation scales (BAS) drive, fun seeking, and reward responsiveness, or behavioural inhibition scale (BIS) (all group x factor interactions: $p\geq.053$; see **Supplementary Table S7**).

Moderating role of individual characteristics and personality in conditioning the effects of antihistamines for itch: separate groups

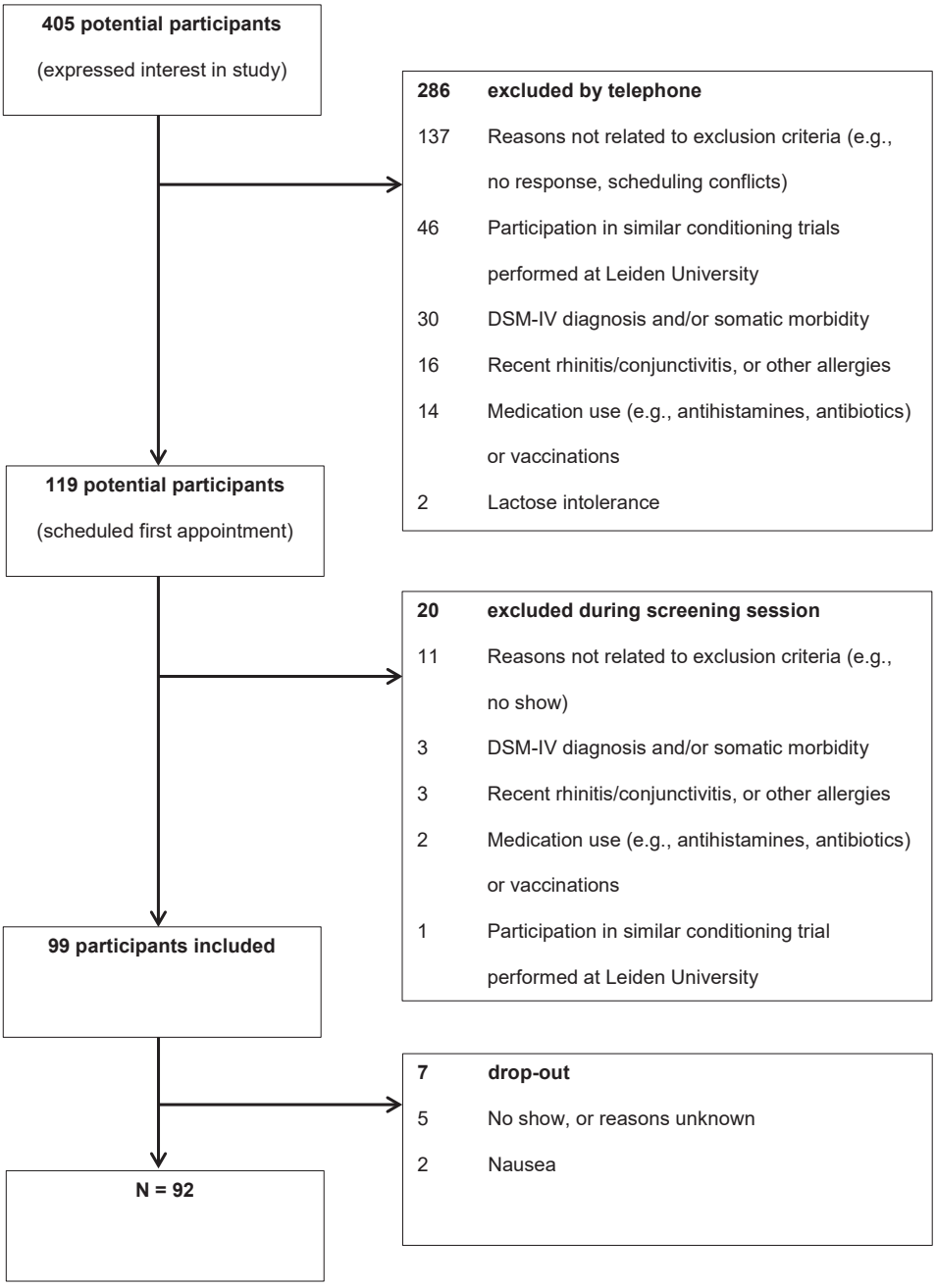
Optimism was found to moderate the effects of closed-label conditioning on mean itch in response to iontophoresis during evocation, compared to the other groups (closed-label conditioning dummy variable x optimism interaction: p=.021; see **Supplementary Table S8**). Higher levels of optimism were related to lower levels of mean itch in the closed-label conditioned group, compared to the other groups (see **Supplementary Figure S2**). However, post-hoc conditional effects of group at various levels of optimism were not significant (p≥.12). For the other dummy group factors, no effects were found (all $p_{interaction}$≥ .29).

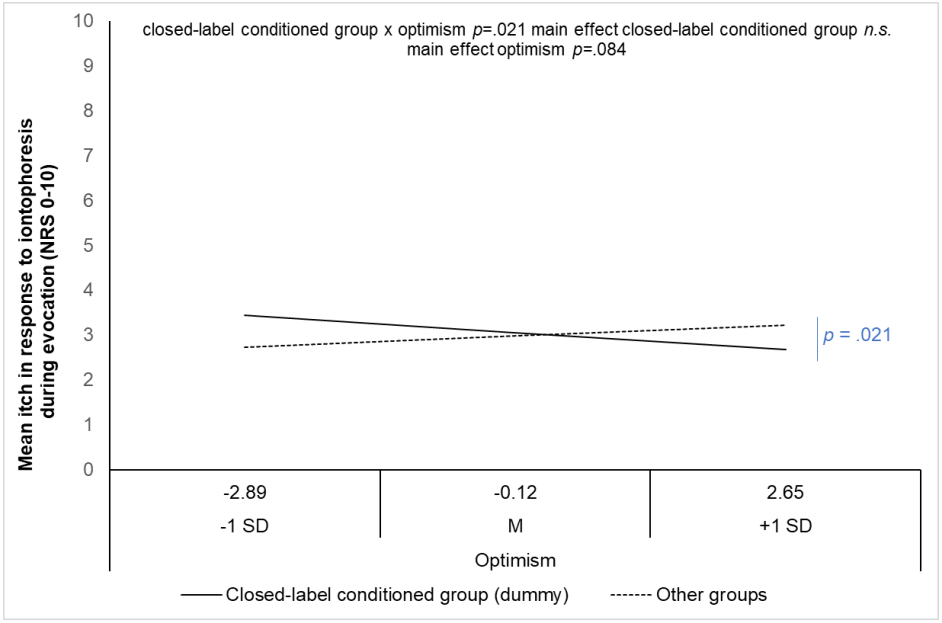
BAS reward responsiveness was found to significantly moderate the effect of the conditioned-not-evoked group on mean itch in response to iontophoresis during evocation, compared to the other groups (conditioned-not-evoked dummy variable x BAS reward responsiveness: p=.020). Higher levels of reward responsiveness were significantly associated with higher levels of mean itch in the conditioned-not-evoked group, compared to other groups (conditional effect at +1 SD of BAS reward responsiveness: t=2.18, p=.032; see **Supplementary Figure S3**). For the other dummy group factors, no effects were found (all $p_{interaction}$≥ .087). Finally, group effects were not significantly moderated by worrying, perceived stress, behavioural activation scales (BAS) drive and fun seeking, or behavioural inhibition scale (BIS) (all group x factor interactions: p≥.077; see **Supplementary Table S8**).

Concluding note on the moderating role of individual characteristics and personality in conditioning the effects of antihistamine for itch

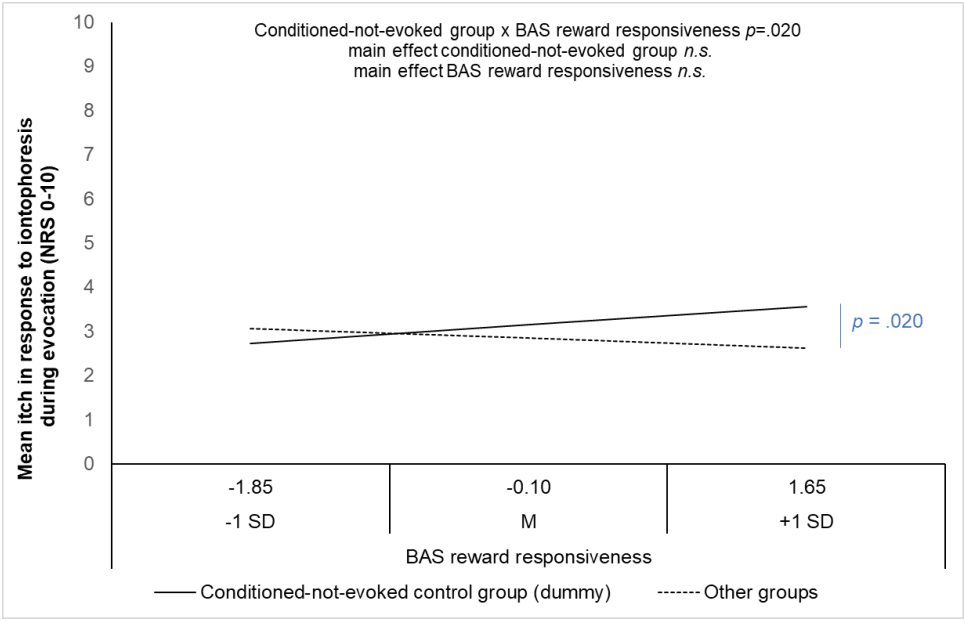
Some evidence was found for a moderating role for optimism in the closed-label conditioned group compared to others, however, post-hoc conditional effects at various levels of optimism were not significant, illustrating that such an effect may be limited. These results need to be interpreted very cautiously, especially given that the groups differed in optimism at baseline. Finally, a potential moderating effect of BAS reward responsiveness within one of the control groups was shown, with higher reward responsiveness being related to higher itch compared to other groups. This moderation is likely not related to the conditioning procedure, as this moderation also encompassed differences compared to the other control group.

Supplementary Figure S1. In- and exclusion of participants according to protocol criteria and drop-out specifications.





Supplementary Figure S2. Conditional effect of the closed-label conditioned group versus other groups on mean itch during iontophoresis in the evocation phase, controlled for itch during baseline, moderated by optimism.



Supplementary Figure S3. Conditional effect of the conditioned-not-evoked control group versus other groups on mean itch during iontophoresis in the evocation phase, controlled for itch during baseline, moderated by behavioural activation scale (BAS) subscale reward responsiveness.

Supplementary Table S1. Analyses of (co)variance results, means, and standard deviations for the separate groups comparisons

	Open-label conditioned group (n=23)	Closed-label conditioned group (n=23)	Conditioned-not-evoked control group (n=23)	Non-conditioned control group (n=22)	ANCOVA results: effects of group on outcome parameter	
					p	$\eta^2_{partial}$
Demographic factors						
Age [A]	21.87 ± 2.93	23.30 ± 2.96	21.30 ± 1.52	21.59 ± 2.09	.10	
Body Mass Index [B]	23.09 ± 3.25	23.98 ± 3.34	22.99 ± 2.68	22.80 ± 3.97	.64	
Sex [male]: n(%)	3 (13.0)	6 (26.1)	3 (13.0)	3 (13.6)	.56	
Ethnicity [Caucasian]: n(%) [C]	20 (90.9)	21 (95.5)	21 (100.0)	20 (90.9)	.64	
Allergy – anamnesis [yes]: n(%)	6 (26.1)	8 (34.8)	7 (30.4)	7 (31.8)	.94	
Allergy – IgE response [positive]: n(%) [D]	7 (30.4)	9 (39.1)	9 (42.9)	9 (40.9)	.84	
Eosinophilic profile [within normal range]: n(%)	23 (100.0)	22 (95.7)	20 (87.0)	22 (100.0)	.44	
History of antihistamine use [E]	6 (26.1)	6 (26.1)	5 (21.7)	3 (13.6)	.72	
Pre-conditioning histamine iontophoresis (baseline)						
Process measure						
Expected itch pre-iontophoresis	4.57 ± 2.12	3.96 ± 2.01	4.78 ± 1.81	3.54 ± 2.11	.16	.06
Expected itch post-iontophoresis	3.59 ± 1.77	3.99 ± 1.98	4.10 ± 1.78	3.73 ± 2.11	.79	.01
Primary outcome measure						
Mean self-reported itch	3.43 ± 1.82	3.88 ± 2.07	3.62 ± 1.43	3.15 ± 1.87	.58	.02
Secondary outcome measures						
Subjective skin response	22.70 ± 13.04	25.68 ± 15.45	25.35 ± 12.21	23.85 ± 11.58	.86	.01
Wheal area (cm^2) [F]	12.77 ± 2.68	11.90 ± 3.39	10.47 ± 3.83	10.79 ± 3.32	.08	.07
Flare area (cm^2) [F]	48.12 ± 12.34	47.84 ± 12.85	44.66 ± 11.81	49.24 ± 8.91	.63	.02
Change in skin temperature (°C) [G]	1.39 ± 1.44	1.92 ± 1.69	1.36 ± 2.05	1.92 ± 1.69	.50	.03

Supplementary Table S1. *Continued (2/2)*

	Open-label conditioned group (n=23)	Closed-label conditioned group (n=23)	Conditioned-not-evoked control group (n=23)	Non-conditioned control group (n=22)	ANCOVA results: effects of group on outcome parameter	
					p	$\eta^2_{partial}$
Post-conditioning histamine iontophoresis (evocation)						
Process measure						
Expected itch [H]	3.21 ± 2.15	4.37 ± 2.24	4.56 ± 1.59	3.94 ± 1.82	.037	.09
Remembered itch from baseline	3.80 ± 2.07	4.11 ± 2.21	3.96 ± 1.85	3.84 ± 2.18	.96	< .01
Expected medication efficacy	5.27 ± 2.29	3.94 ± 2.23	3.81 ± 2.48	3.81 ± 2.37	.11	.07
Primary outcome measure						
Mean self-reported itch [H]	2.50 ± 1.59	3.27 ± 2.24	3.32 ± 1.40	2.70 ± 1.66	.23	.05
Secondary outcome measures						
Subjective skin response	22.58 ± 13.16	25.04 ± 15.52	27.28 ± 11.96	23.41 ± 10.62	.66	.02
Wheal area (cm^2) [I]	11.05 ± 2.94	11.00 ± 3.30	9.46 ± 3.35	10.56 ± 3.46	.61	.02
Flare area (cm^2) [I]	46.03 ± 13.23	44.56 ± 12.66	44.81 ± 11.01	45.84 ± 13.54	.74	.02
Change in skin temperature (°C) [G H]	1.46 ± 1.75	1.21 ± 1.70	1.16 ± 1.36	0.96 ± 1.60	.67	.02

Note. [A] As tested by non-parametric Kruskal Wallis test (ANOVA assumptions were violated). [B] n=1 is missing. [C] n=4 missing. [D] n=2 missing. [E] Not within past 2 months, moreover, an extensive history of levocetirizine use was considered ground for exclusion. [F] Analysis corrected for the amount of time passed between histamine iontophoresis and measurement of the variable. [G] Calculated as post-histamine iontophoresis skin temperature – control. [H] Analysis corrected for pre-conditioning (baseline) variable. [I] Analysis corrected for pre-conditioning (baseline) variable, as well as for the amount of time passed between histamine iontophoresis and measurement of the variable.

Supplementary Table S2. Mixed between-within subjects repeated measures (RMA) results, means, and standard deviations for the combined conditioned groups vs the combined control groups

									Mixed between-within subjects RMA results								
Variables	**Evocation day 1**			**Evocation day 2**		**Evocation day 3**			**Group**			**Group x time**			**Time**		
	Pre-CS	**+30 min**	**+60 min**	**+30 min**	**+60 min**	**+30 min**	**+60 min**	**+90 min**	**F**	***p***	**η^2**	**F**	***p***	**η^2**	**F**	***p***	**η^2**
Physiological outcome parameters																	
Spirometry: FVC $_{\%\,predicted}$																	
Combined conditioned groups (n=24)	101.8 ± 11.0	101.7 ± 11.4	102.5 ± 12.2	100.9 ± 11.7	100.6 ± 11.7	100.5 ± 12.4	100.4 ± 11.9	100.8 ± 11.8	2.4	.13	.05	0.6	.75	.10	2.1	.06	.28
Combined control groups (n=23)	107.1 ± 12.0	107.5 ± 12.0	107.7 ± 11.7	105.3 ± 12.2	105.4 ± 12.7	105.7 ± 12.6	106.1 ± 11.9	106.7 ± 11.6									
Spirometry: FEV$_{1\%\,predicted}$																	
Combined conditioned groups (n=24)	94.7 ± 8.8	94.4 ± 9.7	94.8 ± 9.9	95.2 ± 10.5	94.0 ± 9.9	93.5 ± 9.9	93.7 ± 9.5	93.7 ± 9.9	2.3	.14	.05	1.0	.43	.16	1.5	.20	.21
Combined control groups (n=23)	99.4 ± 10.1	99.5 ± 10.0	98.8 ± 10.6	98.4 ± 10.3	97.8 ± 11.0	98.2 ± 11.4	98.5 ± 10.8	98.3 ± 10.7									
Mean heart rate (in BPM)																	
Combined conditioned groups (n=44)	76.3 ± 11.1	71.6 ± 9.6 ***	72.1 ± 8.5 ***	73.6 ± 8.0	73.5 ± 8.0	74.5 ± 8.9	73.4 ± 8.0	66.1 ± 8.3 ***	3.0	.084	.03	2.4	.03	.17	25.4	<.001	.69
Combined control groups (n=44)	74.7 ± 10.9	71.0 ± 9.4 ***	69.7 ± 8.9 ***	70.6 ± 9.1 ***	69.3 ± 8.3 ***	71.0 ± 9.3 †	68.4 ± 9.5 ***	69.8 ± 8.3 ***									
Skin conductance level																	
Combined conditioned groups (n=41)	3.3 ± 2.0	4.2 ± 2.3	4.2 ± 2.2	4.4 ± 2.9	4.4 ± 2.8	4.4 ± 2.6	4.4 ± 2.6	4.2 ± 2.4	0.6	.43	<.01	1.0	.44	.08	8.2	<.001	.43
Combined control groups (n=44)	3.9 ± 2.3	4.9 ± 2.6	4.8 ± 2.1	4.6 ± 2.2	4.4 ± 2.0	4.9 ± 2.1	4.6 ± 1.9	4.2 ± 1.8									
Psychological outcome parameters																	
Positive Affect (PANAS PA)																	
Combined conditioned groups (n=46)	25.5 ± 7.9	25.1 ± 8.2	25.7 ± 8.8	24.0 ± 6.8	25.3 ± 7.6	23.7 ± 7.1	24.6 ± 7.9	24.9 ± 7.9	0.8	.36	<.01	0.6	.78	.05	5.3	<.001	.31
Combined control groups (n=45)	23.9 ± 7.5	22.7 ± 7.8	24.9 ± 7.6	22.2 ± 7.8	23.7 ± 9.3	22.5 ± 7.9	23.8 ± 8.8	24.1 ± 7.8									
State anxiety (STAI-S-s)																	
Combined conditioned groups (n=46)	31.0 ± 9.4	29.6 ± 8.8	31.0 ± 8.3	29.6 ± 7.8	30.4 ± 7.3	29.2 ± 7.4	31.2 ± 8.3	30.1 ± 7.5	1.1	.30	.01	0.7	.69	.05	2.8	.01	.19
Combined control groups (n=45)	31.9 ± 8.1	30.5 ± 6.6	32.5 ± 8.2	31.1 ± 7.6	32.0 ± 8.1	31.8 ± 8.8	31.8 ± 7.5	32.4 ± 7.1									
General wellbeing (NRS)																	
Combined conditioned groups (n=46)	5.7 ± 0.8	5.9 ± 0.8	5.9 ± 0.8	6.0 ± 0.7	5.9 ± 0.6	6.1 ± 0.7	6.0 ± 0.7	5.9 ± 0.7	<0.01	.96	<.01	1.4	.23	.10	10.3	<.001	.46
Combined control groups (n=45)	5.9 ± 0.8	6.0 ± 0.8	5.9 ± 0.9	6.0 ± 0.7	6.0 ± 0.8	6.0 ± 0.8	6.0 ± 0.8	5.8 ± 0.7									

Note. † p<.10, * p<.05, ** p<.01, and *** p<.001 vs variables at the pre-CS level on evocation day 1 (post-hoc within subjects RMA for separate groups).

CS = conditioned stimulus, RMA=repeated measures analysis, $FVC_{\%\ predicted}$ = forced volume capacity (as calculated percentage of predicted values), $FEV_{1\ \%\ predicted}$ = forced expiratory volume in 1 second (as calculated percentage of predicted values), BPM = beats per minute, PANAS PA = Positive Affect and Negative Affect Schedule – Positive Affect, STAI-S-s = State Trait Anxiety Index – State Anxiety, NRS = Numeric Rating Scales

Supplementary Table S3. Mixed between-within subjects repeated measures (RMA) results, means, and standard deviations for the separate group comparison

									Mixed between-within subjects RMA results								
Variables	**Evocation day 1**			**Evocation day 2**		**Evocation day 3**			**Group**			**Group x time**			**Time**		
	Pre-CS	**+30 min**	**+60 min**	**+30 min**	**+60 min**	**+30 min**	**+60 min**	**+90 min**	**F**	***p***	**η^2**	**F**	***p***	**η^2**	**F**	***p***	**η^2**
Physiological outcome parameters																	
Spirometry: FVC % predicted																	
Open-label conditioned group (n=12)	99.9 ± 10.1	99.7 ± 11.4	99.8 ± 11.3	99.9 ± 9.8	99.2 ± 9.8	97.8 ± 10.9	98.8 ± 9.9	99.4 ± 11.4									
Closed-label conditioned group (n=12)	103.8 ± 11.8	103.7 ± 11.6	105.2 ± 13.0	101.9 ± 13.7	102.1 ± 13.6	103.2 ± 13.6	102.1 ± 13.9	102.3 ± 12.5	1.0	.40	.07	1.1	.34	.17	2.1	.072	.28
CNE control group (n=12)	106.6 ± 10.3	106.9 ± 10.5	106.9 ± 10.0	104.4 ± 10.3	104.1 ± 10.8	105.3 ± 9.9	105.7 ± 9.5	106.7 ± 8.8									
Non-conditioned control group (n=11)	107.7 ± 14.1	108.1 ± 14.0	108.5 ± 13.7	106.2 ± 14.4	106.9 ± 14.9	106.2 ± 15.5	106.6 ± 14.6	106.6 ± 14.5									
Spirometry: FEV_1 % predicted																	
Open-label conditioned group (n=12)	93.8 ± 8.4	92.9 ± 9.6	93.1 ± 9.4	93.8 ± 9.0	92.8 ± 9.1	91.8 ± 8.7	92.3 ± 7.3	92.1 ± 9.2									
Closed-label conditioned group (n=12)	95.7 ± 9.4	95.9 ± 10.0	96.5 ± 10.4	96.6 ± 12.1	95.3 ± 10.9	95.3 ± 11.1	95.0 ± 11.5	95.3 ± 10.6	2.0	.13	.12	0.6	.89	.10	1.4	.23	.21
CNE control group (n=12)	95.9 ± 8.5	96.3 ± 8.3	95.3 ± 9.0	94.9 ± 9.2	93.9 ± 9.3	95.0 ± 10.6	95.0 ± 9.4	95.3 ± 9.7									
Non-conditioned control group (n=11)	103.3 ± 10.7	103.1 ± 10.9	102.6 ± 11.2	102.2 ± 10.6	102.0 ± 11.5	101.6 ± 11.6	102.4 ± 11.2	101.6 ± 11.1									
Mean heart rate (in BPM)																	
Open-label conditioned group (n=21)	78.9 ± 10.1	73.9 ± 9.7 **	73.8 ± 9.1 *	74.7 ± 7.5	74.4 ± 7.4	75.4 ± 8.9	73.5 ± 8.3 *	70.1 ± 7.3 ***									
Closed-label conditioned group (n=23)	73.9 ± 11.8	69.5 ± 9.2 *	70.6 ± 7.7	72.7 ± 8.4	72.6 ± 8.5	73.8 ± 9.1	73.2 ± 7.9	69.5 ± 7.7	1.4	.25	.05	1.7	.026	.13	25.1	<.001	.69
CNE control group (n=23)	74.4 ± 11.2	70.3 ± 9.6 **	68.1 ± 8.8 ***	71.0 ± 8.5	68.8 ± 8.3 **	71.1 ± 9.8	67.1 ± 9.2 **	65.6 ± 7.9 ***									
Non-conditioned control group (n=21)	75.0 ± 10.8	71.7 ± 9.3	71.5 ± 8.9	70.3 ± 9.8	69.7 ± 8.4	70.9 ± 9.0	69.8 ± 9.8	66.6 ± 8.9 ***									
Skin conductance level																	
Open-label conditioned group (n=18)	3.2 ± 1.8	3.9 ± 1.8	3.9 ± 1.7	4.6 ± 3.2	4.6 ± 3.1	4.4 ± 2.8	4.1 ± 2.4	4.1 ± 2.3									
Closed-label conditioned group (n=23)	3.4 ± 2.2	4.4 ± 2.7	4.4 ± 2.6	4.2 ± 2.8	4.2 ± 2.5	4.5 ± 2.5	4.6 ± 2.7	4.3 ± 2.5	0.2	.87	<.01	1.0	.53	.08	7.9	<.001	.43
CNE control group (n=23)	3.8 ± 2.3	5.1 ± 2.8	4.8 ± 1.9	4.7 ± 2.3	4.6 ± 1.9	4.9 ± 1.8	4.6 ± 1.6	4.3 ± 1.6									
Non-conditioned control group (n=21)	4.0 ± 2.4	4.7 ± 2.4	4.8 ± 2.4	4.6 ± 2.3	4.3 ± 2.2	4.8 ± 2.5	4.6 ± 2.2	4.2 ± 2.1									
Psychological outcome parameters																	
Positive Affect (PANAS PA)																	
Open-label conditioned group (n=23)	23.2 ± 8.1	22.3 ± 7.7	23.2 ± 8.4	21.8 ± 6.9	22.6 ± 7.0	22.0 ± 7.4	21.7 ± 6.9	23.1 ± 7.3									
Closed-label conditioned group (n=23)	27.9 ± 7.0	27.9 ± 7.8	28.2 ± 8.7	26.1 ± 6.3	28.0 ± 7.3	25.5 ± 6.5	27.6 ± 7.8	26.7 ± 8.3	2.1	.11	.07	0.7	.88	.05	5.2	<.001	.31
CNE control group (n=23)	23.6 ± 6.3	22.7 ± 6.7	24.7 ± 7.3	21.7 ± 6.9	23.3 ± 9.2	22.1 ± 7.2	22.6 ± 8.6	23.4 ± 7.3									
Non-conditioned control group (n=22)	24.3 ± 8.7	22.7 ± 9.0	25.1 ± 8.1	22.8 ± 8.8	24.2 ± 9.5	23.0 ± 8.7	25.0 ± 9.1	24.9 ± 8.3									

Supplementary Table S3. *Continued (2/2)*

Variables	Evocation day 1			Evocation day 2		Evocation day 3			Mixed between-within subjects RMA results								
	Pre-CS	+30 min	+60 min	+30 min	+60 min	+30 min	+60 min	+90 min	Group			Group x time			Time		
									F	*p*	η^2	F	*p*	η^2	F	*p*	η^2
Psychological outcome parameters																	
State anxiety (STAI-S-s)																	
Open-label conditioned group (n=23)	32.9 ± 10.6	31.6 ± 9.3	32.3 ± 9.3	30.3 ± 8.5	30.1 ± 8.7	28.8 ± 7.8	30.3 ± 8.4	29.1 ± 8.2									
Closed-label conditioned group (n=23)	29.1 ± 7.9	27.7 ± 8.1	29.7 ± 7.0	29.0 ± 7.1	30.6 ± 5.8	29.6 ± 7.1	32.2 ± 8.3	31.0 ± 6.8									
CNE control group (n=23)	30.7 ± 8.4	28.7 ± 6.7	31.0 ± 9.6	29.4 ± 7.1	30.7 ± 8.5	30.7 ± 9.6	31.2 ± 8.0	32.8 ± 7.2	0.8	.49	.03	1.1	.33	.09	2.8	.013	.19
Non-conditioned control group (n=22)	33.2 ± 7.8	32.4 ± 6.1	34.1 ± 6.3	32.9 ± 7.8	33.3 ± 7.5	32.9 ± 8.1	32.4 ± 7.1	32.1 ± 7.1									
General wellbeing (NRS)																	
Open-label conditioned group (n=23)	5.5 ± 0.9	5.8 ± 0.9	5.8 ± 0.9	6.0 ± 0.7	5.9 ± 0.7	6.1 ± 0.8	6.0 ± 0.8	5.9 ± 0.7									
Closed-label conditioned group (n=23)	5.8 ± 0.7	6.1 ± 0.8	6.0 ± 0.8	6.1 ± 0.6	6.0 ± 0.6	6.1 ± 0.6	6.0 ± 0.7	5.9 ± 0.7									
CNE control group (n=23)	5.9 ± 0.8	6.0 ± 0.8	5.9 ± 1.0	6.1 ± 0.7	6.0 ± 0.8	6.0 ± 0.9	6.0 ± 0.9	5.8 ± 0.8	0.2	.89	<.01	1.0	.47	.08	10.5	<.001	.48
Non-conditioned control group (n=22)	5.8 ± 0.7	5.9 ± 0.7	5.8 ± 0.7	5.9 ± 0.7	5.9 ± 0.8	5.9 ± 0.6	5.9 ± 0.8	5.9 ± 0.7									

Note. † p<.10, * p<.05, ** p<.01, and *** p<.001 vs variables at the pre-CS level on evocation day 1 (post-hoc within subjects RMA for separate groups).

CS = conditioned stimulus, RMA=repeated measures analysis, CNE = conditioned-not-evoked, $FVC_{\%\ predicted}$ = forced volume capacity (as calculated percentage of predicted values), $FEV_{1\ \%\ predicted}$ = forced expiratory volume in 1 second (as calculated percentage of predicted values), BPM = beats per minute, PANAS PA = Positive Affect and Negative Affect Schedule – Positive Affect, STAI-S-s = State Trait Anxiety Index – State Anxiety, NRS = Numeric Rating Scales

Supplementary Table S4. Suspected medication intake in each session, and comparison of evocation vs. baseline itch by group.

		Open-label conditioned group (n=23) [A]	Closed-label conditioned group (n=23) [A]	Conditioned-not-evoked control group (n=23) [A]	Non-conditioned control group (n=22) [A]	Group comparison [C]			
						Open-label conditioned group included		Open-label conditioned group excluded	
						χ^2	*p*	χ^2	*p*
Acquisition									
Session 1.	Levocetirizine	73.9 (17)	30.4 (7)	34.8 (8)	59.1 (13)	11.63	.009	4.40	.11
	Placebo	26.1 (6)	69.6 (16)	65.2 (15)	40.9 (9)				
Session 2	Levocetirizine	73.9 (17)	39.1 (9)	34.8 (8)	45.5 (10)	8.57	.036	0.54	.76
	Placebo	26.1 (6)	60.9 (14)	65.2 (15)	54.5 (12)				
Session 3.	Levocetirizine	69.6 (16)	30.4 (7)	47.8 (11)	45.5 (10)	7.18	.066	1.68	.43
	Placebo	30.4 (7)	69.6 (16)	52.2 (12)	54.5 (12)				
Evocation									
Session 1.	Levocetirizine	17.4 (4)	39.1 (9)	34.8 (8)	50.0 (11)	5.47	.14	1.14	.57
	Placebo	82.6 (19)	60.9 (14)	65.2 (15)	50.0 (11)				
Session 2	Levocetirizine	17.4 (4)	47.8 (11)	39.1 (9)	54.5 (12)	7.45	.059	1.08	.58
	Placebo	82.6 (19)	52.2 (12)	60.9 (14)	45.5 (10)				
Session 3.	Levocetirizine	13.0 (3)	47.8 (11)	34.8 (8)	45.5 (10)	7.58	.056	0.91	.64
	Placebo	87.0 (20)	52.2 (12)	65.2 (15)	54.5 (12)				
Comparison of evocation vs. baseline itch									
Mean itch	A lot less itch	4.3 (1)	8.7 (2)	4.5 (1) [B]	9.1 (2)				
	Somewhat less itch	65.2 (15)	56.5 (13)	36.4 (8) [B]	54.5 (12)				
	Comparable itch	30.4 (7)	8.7 (2)	36.4 (8) [B]	13.6 (3)	13.41	.15	6.56	.36
	Somewhat more itch	0.0 (0)	26.1 (6)	22.7 (5) [B]	22.7 (5)				
	A lot more itch	0.0 (0)	0.0 (0)	0.0 (0) [B]	0.0 (0)				

Note. [A] depicted as % (n). [B] Corrected for n=1 missing values. [C] Groups were compared using Chi-Square tests.

Supplementary Table S5. Relation between suspected medication intake during the final evocation session and histamine iontophoresis outcome measures.

	Suspected medication intake during the final evocation session											
	Open-label conditioned group included						Open-label conditioned group excluded					
	Levocetirizine (n=32)		Placebo (n=59)		AN(C)OVA outcomes		Levocetirizine (n=29)		Placebo (n=39)		AN(C)OVA outcomes	
					p	$\eta^2_{partial}$					p	$\eta^2_{partial}$
Process measure												
Expected itch [A]	4.42	± 1.94	3.80	± 2.02	.76	< .01	4.45	± 1.90	4.18	± 1.90	.29	.02
Primary outcome												
Mean itch [A]	2.82	± 1.93	3.02	± 1.67	.054	.04	2.93	± 1.96	3.23	± 1.68	.016	.09
Secondary outcomes												
Subjective skin response [A]	23.06	± 11.82	25.42	± 13.44	.017	.06	24.00	± 12.01	26.21	± 13.44	.030	.07
Wheal area (cm^2) [B]	10.87	± 2.97	10.33	± 3.44	.59	< .01	10.85	± 3.11	9.96	± 3.56	.88	< .01
Flare area (cm^2) [B]	46.34	± 14.12	44.74	± 11.52	.59	< .01	45.15	± 13.92	44.99	± 11.05	.86	< .01
Change in skin temperature (°C) [A,C]	1.33	± 1.70	1.13	± 1.54	.54	< .01	1.28	± 1.76	0.99	± 1.36	.43	.01

Note. [A] Analysis corrected for pre-conditioning (baseline) variable. [B] Analysis corrected for pre-conditioning (baseline) variable, as well as for the amount of time passed between histamine iontophoresis and measurement of the variable. [C] Calculated as post-histamine iontophoresis skin temperature – control.

Supplementary Table S6. Means and standard deviations of the individual characteristics of the sample group, with analysis of variance (ANOVA) outcome and calculated Cronbach's alpha for the subscales.

	Combined groups				**Separate groups**						
			ANOVA						ANOVA		
	Conditioned groups (*n*=46)	Control groups (*n*=45)	F	*p*	Open-label conditioned group (*n*=23)	Closed-label conditioned group (*n*=23)	Conditioned-not-evoked control group (*n*=23)	Non-conditioned control group (*n*=22)	F	*p*	Cronbach's α scale
Optimism [A]	18.33 ± 2.72	16.93 ± 2.67	6.07	.016	18.17 ± 2.67	18.48 ± 2.81	16.65 ± 2.96	17.23 ± 2.37	2.21	.093	.68
Perceived stress [B]	8.83 ± 4.28	9.76 ± 4.26	1.08	.30	8.52 ± 4.09	9.13 ± 4.54	9.61 ± 4.08	9.91 ± 4.55	0.45	.72	.78
Worrying [C]	37.93 ± 10.14	38.84 ± 10.90	0.17	.68	38.39 ± 9.57	37.48 ± 10.88	37.87 ± 10.91	39.86 ± 11.05	0.22	.89	.92
Behavioral activation: drive [D]	10.30 ± 2.44	11.02 ± 1.94	2.40	.13	10.13 ± 2.77	10.48 ± 2.11	10.74 ± 1.91	11.32 ± 1.99	1.14	.34	.70
Behavioral activation: fun seeking [D]	10.50 ± 1.72	10.91 ± 1.92	1.16	.29	10.39 ± 1.73	10.61 ± 1.75	10.87 ± 2.18	10.95 ± 1.65	0.44	.73	.46
Behavioral activation: reward responsiveness [D]	17.24 ± 1.77	16.76 ± 1.72	1.75	.19	17.43 ± 1.70	17.04 ± 1.85	17.30 ± 1.77	16.18 ± 1.50	2.42	.072	.53
Behavioral inhibition [D]	18.57 ± 4.03	18.44 ± 4.11	0.02	.89	19.35 ± 4.18	17.78 ± 3.80	18.35 ± 4.01	18.55 ± 4.31	0.58	.63	.83

Note. [A] Assessed by the Life Orientation Test – revised (LOT-R [24], [B] Assessed by the Perceived Stress Scale (PSS [25], [C] Assessed by the Penn State Worry Questionnaire (PSWQ [26], [D] Assessed by the Behavioural Inhibition System / Behavioural Approach System scales (BIS/BAS scales [21]

Supplementary Table S7. Moderation by individual characteristics for the effects of the combined conditioned groups on self-reported itch during iontophoresis in the evocation phase, controlled for baseline, using the PROCESS moderation method.

Variable	Coefficient	t	*p*	Bootstrap LLCI	Bootstrap ULCI	R-square model
Model 1: moderation by optimism [A]						
Conditioning (group)	-0.39	-1.67	.11	-0.88	0.09	
Optimism [B]	0.07	1.14	.26	-0.05	0.20	.62
Conditioning x optimism	-0.09	-1.01	.31	-0.27	0.09	
Model 2: moderation by perceived stress [A]						
Conditioning (group)	-0.34	-1.41	.16	-0.81	0.14	
Perceived stress [C]	0.03	0.79	.43	-0.05	0.11	.61
Conditioning x perceived stress	-0.05	-0.90	.37	-0.16	0.06	
Model 3: moderation by worrying [A]						
Conditioning (group)	-0.33	-1.40	.16	-0.80	0.14	
Worrying [D]	-0.02	-1.16	.25	-0.05	0.01	.61
Conditioning x worrying	0.03	1.15	.25	-0.02	0.07	
Model 4: moderation by BAS drive [A]						
Conditioning (group)	-0.38	-1.59	.12	-0.85	0.10	
BAS drive [E]	0.07	0.85	.40	-0.10	0.25	.61
Conditioning x BAS drive	-0.15	-1.38	.17	-0.37	0.07	
Model 5: moderation by BAS fun seeking [A]						
Conditioning (group)	-0.36	-1.51	.13	-0.84	0.11	
BAS fun seeking [E]	-0.06	-0.70	.49	-0.25	0.12	.61
Conditioning x BAS fun seeking	0.04	0.27	.78	-0.23	0.30	
Model 6: moderation by BAS reward responsiveness [A]						
Conditioning (group)	-0.36	-1.52	.13	-0.82	0.11	
BAS reward responsiveness [E]	0.12	1.21	.23	-0.08	0.31	.63
Conditioning x BAS reward responsiveness	-0.27	-1.96	.053 †	-0.54	0.003	
Model 7: moderation by behavioral inhibition (BIS) [A]						
Conditioning (group)	-0.34	-1.44	.15	-0.81	0.13	
BIS [E]	0.01	0.24	.81	-0.07	0.09	.61
Conditioning x BIS	0.03	0.50	.62	-0.09	0.15	

Note. [A] Model controlled for mean itch during baseline histamine iontophoresis. In all models, itch during baseline iontophoresis was strongly related to itch during evocation (all $p < .001$). This association causes the high explained variance in the model. [B] Assessed by the Life Orientation Test – revised (LOT-R [24], [C] Assessed by the Perceived Stress Scale (PSS [25], [D] Assessed by the Penn State Worry Questionnaire (PSWQ [26], [E] Assessed by the Behavioural Inhibition System / Behavioural Approach System scales (BIS/BAS scales [21]. † $p<.10$. LLCI = lower limit confidence interval. ULCI = upper limit confidence interval.

Supplementary Table S8. Moderation by individual characteristics for the effects of the separate groups on self-reported itch during iontophoresis in the evocation phase, controlled for baseline, using the PROCESS moderation method.

Variable	Coefficient	t	*p*	Bootstrap LLCI	Bootstrap ULCI	R-square model
Model 1: moderation by optimism:						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.46	-1.36	.18	-1.13	0.21	
Optimism [B]	> -0.01	-0.01	.99	-0.10	0.10	.62
Conditioning x optimism	0.11	1.06	.29	-0.10	0.31	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	0.05	0.15	.88	-0.62	0.72	
Optimism [B]	0.09	1.75	.084 †	-0.01	0.19	.64
Conditioning x optimism	-0.23	-2.35	.021 *	-0.42	-0.04	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.35	1.00	.32	-0.35	1.04	
Optimism [B]	< 0.01	0.09	.93	-0.10	0.11	.62
Conditioned-not-evoked x optimism	0.07	0.73	.47	-0.12	0.26	
Model 2: moderation by perceived stress						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.47	-1.41	.16	-1.14	0.94	
Perceived stress [C]	0.03	1.01	.32	-0.03	0.09	.63
Conditioning x perceived stress	-0.12	-1.79	.077 †	-0.25	0.01	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	0.02	0.05	.96	-0.66	0.70	
Perceived stress [C]	< 0.01	-0.13	.90	-0.07	0.06	.62
Conditioning x perceived stress	0.04	0.54	.59	-0.09	0.16	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.27	0.80	.43	-0.40	0.94	
Perceived stress [C]	< 0.01	0.10	.92	-0.06	0.07	.62
Conditioned-not-evoked x perceived stress	0.01	0.16	.87	-0.12	0.14	
Model 3: moderation by worrying						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.42	-1.24	.22	-1.09	0.25	
Worrying [D]	-0.01	-0.45	.65	-0.03	0.02	.62
Conditioning x worrying	0.01	0.18	.86	-0.05	0.06	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	0.02	0.07	.94	-0.65	0.70	
Worrying [D]	-0.01	-0.94	.35	-0.04	0.01	.62
Conditioning x worrying	0.03	1.13	.26	-0.02	0.08	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.25	0.75	.45	-0.42	0.92	
Worrying [D]	> -0.01	-0.04	.97	-0.03	0.03	.62
Conditioned-not-evoked x worrying	-0.02	-0.61	.54	-0.07	0.04	
Model 4: moderation by BAS drive						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.46	-1.33	.19	-1.14	0.23	
BAS drive [E]	0.01	0.08	.94	-0.13	0.14	.62
Conditioning x BAS drive	-0.06	-0.57	.57	-0.28	0.16	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	-0.03	-0.09	.93	-0.71	0.65	
BAS drive [E]	0.01	0.22	.83	-0.11	0.14	.62
Conditioning x BAS drive	-0.15	-1.12	.26	-0.40	0.11	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.27	0.79	.43	-0.40	0.94	
BAS drive [E]	-0.04	-0.67	.50	-0.16	0.08	.62
Conditioned-not-evoked x BAS drive	0.11	0.80	.43	-0.17	0.39	

Supplementary Table S8. *Continued (2/2)*

Variable	Coefficient	t	*p*	Bootstrap LLCI	Bootstrap ULCI	R-square model
Model 5: moderation by BAS fun seeking						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.43	-1.28	.20	-1.11	0.24	
BAS fun seeking [E]	-0.05	-0.72	.47	-0.20	0.10	.62
Conditioning x BAS fun seeking	0.03	0.20	.84	-0.28	0.34	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	-0.01	-0.02	.98	-0.68	0.67	
BAS fun seeking [E]	-0.05	-0.59	.55	-0.20	0.11	.62
Conditioning x BAS fun seeking	> -0.01	-0.01	.99	-0.33	0.33	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.26	0.78	.44	-0.41	0.93	
BAS fun seeking [E]	-0.05	-0.59	.56	-0.21	0.11	.62
Conditioned-not-evoked x BAS fun seeking	< 0.01	0.02	.97	-0.27	0.28	
Model 6: moderation by BAS reward responsiveness						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.37	-1.08	.28	-1.05	0.31	
BAS reward responsiveness [E]	0.04	0.52	.61	-0.12	0.20	.63
Conditioning x BAS reward responsiveness	-0.28	-1.73	.087 †	-0.60	0.04	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	0.03	0.09	.93	-0.66	0.72	
BAS reward responsiveness [E]	-0.02	-0.21	.83	-0.18	0.15	.62
Conditioning x BAS reward responsiveness	-0.03	-0.22	.83	-0.34	0.27	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.34	1.00	.32	-0.33	1.01	
BAS reward responsiveness [E]	-0.13	-1.58	.12	-0.29	0.03	.64
Conditioned-not-evoked x BAS reward responsiveness	0.37	2.37	.020 *	0.06	0.67	
Model 7: moderation by behavioral inhibition (BIS)						
Open-label conditioned group dummy [A]						
Open-label conditioning	-0.41	-1.22	.23	-1.08	0.26	
BIS [E]	0.04	1.28	.21	-0.02	0.11	.62
Conditioning x BIS	-0.05	-0.72	.47	-0.18	0.08	
Closed-label conditioned group dummy [A]						
Closed-label conditioning	0.12	0.36	.72	-0.55	0.79	
BIS [E]	0.01	0.19	.85	-0.06	0.07	.63
Conditioning x BIS	0.12	1.64	.10	-0.02	0.25	
Conditioned-not-evoked control group dummy [A]						
Conditioned-not-evoked	0.29	0.83	.41	-0.39	0.95	
BIS [E]	0.03	0.95	.35	-0.04	0.10	.62
Conditioned-not-evoked x BIS	> -0.01	-0.06	.95	-0.14	0.13	

Note. Dummy variables were computed with the non-conditioned control group as reference category. [A] Models controlled for mean itch during baseline histamine iontophoresis, and other dummy variables. In all models, itch during baseline iontophoresis was strongly related to itch during evocation (all $p < .001$). This association causes the high explained variance in the model. [B] Assessed by the Life Orientation Test – revised (LOT-R [24], [C] Assessed by the Perceived Stress Scale (PSS [25], [D] Assessed by the Penn State Worry Questionnaire (PSWQ [26], [E] Assessed by the Behavioural Inhibition System / Behavioural Approach System scales (BIS/BAS scales [21]. † $p<.10$; * $p<.05$. LLCI = lower limit confidence interval. ULCI = upper limit confidence interval.

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